UNIVERSITÉ FRANÇOIS - RABELAIS

DE TOURS

ÉCOLE DOCTORALE : Santé, Sciences et Techniques

Inserm U930 Equipe 4

THÈSE présentée par :

Nylza Yadira IBARGUEN VARGAS

soutenue le : **4 juillet 2008**

pour obtenir le grade de : **Docteur de l'université François - Rabelais**

Discipline/ Spécialité de : Sciences de la vie et santé / Neurosciences

Implication du BDNF dans l'etiopathogenèse et le traitement des troubles anxio-dépressifs

Aspects précliniques

THÈSE dirigée par :

Mme BELZUNG Catherine	Professeur des universités, Université F. Rabelais (Tours)

RAPPORTEURS :

Mr LASSALLE Jean-Michel	Professeur des universités, Université P. Sabatier (Toulouse)
Mme THIEBOT Marie-Hélène	Directeur de recherche, Inserm (Paris)

JURY :

Mme BELZUNG Catherine	Professeur des universités, Université F. Rabelais (Tours)
Mr CAMUS Vincent	Professeur des universités (PU-PH), Université F.Rabelais (Tours)
Mr GARDIER Alain	Professeur des universités, Université Paris-Sud (Chatenay-Malabry)
Mr LASSALLE Jean-Michel	Professeur des universités, Université P. Sabatier (Toulouse)
Mme THIEBOT Marie-Hélène	Directeur de recherche, Inserm (Paris)
Mr SPEDDING Michaël	Professeur, Institut de Recherche International, Servier (Paris)

REMERCIEMENTS

Le Professeur Catherine BELZUNG a dirigé ce travail avec dynamisme et professionnalisme. Je tiens à lui exprimé toute ma gratitude pour avoir cru en moi et m'avoir acceptée dans son équipe, ainsi qu'à la scientifique éminente pour son enthousiasme, pour ses conseils toujours pertinents, pour son indulgence par rapport à ma maîtrise approximative du français (surtout au début) et ses encouragements continus ; mais surtout merci pour ton amitié.

****

Le Docteur Marie-Hélène THIEBOT et le Professeur Jean-Michel LASSALLE ont accepté d'être les rapporteurs de cette thèse. Je les remercie d'avoir accordé du temps et toute leur attention à ce travail. Je tiens surtout à leur exprimer toute mon estime pour l'honneur qu'ils me font d'éclairer ce travail de leur excellence scientifique. Nul doute que leurs commentaires et leurs critiques enrichiront ce manuscrit et mon questionnement scientifique.

****

Le Professeur Vincent CAMUS, le Professeur Alain GARDIER et le Professeur Michael SPEDDING ont accepté de rejoindre le jury de cette thèse. Je me sens extrêmement honorée de leur participation et pour cela je tiens à leur exprimer toute ma reconnaissance.

****

Je tiens à exprimer spécialement toute ma gratitude au Docteur Wissam EL-HAGE pour ses conseils avisés et sa présence dans les bons moments mais aussi son soutien dans les moments plus difficiles. Encore merci pour tes encouragements et ton positivisme en toutes occasions !

****

Je remercie Dominique LEGLAUNEC et Anne-Marie LEGUISQUET pour leur disponibilité et leur aide précieuse au quotidien mais aussi le Docteur Véronique BOZON et Maryse PINGAUD pour leur gentillesse dès mon arrivée à l'Université F.Rabelais et depuis au laboratoire.

****

Merci au Professeur Christian ANDRES et à son équipe de m'avoir permis de travailler dans son laboratoire lors de nos projets collaboratifs. Je tiens plus particulièrement à remercier le Docteur Patrick VOURC'H pour sa disponibilité et sa participation à plusieurs de nos expériences. Un grand merci aussi à Catherine ANTAR, Rose-Anne THEPAULT et Sylviane VEDRINE.

****

Je remercie également très sincèrement le Docteur Pascal BARONE, Raymond JEGAT, le Docteur Samuel LEMAN ainsi que les autres membres du laboratoire Bruno BRIZARD, le Docteur Frédéric MINIER, tous les doctorants et stagiaires. Je souhaite exprimer particulièrement toute ma gratitude au Docteur Caroline VEUILLE et au Professeur LE PAPE d'avoir partagé leur bureau et pour nos échanges instructifs et amicaux. Et je n'oublie pas les autres membres du DESCO dont le Professeur Christine ERRARD, le Docteur Nadine FRESQUET, le Professeur Alain LENOIR et le Docteur Jean-Luc MERCIER. Merci beaucoup à vous tous pour votre accueil et de rendre plus agréable le laboratoire.

****

À Boriana ATANASOVA, François CHRISTMANN, Stephanie COUBARD, Séverine DEVERS, Walter SAULQUIN, Danival SOUZA et Ipek YALCIN. Merci parce que vous êtes la preuve que partout et dans toutes les circonstances on peut trouve des amis (l'ordre n'est qu'alphabétique !!).

****

Alexandre SURGET j'ai tout plein de mercis pour toi ; merci de m'avoir appris le français, de m'avoir aidé en toutes circonstances et de me communiquer ton enthousiasme, de ta joie de

vivre et d’avoir changé ma vie. Gracias, porque tú eres un regalo precioso que me ha dado la vida.

****

À ma famille “d’ici” et sur tout à mes beaux-parents, Dominique et Jacques, pour leur soutien inconditionnel et pour m’accompagner depuis le début dans ce pays.

****

Gracias Anissa SURGET-IBARGUEN porque así tú no lo sepas aun, llegaste a mi vida para darle alegría, para enseñarme a disfrutar de las pequeñas y de las grandes cosas de la vida de todos los días.

****

Gracias Berardo de Jesús RODRIGUEZ porque desde siempre, has estado a mi lado para apoyarme y quererme, porque tú tienes razón cuando dices que el tiempo y las circunstancias cambian, pero nosotros vivimos como dando la vuelta en el bus y seguimos siendo amigos.

****

Gracias Héctor José CARO BOTERO por todos estos años de amistad, que me han permitido ver la vida desde otra perspectiva y por mostrarme que la vida, hay que vivirla con tranquilidad como si siempre fuéramos de tenis blancos.

****

Gracias a todos mis amigos de mi país por seguirlo siendo a pesar de la distancia, por darme animo, apoyarme, escribirme y tenerme presente en todos estos años de ausencia.

****

Gracias Familia, a todos y cada uno de ustedes, porque desde la distancia (que no es sino geográfica) me acompañan cada uno de mis días, con su amor y sus oraciones me dan la fuerza para seguir adelante y disfrutar de la vida. Gracias también por mantenerme presente en la vida cotidiana de la familia, con fotos, webcam, mensajes, llamadas y todos los medios posibles.

****

Sommaire

RESUME **4**

LISTE DES ABREVIATIONS **5**

INTRODUCTION **6**

1. Les troubles anxio-dépressifs 7

1.1. Le trouble dépressif *7*

1.2. Les troubles anxieux *8*

1.3. Comorbidité anxiété-dépression *10*

2. Neurobiologie des troubles anxio-dépressifs 10

2.1. Neurobiologie de la dépression *10*

2.2. Neurobiologie de l'anxiété *17*

3. Les traitements des troubles anxio-dépressifs 22

3.1. Les traitements des troubles dépressifs *23*

3.2. Les traitements des troubles anxieux *27*

4. Le BDNF 30

4.1. Les Neurotrophines *30*

4.2. Caractérisation du BDNF *32*

4.3. Fonction du BDNF *36*

5. Les modèles animaux 44

5.1. Modéliser la dépression *45*

5.2. Les modèles animaux d'anxiété *51*

Objectifs de la thèse 58

Article 1 - Association of a mouse BDNF polymorphism (Leu32Met) and the behavioral phenotypes in six inbred strains **60**

Article 2 - Multifaceted strain-specific effects in a chronic mouse model of depression and of antidepressant reversal **80**

Discussion complémentaire de l'article 2 108

Article 3 - Deficit in BDNF level does not increase vulnerability to stress but dampens antidepressant-like effects in the unpredictable chronic mild stress **111**

DISCUSSION GENERALE **135**

1. Le polymorphisme Leu32Met du BDNF 137

2. BDNF et anxiété 139

3. BDNF et dépression 142

3.1. BDNF et état dépression-like *143*

3.2. BDNF et antidépresseurs *145*

Conclusion 148

BIBLIOGRAPHIE **150**

ANNEXES **180**

Article 4 - Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. 181

Article 5 - Corticolimbic transcriptome changes are state-dependent and region-specific in a rodent model of depression and of antidepressant reversal. 182

Article 6 - Lack of serotonin1B receptor expression leads to age-related motor dysfunction, early onset of brain molecular aging and reduced longevity 183

RESUME

Bien que les troubles anxio-dépressifs représentent une des principales causes d'invalidité et un des plus sérieux problèmes de santé dans le monde, les mécanismes neurobiologiques à l'origine de ces affections demeurent méconnus. Les processus mis en jeu semblent multiples et complexes : passant par des déséquilibres au niveau des neurotransmetteurs jusqu'à des modifications de la plasticité neurale et du remodelage cellulaire. Les neurotrophines étant considérées comme les principaux régulateurs de la plasticité, l'hypothèse d'un lien causal entre le niveau de neurotrophine, principalement de l'une d'entre elles, le Brain-Derived Neurotrophic Factor (BDNF) et l'apparition de troubles anxio-dépressifs a ainsi été proposée. Notre travail a donc eu pour objectif l'étude de l'implication du BDNF dans l'étiopathogenèse et le traitement des troubles anxio-dépressifs à travers l'utilisation de modèles murins.

Etant donné que plusieurs études cliniques et précliniques ont montré une implication du BDNF dans plusieurs traits psychologiques et comportementaux, nous avons cherché à déterminer si des différences dans le gène BDNF pouvaient être à l'origine de la grande hétérogénéité comportementale des différentes souches de souris. Nous rapportons dans cette étude l'existence d'un polymorphisme sur un seul nucléotide (SNP) à l'origine d'un changement d'acide aminé (une leucine est remplacé par une méthionine) en position 32 dans la séquence du prodomaine du BDNF. Nous démontrons ensuite que, bien que ce SNP ne modifie par l'expression basale de BDNF dans le cerveau, ce polymorphisme est associé au phénotype « anxieux » des souris. Par contre, il n'est pas impliqué dans le comportement alimentaire, le toilettage, l'activité, l'apprentissage et la mémoire. Une analyse précise des données montre que les souris portant l'allèle Met présentent une propension plus importante à développer des réactions néophobiques et des comportements d'anxiété de trait. Ainsi, ce polymorphisme pourrait contribuer aux différences de profils comportementaux des différentes lignées de souris dans les tests d'anxiété.

Ensuite, nous avons réalisé une comparaison de sept lignées de souris dans un modèle chronique de la dépression et de l'action thérapeutique des antidépresseurs. Ce paradigme, le stress chronique léger imprédictible (UCMS), modélise l'influence des stress socio-environnementaux dans l'étiopathogenèse de la dépression et est sensible à l'action chronique des antidépresseurs. Nous montrons dans cette étude que l'application d'un même facteur étiologique (les stresseurs) est capable d'induire presque autant de profils différents d'altérations physiques, comportementales et physiologiques, et de réponses au traitement que de lignées de souris testées. De plus, les différents profils obtenus se rapprochent des différents sous-types de la pathologie humaine suggérant que le patrimoine génétique des patients pourrait être déterminant dans l'apparition clinique d'un sous-type particulier. Par contre, aucune association entre le polymorphisme Leu32Met du BDNF et les altérations « dépression-like » ou la réponse au traitement antidépresseur n'émerge de notre étude.

Nous avons enfin étudié l'effet d'un déficit en BDNF sur la vulnérabilité au stress chronique et la réponse aux antidépresseurs par l'utilisation de souris hétérozygotes BDNF $^{+/-}$. Nous montrons ici que l'expression monoallélique du BDNF n'induit pas de différences dans la sensibilité à l'UCMS sur toutes les mesures physiques (état du pelage, poids), comportementales (alimentations, activité, anxiété, agressivité, résignation) et neuroendocrines (corticostéronémie). Par contre, certains effets du traitement sont perturbés chez les souris BDNF $^{+/-}$. Finalement, notre étude suggère que le BDNF n'est pas impliqué dans l'apparition des altérations « dépression-like » mais qu'il est nécessaire à l'efficacité des antidépresseurs.

Ce travail a donc permis de mettre en évidence une relation entre le BDNF et les comportements anxieux ainsi que l'action des antidépresseurs. De plus, en utilisant deux modèles différents (le polymorphisme d'un gène ou son ablation partielle), nous avons montré l'absence de lien entre le BDNF et l'étiopathogenèse de la dépression.

LISTE DES ABREVIATIONS

5-HT : 5-hydroxytryptamine

ACTH : adrenocorticotropin hormone (hormone adrénocorticotrope)

BDNF : brain-derived neurotrophic factor

BZDs : benzodiazépines

CCK : cholécystokinine

CRF : corticotrophin-releasing factor or hormone (corticolibérine)

DA : dopamine

GABA : acide γ(gamma)-amino butyrique

HPA: hypothalamus-pituitary-adrenals (hypothalamus-hypophyse-surrénale)

IMAO : inhibiteurs de la monoamine oxydase

ISRS : inhibiteur de la recapture de sérotonine

LTP : long term potentiation (potentialisation à long terme)

MDTB : mouse defense battery test

NA : noradrénaline

NSF test : novelty-suppressed feeding test

NT : neurotrophine

PTSD : trouble du stress post-traumatique

PVN : noyau paraventriculaire

SNP : single nucleotide polymorphism (polymorphisme sur un seul nucléotide)

TrK : récepteur à tyrosine kinase

UCMS: unpredictable chronic mild stress (stress chronique léger impredictible)

INTRODUCTION

1. Les troubles anxio-dépressifs

Les troubles anxio-dépressifs sont les désordres psychiatriques ayant la plus forte prévalence dans la population générale. En effet, on estime que plus de 20% de la population des pays industrialisés va souffrir de ce trouble à un moment de sa vie (Nestler *et coll.*, 2002a, Valle Fernandez, 2002). En outre, parmi les patients atteints d'un trouble anxieux, 30% développeront une dépression secondaire ou bien même présentent une dépression comorbide au trouble anxieux. Habituellement, on considère que la dépression et l'anxiété sont des entités nosologiques indépendantes mais dans la clinique il est plutôt rare de trouver une pathologie strictement confinée à l'un des deux troubles. En effet, ces deux désordres partagent des symptômes communs; ce qui contribue sans doute à leur comorbidité. Ces observations ont conduit à une modification de la classification des troubles anxio-dépressifs au cours de ces dernières années (Valle Fernandez, 2002).

1.1. Le trouble dépressif

La notion de dépression, en tant qu'entité pathologique, s'est construite au cours du temps. Hippocrate, au Vème siècle avant JC, a été l'un des premiers auteurs à avoir décrit cette pathologie. Pour lui, il s'agissait d'un déséquilibre des fluides du corps qu'il attribuait à un excès de bile noire, d'où son nom : la mélancolie. Au Ier siècle après JC, Galien et Arète de Cappadoce considèrent la mélancolie et la manie comme des troubles associés. Plus tard, au cours du Moyen Age, cette maladie est considérée comme liée à une cause diabolique. Il a fallu attendre 1725 pour trouver pour la première fois le mot « dépression » sous la plume du britannique Richard Blackmore. Au cours du XXème siècle, l'intérêt se porte sur la classification des troubles psychiatriques. L'une des premières contributions dans ce domaine est un article d'Emil Kraepelin, paru en 1921 et intitulé « *Maniac depressive insannity and paranoia* ». Bien que ne mentionnant pas explicitement la dépression, (Krapelin distingue la folie, la schizophrénie et la psychose maniaco-dépressive), il s'agit d'un premier pas dans la direction de la classification de ces désordres en entités nosologiques distinctes. Ces travaux allaient aboutir à des outils diagnostiques comme ceux proposés par l'Organisation Mondiale de la Santé (OMS) ou par l'Association Américaine de Psychiatrie (les différentes versions du DSM). Ces derniers sont les plus utilisés actuellement dans le domaine de la recherche. Ce n'est que dans la version la plus récente, le DSM-IV, qu'apparaît l'idée de distinguer les

différents types de dépression. Ainsi, actuellement, on distingue la dépression majeure, la dépression aigüe, la dépression épisodique, etc. (American Psychiatric Association, 2000).

Le DSM-IV (1994) définit le trouble dépressif majeur par la présence de un ou plusieurs épisodes de dépression majeure sans antécédent d'épisode maniaque, mixte ou hypomaniaque. Un épisode majeur doit durer au moins deux semaines et se caractérise par la présence d'au moins cinq symptômes sur les neuf suivants:

1. Humeur dépressive,
2. Diminution de l'intérêt et du plaisir,
3. Perte d'appétit et de poids d'au moins 5 % par mois,
4. Insomnie ou hypersomnie,
5. Agitation ou retard au niveau psychomoteur,
6. Fatigue et perte d'énergie,
7. Sentiment de culpabilité ou manque de valorisation de soi,
8. Trouble de la concentration,
9. Pensée de mort et de suicide.

La dépression majeure est un trouble psychiatrique caractérisé par une forte prévalence et fréquemment sous diagnostiqué. En outre, lorsqu'il a été diagnostiqué, le traitement préconisé est parfois mal adapté. Son incidence est estimée à 5% de la population mondiale, et, dans les pays développés, la prévalence peut atteindre de 17 à 20% (Nestler *et coll.*, 2002b, Tamminga *et coll.*, 2002). La dépression est plus fréquente dans le sexe féminin. D'après l'OMS, la dépression est la principale cause de handicap[1] mondial. Ce même organisme estime que la dépression est actuellement la 4ème pathologie la plus fréquente et qu'en 2020 il s'agira de la 2ème pathologie la plus fréquente après les troubles cardiovasculaires. Ainsi, la dépression est à la fois un vrai problème de santé publique et un problème économique en raison de l'absentéisme au travail induit par la maladie et du coût des traitements. En effet, les antidépresseurs représentent 51% des prescriptions de psychotropes et, d'après une étude pilotée par la SOFRES et parue en 1996, 3.4% des français consomment des antidépresseurs.

1.2. Les troubles anxieux

L'anxiété est un état émotionnel susceptible d'apparaître chez chaque individu lorsqu'il est confronté à une situation comportant une menace potentielle. Lorsque cet état perdure dans le temps et se manifeste dans des situations non anxiogènes et sans proportion avec des

[1] Il s'agit ici de la définition du handicap de l'OMS

causes objectives, on considère qu'il s'agit d'un trouble anxieux. Ces troubles sont décrits dans le DSM-IV et concerne les pathologies suivantes :

-Trouble panique sans agoraphobie

-Trouble panique avec agoraphobie

-Agoraphobie sans antécédent de trouble panique

- Phobie spécifique

- Phobie sociale

- Trouble obsessionnel compulsif

- Trouble de stress post-traumatique

- Trouble lié à un état de stress aigu

- Trouble de l'anxiété généralisée

- Trouble anxieux dû à une affection médicale générale

- Trouble anxieux induit par une substance

- Trouble anxieux non spécifié.

Parmi les troubles décrits ci-dessus, l'anxiété généralisée est la pathologie ayant la plus forte prévalence (Tamminga *et coll.*, 2002). En effet, on estime que 30% des patients ayant un trouble anxieux sont atteints d'anxiété généralisée. Les autres troubles anxieux sont caractérisés par une fréquence moindre : le troubles obsessionnel-compulsif touche 1.7% des patients ayant un trouble anxieux, la phobie sociale concerne de 1 à 3.5 % des patients, l'agoraphobie concerne de 1.3 à 3% des patients, le stress post-traumatique touche 17% de ces patients. Ceci montre qu'il s'agit d'un groupe de pathologies très fréquentes dans la population(Hunkeler *et coll.,* 2003).

1.3. Comorbidité anxiété-dépression

La relation entre la dépression et les troubles anxieux est mal évaluée. L'anxiété n'est pas un critère de diagnostic pour la dépression majeure (DSM-IV) alors que la dépression et les troubles anxieux sont clairement reconnus comme des maladies distinctes. Cependant, la question d'un continuum n'a jamais été abandonnée, et a été décrite chez de nombreux patients où coexistent des symptômes anxieux et dépressif (Angst *et coll.*, 1997).

Leibowitz a bien décrit la co-occurrence des deux troubles anxieux et de la « dépression », et recommande la prise en compte de symptômes anxieux pour une évaluation plus appropriée de la dépression dans le but d'optimiser les stratégies thérapeutiques et le taux de guérison. En effet, les patients dépressifs qui présentent des niveaux élevés d'anxiété mettent plus de temps à se rétablir, présentent une récurrence plus forte des épisodes dépressifs, un taux plus important de traitements médicamenteux multiples et un risque de suicide plus élevé que les personnes souffrant de dépression sans symptômes anxieux (Liebowitz, 1993).

La prévalence du trouble comorbide anxiété/dépression serait de 10 à 20% ; toutefois, d'autres travaux rapportent une prévalence plus faible (Rouillon, 1999). L'épidémiologie des troubles psychiatriques montre que 50 à 60% de patients diagnostiques pour une dépression majeure disent avoir expérimenté un ou plusieurs troubles anxieux antérieurement à la survenue de leur trouble dépressif (Fava *et coll.*, 2000), suggérant qu'il pourrait s'agir d'un facteur de vulnérabilité favorisant une dépression. Des études sur les sous-types d'anxiété et de dépression montrent une forte association entre les troubles de panique ou une anxiété généralisée et un trouble dépressif majeure (comorbidité respective de 56-73% et 62-67%) (Kaufman et Charney, 2000), ce qui précise un peu la nature de ce trouble.

2. Neurobiologie des troubles anxio-dépressifs

2.1. Neurobiologie de la dépression

2.1.1. Bases neuro-anatomiques

Les modèles neurobiologiques actuels du trouble dépressif sont le résultat de plusieurs décennies d'investigations ayant pour objectif d'éclairer l'étiologie et le traitement de ce trouble. Les recherches initiales s'appuyaient sur l'observation de l'efficacité clinique des antidépresseurs et se sont donc focalisées sur les cibles de ces traitements, en particulier sur l'étude des récepteurs et des neurotransmetteurs. Les aires cérébrales d'intérêt étaient donc

celles dans lesquelles se trouvaient ces cibles moléculaires. Au fur et mesure de la progression dans la connaissance des altérations associées aux états dépressifs, la caractérisation du réseau cérébral altéré dans ce trouble s'est élargi. Ainsi, l'observation d'un déficit de l'axe neuroendocrinien du stress a conduit à la notion de l'implication de l'hypothalamus, l'aire cérébrale qui orchestre la libération des hormones du stress (Van Den Hoofdakker, 1994, Zhou *et coll.*, 2001). D'autres progrès sont liés à l'apparition de nouvelles techniques d'exploration cérébrale comme la Tomographie par Emission de Positons (TEP) ou l'Imagerie par Résonance Magnétique (IRM) qui ont permis de montrer l'implication du cortex préfrontal, du striatum ou de l'amygdale (Dougherty *et coll.*, 2004, Driessen *et coll.*, 2000, Thomas *et coll.*, 2001). Petit à petit, les connaissances sur ce réseau se sont précisées, aboutissant à la description de l'implication de zones de plus en plus spécifiques, comme par exemple l'implication de la partie la plus antérieure du gyrus cingulaire dans la dépression familiale (Drevets *et coll.*, 1997, Hirayasu *et coll.*, 1999), celle des aires dorsolatérale et dorsomédiale du cortex préfrontal dans la modulation de l'expression émotionnelle et de l'anxiété dans les états dépressifs ou celle du striatum ventral dans les mécanismes de récompense et la régulation de l'humeur (Cotter *et coll.*, 2002, Drevets, 1999).

2.1.2. Hypothèses

Pour comprendre la physiopathologie des troubles anxio-dépressifs et éviter des interprétations trop réductionnistes, il est nécessaire d'avoir une vision de l'intrication des différents niveaux d'intégration (systémique, cellulaire, moléculaire) dans les événements impliqués dans l'étiologie et le déroulement de la pathologie. Les facteurs impliqués dans ces différents niveaux interagissent entre eux et contribuent tous à l'explication de la maladie. Par exemple, l'altération de l'expression d'une protéine ou d'un groupe de protéines peut affecter le fonctionnement cellulaire, aboutissant à une altération de la neuroplasticité qui, à son tour, va agir sur les circuits neuronaux chargés de réguler l'humeur. Ces dysfonctionnements moléculaires peuvent donc produire des symptômes, qui se traduisent par une pathologie. La figure 1 fournit une illustration de ces mécanismes (Manji et Lenox, 2000).

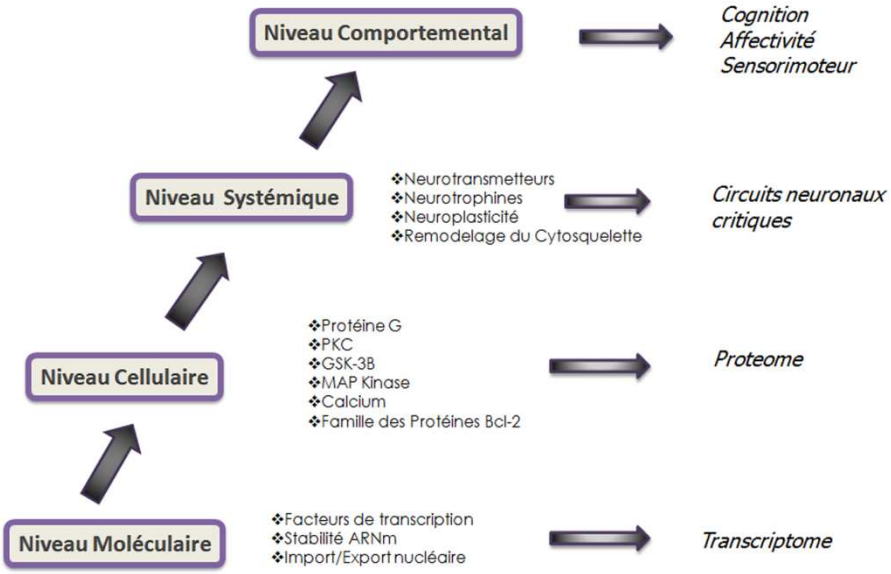

Figure 1. Modèle étiophysiopathologique des troubles anxio-dépressif, montrant différents niveaux d'organisation impliqués (non exhaustif). Modifié d'après Manji et Lenox (2000).

2.1.2.1. Hypothèse monoaminergique

L'explication de l'étiologie du trouble dépressif a commencé au milieu du XXème siècle grâce à l'utilisation de la réserpine, un alcaloïde utilisé dans le traitement de l'hypertension artérielle. En effet, l'administration de cette substance produisait une altération de l'humeur et des symptômes dépressifs chez 15% des patients traités associée à une déplétion de la neurotransmission monoaminergique cérébrale. Ces observations ont abouti à l'hypothèse qu'un déficit monoaminergique pourrait être à l'origine de la dépression. Cette hypothèse allait recevoir un crédit supplémentaire avec la mise en évidence des propriétés antidépressives d'inhibiteurs de la monoamine oxydase (IMOA) comme celle de l'isoniazide, un traitement antituberculeux. En effet, les IMAOs agissent en augmentant la concentration de monoamines comme la sérotonine et la noradrénaline dans le cerveau. Les effets de ces substances ont d'abord été testés chez des patients atteints de tuberculose, et ont ensuite été étudiés chez des dépressifs non tuberculeux, aboutissant à l'observation que cette substance était efficace dans le traitement de la dépression.

2.1.2.2. Les nouvelles hypothèses concernant l'étiopathogénie et la pathophysiologie de la dépression

Plus récemment, de nouvelles hypothèses permettant de rendre compte de l'étiopathogénie et la pathophysiologie de la dépression ont été proposées, en particulier l'hypothèse d'une dys-régulation de l'axe hypothalamus-hypophyse-surrénale (hypothalamus-pituitary-adrenals : HPA), d'un déficit immunitaire ou un déficit de la plasticité cérébrale. En ce qui concerne l'axe HPA, nous aborderons plus en détail les anomalies du Corticotropin Releasing Factor (CRF) et des glucocorticoïdes et, pour ce qui est de la plasticité, nous aborderons plus particulièrement des altérations des facteurs neurotrophiques comme le BDNF

2.1.2.2.1. Dysfonctionnement de l'axe HPA

Chez les mammifères, l'axe HPA est l'un des systèmes permettant la régulation des hormones du stress. Différentes structures, cérébrales et endocrines, sont impliquées dans ce système. Au niveau cérébral, l'hypothalamus intègre les stimuli émotionnels, cognitifs et endocriniens, et détermine l'amplitude et la durée des réponses neuronales et hormonales dans des situations de stress. Lors de l'activation de l'axe HPA, les neurones du noyau paraventriculaire (paraventricular nucleus : PVN) de l'hypothalamus sécrètent le facteur de libération de l'hormone corticotrope, le CRF, qui stimule la synthèse et la libération de l'adrénocorticotropine (ACTH : adrenocorticotropin hormon) par l'hypophyse antérieure. Une fois sécrétée, l'ACTH parvient, par la circulation sanguine, au niveau des glandes surrénales induisant la libération de glucocorticoïdes (cortisol chez les Primates, corticostérone chez les Rongeurs)(Nestler *et coll.*, 2002a, Sapolsky, 2001). Les glucocorticoïdes régulent la physiologie cellulaire et le comportement par l'activation de différents mécanismesconduisant à une mise en alerte de l'organisme: augmentation du tonus cardiovasulaire, suppression de l'activité anabolique, néoglucogenèse, etc. Ce mécanisme est présenté en détail dans la figure 2. L'ensemble de ces processus sont adaptatifs s'ils surviennent de façon aigüe et pendant une durée limitée; par contre, s'ils surviennent de façon prolongée, ils produisent des conditions défavorables pour l'individu, conduisant à des pathologies (Sapolsky *et coll.*, 2000). Par le biais des récepteurs aux glucocorticoïdes, les glucocorticoïdes exercent un rétrocontrôle négatif sur l'axe HPA, si bien qu'une forte libération de ces hormones aboutit, par le biais d'une cascade impliquant l'hypothalamus puis l'hypophyse antérieure, à une réduction de leur sécrétion (Nestler *et coll.*, 2002a, Sapolsky *et coll.*, 2000).

La dépression se caractérise par des altérations neuroendocrines, en particulier une hyper-activité de l'axe HPA et un déficit du rétrocontrôle négatif. Ces altérations peuvent être mises en évidence grâce à différents tests neuroendocriniens. Le premier, le test de suppression à la dexaméthasone, consiste à injecter un corticoïde exogène, la dexaméthasone, ce qui induit dans les heures suivantes une diminution des glucocorticoïdes endogènes grâce à l'activation du rétrocontrôle négatif. Ce test montre qu'une grande proportion de patients dépressifs conserve une concentration en glucocorticoïdes endogènes élevée, indiquant une altération du rétrocontrôle négatif de l'axe HPA. Un autre test, basé sur le même principe mais plus sensible pour détecter les dysfonctionnements de l'axe HPA est le test de suppression de dexaméthasone-CRF qui combine l'injection de dexaméthasone suivi plusieurs heures après d'une injection de CRF. Si l'intégrité du rétrocontrôle négatif est conservée, l'injection de CRF doit entraîner une augmentation limitée d'ACTH et de cortisol. Il a été montré qu'un grand nombre de patients dépressifs présente une augmentation importante d'ACTH et de cortisol après dexaméthasone et CRF (Holsboer, 2000, Holsboer, 2001c).

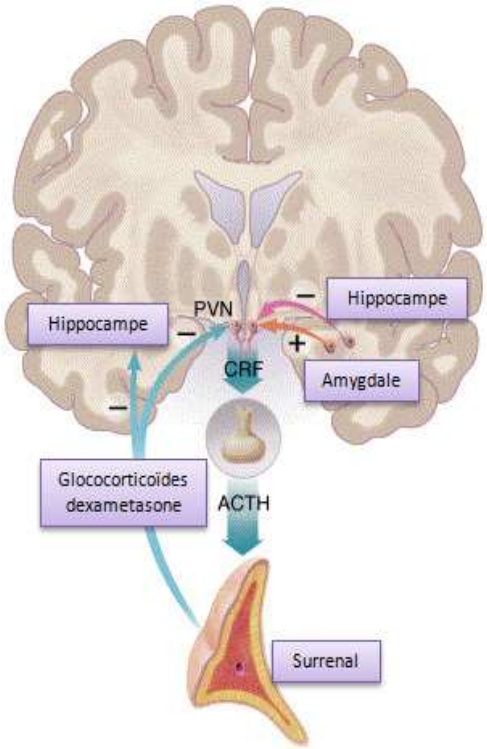

Figure 2. Régulation de l'axe hypothalamique-hypophysaire-surrénalien (HPA). Les neurones à CRF (Corticotropin Releasing Factor) du PVN (paraventricular nucleus) intègrent l'information liée au stress. Les entrées neuronales incluent des afférences excitatrices de l'amygdale et des afférences inhibitrices à partir de l'hippocampe. D'autres entrées importantes peuvent provenir de la voie monoaminergique ascendante (non indiqué). Le CRF est libéré par ces neurones dans le système porte hypophysaire et agit sur les récepteurs CRF de l'hypophyse antérieure ; leur activation va entraîner la libération de l'ACTH (adrenocorticotropin hormone). L'ACTH atteint le cortex surrénalien par voie sanguine, où elle stimule la libération de glucocorticoïdes. En plus de ses nombreuses fonctions, les glucocorticoïdes (y compris les formes synthétiques comme la dexaméthasone) répriment la synthèse et la libération de CRF et d'ACTH. De cette façon, les glucocorticoïdes peuvent inhiber leur propre synthèse. À des niveaux supérieurs, les glucocorticoïdes peuvent aussi affaiblir, et même endommager en cas d'excès, l'hippocampe ce qui pourrait initier ou maintenir les états hypercortisolémiques retrouvées fréquemment dans la dépression. Modifié d'après Nestler *et coll.* (2002).

Il a été remarqué que le maintien des perturbations de l'axe HPA sous traitement était associé à un risque élevé de rechutes ou de résistance aux antidépresseurs tandis que la normalisation de l'axe HPA était la condition nécessaire à la rémission clinique. Ainsi, il semble que la perturbation de l'axe soit un mécanisme clef dans l'établissement d'un état dépressif. Plusieurs études chez l'animal ont confirmé et affiné cette hypothèse. Il a en effet été montré que le stress chronique, qui est souvent utilisé dans la modélisation des états dépressifs chez les murins, atténue le rétrocontrôle négatif que les glucocorticoïdes exercent sur l'activation de l'axe HPA. Des structures comme le cortex préfrontal et l'hippocampe possèdent de nombreux récepteurs aux corticostéroïdes et participent significativement à cette boucle de rétrocontrôle négatif. Leur capacité d'inhiber cet axe est atténuée par un stress chronique. Enfin, des souris déficientes en certains éléments de l'axe HPA comme les récepteurs aux glucocorticoïdes sont aujourd'hui utilisées comme modèles de dépression par plusieurs équipes. Par conséquent, cette hypothèse a été à l'origine du développement de nouvelles molécules qui ciblent notamment les neuropeptides de l'axe HPA, comme par exemple des antagonistes des récepteurs au CRF de type 1 ou de la Vasopressine de type 1b. En effet, des antagonistes CRF1 ont montré des potentialités antidépressives et anxiolytiques dans des études chez l'animal et chez l'Homme (Holsboer, 2000).

2.1.2.2.2. Hypothèse du système immunitaire

Aujourd'hui, il est clair qu'il existe une communication entre le cerveau, le système immunitaire et le système endocrinien. Ces interactions sont importantes pour comprendre comment une dysfonction du système immunitaire peut expliquer la pathogenèse d'affections psychiatriques et, à son tour, comment ces pathologies psychiatriques peuvent altérer la fonction immunitaire (Mcewen, 2000b, Pollak et Yirmiya, 2002, Raison et Miller, 2001). Une dysfonction des cytokines (messagers chimiques sécrétés par les cellules du système immununitaire) et des autres médiateurs d'inflammation libérés par le système immunitaire sont associés à la dépression (Leonard, 2001a, Maes, 1995, Pollak et Yirmiya, 2002). Ainsi, il existe des évidences qui associent l'augmentation des cytokines pro-inflammatoires dans le cerveau avec la production des symptômes caractéristiques du comportement de maladie comme l'anhédonie, l'anorexie, les altérations du sommeil et la diminution de la libido, symptômes qui sont aussi présents dans les états dépressifs. L'augmentation périphérique des cytokines peut être impliquée dans d'autres maladies comme l'ostéoporose, les maladies cardiovasculaires et les pathologies auto-immunes qui sont des maladies associées fréquemment à la dépression (Carney *et coll.*, 2002, Mcewen, 2000a). En outre,

l'augmentation des cytokines pro-inflammatoires libérées par la microglie et les astrocytes conduit à une augmentation de l'activité de l'axe HPA, en particulier de la production de glucocorticoïdes, ce qui dans les conditions normales aboutit à un effet immunosuppresseur (Leonard, 2001a, Leonard, 2001b). Cette augmentation des glucocorticoïdes et des cytokines pro-inflammatoires conduit à une altération des systèmes noradrénergiques et sérotoninergiques dans le cerveau. De plus, la présence de récepteurs des cytokines a été mise en évidence dans les neurones sérotoninergiques, ce qui contribue à modifier la production de sérotonine et à altérer le fonctionnement de ces systèmes. Cette hypothèse immunitaire de la dépression prend aussi en compte les changements endocriniens et immunitaires qui jouent un rôle important dans l'étiologie de la dépression. En outre, elle peut expliquer l'action thérapeutique des antidépresseurs, en raison de leur action sur le système endocrinien et sérotoninergique. Cependant, malgré ce constat, les résultats sur le rôle de l'immunité dans la dépression sont parfois contradictoires. Il est donc nécessaire de déterminer si les altérations du système immunitaire sont la cause ou la conséquence de l'état dépressif (Leonard, 2001b).

2.1.2.2.3. Altération de la neuroplasticité

La neuroplasticité correspond à un grand nombre de processus comme la production de nouvelles cellules (neurones ou glie), le développement de l'arborisation dendritique neuronale et de nouvelles connexions synaptiques, le changement de volume de régions du cerveau, des altérations moléculaires qui peuvent produire une altération dans la réponse cellulaire. Elle correspond à la capacité des cellules cérébrales de s'adapter aux différentes situations et reflète l'interaction du sujet avec son milieu. Il existe une grande quantité d'études qui portent sur le remodelage de la cytoarchitecture cérébrale chez les sujets dépressifs. En effet, une grande partie des hypothèses actuelles expliquant l'étiopathogenèse de la dépression postulent une altération de la plasticité neuronale.

Des études d'imagerie cérébrales et des études *post-mortem* ont révélé de nombreuses anomalies neuroanatomiques chez des patients souffrant de dépression : diminution du volume de l'hippocampe corrélée à la durée de la dépression, atrophie ou perte de neurones de l'hippocampe ou dans le cortex préfrontal (Kempermann et Kronenberg, 2003), réduction de la myéline ou des cellules gliales (Sapolsky *et coll.*, 1985) -en particulier des oligodendrocytes- dans le cortex préfrontal et l'amygdale, anomalie du flux sanguin cérébral et du métabolisme du glucose au niveau des aires limbiques et du cortex préfrontal

(Magarinos *et coll.*, 1996). En outre, les antidépresseurs, administrés de manière chronique, contrecarrent certaines de ces altérations (Brown *et coll.*, 1999, Santarelli *et coll.*, 2003, Sapolsky *et coll.*, 1985, Sheline, 1996).

De nombreuses études précliniques et des recherches fondamentales ont permis de déterminer différents facteurs capables d'interagir avec la plasticité cellulaire, en relation avec le stress et avec les antidépresseurs. Ainsi, il a été montré que le stress était capable de produire atrophie, mort cellulaire et diminution de la neurogenèse au niveau de l'hippocampe ainsi que de réduire le volume de l'hippocampe et d'entraîner des dysfonctions du cortex préfrontal chez les rongeurs et des primates non humains. Des études ont démontré que l'administration chronique d'antidépresseurs atténue les changements morphologiques et structuraux induits par le stress dans l'hippocampe (Malberg *et coll.*, 2000, Malberg et Schechter, 2005). En particulier, les antidépresseurs augmentent la prolifération et la survie des cellules granulaires de l'hippocampe. Enfin, il a été montré que l'abolition de la neurogenèse hippocampique induisait une inefficacité des antidépresseurs dans un modèle murin de dépression (Santarelli *et coll.*, 2003). Le fait que la régulation positive de ce phénomène de remodelage cérébral par les antidépresseurs nécessite une administration à long terme (plus de 2 ou 3 semaines) est à mettre en parallèle avec le délai nécessaire en clinique pour l'apparition des premiers effets bénéfiques lors des traitements des troubles dépressifs.

2.2. Neurobiologie de l'anxiété

Certains auteurs comme Gray et McNaughton (Mcnaughton et Gray, 2000) proposent un modèle théorique associant les différentes pathologies anxieuses avec différentes structures cérébrales, différents types de relation avec le danger (réel ou potentiel), différentes réponses possibles (évitables ou non) et différents états psychologiques. En résumé, on peut dire que si le danger est réel et qu'il est possible d'y échapper, on observe une réponse de fuite associée à de la peur. En cas d'excès d'activation de l'amygdale, cette réponse devient excessive et la personne présenterait une phobie. Si par contre le danger est réel mais qu'il n'est pas possible de s'y soustraire, le sujet présente une réponse de freezing. En cas d'anomalie de ce système, le sujet serait atteint de trouble de panique. Par contre, lorsque le danger est vague, potentiel et qu'il est possible d'y échapper, deux situations sont possibles : soit le danger est détectable, soit il ne l'est pas. Dans le premier cas, on observe des réponses d'exploration qui mettent en œuvre le septum et l'hippocampe ; en cas d'anomalie, on observerait un état d'anxiété

généralisée. Dans le second cas, le cortex cingulaire est sollicité et une anomalie de ce système induirait un trouble obsessionnel-compulsif.

Les différentes pathologies anxieuses, même si elles ne se traduisent pas par une phénoménologie identique, impliquent des modifications neurobiologiques et des facteurs étiologiques communs. En outre, elles sont soignées par les mêmes molécules. Cela souligne à l'évidence que les facteurs neurobiologiques sous-tendant des pathologies n'agissent pas de façon mécanique. En effet, si c'était le cas, les modifications comportementales et physiologiques induites seraient toujours les mêmes (phénoménologie identique). Or cela est loin d'être le cas. Bien au contraire, ces facteurs sont interprétés par l'organisme en fonction du contexte (environnement psychologique, nutritionnel, etc.), aboutissant à diverses expressions phénoménologiques.

Etant donné la grande diversité des troubles anxieux, la description précise des bases neurobiologiques de chaque sous-type de troubles anxieux se serait avérée fastidieuse et inadaptée par rapport à nos travaux. Nous nous sommes donc restreints aux troubles les plus communs et les plus appropriés par rapport à nos travaux, particulièrement l'anxiété généralisée.

2.2.1. L'anxiété généralisée

Peu d'études d'imagerie ont été réalisées chez des patients atteints d'anxiété généralisée. On peut mentionner une étude d'IRM morphologique qui a montré une augmentation du volume de l'amygdale droite chez les patients sans que d'autres aires du système limbique (hippocampe par exemple) ne soient modifiées (De Bellis *et coll.*, 2000). Ceci suggère qu'il existe chez ces patients une augmentation du volume du circuit cérébral sollicité par les situations de peur, ce qui est tout à fait cohérent avec la symptomatologie observée. Plus récemment, peu d'études se sont focalisées sur cette question et les rares expériences réalisées concernent un nombre trop restreint de sujets pour fournir des données indiscutables.

Les hypothèses de la pathophysiologie de l'anxiété sont principalement basées sur les cibles pharmacologiques des traitements utilisées. Etant donné que l'anxiété généralisée est traitée des benzodiazépines (BDZs), des β-bloquants ou des facteurs agissant sur la neurotransmission sérotoninergique, on peut légitimement se demander si les patients atteints d'anxiété généralisé présentent des anomalies soit de la neurotransmission GABAergique (le récepteur aux BDZs est associé au récepteur $GABA_A$), soit de la neurotransmission noradrénergique, soit de la neurotransmission sérotoninergique. Peu de données existent pour

l'instant mais on peut signaler que les sujets atteints d'anxiété généralisée présentent une diminution de la fixation des BDZs dans le lobe temporal gauche (Tiihonen *et coll.*, 1997). En outre, le nombre de récepteurs aux BDZs périphériques dans les plaquettes est augmenté chez les patients (Chiu *et coll.*, 2001), une anomalie qui est corrélée avec la sévérité du trouble anxieux. Par contre, en ce qui concerne la neurotransmission monoaminergique, aucune étude n'a essayé de vérifier l'hypothèse d'une anomalie de la neurotransmission sérotoninergique ou noradrénergique chez les patients atteints d'anxiété généralisé.

Les points communs des mécanismes neurobiologiques de l'anxiété et de la dépression sont nombreux et confortent l'observation d'une comorbidité très fréquente entre troubles anxieux et dépression. Il est donc envisageable que ces différentes pathologies ne constituent pas des réalités indépendantes.

2.2.2. Les états de stress post-traumatique (ESPT)

Plusieurs études (Liberzon et Phan, 2003) utilisant des paradigmes expérimentaux de provocation des symptômes de l'ESPT montrent que ce désordre est associé à une diminution du flux sanguin dans l'hippocampe et à une augmentation du flux sanguin dans le cortex cingulaire antérieur, le cortex préfrontal, le cortex orbito-frontal, l'insulae et l'amygdale.

En particulier, les patients atteints d'un ESPT présentent une réduction importante du volume de l'hippocampe, allant de 5 à 8% selon des études. Les mécanismes de cette réduction ont été largement discutés: il a d'abord été proposé qu'elle soit la conséquence d'une libération excessive de cortisol. On sait en effet que le stress induit une forte libération de glucocorticoïdes (cortisol chez l'Homme), qui sont des substances possédant des propriétés neurotoxiques. L'hippocampe est riche en récepteurs aux glucocorticoïdes. L'idée généralement admise est donc qu'un excès de cortisol, sécrété en réponse à l'événement traumatique, est libéré au niveau de l'hippocampe, générant la mort des neurones et, par la suite, une réduction du volume de cette aire cérébrale. Cependant, les résultats d'une étude de comparaison de jumeaux monozygotes discordants pour l'ESPT montrent que cette hypothèse est incorrecte(Gilbertson *et coll.*, 2002). En effet, cette étude montre que la réduction du volume de l'hippocampe n'est pas une conséquence de l'événement traumatique, mais un facteur déterminant la susceptibilité pour le développement de l'ESPT suite à un événement traumatique.

De plus, une réduction du nombre des récepteurs aux BDZs a été trouvée par la technique du SPECT (=Single Photon Emission Computer Tomography, une technique de tomographie

détectant des rayonnements γ comme la PET, mais avec une résolution moindre) dans le cortex préfrontal(Bremner *et coll.*, 2000). On trouve aussi des modifications de la fonction sérotoninergique chez les patients. Ces patients présentent une diminution des transporteurs à la sérotonine au niveau plaquettaire, qui est corrélé avec l'intensité des symptômes. Néanmoins, le neurotransmetteur le plus étudié dans les ESPT est la noradrénaline. En effet, de très nombreuses études ont montré une hyper-sécrétion de noradrénaline chez ces patients. Par exemple, le taux urinaire de noradrénaline est plus élevé chez les sujets atteints de cette pathologie. Le niveau plasmatique est lui aussi modifié lorsque l'on présente des stimuli associés au traumatisme. En outre, il y a une diminution du nombre de récepteurs à la noradrénaline de type α2 plaquettaire, qui est à mettre en relation avec l'augmentation du taux de noradrénaline. En effet, l'augmentation du taux d'un neurotransmetteur entraîne souvent, grâce à un mécanisme compensatoire, une régulation du nombre de récepteurs. Ainsi, les modifications neurochimiques les plus marquantes chez les patients atteints d'ESPT sont celles concernant le système noradrénergique.

2.2.3. Les attaques de panique

Il a été constaté que la stimulation électrique du locus coeruleus et de régions voisines comme la substance grise périaqueducale dorsale ou de l'hypothalamus médial induisent des états de peur intense ressemblant à une attaque de panique. De plus, ceci est associé à des modifications de l'activité du système nerveux autonome, ressemblant à celles existant dans les attaques de panique. Des études morphologiques reportent une diminution du volume des lobes temporaux des patients atteints de trouble de panique. Cette anomalie est corrélée avec la sévérité de la maladie. Une région particulièrement touchée est l'amygdale, qui est curieusement atrophiée chez les patients. Par contre, cette diminution ne concerne pas l'hippocampe. Des études fonctionnelles permettent surtout de montrer des asymétries gauche/droite chez ces patients : ces anomalies touchent en particulier l'hippocampe, le gyrus parahippocampique et toutes les régions du cortex, y compris le cortex préfrontal. Les auteurs interprètent ce résultat en suggérant que cela est dû à une augmentation anormale de l'activité droite.

En outre, on constate une hyperactivation noradrénergique qui concerne en particulier un sous-type de récepteurs à la noradrénaline, les récepteurs α2 : cela se traduit en particulier par une hyper-sensibilité pour les antagonistes des récepteurs α2 comme la yohimbine et une hyposensibilité pour les agonistes α2(Abelson *et coll.*, 1992, Brambilla *et coll.*, 1995,

Charney *et coll.*, 1992). Une autre explication concerne l'implication des récepteurs aux BDZs. En effet, on trouve chez les patients une diminution du nombre de sites de liaison aux BDZs, en particulier dans le cortex temporal et le cortex frontal (Kaschka *et coll.*, 1995, Schlegel *et coll.*, 1994). L'une des hypothèses avancée est celle d'une altération de la sensibilité des récepteurs aux BDZs (Nutt *et coll.*, 1990). D'après cette hypothèse, il y a chez les patients une anomalie des récepteurs dans le sens d'un décalage de la pharmacologie des récepteurs: les antagonistes se comportent comme des agonistes inverses et les agonistes voient leur efficacité très réduite, voire nulle. On trouve aussi une association entre ce trouble et un polymorphisme du transporteur d'un neuropeptide, la cholécystokinine, induisant une modification du nombre de transporteurs, et, par conséquent, du taux de cholécystokinine. Ce polymorphisme est cohérent avec l'observation que certaines formes de cholécystokinine déclenchent des crises de panique. En outre, des anomalies dans la zone du promoteur du gène codant le récepteur à la cholécystokinine de type 2 (CCK-2, anciennement récepteurs CCK-B), qui est le principal type de récepteur à la cholécystokinine exprimé au niveau cérébral, sont aussi observées chez les sujets atteints d'attaques de panique. Par contre, aucune anomalie dans le gène codant le récepteur à la cholécystokinine de type 1 (CCK-1, ex récepteur CCK-A) n'est décrite, ce qui suggère que le déficit est central, et non périphérique. Une anomalie du gène codant le récepteur à l'adénosine de type A2 est aussi décrite. Là encore, ce résultat est parfaitement cohérent avec l'observation que la caféine, qui est un antagoniste des récepteurs à l'adénosine de type A2, déclenche des crises de panique chez ces patients. Finalement, des associations significatives sont aussi observées entre trouble de panique et modification des gènes codant pour le transporteur de cholécystokinine, le récepteur à l'adénosine du type A2, et des enzymes de dégradation des monoamines, comme le gène de la MAO_A (Monoamine oxydase de type A) et celui de la COMT (Catéchol-O-méthyl-transférase).

Ces différentes études démontrent que différents systèmes de neurotransmetteurs et différents gènes sont impliqués dans les troubles de panique. Il est vraisemblable que ces facteurs interagissent entre eux de façon complexe, conférant une susceptibilité accrue à des agents ou à des environnements panicogènes.

2.2.4. Les troubles obsessionnels-compulsifs (TOC)

Les données des diverses études de neuro-imagerie morphologique sont convergentes et montrent généralement une diminution du volume d'une région des ganglions de la base, le

noyau caudé, chez les patients atteints de TOC. Les études de neuro-imagerie fonctionnelle peuvent être réalisées soit chez des patients au repos, soit lorsque l'on présente aux patients des stimuli déclenchant les compulsions. Dans ce dernier cas, on observe une augmentation de l'activité dans le cortex orbito-frontal et le cortex cingulaire antérieur plus importante. L'implication du cortex cingulaire est confirmée par le fait que son ablation chirurgicale constitue un excellent traitement des TOC. Par contre, aucune anomalie n'est détectée dans d'autres régions généralement impliquées dans les troubles anxieux, comme l'amygdale et l'hippocampe. (Martis *et coll.*, 2002)

Une hypothèse sérotoninergique des TOC a été avancée d'après l'observation d'une corrélation positive entre l'aptitude des traitements à soigner les symptômes du TOC et la diminution induite par le traitement du taux de sérotonine dans les plaquettes et du taux de son métabolite dans le liquide céphalo-rachidien. Une autre hypothèse postule l'implication du système dopaminergique. En effet, ce neurotransmetteur est très abondant dans les structures des ganglions de la base qui présentent des anomalies chez ces patients et, lorsque l'on administre des agents augmentant la neurotransmission dopaminergique (amphétamine, apomorphine, L-DOPA) à des animaux, des comportements stéréotypés, répétitifs, assez semblables à des comportements compulsifs apparaissent. En outre, la fréquence des TOC est beaucoup plus élevée parmi les patients souffrant de certaines maladies neurologiques impliquant le système dopaminergique, comme par exemple le syndrome de Gilles de la Tourette. En effet, 50% des patients atteints d'un syndrome de Gilles de la Tourette développent un TOC. Par conséquent, ceci a donné lieu à l'hypothèse d'un déséquilibre entre dopamine et sérotonine dans la maladie. Les ganglions de la base, qui sont un maillon essentiel dans la motricité et qui sont perturbés chez les patients atteints d'un TOC, sont sous la double influence de la sérotonine et la noradrénaline.(Martis *et coll.*, 2002, Park *et coll.*, 2000, Weizman et Weizman, 2002).

3. Les traitements des troubles anxio-dépressifs

Dans l'histoire du traitement de la dépression, on trouve, vers la fin des années 1880, les premiers essais pharmacologiques avec l'amphétamine dans sa forme racémique, un médicament qui avait été initialement proposé pour le traitement de la narcolepsie. Ensuite, dans les années 1920, la teinture d'opium que l'on administrait combinée avec des vitamines, est devenue le traitement phare de la dépression. Entre 1957 et 1970, une tentative infructueuse pour améliorer les phénothiazines a conduit à la découverte des Antidépresseurs

Tricycliques. A la même époque et pour améliorer l'efficacité des substances antituberculeuses comme l'iproniazide, la recherche a permis la synthèse des inhibiteurs de la monoamine-oxydase. Dans les décennies 1970 et 1980, les inhibiteurs sélectifs de la recapture de la 5-HT (ISRS) ont été découverts dans le but initialement de diminuer les effets secondaires des antidépresseurs tricycliques. Ces dernières années, se sont développés les inhibiteurs réversibles de la monoamine-oxydase de type A (IRMAs), les molécules dotées d'une action mixte (5-HT et NA), les antidépresseurs spécifiquement noradrénergiques ou sétotoninergiques et les inhibiteurs sélectifs de la recapture de NA (ISRN).

En ce qui concerne l'anxiété, on peut mentionner l'introduction, au début du XIXe siècle, de l'hydrate de chloral et le paraldéhyde dans la thérapie de cette pathologie. Au XXe siècle, des protocoles de traitement ont été proposés basés sur l'utilisation de barbituriques, d'antihistaminiques et de BDZs. Les BDZs, créées en principe pour traiter d'autres pathologies, se sont avérées dotées de propriétés anxiolytiques. Plus récemment, l'utilisation d'agonistes des récepteurs de 5-HT1a, d'Antidépresseurs Tricycliques, l'ISRS et les antidépresseurs mixtes NA/5-HT se sont développés comme alternative thérapeutique. (Ban, 2001).

3.1. Les traitements des troubles dépressifs

3.1.1. Les antidépresseurs tricycliques

Découverts par hasard au milieu du XXème siècle, ces médicaments ont été certainement efficaces pour produire une amélioration chez les patients déprimés ainsi que chez ceux souffrant de diverses pathologies anxieuses. Leur effet typique est d'augmenter immédiatement la fonction monoaminergique en inhibant la recapture des monoamines : certains ont une action préférentiellement sérotoninergique (comme la clomipramine) et d'autres une action préférentiellement noradrénergique (comme la désipramine, la maprotiline ou la nortriptiline). En outre, tous les tricycliques bloquent les récepteurs muscariniques cholinergiques, les récepteurs H1 de l'histamine et les récepteurs α-adrenérgiques. Cependant cette action n'est pas suffisante pour expliquer les effets thérapeutiques de cette substance puisque des adaptations cérébrales spécifiques, survenant plusieurs semaines après le début du traitement et après la stimulation monoaminergique doivent se mettre en place pour observer l'effet thérapeutique.(Artigas *et coll.*, 2002)

Le blocage de la recapture des monoamines est responsable de l'effet thérapeutique de ces substances alors que leur action sur les autres récepteurs est responsable de leurs effets secondaires. Certains antidépresseurs tricycliques bloquent aussi les récepteurs de type 5-

HT2A, ainsi que les canaux Na^+ dans le cerveau et dans le cœur, causant des arythmies cardiaques, des arrêts cardiaques et des convulsions (en cas de surdosage). En particulier, le blocage des récepteurs α1 provoque une hypotension orthostatique et des vertiges, les effets anticholinergiques sur les récepteurs muscariniques produisent des troubles de la mémoire, le blocage des récepteurs H1 peut causer de la sédation et une augmentation du poids corporel (Holsboer, 2001a, Holsboer, 2001b). Cependant, en dépit de leurs effets secondaires, les AD Tricycliques continué à être des AD de choix dans plusieurs pays, surtout en raison de leur coût.

3.1.2. Les inhibiteurs de la monoamine oxydase

Les inhibiteurs de monoamine-oxidase (IMAOs) ont été les premiers ADs découverts et cliniquement efficaces. Ils ont été découverts accidentellement lorsque, en observant l'action de certains médicaments antituberculeux comme l'iproniazide, il s'avéré qu'ils amélioraient la dépression qui coexistait chez quelques uns de ces patients. Par la suite, il fut montré que ces substances agissaient en inhibant la monoamine oxydase (MAO). Les IMAOs utilisés dans les années 50-60 étaient des inhibiteurs irréversibles de la MAO, ce qui signifie que l'activité enzymatique de la MAO redevenait normale uniquement lorsque de l'enzyme était synthétisée de *Nouvo*. (Moncrieff, 2008, Slattery *et coll.*, 2004)

La MAO peut être subdivisée en deux sous-classes : la forme A et la forme B. Les premiers ADs agissaient en inhibant les deux formes de MAO puisqu'ils n'étaient pas sélectifs. La MAO de type A interagit préférentiellement avec le métabolisme de monoamines impliquées dans les états dépressifs, comme la 5-HT, la NA et la DA; le type B étant plutôt impliqué dans le métabolisme des protoxines en toxines causant la dégradation neuronale.
L'inhibition de la MAO-A produit des effets antidépresseurs mais aussi un effet secondaire hypertenseur alors que l'inhibition de la MAO B est en relation avec l'interruption de processus dégénératif comme ceux survenant dans la maladie de Parkinson. L'un des effets secondaires les plus ennuyeux des IMAOs non sélectifs et irréversibles consiste dans le fait qu'ils puissent causer de graves altérations de la pression artérielle, aboutissant par exemple à une maladie cérébrovasculaire Ce risque peut être contrôlé par la restriction diététique et en empêchant l'usage de certains médicaments (par ex., ISRS, agents sympathicomimétiques). Par la suite, des inhibiteurs de synthèse de la MAO sélectifs et réversibles comme le moclobémide sont apparus sur le marché. Ces substances sont plus adéquates que les premières et, en outre, ils possèdent des propriétés anxiolytiques, ce qui élargit leur spectre d'action. (Moncrieff, 2008, Youdim, 2006)

3.1.3. Les nouveaux antidépresseurs

Au cours, de la décennie 1980s, des substances de diverses structures commencent à se développer. Souvent, ils sont aussi efficaces les IMAOs, mais avec moins d'effets collatéraux et avec une action thérapeutique plus rapide. Ces substances agissent elles aussi en augmentant la disponibilité des monoamines dans la synapse. Cependant, malgré l'énorme développement de la recherche, il y a eu peu de progrès dans la recherche de nouvelles cibles dans le traitement de la dépression, ceci peut-être en raison des gains astronomiques obtenus grâce à la commercialisation des inhibiteurs de la recapture de la 5-HT, des médicaments de maniement facile et pourvus de peu d'effets collatéraux

3.1.3.1. Les inhibiteurs sélectifs de la recapture de 5-HT (ISRS)

Dans ce groupe de médicaments, on trouve la Fluoxétine (qui est apparue sur le marché en 1988 et qui est devenue l'un des médicaments les plus vendus dans le monde), la sertraline, la paroxétine et le citalopram. Bien qu'ils appartiennent à des familles chimiques distinctes, toutes ces substances ont des caractéristiques communes : leur aptitude à la recapture de 5-HT. Bien qu'agissant aussi sur la neurotransmission noradrénergique, cholinergique ou histaminique, leurs effets thérapeutiques sont dus à l'action sur la recapture de 5-HT. Ils ont peu à peu remplacé les tricycliques, spécialement chez des patients âgés.(Carrasco et Sandner, 2005, Rudolph, 2002, Stahl *et coll.*, 2002)

3.1.3.2. Les substances agissant préférentiellement sur la NA

Ces substances agissent en stimulant la neurotransmission noradrenergique, ce qui peut être obtenu de plusieurs façons : l'inhibition sélective des récepteurs $\alpha 2$ à la noradrénaline présynaptique ou l'inhibition de la recapture de NA. Les effets thérapeutiques de ces substances ont été expliqués par l'action de la NA sur les récepteurs β1 post-synaptiques localisés sur les neurones projetant du noyau du locus coeruleus jusqu'au cortex frontal. Une amélioration des fonctions cognitives a aussi été observée qui peut être due à l'action de la NA sur les récepteurs post-synaptiques α2 noradrenérgiques dans la voie qui va du locus coeruleus vers d'autres aires du cortex frontal. Cependant, ces données sont exclusivement basées sur des modèles animaux. Les effets secondaires de ces substances ont

été expliqués par la stimulation des récepteurs β1 situés dans le cervelet, le Système Nerveux Périphérique, le système limbique, le tronc cérébral, les récepteurs de l'aorte et dans la moelle épinière. (Invernizzi et Garattini, 2004, Kobayashi *et coll.*, 2008, Weiss *et coll.*, 2007)

3.1.3.3. Les inhibiteurs mixtes de la recapture de 5-HT et de NA

Les inhibiteurs sélectifs de la recapture de 5-HT et NA, tout comme les AD tricycliques, stimulent la neurotransmission 5-HT et NA, et donc les récepteurs de ces deux neurotransmetteurs. On peut noter qu'à la différence des autres molécules précédemment citées, ces substances sont dépourvues d'effet sur les récepteurs α1, sur les récepteurs cholinergiques ou sur les récepteurs histaminiques, si bien que leur effet sur la 5-HT et la NA est sélectif. L'action mixte, à la fois sur la neurotransmission NA et sur la neurotransmission 5-HT, associée à l'absence d'action sur les autres cibles moléculaires, induit une synergie de l'action que ces composés exercent sur l'expression génétique, synergie qui n'est pas observées en cas d'action moins ciblée. La Venlafaxine est le prototype des inhibiteurs sélectifs de la recapture mixte 5-HT/NA : administrée à faibles doses, elle inhibe la 5-HT. Lorsque la dose augmente, on observe en outre le blocage de la recapture NA. La Venlafaxine est un AD d'action plus rapide que les autres, utilisé chez des patients atteints d'une dépression réfractaire à d'autres traitements. (Andrews *et coll.*, 1996, Montgomery *et coll.*, 2007)

3.1.3.4. Les antidépresseurs atypiques

Ce sont des médicaments avec différents profils neurochimiques. La tianeptine est un représentant de cette catégorie de substances : curieusement, elle augmente la recapture de 5-HT et qui module la transmission glutamatergique (Fuchs *et coll.*, 2004). On peut aussi citer la Mirtazapine (dérivée de la Miansérine) qui agit par le biais d'un effet antagoniste sur les récepteurs de type α_2 situés sur les neurones noradrénergiques, ce qui provoque une augmentation de la libération de NA, cette NA agissant sur les récepteurs α1 situés sur les neurones 5-HT, augmentant la libération de 5-HT. De plus, ce médicament inhibe les hétérorécepteurs α_2 localisés sur des terminaisons 5-HT, il exercent aussi une action inhibitrice sur des hétérorécepteurs 5-HT_{2A} et 5-HT_3 (Holsboer, 2001b, Quitkin *et coll.*, 2001, Tamminga *et coll.*, 2002)

3.2. Les traitements des troubles anxieux

Différents types de pharmacothérapies sont utilisées dans le traitement des troubles des patients atteints d'anxiété généralisée : des pharmacothérapies à action rapide comme les BDZs et les β-bloquants, et des pharmacothérapies à action différée comme la buspirone et les antidépresseurs. Ces composés agissent sur différents symptômes de l'anxiété généralisée. Ainsi, les BDZs réduisent l'hypervigilance et certains symptômes liés à l'activation du système nerveux autonome, alors que les β-bloquants agissent exclusivement sur la composante autonome. Par contre, les agents sérotoninergiques comme les inhibiteurs de la recapture sélectifs de la sérotonine (qui sont des antidépresseurs) et la buspirone, réduisent, après un traitement chronique, l'anxiété et les soucis excessifs que présentent ces patients. En réalité, l'idée de traiter l'anxiété généralisée avec des antidépresseurs est une idée qui est apparue dans les années 90 et à présent les antidépresseurs de la classe des inhibiteurs de recapture de la sérotonine comme la fluoxétine (Prozac) sont le traitement le plus fréquent de la plupart des troubles anxieux, incluant les attaques de panique, les troubles obsessionnels compulsifs, les états de stress-post-traumatique et les phobies. Les anxiolytiques classiques tels que les BDZs ne viennent que compléter le traitement.

3.2.1. Benzodiazépines

Depuis le début des années 1960, les BZDs sont devenues disponibles et de plus en plus prescrits à partir des années 1970 (Lader, 1991). Les BZDs agissent en se liant au récepteur l'acide γ-aminobutyrique (GABA) et vont alors accroître l'efficacité du GABA lui-même à induire des afflux de chlorure inhibiteur (Nutt et Malizia, 2001). Le GABA est le principal neurotransmetteur responsable de l'inhibition neuronal. Ainsi, les BZDs en renforçant l'action du GABA aura comme action de diminuer l'activité globale du système nerveux central. Par conséquent, les BZDs auront des propriétés myorelaxantes, sédatives, amnésiantes, antiépileptiques et anxiolytiques.

Dans la lutte contre les troubles anxieux, les BZDs ont plusieurs avantages dont celui de soulager rapidement et efficacement les patients, tout en ayant peu d'effets secondaires (Rickels et Rynn, 2002), au contraire des barbituriques, molécules à la pharmacologie similaire auxquelles les BZDs ont succédé. Elles peuvent aider à réduire la gravité, la durée et la fréquence des symptômes (Association Des Psychiatres Du Canada, 2006). Il semble que les personnes susceptibles de tirer le meilleur parti d'un traitement à l'aide d'une BZD sont celles ayant des symptômes somatiques aigus, chez qui il est nécessaire d'obtenir un effet

anxiolytique rapide (Goodman, 2004, Rickels et Rynn, 2002, Sramek *et coll.*, 2002). La place des BZDs en monothérapie est controversée et devrait être réservée à un traitement à court terme en l'absence de symptômes dépressifs ou d'autres comorbidités (Goodman, 2004, Rickels et Rynn, 2002). Il faut garder à l'esprit qu'entre 33 et 50 % des patients n'auront pas de rémission avec une BZD seule et qu'il y a un risque de récurrence plus élevé qu'avec un autre type de traitement lorsqu'on cesse le traitement (Goodman, 2004). Les patients qui ne répondent pas à une BZD après deux semaines de traitement devraient recevoir un autre type de thérapie (Rickels et Rynn, 2002). Pour ces raisons, les BZDs demeurent un traitement à privilégier comme thérapie associée aux antidépresseurs surtout dans les cas de comorbidité anxio-dépressive, avant que l'efficacité de l'antidépresseur commence à se manifester adéquatement (Association Des Psychiatres Du Canada, 2006).

Par contre, plusieurs effets indésirables restent associés aux BZDs. En effet, ils peuvent causer de la sédation et la relaxation musculaire, mais aussi avec des déficits de l'attention, de déficit cognitif, de la mémoire et des capacités psychomotrices (Shader et Greenblatt, 1993). Surtout, lors de traitement à long-terme, l'utilisation des BZDs est associée à un risque de dépendance (Rickels *et coll.*, 1999), un risque plus élevé d'accidents et de chutes, et des perturbations cognitives (Taylor *et coll.*, 1998). Les BZDs ont également été associés à plusieurs autres inconvénients en lien avec la dépendance, comme une tolérance et surtout l'apparition de symptômes de sevrage à l'arrêt de traitement, en particulier si cela se produit soudainement (Romach *et coll.*, 1995).

Au cours des dernières années, le taux de prévalence de la consommation de BDZs dans un grand nombre des pays est estimé être stable ou en légère baisse, mais reste à des niveaux variant entre 2% et 3% de la population générale (Zandstra *et coll.*, 2002). Bien que l'utilisation thérapeutique à long-terme des BDZs est controversée, il semble que son efficacité soit limité aux troubles paniques et à la phobie sociale (Schweizer et al, 1993; Otto et al, 2000). Néanmoins, la prévalence de ces troubles toutefois, est relativement faible par rapport à d'autres troubles anxio-dépressifs (Zandstra *et coll.*, 2004).

3.2.2. Les autres molécules utilisées contre les troubles anxieux

La buspirone, un agoniste partiel des récepteurs sérotoninergiques 5-HT1A, peut être une solution de remplacement intéressante des BDZs. Elle possède l'avantage de ne pas entraîner de dépendance physique, de symptômes de sevrage ou de troubles cognitifs. Plusieurs études ont démontré que son efficacité était supérieure à celle du placebo et semblable à celle des BDZs (Gammans *et coll.*, 1992, Grimsley, 2005, Rickels et Rynn,

2002). Cependant, à l'inverse de ceux-ci, la buspirone serait plus efficace pour traiter les symptômes psychiques plutôt que somatiques et pourrait être envisagée chez les patients ayant des symptômes dépressifs associés à l'anxiété (Gammans *et coll.*, 1992, Rickels et Rynn, 2002). Son début d'action est cependant plus lent et se rapproche de celui des antidépresseurs, soit environ deux semaines (Rickels et Rynn, 2002). Elle doit être administrée de façon régulière en trois doses quotidiennes Il semblerait que les patients récemment traités (< 1 mois) avec une benzodiazépine répondraient moins bien à la buspirone et auraient plus d'effets secondaires (Rickels *et coll.*, 2000). On peut cependant éviter cette situation en diminuant très lentement la benzodiazépine lorsqu'on envisage l'administration de buspirone3. Son profil d'effets secondaires avantageux fait de la buspirone un choix de traitement intéressant lorsque la dépendance physique et l'altération des fonctions psychomotrices et cognitives sont préoccupantes, mais les avantages cliniques par rapport aux antidépresseurs n'est pas toujours clairs (Association Des Psychiatres Du Canada, 2006).

L'hydroxyzine, un antihistaminique, a démontré une certaine utilité dans le traitement de trouble anxieux. Son effet sédatif pourrait être une explication à son effet anxiolytique, mais rien n'a été clairement démontré. Il aurait une efficacité à long terme, mais son début d'action serait encore plus lent (Goodman, 2004, Grimsley, 2005).

Les β-bloquants, comme le propranolol, peuvent aider à soulager certains symptômes physiques de l'anxiété, tels les tremblements, les bouffées congestives et la tachycardie.
Par contre, les β-bloquants ne sont pas aussi efficaces que les BDZs, et ils ne sont plus recommandés pour le traitement du trouble d'anxiété généralisée (Association Des Psychiatres Du Canada, 2006, Grimsley, 2005).

Quelques anticonvulsivants ont été étudiés dans le traitement de divers troubles anxieux. Particulièrement, la prégabaline a démontré une efficacité supérieure au placebo et équivalente aux BZDs sur des suivis de quatre à six semaines (Pohl *et coll.*, 2005, Rickels *et coll.*, 2005). Elle possède un début d'action rapide et un profil d'effets indésirables plus favorable que les BZDs.

Les recherches actuelles tentent également de développer de nouvelles stratégies thérapeutiques pour les troubles anxio-dépressifs. Ainsi, les antagonistes de la cholécystokinine (Charrier *et coll.*, 1995, Harro, 2006), des neurokinines (Ebner et Singewald, 2006, Mclean, 2005), du CRF (Griebel *et coll.*, 2002b), de la vasopressin (Griebel *et coll.*, 2002a) et du neuropeptide Y (Heilig, 2004) ainsi que les agonistes de la mélatonine (Delagrange et Boutin, 2006, Loiseau *et coll.*, 2005, Loiseau *et coll.*, 2006, Mocaer *et coll.*,

2005) et des endocannabinoïdes (Chaperon et Thiebot, 1999, Hill et Gorzalka, 2005, Vinod et Hungund, 2006) s'imposent comme de futures cibles potentielles.

4. Le BDNF

4.1. Les Neurotrophines

La diversité cellulaire du système nerveux trouve son origine dans l'action de différents processus de différenciation, de maturation et de migration cellulaire, ainsi que dans la synaptogenèse. L'ensemble de ces processus ne sont possibles que grâce à des facteurs neurotrophiques, c'est-à-dire grâce à des protéines qui contrôlent la survie et la fonctionnalité de populations spécifiques de neurones. Ces facteurs neurotrophiques sont groupés dans la superfamille des neurotrophines, qui sont présents dans des neurones cholinergiques, dopaminergiques et noradrenergiques du système nerveux central (SNC) ainsi que dans les neurones sympathiques et sensoriels du système nerveux périphérique. Six protéines structurelles font partie de la famille des neurotrophines : le NGF (Nerve Growth Factor), le BDNF (Brain Derived Neurotrophic Factor), la NT3, la NT4/5, la NT6 et la NT7 (respectivement, neurotrophines 3, 4/5, 6 et 7) (Nilsson *et coll.*, 1998). Initialement, les neurotrophines sont synthétisées à partir d'un précurseur, la pro-neurotrophine qui est clivé par différentes enzymes, donnant origine à des protéines matures une fois qu'ils sont libérés dans le milieu extra-cellulaire. Dans sa forme mature, la protéine constitue un complexe avec une protéine jumelle, formant ainsi un dimère, qui permet l'activation de récepteurs spécifiques (Lessmann *et coll.*, 2003).

Les neurotrophines agissent grâce à deux types de récepteurs : les récepteurs TrK (tyrosine kinase), qui présentent une grande affinité pour les neurotrophines matures, et les récepteurs p75, qui présentent une faible affinité pour les protéines matures mais une forte affinité pour les protéines immatures et les protéines précurseurs (Lu *et coll.*, 2005). Il existe plusieurs récepteurs TrK (TrK A, TrKB, TrK C) ; les différents types de récepteurs TrK identifiés jusqu'à présent sont activés principalement par un ou plusieurs membres de la famille des neurotrophines. Ainsi, les récepteurs TrK A ont une affinité préférentielle pour le NGF, les récepteurs TrK B fixent principalement le BDNF et la NT 4/5 et les récepteurs TrK C ont une forte affinité pour la NT 3 (Chao, 2003). Ceci est illustré sur la figure 3, qui présente les neurotrophines et l'interaction avec leurs récepteurs d'une façon détaillée. Il existe aussi des récepteurs TrK tronqués car il leur manque un résidu de tyrosine kinase. Les récepteurs tronqués sont capables de fixer les neurotrophines mais ne peuvent pas initier la

phosphorylation nécessaire pour la traduction du signal. Ainsi, la distribution et la concentration dans la membrane des récepteurs tronqués pourraient potentiellement moduler l'activité des neurotrophines en limitant leur possibilité d'interagir avec les récepteurs complets. Très longtemps, il était admis que les neurotrophines participaient aux premières étapes du développement du système nerveux mais ces dernières années il a été montré que les neurotrophines, et en particulier le BDNF, sont aussi intriquées dans la plasticité synaptique, la survie et la prolifération neuronale chez le sujet adulte (Bramham et Messaoudi, 2005, Schinder et Poo, 2000).

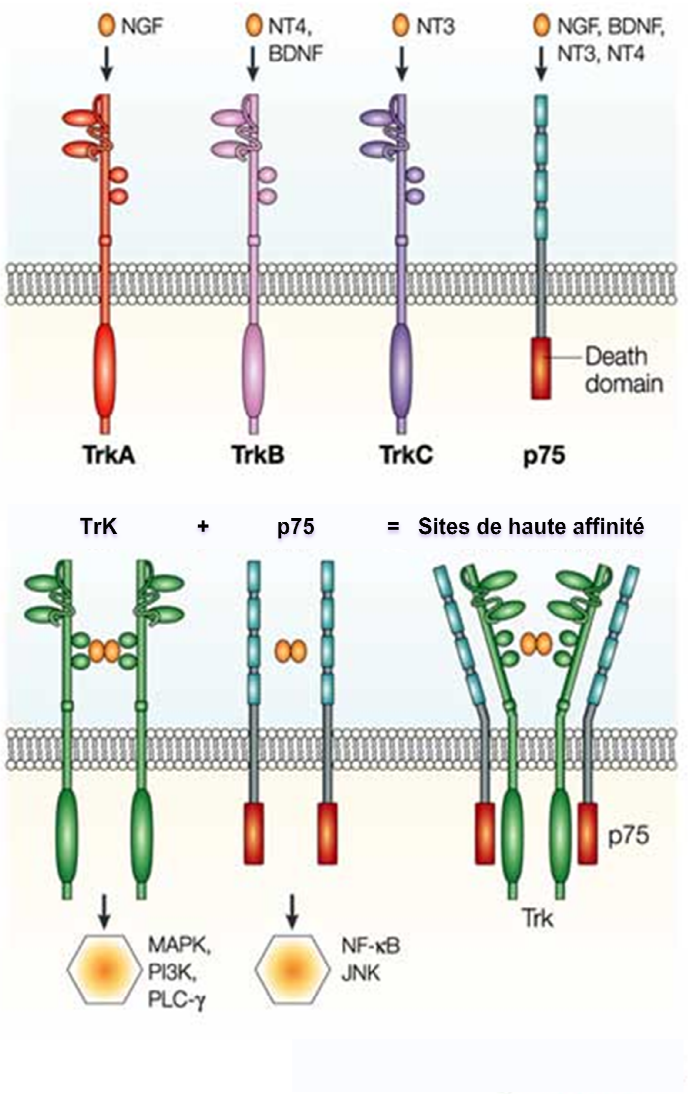

Figure 3. Modèles d'activation des récepteurs Trk et p75. La liaison de neurotophines entraîne la dimérisation de chaque récepteur. Les neurotrophines se lient sélectivement à des récepteurs Trk, alors que toutes les neurotrophins se lient à p75. Les récepteurs Trk contiennent des domaines extracellulaires à immunoglobuline G (IgG) pour la liaison du ligand et un domaine intracellulaire avec plusieurs tyrosines kinases catalytiques. Chaque récepteur active plusieurs voies de transduction du signal. La partie extracellulaire de p75 contient quatre sequences riches en cystéine tandis que la partie intracellulaire contient un « death domain ». L'interaction entre les récepteurs Trk et p75 peuvent entraîner des modifications dans l'affinité pour les neurotrophines. BDNF, brain-derived neurotrophic factor; JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NGF, facteur de croissance nerveuse; NT, Neurotrophine;PI3K, hosphatidylinositol 3-kinase; PLC, phospholipase C. modifié d'après Chao *et coll.* (2003).

4.2. Caractérisation du BDNF

4.2.1. Gène et transcription

Le gène *BDNF* a été cartographié sur le chromosome 11 chez l'humain, sur le chromosome 2 chez la souris et sur le chromosome 3 chez le rat (Ozcelik *et coll.*, 1991). Jusqu'en 2005, les travaux sur le gène *BDNF* étaient incomplets et, par conséquent, la notation des exons n'était pas adéquate. En effet, il était jusqu'alors admis que le gène du *BDNF* comportait 7 exons, la partie codante correspondant à l'exon V. Dans cette version, les épissages alternatifs étaient décrits, mais uniquement dans la partie non codante. En 2005, Liu et coll. montrèrent que le gène comportait en réalité 8 exons et que la partie codante correspondait à l'exon VIII. En outre, en 2006, Liu et coll. ont fourni des études détaillées du gène *BDNF* humain indiquant un modèle de régulation beaucoup plus complexe. En effet, ils ont montré que des épissages alternatifs sont présents dans la partie codante du gène, aboutissant à 10 transcrits différents (BDNF 1, BDNF 2A, BDNF 2B, BDNF 2C, BDNF 3, BDNF 4 ; BDNF 5, BDNF 6A, BDNF 6B, BDNF 7) (Liu *et coll.*, 2006, Liu *et coll.*, 2005b).

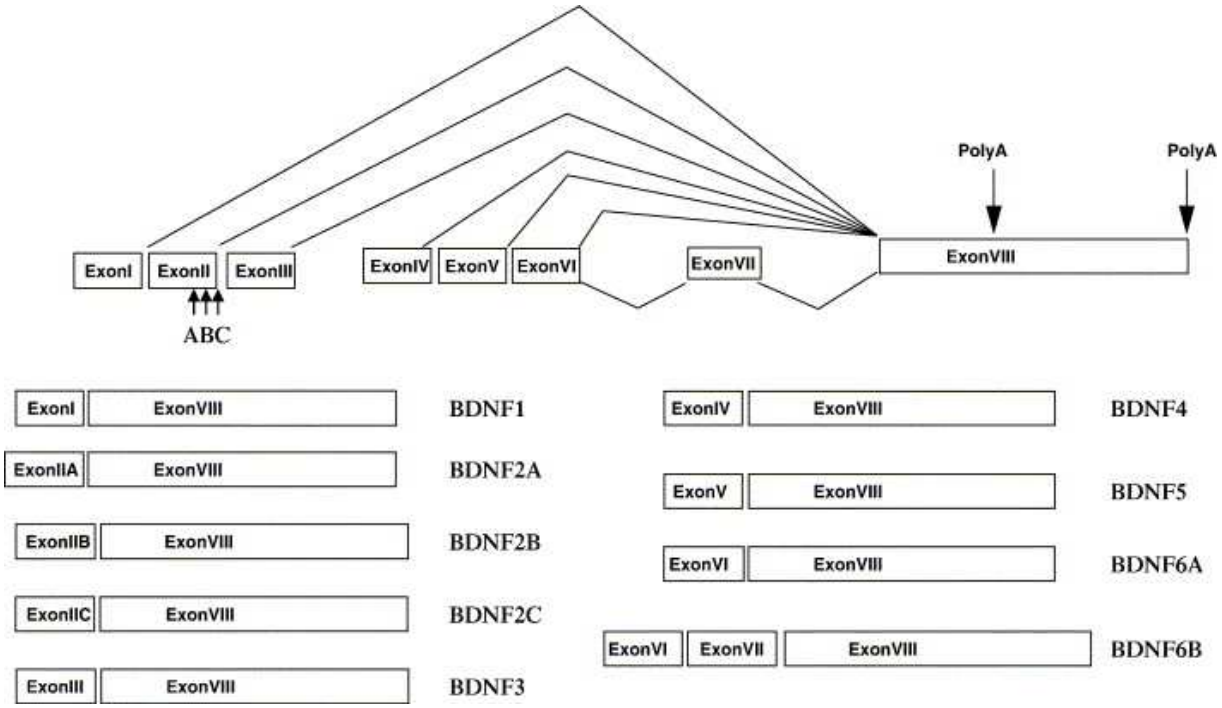

Figure 4. Gène du *BDNF* de souris et de rat et l'épissage alternatif. Les boîtes représentent les exons. Les lignes connectent deux et trois exons formant les différents variants selon l'épissage alternatif. Les flèches indiquent les sites d'épissage et de polyadenylation alternative à l'intérieur des exons. D'après Liu *et coll.* (2006).

La structure génomique du gène *BDNF* de la souris et du rat est très proche de celle du gène humain. Le gène du BDNF du rongeur possède lui aussi 8 exons dont 1 seul exon codant pour la protéine, l'exon VIII. La nomenclature va donc refléter la structure du gène humain. On trouve une homologie étroite entre l'Homme et les murins pour toutes les sections sauf pour l'exon VII qui ne possède pas de site d'initiation de la transcription chez les rongeurs, mais qui peut être associé à l'exon VI, qui lui en possède un. Chez ces espèces, le gène du BDNF est à l'origine non pas de 10 mais de 8 transcrits différents liés à des associations d'exons (BDNF1 (exons I +VIII), BDNF2A, BDNF2B, BDNF2C (exon II+ VIII), BDNF3 (exons II + VIII), BDNF4 (exons IV + VIII) ; BDNF5 (exons V + VIII), BDNF6A (exons VI + VIII) et BDNF6B (VI + VII + VIII) (Liu *et coll.*, 2006). Pour plus de détails sur la structure du gène murin, voir la figure 4.

Chez l'Homme, quelques polymorphismes du gène *BDNF* ont été répertoriés mais un seul variant génétique fonctionnel a été identifié jusqu'à présent, le SNP (single nucléotide polymorphism : polymorphisme du à une modification d'un seul nucléotide) Val^{66}-Met (rS6265)(Chen *et coll.*, 2006, Egan *et coll.*, 2003, Jiang *et coll.*, 2005, Schumacher *et coll.*, 2005, Szeszko *et coll.*, 2005, Tsai *et coll.*, 2003). Ce polymorphisme est en effet localisé dans la séquence codante pour la proforme du BDNF. Aucun changement au niveau de l'activité transcriptionnelle associé à ce polymorphisme n'a été détecté jusqu'à présent. Il ne produit pas non plus d'effet sur le ratio pro BDNF/BDNF mature mais perturbe le transport et la distribution cellulaire ainsi que la sécrétion du BDNF, notamment vers les synapses.

4.2.2. La protéine BDNF

Le BDNF est synthétisé à partir de l'exon VIII du gène qui code pour le pré-propeptide contenant le peptide signal (18 acides aminés), le pré-domaine (110 et 112 acides aminés respectivement chez les rongeurs et chez l'Homme) et la séquence mature (119 acides aminés) (Bishop *et coll.*, 1994, Timmusk *et coll.*, 1993). Le polypeptide mature présente autour de 55% de ressemblance avec les acides aminés des autres membres de la famille des neurotrophines. Le BDNF est une protéine de 27 kDalton dans la forme dimérique qui possède 6 résidus cystéine qui permettent la constitution de ponts disulfure, lesquels sont d'une importance primordiale pour la structure tridimensionnelle et donc pour l'activité du BDNF. Une faible immunogénicité a été décrite pour le BDNF, qui est probablement due au fait que la protéine mature est extraordinairement conservée entre les espèces (Acheson *et coll.*, 1991, Barde *et coll.*, 1982, Leibrock *et coll.*, 1989, Murphy *et coll.*, 1993). Le BDNF est

initialement produit sous une préforme (le pro-BDNF), la protéine précurseur qui, au sein de la cellule, peut être clivée en protéine mature (le BDNF). Néanmoins, il a été montré récemment que les neurotrophines et en particulier le BDNF peuvent aussi être secrétées sous la forme de pro-neurotrophines pour être ensuite clivées en dehors de la cellule. Une mutation peut agir spécifiquement sur le clivage intra- ou extra-cellulaire. Par exemple, elle peut ne concerner que le clivage intra-cellulaire, auquel cas le BDNF sera toujours produit au niveau extra-cellulaire. Ainsi, la protéine pourra être toujours présente, en dépit de la mutation. En effet, des études récentes ont confirmé un rôle fonctionnel pour les pro-neurotrophines, en particulier pour le pro-BDNF. Notamment, le prodomaine est impliqué dans l'établissement de la structure tridimensionnelle de la protéine mature. En outre, la proneurotrophine pourrait également agir au niveau extracellulaire sur les structures cibles des neurotrophines, en se fixant sur les récepteurs aux neurotrophines, bien qu'ayant une affinité moindre que la forme mature (Chao, 2003, Chao et Bothwell, 2002, Farhadi *et coll.*, 2000, Kolbeck *et coll.*, 1994, Lessmann *et coll.*, 2003). Les différentes voies de stockage et de sécrétion sont illustrées figure 5.

4.2.3. Les récepteurs du BDNF

Les neutrophines se fixent à un même récepteur de faible affinité (p75NTR), mais il existe également des récepteurs plus sélectifs à haute affinité, à l'origine des spécificités fonctionnelles de chaque neurotrophine. Le BDNF peut se lier au récepteur de faible affinité pour le NGF (p75NTR), de façon similaire au NGF. Néanmoins, la découverte d'une part que le BDNF et le NGF peuvent agir sur des populations de neurones différentes et d'autre part que les neurones qui répondent au NGF n'expriment en général pas de récepteurs de haute affinité pour le BDNF, a suggéré que le BDNF pouvait se lier, lui aussi, à un récepteur de haute affinité spécifique. Le récepteur TrkB a ainsi été caractérisé. Le proBDNF, quant à lui, présente une affinité opposée : haute pour le p75NTR et faible pour le TrkB.

Le récepteur p75NTR fixe avec une faible affinité et de façon non sélective toutes les neurotrophines matures, tandis que les récepteurs de la classe TrK sont des récepteurs sélectifs de haute affinité. L'activation des récepteurs p75NTR peut susciter des événements pro-apoptotiques (Bamji *et coll.*, 1998, Yoon *et coll.*, 1998) ce qui ne survient pas dans la signalisation des récepteurs de type TrK. Toutefois, lorsqu'ils sont co-exprimés avec les récepteurs TrK, les récepteurs p75NTR peuvent interagir avec les récepteurs TrK, ce qui a comme effet d'augmenter l'affinité des deux types de récepteurs pour les neurotrophines

matures. En outre, dans ce cas, les récepteurs p75NTR acquièrent des caractéristiques qu'ils ne présentent pas lorsqu'ils sont exprimés seuls, comme la possibilité d'interférer avec la survie cellulaire, la croissance des neurites et la plasticité synaptique (Lu *et coll.*, 2005, Martinowich *et coll.*, 2007).

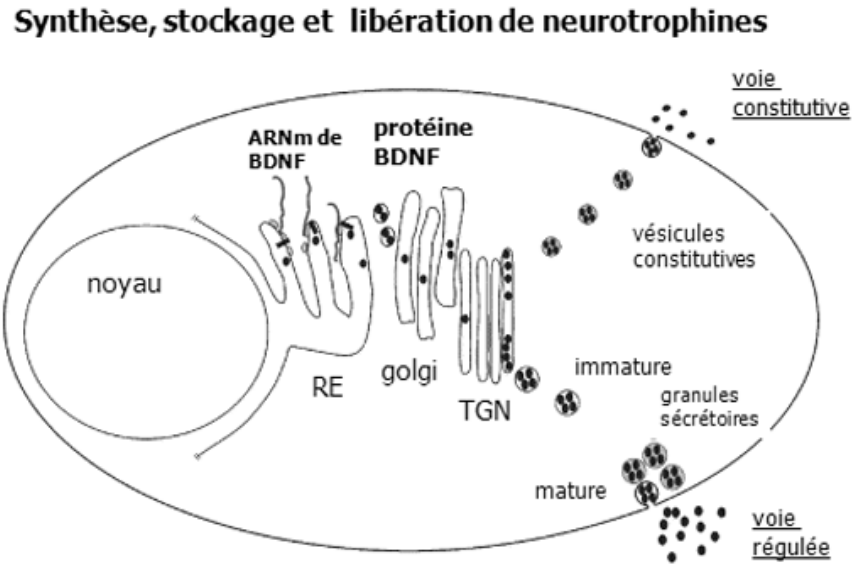

Figure 5. De la synthèse à la sécrétion du BDNF. L'ARNm du BDNF est transporté des ribosomes (gris clairs) au réticulum endoplasmique (RE) et le pré-pro-protéine naissant est alors séquestrée dans le RE (symboles noirs). Le BDNF atteint l'appareil de Golgi, puis le réseau Trans-Golgien (TGN) ; il est ensuite accumulé dans des vésicules. Des convertases présentes dans le TGN peuvent alors cliver la pro-séquence, puis le BDNF mature est groupé dans des vésicules qui seront libérées constitutivement. Alternativement, le pro-BDNF peut quitter le TGN dans des granules de sécrétion immatures (blanches) qui contiennent un type différent de convertases. Le BDNF mature pourra alors être produit dans les granules de sécrétion en route vers la membrane plasmique et donner des granules de sécrétion matures (gris). Les granules de sécrétion peuvent s'accumuler près de la membrane plasmique et être finalement libérés à la suite d'un signal approprié. Modifié d'après Lessmann *et coll.* (2003).

4.2.4. La distribution du BDNF et de ses récepteurs

Différents patterns d'expression de l'ARNm codant pour le BDNF ont été décrits dans les régions du cerveau au cours du développement post-natal, suggérant un gradient caudo-rostral dans l'expression du BDNF pendant le développement du cerveau ; ceci peut être mis en parallèle avec le développement du cerveau, lui aussi caudo-rostral et suggère l'implication du gène du BDNF dans la prolifération et la différentiation neuronale. Chez l'adulte, le BDNF

est particulièrement exprimé dans différentes zones du cerveau antérieur, en particulier l'hippocampe, l'amygdale et différentes zones du cortex. On le trouve aussi dans le clastrum, le noyau endopiriforme ou le cervelet (Ernfors *et coll.*, 1990b, Wetmore *et coll.*, 1990). En outre, chez l'Homme adulte, le BDNF a aussi été caractérisé en dehors du Système Nerveux Central (SNC) dans le cœur, les poumons et les muscles squelettiques (Hofer *et coll.*, 1990), les reins, la glande submaxillaire, les ovaires (Ernfors *et coll.*, 1990a), la rétine (Scarisbrick *et coll.*, 1993) et les plaquettes (Leibrock *et coll.*, 1989, Yamamoto et Gurney, 1990). Finalement, chez le rat, on le trouve aussi au niveau de l'aorte (Scarisbrick *et coll.*, 1993).

4.3. Fonction du BDNF

Actuellement, plusieurs fonctions du BDNF ont été décrites: la régulation de l'activité de la synapse par des phénomènes de plasticité facilitant la transmission synaptique (Lessmann, 1998, Lu, 2003, Lu et Chow, 1999), une fonction trophique, le BDNF agissant comme un facteur promoteur de la survie de populations neuronales lors du développement et chez l'adulte ; une fonction dans le remodelage de populations neuronales (Connor et Dragunow, 1998, Murer *et coll.*, 2001). Les neurones libèrent le BDNF au niveau des dendrites, agissant soit sur le neurone qui le sécrète, soit au niveau des neurones adjacents (respectivement boucles autocrines ou paracrines), soit encore au niveau des neurones afférents (BDNF transporté de façon antérograde), soit enfin au niveau des tissus cibles (BDNF transporté de façon rétrograde) (Altar et Distefano, 1998, Mufson *et coll.*, 1999). Ceci est illustré dans la figure 6.

4.3.1. Fonction dans la plasticité cellulaire

Le BDNF n'est pas seulement impliqué dans une régulation au cours du développement mais aussi dans le maintien des synapses chez l'adulte. En effet, l'application chronique de BDNF module la croissance des axones et des dendrites dans le SNC de l'adulte, particulièrement dans le cortex visuel (Akaneya *et coll.*, 1996, Lein et Shatz, 2000). Le BDNF participe aussi à la mise en place des synapses glutamategiques et GABAergiques dans le SNC (Luikart et Parada, 2006, Seil, 2003).

En ce qui concerne le développement, les travaux avec des souris génétiquement invalidées pour le gène du BDNF (BDNF -/-) ont montré que ces animaux souffrent d'altérations du développement cérébral, aboutissant entres autres à des déficits sensoriels incompatible avec la survie des jeunes. Cela confirme le rôle primordial joué par le BDNF dans le développement normal du système nerveux (Ernfors *et coll.*, 1994, Jones *et coll.*, 1994).

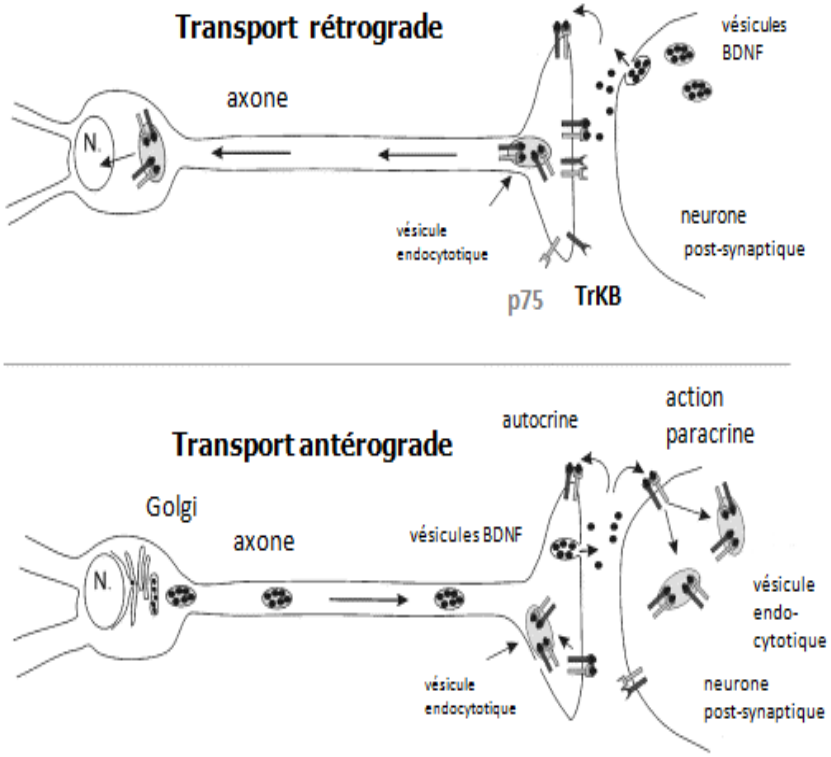

Figure 6. Transport antérograde et rétrograde du BDNF. *En haut*: selon la vue classique, le BDNF est sécrété par la cellule cible innervée (par la voie de sécrétion constitutive ou régulée), se lie à son récepteur et est ensuite accumulé dans une vésicule d'endocytose avec son récepteur. Après le transport rétrograde de la vésicule endocytotique (appelé « endosome de signalisation »), le complexe BDNF/récepteur commence la signalisation dans le soma de la cellule, en plus des processus de signalisation au niveau des terminaisons axonales. *En bas*: Les vésicules contenant du BDNF peuvent aussi être transportés du soma de la cellule aux terminaisons axonales des neurones à BDNF. A la suite d'une sécrétion présynaptique, le BDNF peuvent avoir une action autocrine ou paracrine, selon le site d'expression du récepteur TrkB. Les endosomes de signalisation (vésicules endocytotiques) peuvent donc être générés dans des compartiments pré- ou post-synaptiques. Un transport antérograde n'exclut pas la possibilité d'un transport rétrograde dans le même neurone. Modifié d'après Lessmann *et coll*. (2003).

Chez l'adulte, le BDNF est impliqué à la fois dans des changements structurels et fonctionnels. En effet, il participe à l'activation de voies de signalisation intracellulaires

complexes qui conduisent non seulement à réguler l'intégrité structurale des neurones dans le cerveau, leur survie, la prolifération et la croissance cellulaire mais aussi à moduler la plasticité synaptique, la potentialisation à long terme (LTP), la dépression à long terme (LTD) et le remodelage d'assemblées de neurones (Bramham et Messaoudi, 2005, Spedding et Lestage, 2005, Spedding *et coll.*, 2003, Tapia-Arancibia *et coll.*, 2004).

4.3.2. BDNF et mémoire

Certains de ces processus sont essentiels dans l'apprentissage et la mémoire. Ceci est confirmé par la localisation du BDNF : en effet, la présence d'un taux élevé de BDNF dans l'hippocampe, une région cérébrale clef dans la mémoire déclarative, souligne une implication probable du BDNF dans les processus qui sous-tendent l'apprentissage et la mémoire. Plusieurs données expérimentales viennent à l'appui de cette hypothèse. En effet, il existe une bonne corrélation entre l'apprentissage à court ou à long terme, la formation de la mémoire et l'expression de l'ARNm du BDNF dans l'hippocampe (Alonso *et coll.*, 2002, Mizuno *et coll.*, 2000, Yamada *et coll.*, 2002, Yamada et Nabeshima, 2003). En outre, chez le rongeur, l'apprentissage spatial (labyrinthe de Morris) et le conditionnement de peur contextuelle (deux apprentissages qui dépendent de l'hippocampe) augmentent l'expression des ARNm du BDNF dans l'hippocampe (Chen *et coll.*, 2006). D'autre part, les stratégies expérimentales visant à réduire l'expression du BDNF ou de la signalisation des récepteurs TrkB (utilisation d'anticorps antisens ou de souris génétiquement invalidées) induisent un déficit de la formation, du stockage et de la récupération de la mémoire spatiale, de la mémoire de référence ou de la mémoire de travail (Alonso *et coll.*, 2002, Linnarsson *et coll.*, 1997, Minichiello *et coll.*, 1999, Mizuno *et coll.*, 2000, Mu *et coll.*, 1999, Saarelainen *et coll.*, 2000). En outre, chez l'Homme, le polymorphisme BDNF Val66Met a une incidence sur la mémoire, ainsi que sur la fonction et le volume hippocampique (Egan *et coll.*, 2003, Hariri *et coll.*, 2003, Montkowski et Holsboer, 1997, Szeszko *et coll.*, 2005). Ainsi, la perturbation de la signalisation et de la fonction du BDNF pourrait servir de substrat aux déficits d'apprentissage et de mémoire qui se produisent dans plusieurs neuro-et/ou psychopathologies.

4.3.3. BDNF et addiction

Par ailleurs, le BDNF joue un rôle important dans la fonction d'un certain nombre de circuits cérébraux, notamment ceux impliqués dans les effets comportementaux et physiologiques de l'addiction (Bolanos et Nestler, 2004). On peut mentionner les effets du BDNF sur la morphologie et la fonction des neurones dopaminergiques impliqués dans les circuits de récompense cérébraux. Ceci a été montré à la fois dans des études *in vitro* (Hyman *et coll.*, 1991, Kontkanen et Castren, 1999) et dans des études *in vivo (Shen et coll., 1994).* Des perfusions de BDNF dans le noyau accumbens ou l'aire tegmentale ventrale, deux régions fortement impliquées dans les processus d'addiction et/ou de récompense, renforcent les effets locomoteurs, hédoniques ou consommatoires de la cocaïne (Horger *et coll.*, 1999, Lu *et coll.*, 2004). En outre, l'inhibition du BDNF dans le noyau accumbens réduit la libération de dopamine induite par les amphétamines (Narita *et coll.*, 2003). De plus, l'auto-administration de cocaïne et son sevrage induisent des changements du niveau de BDNF dans les aires cérébrales impliquées dans l'addiction comme la zone tegmentale ventrale, le noyau accumbens et l'amygdale ; ces changements persistent pendant 90 jours après la dernière exposition à la cocaïne (Grimm *et coll.*, 2003). Des souris hétérozygote pour le gène du BDNF (BDNF +/-), qui produisent environ la moitié des niveaux normaux de l'expression du BDNF, présentent une diminution de moitié du niveau de dopamine dans le striatum par rapport aux souris sauvages (Dluzen *et coll.*, 1999) et sont moins sensibles aux effets locomoteurs et hédoniques de la cocaïne (Hall *et coll.*, 2003b). Finalement, chez l'Homme, des marqueurs génétiques liés au locus du BDNF ont été décrits et associées aux différences individuelles dans la vulnérabilité à l'abus de poly-substances (Liu *et coll.*, 2005a, Uhl *et coll.*, 2001).

4.3.4. BDNF et troubles anxio-dépressifs

Durant la dernière décennie, des perturbations de la plasticité cérébrale ont été soulignées dans un nombre significatif de trouble psychiatriques dont la dépression majeure (D'sa et Duman, 2002), les troubles bipolaires (Manji *et coll.*, 2000), les états de stress post-traumatique (PTSD post-traumatic stress disorder) (Bremner *et coll.*, 2008), l'anxiété généralisée (Kalueff, 2007, Martinowich *et coll.*, 2007) et les troubles obsessionnels compulsifs (OCD : obsessive-compulsive disorder) (Taylor et Liberzon, 2007). Les phénomènes de plasticité neurale doivent être vus aujourd'hui comme un processus adaptatif permettant au cerveau de réaliser les changements morphologiques et fonctionnels nécessaires

au sujet pour répondre de façon adéquate à une variété de signaux complexes provenant de différentes régions cérébrales, du système nerveux périphérique et de l'environnement. Ainsi, les altérations de ces processus pourraient affecter un large spectre de fonctions cérébrales et pourrait être un facteur étiologique menant à un état pathologique, en particulier à certains troubles neuropsychiatriques.

En particulier, en ce qui concerne les troubles anxieux et la dépression, on peut signaler qu'une diminution du niveau de BDNF a été caractérisée chez des patients dépressifs dans une étude *post-mortem* (Chen *et coll.*, 2001). De plus, plusieurs études ont trouvé une diminution des taux sanguins de BDNF chez les dépressifs tandis qu'un traitement aux AD semble rétablir les niveaux de BDNF au niveau de celui trouvé chez des sujets sains. Cependant ces résultats ne sont pas toujours confirmés (Lee *et coll.*, 2007, Shimizu *et coll.*, 2004), si bien que l'on peut s'interroger sur la pertinence de ces données. Par ailleurs, un polymorphisme dans la région codante du gène du BDNF, qui produit du pro-BDNF avec soit de la méthionine soit de la valine en position 66, a été associé à plusieurs troubles de l'humeur. Fait intéressant, ce polymorphisme est également associée à d'autres troubles, notamment l'anxiété pathologique (Chen *et coll.*, 2006, Hong *et coll.*, 2003c, Hwang *et coll.*, 2006, Jiang *et coll.*, 2005), les OCD et les troubles de l'alimentation, qui sont tous traités avec succès par des antidépresseurs (Hall *et coll.*, 2003a, Hemmings *et coll.*, 2007, Lebrun *et coll.*, 2006). Néanmoins, ici aussi, la littérature offre un nombre non négligeable de résultats contradictoires (Hong *et coll.*, 2003b, Hwang *et coll.*, 2006, Schumacher *et coll.*, 2005, Sen *et coll.*, 2003, Tsai *et coll.*, 2003). En outre, récemment Chen et coll en 2006 ont généré des souris knock-in pour le gène BDNF humain avec soit l'allèle Val66 soit l'allèle Met66. Les souris BDNFMet/Met ont reproduit plusieurs phénotypes qui rappellent ceux associés à ce polymorphisme chez l'homme. En particulier, les souris BDNFMet/Met présentent une diminution du volume de l'hippocampe, une augmentation significative des comportements de type anxieux dans plusieurs tests comportementaux et une résistance aux effets anxiolytiques de la fluoxétine (Chen *et coll.*, 2006). Ainsi, tout comme pour l'étude précédente, l'utilisation de modèles animaux permet d'apporter des indications utiles quant à l'implication du BDNF dans les troubles anxio-dépressifs. Etant donné que le stress est une cause principale capable d'induire des troubles de l'humeur, des paradigmes basées sur l'application de stresseurs ou de ses médiateurs hormonaux ont été utilisés comme modèle animaux des troubles affectifs (Fuchs et Fliugge, 2006, Santarelli *et coll.*, 2003, Willner, 1986). Ces modèles peuvent eux aussi contribuer à éclairer l'implication du BDNF dans ces troubles affectifs. En effet, l'administration de corticostérone ou l'exposition d'animaux à des

stress aigus ou chroniques induisent une diminution de l'expression des transcrits d'ARNm du BDNF et du taux de BDNF, en particulier dans l'hippocampe (Murakami *et coll.*, 2005).

Par contre, des résultats divergents ont émergé d'études avec des souris génétiquement modifiées pour le gène du BDNF. Les souris KO hétérozygotes (+/-) pour le BDNF, qui présentent environ moitié moins de BDNF dans le cerveau que les souris sauvages, présentent différentes altérations (changements dans le volume hippocampique, dans l'arborisation dendritique, dans le système monoaminergique, hyperphagie, obésité, hyperactivité, agressivité, perturbation de la potentialisation à long terme, déficit d'apprentissage) mais aucune d'entres elles n'est en relation directe avec un éventuel état de type dépressif ou anxieux (Bartoletti *et coll.*, 2002, Chen *et coll.*, 2006, Chourbaji *et coll.*, 2004, Deltheil *et coll.*, 2007, Duan *et coll.*, 2003, Guiard *et coll.*, 2008, Hall *et coll.*, 2003a, Hall *et coll.*, 2003b, Kernie *et coll.*, 2000, Linnarsson *et coll.*, 1997, Liu *et coll.*, 2004a, Liu *et coll.*, 2004b, Lyons *et coll.*, 1999, Macqueen *et coll.*, 2001, Montkowski et Holsboer, 1997, Ren-Patterson *et coll.*, 2006, Saarelainen *et coll.*, 2003, Saylor *et coll.*, 2006). De la même manière, un modèle murin utilisant la méthode du knock-out inductible dans le cerveau antérieur ne produit pas de comportements « dépressif-like » chez les souris mutées (Monteggia *et coll.*, 2004). Par contre, des délétions conditionnelles postnatales du BDNF dans le cerveau antérieur induisent une augmentation des comportements anxieux dans le test des boîtes claire/obscure (Rios *et coll.*, 2001) ainsi qu'une augmentation de l'immobilité dans le test de Porsolt mais uniquement chez les femelles.

Ainsi, une relation entre BDNF et troubles anxio-dépressifs est clairement établie à travers ces études. Néanmoins, l'existence d'un lien causal entre les changements de niveau de BDNF et l'apparition de ces troubles parait douteux étant donné le nombre relativement élevé de résultats contradictoires.

4.3.5. BDNF et les effets des ADS

Il a été constaté récemment que les réponses comportementales produites par les antidépresseurs dans le test de la nage forcée sont supprimées chez les souris hétérozygotes pour le gène BDNF ainsi que chez des souris dont la signalisation du TrkB est inhibé (Saarelainen *et coll.*, 2003). Ces observations ont par la suite été confirmées avec des souris KO inductibles ou conditionnelles pour le BDNF dans le cerveau antérieur (Monteggia *et coll.*, 2004, Monteggia *et coll.*, 2007). Ces données suggèrent que l'augmentation de la signalisation du BDNF est nécessaire pour l'efficacité des antidépresseurs, au moins dans des

modèles animaux. De plus, la signalisation du BDNF semble être suffisante pour induire cette action, comme le montre le fait que l'injection directe de BDNF dans le cerveau médian ou dans l'hippocampe induise des effets de type antidépressifs (Duman et Monteggia, 2006). En outre, *in vitro* l'augmentation de la signalisation de TrkB, produites par la sur-expression du gène du TrkB, induit une action similaire aux effets antidépresseurs (Koponen *et coll.*, 2005). On peut du reste noter que les effets des antidépresseurs et de la surexpression de TrkB ne sont pas cumulatifs.

La signalisation normale du BDNF pourrait être à la fois nécessaire et suffisante pour l'action des ADs. L'équipe de Castrén a découvert récemment que des antidépresseurs agissant par différents mécanismes stimulent l'activation des voies de signalisation liées au BDNF/TrkB dans l'heure qui suit l'administration de la substance (Castren *et coll.*, 2007, Rantamaki *et coll.*, 2006).

Après plusieurs jours de traitement, la quasi-totalité des thérapies antidépressives, y compris les électrochocs et les pharmacothérapies comme les antidépresseurs et le lithium, augmentent l'expression de l'ARNm du BDNF dans l'hippocampe et dans le cortex (Duman et Monteggia, 2006, Nibuya *et coll.*, 1995, Russo-Neustadt *et coll.*, 1999, Saarelainen *et coll.*, 2003, Tsankova *et coll.*, 2006). Ainsi, tous les antidépresseurs, indépendamment de leur mécanisme d'action primaire, ont en commun la capacité d'activer rapidement les voies de signalisation du TrkB et d'induire une augmentation durable de la production de BDNF. Le BDNF, lui-même, quand il est administré ou quand la signalisation BDNF/TrkB est génétiquement augmentée, est capable d'induire des effets antidépresseur-like dans plusieurs tests comportementaux (Koponen *et coll.*, 2005, Shirayama *et coll.*, 2002, Siuciak *et coll.*, 1997).

Sachant que la voie de signalisation principale du TrkB implique la voie des MAP kinases (Mitogen-activated protein kinase ou ERK = extracellular signal-regulated kinase), il a été suggéré que celle-ci soit le substrat de l'effet antidepresseur-like du BDNF. Néanmoins, la phosphorylation dépendant de la tyrosine du TrkB induite par les antidépresseurs active non pas la voie des MAP kinases mais celle de la phospholipase-Cγ (gamma) et mène à la phosphorylation du CREB (cAMP-released element binding protein), un facteur de transcription dirigeant l'expression de nombreux gènes.

Une étude a révélé que le stress et/ou les traitements antidépresseurs le rôle excercent un effet sur le remodelage de la chromatine (Tsankova *et coll.*, 2006). En effet, des remodelages de la chromatine, telles que la méthylation ou l'acétylation des sous-unités des histones qui enveloppent l'ADN chromosomique, régulent l'activité de transcription des gènes

en facilitant ou non l'ouverture de la chromatine et par conséquent l'accès au promoteur des complexes multiprotéiques d'initiation de la transcription. En particulier, cette étude a indiqué qu'un modèle chronique de dépression basé sur la défaite sociale chez la souris produit une méthylation à long terme de sous-unités d'histones autour de régions promotrices du gène du BDNF dans l'hippocampe ; cette modification est corrélée avec la répression de la transcription du gène du BDNF. Cela suggère que le stress chronique peut marquer durablement la répression du gène du BDNF, au moins un mois après l'arrêt de l'application de l'agent stressant. Le traitement chronique antidépresseur est capable de reverser les effets comportementaux de la défaite sociale ainsi que de la diminution de l'expression du BDNF.

Néanmoins, il convient de noter que le traitement a agit sur l'expression du BDNF non pas en reversant le méthylation décrite plus haut mais en agissant par le biais d'un autre mécanisme : l'acétylation de cette même sous-unité, ce qui va faciliter l'ouverture de la chromatine. Ainsi, bien que l'imipramine (l'antidépresseur utilisé dans l'étude) rétablisse le niveau d'expression de l'ARNm du BDNF réprimée par le stress social, il s'agissait d'un effet de compensation au niveau de la chromatine et n'ont pas d'une réversion stricto sensu.

Le traitement antidépresseur chronique induit plusieurs changements dans la plasticité du cerveau antérieur chez le rongeur (Castren, 2004a, Castren, 2004b, Castren *et coll.*, 2007, Chen *et coll.*, 2001). En effet, les électrochocs et les antidépresseurs augmentent la neurogenèse hippocampique et le turnover des neurones granulaires dans l'hippocampe (Sairanen *et coll.*, 2005, Warner-Schmidt et Duman, 2006). Lorsque la neurogenèse hippocampique est empêchée, les réponses comportementales aux antidépresseurs sont supprimées (Santarelli *et coll.*, 2003). Bien que la signalisation du BDNF ne semble pas jouer un rôle direct dans la neurogenèse induite par les antidépresseurs, elle est nécessaire pour la survie à long terme des nouveaux neurones granulaires hippocampiques (Sairanen *et coll.*, 2005). En plus de la neurogenèse, les traitements augmentent l'arborisation dendritique dans l'hippocampe, et cet effet est atténué chez les souris ayant des niveaux réduits de BDNF dans le cerveau (Vaidya *et coll.*, 1999). En outre, les traitements antidépresseurs favorisent la maturité morphologique des nouveaux neurones et augmentent la synaptogénèse dans de nombreuses régions de l'hippocampe (en particulier la région CA1) dépourvues de neurogenèse (Hajszan *et coll.*, 2005). Il a été observé récemment que le traitement antidépresseur chronique induit l'expression de la plasticité liée à certaines protéines, comme la CREB phosphorylée et les molécules d'adhésion des cellules neurales, en particulier dans l'hippocampe et le cortex préfrontal (Sairanen *et coll.*, 2007). L'ensemble de ces données suggère que le traitement antidépresseur induit une augmentation relativement importante de

la plasticité neuronale, au moins dans l'hippocampe. La mesure dans laquelle cet effet est tributaire de la signalisation du BDNF reste à être déterminée.

Ainsi, contrairement aux données concernant l'association entre BDNF et troubles anxio-dépressif, les études portant sur le lien entre le BDNF et les effets antidépresseurs sont plus convergents et le traitement de ces troubles pourrait nécessiter un renforcement de l'activité du BDNF. Néanmoins, des évidences directes dans des modèles chroniques de dépression ainsi que des confirmations cliniques restent à établir. En outre, Berton et coll (Berton *et coll.*, 2006) (2006) ont montré que le modèle de défaite sociale induisait une augmentation de BDNF dans l'aire tegmentale ventrale et qu'un knockdown du BDNF dans cette région induit par un vecteur viral génère un effet antidépresseur. Ainsi, une implication du BDNF dans les troubles anxio-dépressifs et dans l'action antidépressive apparaît bien plus complexe qu'une simple diminution ou augmentation du niveau de BDNF dans le cerveau total.

5. Les modèles animaux

Les modèles animaux de maladies psychiatriques prétendent saisir divers aspects de la pathologie humaine, comme les changements physiologiques et comportementaux qui surviennent dans ces désordres, l'étiologie de la maladie et les effets des interventions thérapeutiques. Selon McKinney (Mckinney, 1984), les modèles animaux sont «des préparations expérimentales développées dans une espèce, dans le but d'étudier les phénomènes qui se produisent dans une autre espèce». Dans le cas des modèles animaux de psychopathologie, les chercheurs essayent de développer des syndromes chez les animaux ressemblant aux symptômes humains. Plus tard, d'autres auteurs comme Willner (Willner, 1991) ont proposé des critères supplémentaires que les modèles animaux doivent remplir. Les modèles devraient avoir une *validité de face* (isomorphisme), une *validité de prédiction* (corrélation pharmacologiques), et une *validité de construction* (Homologie et similitude dans les mécanismes neurobiologique). Actuellement, dans l'étude des pathologies psychiatriques, le critère de validité de construction est considéré comme ayant une valeur heuristique, parce que les changements neurobiologiques qui conduisent à l'anxiété ou à la dépression doivent encore être élucidées. Par conséquent, ce critère est considéré comme souhaitable, mais pas essentiel (Cryan *et coll.*, 2002). Ainsi, l'idéal pour qu'un modèle soit proche de la pathologie humaine serait qu'il soit basé sur des causes identiques, qu'il induise des profils des

symptômes similaire et qu'il soit sensible aux mêmes traitements que ceux qui sont efficaces dans la pathologie humaine.

En ce qui concerne les modèles animaux de dépression et d'anxiété pathologique, ils doivent prendre en compte entre autres le phénomène de co-occurrence entre les symptômes : par exemple, des troubles du sommeil, de l'agitation, de l'irritabilité, une difficulté à se concentrer, la perte de contrôle, la fatigue, la peur et la détresse se trouvent dans les deux pathologies. En effet, la comorbidité des troubles anxieux et dépressifs est la règle plutôt que l'exception (Kaufman et Charney, 2000, Nemeroff, 2002)et il est avéré qu'un grand pourcentage des patients souffrant de dépression présentent également des symptômes anxieux ; De plus, la plupart des antidépresseurs existants atténuent avec succès l'anxiété en tant que composante de la dépression (Nelson, 1999).

5.1. Modéliser la dépression

Le diagnostic de la maladie dépressive repose presque exclusivement sur l'observation des comportements et des relations interpersonnelles, ainsi que sur des sentiments subjectifs et des appréciations rapportées par le patient (American Psychiatric Association, 2000). Il est difficile de développer un modèle idéal des troubles dépressifs chez l'animal, puisque que la maladie humaine peut comporter des aspects spécifiquement humains. En particulier, les symptômes typiques des patients déprimés, comme la récurrence des idées de suicide ou de mort ainsi qu'un excès de pensées de culpabilité, sont impossible à modéliser chez l'animal. La création et la validation des modèles animaux des maladies psychiatriques ont été difficiles, principalement en raison de la nature subjective des symptômes à modéliser. C'est par exemple le cas de la tristesse. De plus, on ne connaît pas toujours avec précision les facteurs étiologiques de ces pathologies.

Les premiers modèles d'états dépressifs chez les animaux étaient basés sur des expériences de séparation maternelle infantile chez des primates non humains (Jesberger et Richardson, 1985). Chez les rongeurs, la manipulation de l'environnement autour de la naissance, tels que le stress prénatal et la séparation maternelle, produisent des changements comportementaux qui persistent même à l'âge adulte, ce qui modélise un facteur de risque pour ces psychopathologies (Sanchez *et coll.*, 2001).

Une autre approche pour simuler le comportement humain dans un état dépressif chez les animaux est la résignation acquise (learned helplessness model). Initialement décrite chez

des chiens soumis à des chocs électriques inévitables (Seligman et Maier, 1967), ce modèle induit des altérations comportementales et neurobiologiques voisines de celles existant dans la dépression. Ce modèle a donc été utilisé dans les études sur la «dépression» chez la souris et le rat (Nestler *et coll.*, 2002b, O'neil et Moore, 2003). Plusieurs limites de ce modèle ont été mentionnées comme la difficulté de reproduire les conséquences d'un choc électrique plantaire entre laboratoires (Vollmayr et Henn, 2001) et les inconvénients sur le plan éthique, qui rendent son utilisation difficile dans un certain nombre de pays (O'neil et Moore, 2003).

Un certain nombre de modèles de comportement ont cherché à explorer la dépression en manipulant les relations sociales chez les animaux. On peut évoquer l'utilisation de la perturbation psychosociale comme facteur déclenchant du stress (Blanchard et Blanchard, 1990). Un exemple de cette approche est le stress chronique chez le mâle Tupaia adulte (Tupaia belangeri) qui consiste à faire vivre un animal subordonné en présence d'un animal dominant, les deux étant séparés par une cloison vitrée perméable aux odeurs. Cette situation représente une condition valable pour étudier le comportement ainsi que les changements neurobiologiques et endocriniens qui sous-tendent l'évolution des troubles liés au stress et à la dépression (Fuchs et Flugge, 2002)

Dans le cas spécifique des modèles animaux des troubles psychiatriques comme la dépression chez le rongeur, il est souhaitable que le modèle induise des traits pathologiques mesurables à long terme et de façon durable. Cependant, dans la pratique, les chercheurs utilisent souvent des tests qui permettent d'évaluer des phénomènes ponctuels ; le terme modèle est alors employé par beaucoup d'auteurs bien que les situations utilisées soient des bio-essais plutôt que des modèles de pathologies. L'utilisation du terme « modèle » est dans ce cas probablement un abus de langage. On constate ceci par exemple dans des cas où les effets d'un AD ont été observés dans le test en question après injection aigue.

Dans ce document, on parlera de modèle quand l'animal aura subi un certain nombre de manipulations expérimentales (manipulation génétique, stress survenant au cours du développement du sujet, manipulation pharmacologique, stress chronique chez l'adulte, etc.) aboutissant à certains changements phénotypiques durables qui pourront être détectés à l'aide de certains tests. On appellera « test » une procédure qui permet de déceler un effet pharmacologique, sans que ce test ne mesure nécessairement des comportements homologues à ceux que l'on observerait chez un humain subissant le même traitement.

Il existe plusieurs tests permettant d'évaluer le phénotype dépressif, comme le test de la nage forcée, celui de la suspension par la queue, celui de la préférence au sucrose, etc. Ils sont détaillés ci-dessous.

5.1.1 Tests du phénotype dépressif-like

5.1.1.1. Test de la nage forcée

Ce test crée par Porsolt (Porsolt *et coll.*, 1977a, Porsolt *et coll.*, 1977b) a été très utilisé classiquement dans l'étude de la pharmacologie de la dépression surtout en raison de sa rapidité d'utilisation, de sa facilité de mise en œuvre et de sa reproductibilité. Ainsi, les animaux sont placés dans un bocal de verre rempli d'eau d'une température comprise entre 23 à 25°C et dans lequel ils restent pendant six minutes. Ainsi, les animaux sont mis dans une situation de laquelle ils ne peuvent pas échapper ; dans un premier temps, ils vont se débattre de façon vigoureuse jusqu'au moment où ils vont abandonner cette stratégie et se laisser flotter (immobilité), un comportement qualifié de comportement de « désespoir ». Ce test permet ainsi d'évaluer les effets de certains ADs (Porsolt, 1997, Porsolt *et coll.*, 1977a, Porsolt *et coll.*, 1977b) puisque ces composés réduisent ce comportement d'immobilité. Cependant, il existe plusieurs limites tel que le fait que ce test a été validé avec des animaux non pathologiques et que l'efficacité des substances est généralement observée après un traitement aigu alors que les traitements chez l'Homme ne sont efficaces qu'après plusieurs semaines. Un exemple de dispositif est présenté figure 7.

Figure 7. Dispositif du test de la nage forcée

5.1.1.2. Test de suspension par la queue

Ce test de suspension par la queue est également un standard en pharmacologie du comportement dépressif (Steru *et coll.*, 1985). De la même façon que pour le test de la nage forcée, on peut considérer ce paradigme comme un test permettant d'évaluer les comportements dépressifs-like. Les protocoles restent relativement similaires d'un laboratoire à l'autre, avec des variations de procédures mineures. En général, l'animal est accroché par la queue pendant environ 6 minutes, et on quantifie les périodes d'immobilité (interprété comme des périodes de résignation). Les limites et les conclusions que l'on peut tirer de ce test sont les mêmes que celles formulées pour la nage forcée. Certains auteurs s'accordent à préférer ce paradigme car il serait moins « stressant » que la nage forcée. Bien que l'eau soit un élément très aversif pour les souris qui sont des animaux terrestres (Whishaw et Tomie, 1996), peu d'informations permettent cependant de confirmer ou d'infirmer cette conjecture. Un exemple de dispositif est présenté figure 8.

Figure 8. Dispositif du test de suspension par la queue

5.1.1.3. Test de préférence au sucrose

Ce test repose sur les observations faites chez l'Homme qui montrent la présence d'une anhédonie dans les pathologies dépressives. Paul Willner a été l'un des premiers à introduire et valider ce test comme « détecteur » d'un comportement mimant la dépression

chez le rat. Les animaux peuvent accéder à deux bouteilles de liquide. L'une contient de l'eau ordinaire, l'autre contient une solution sucrée. On peut ainsi comparer la préférence gustative.

Après un stress chronique, la préférence pour la solution sucrée est en général réduite (Forbes *et coll.*, 1996, Willner *et coll.*, 1992, Willner *et coll.*, 1987). L'administration chronique, mais pas aiguë, d'antidépresseurs permet l'inversion de ces effets (Monleon *et coll.*, 1995, Willner *et coll.*, 1987). Ce test peut se révéler conceptuellement délicat dans son interprétation et sa mise en place (Weiss, 1997). Sa réplication semble problématique (Cryan et Mombereau, 2004). L'expérimentation peut, malgré son apparente simplicité, être l'objet de variations qui pourront entraîner des différences de résultats comme la façon de placer les bouteilles, la concentration en sucrose, la durée du test (quelques heures ou tout au long de la journée), le moment de la journée (pendant la période d'activité ou non), etc. Le concept d'anhédonie est en lui–même discutable. Il serait difficile de différencier «l'anticipation de la stimulation plaisante», la « sensation de plaisir» et la « conscience du plaisir » (Berrios et Olivares, 1995). De plus, bien que l'anhédonie soit observée chez une portion élevée de dépressifs, c'est également le cas dans d'autres troubles psychiatriques comme la schizophrénie et le désordre bipolaire (Silverstone, 1991). L'utilisation de sucrose peut aussi induire en erreur : l'attrait des animaux ne concerne peut-être pas l'aspect de plaisir du goût sucré, mais plutôt l'attrait pour l'aspect calorique. C'est pourquoi certains auteurs utilisent de la saccharine, sans calorie. Cependant, des travaux indiquent que ces deux phénomènes sont indépendants (Willner *et coll.*, 1996).

5.1.2. Les modèles empiriques

Il s'agit de modèles qui ont été fondés non pas en tentant d'imiter l'étiologique de la pathologie humaine mais sur l'apparition d'un phénotype « dépressif » ou « antidepressif-like ». Ainsi ces modèles empiriques ont l'inconvénient de présenter peu de relation avec les étiologies cliniques de ce trouble.

L'hypomotilité induite par l'administration de réserpine ou le traitement antidépresseur néonatal (administration de clomipramine) se basent sur une étiologie pharmacologique, ce qui ne représente qu'une portion faible des causes de dépression chez l'homme.

Le modèle de récompense retardée consiste à apprendre à des rongeurs à différer leur appui sur un levier pour obtenir une récompense. Leur performance serait améliorée par l'administration des antidépresseurs. L'autostimulation facilitée consiste à augmenter la

sensibilité des rongeurs à une procédure d'autostimulation de zones cérébrales de récompense par administration des antidépresseurs. Bien que les sujets dépressifs présent des perturbations de leur réponse à la récompense, ces deux modèles n'ont pas de réel équivalent étiologique chez l'homme.

Le modèle de bulbectomie olfactive consiste à léser le bulbe olfactif de rongeurs. L'anosmie qui en résulte entraîne des modifications comportementales (hyperactivité, diminution de l'apprentissage d'un évitement passif, agression) assimilées à des comportements de type dépressif et reversés par un traitement antidépresseur.

Les modèles génétiques sont des lignées consanguines ou des lignées sélectionnées de rats : souches Flinders sensitives (FSL), Fawn-Hooded (FH) et Roman Low-Avoidance (RLA) qui présent des caractéristiques physiologiques et comportementales proches de celles observées chez les patients dépressifs. Cependant, ces souches sont sélectionnées initialement pour un phénotype précis ou un gène unique et ne sont pas représentatifs d'une population générale.

Les modèles génomiques s'appuient quant à eux sur l'invalidation d'un unique caractère génétique (gène des récepteurs aux glucocorticoïdes, au CRF, gène du transporteur de la sérotonine). Or, l'ablation d'un gène unique est rarement un facteur impliqué dans l'étiologie d'un état dépressif chez l'Homme.

5.1.3. Le stress chronique léger imprédictible (Unpredictable Chronic Mild Stress, UCMS)

Parmi les facteurs les plus puissants connus pour provoquer ou déclencher des épisodes dépressifs, on trouve les événements stressants (Kendler *et coll.*, 1999, Kessler, 1997, Paykel, 2001) Le stress cause une perturbation de l'homéostasie de l'organisme, mais seulement lorsqu'il est de longue durée ; il peut alors conduire entres autres à un déséquilibre des neurotransmetteurs cérébraux, à des dysfonctionnements neuroendocriniens, favorisant l'apparition d'une maladie psychiatrique comme la dépression et l'anxiété (Willner *et coll.*, 1992, Yadid *et coll.*, 2000).

Le modèle d'UCMS est basé sur l'exposition des animaux à des stresseurs imprédictibles. Ainsi, ils sont soumis à une succession de stresseurs comme une légère perturbation du cycle de lumière-obscurité, l'hébergement sur de la sciure humide, l'inclinaison de la cage, etc (Ducottet *et coll.*, 2004, Ducottet et Belzung, 2005, Mineur *et coll.*, 2003, Willner *et coll.*, 1992). Ces stresseurs sont dans tous les cas de faible intensité et

la caractéristique fondamentale du protocole réside essentiellement dans la chronicité et l'imprédictibilité de l'application des stresseurs. Il faut noter que les caractéristiques de l'application des stresseurs peuvent varier d'un laboratoire à l'autre (O'neil et Moore, 2003).

5.2. Les modèles animaux d'anxiété

En ce qui concerne les modèles d'évaluation de l'anxiété, une distinction s'impose entre anxiété normale et anxiété pathologique. En effet, l'anxiété normale est une réponse à une situation menaçante ou potentiellement dangereuse qui accompagne de nombreux aspects de la vie quotidienne ; en revanche, l'anxiété pathologique est une réponse inappropriée à un stimulus externe ou interne. Compte tenu de la grande complexité des troubles de l'anxiété et de la comorbidité avec la dépression, il semble impossible de mettre au point un paradigme expérimental qui modéliserait uniquement un trouble anxieux particulier, comme les attaques de panique, l'anxiété généralisée, etc (Shekhar *et coll.*, 2001). Là encore, la modélisation des états pathologiques nécessiterait sans doute de disposer de sujets expérimentaux ayant subi un certains nombreux de manipulations isomorphiques à celles contribuant à la vulnérabilité à l'anxiété pathologique chez l'Homme. Cependant, bien souvent le terme de « modèles animaux d'anxiété » est utilisé à tort pour désigner des tests d'anxiété normale, permettant de révéler les effets de certains anxiolytiques.

5.2.1. Test d'anxiété normale

Chez les rongeurs, différents systèmes de catégorisation ont été proposés pour classer ces tests. L'un des plus répandus est celui qui consiste à séparer les tests fondés sur les réponses inconditionnées de ceux fondés sur les réponses conditionnées. La première catégorie est divisée en trois sous-groupes: les tests basés sur le comportement exploratoire (par exemple, le labyrinthe en croix surélevé ou en zéro et le test de la boîte claire-obscure), les tests basés sur le comportement social (test de l'interaction sociale) et les modèles fondés sur les réactions de stress somatique (par exemple, l'hyperthermie induite par le stress). Il existe d'autres paradigmes qui ne rentrent pas facilement dans ces sous-groupes tels que les batteries de tests d'anxiété/peur.

5.2.1.1. Le labyrinthe en croix ou en zéro surélevé

Aujourd'hui, la majorité des études utilisant des modèles animaux d'anxiété normale sont des procédures basées sur le comportement naturel des animaux. Parmi ceux-ci, le

labyrinthe en croix surélevé est devenu l'un tests de comportement des plus populaires.(Lister, 1987, Rodgers *et coll.*, 1997) . Sa popularité est principalement due à des raisons pratiques, parce que ce test permet un dépistage rapide du potentiel anxiogène ou anxiolytique d'une substance ou de modifications génétiques. Le labyrinthe en croix surélevé se compose de deux bras ouverts et de deux bras fermés (figure 9). Lorsque l'animal est pris directement de la cage, il explore les différents bras et le nombre total d'entrées dans chaque bras est compté. Le comportement de peur se traduit dans cette situation par une inhibition de l'exploration des bras ouverts. Les traitements anxiolytiques permettront de surmonter la peur, induisant une augmentation des entrées dans les bras ouverts tandis que les agents anxiogéniques ont l'effet opposé. Malheureusement, dans ce test, les comportements peuvent être influencés par différents facteurs comme, par exemple, la souche étudiée, les conditions d'élevage, l'intensité de la lumière. Pour surmonter ces problèmes, Rodgers et Johnson (Rodgers et Johnson, 1995) , ont développé une version "éthologique" de ce labyrinthe qui consiste à inclure la mesure de postures spécifiques (par exemple, l'évaluation des risques) en plus des mesures spatio-temporelle comme l'évitement des bras ouverts.

Il existe une version modifiée de ce test : le labyrinthe en zéro surélevé (Shepherd *et coll.*, 1994). Il est similaire au labyrinthe en croix surélevée par le fait qu'il est aussi surélevé et qu'il contient des zones fermées avec des murs et des régions ouvertes sans murs (figure 9). Dans les deux cas, le rongeur montre une éviction des zones ouvertes (Shepherd *et coll.*, 1994). Néanmoins, le labyrinthe en zéro surélevé présente plusieurs avantages par rapport au labyrinthe en croix surélevée : il n'a pas de zone centrale qui peut conduire à des mesures ambigües et il a une forme circulaire ce qui donne au rongeur la possibilité d'exploration ininterrompue.

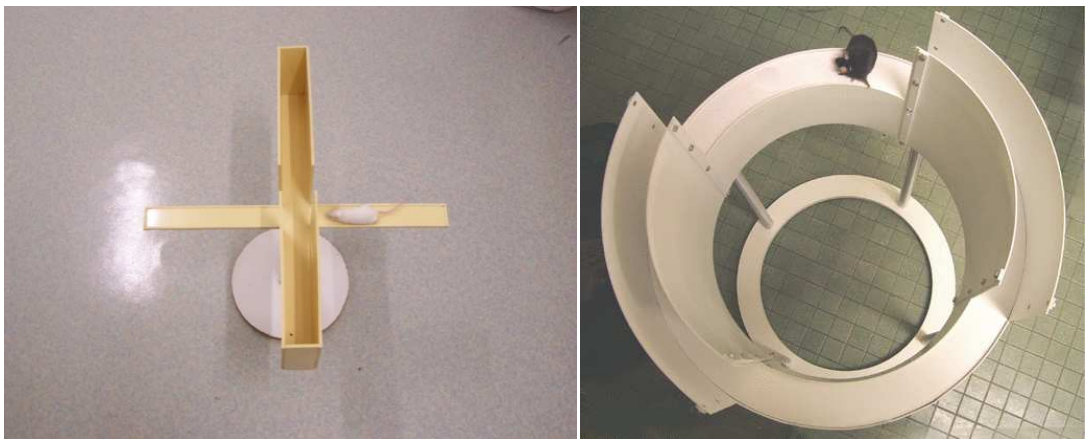

Figure 9. Dispositif du labyrinthe en croix surélevée (à gauche) et en zéro surélevé (à droite)

5.2.1.2. La boîte claire-obscure

La boîte claire-obscure se fonde également sur une opposition entre une partie aversive (boîte vivement éclairée) et une partie plus attractive (boîte obscure) (Belzung *et coll.*, 1987, Crawley et Goodwin, 1980). Les deux cages sont parfois reliées par un tunnel. En général, l'animal est observé pendant cinq minutes. Un temps moins importants dans le partie éclairée est considéré comme un comportement de type anxiété-like. Un exemple de dispositif est présenté figure 10.

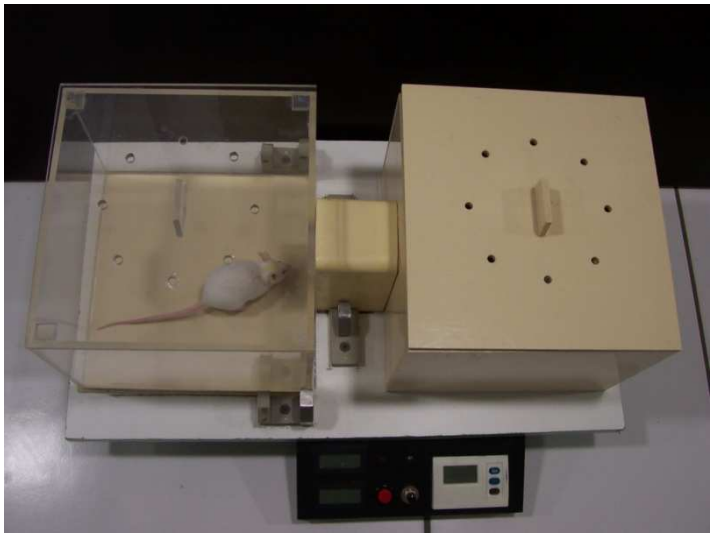

Figure 10. Dispositif du test de da boîte claire-obscure

5.2.1.3. Novelty-Suppressed Feeding Test (NSF)

Le NSF test consistait en une boîte en bois de dimensions qui varient entre les expériences et les différentes laboratoires comme par exemple 33x33x30 cm (Surget *et coll.*, 2008) ou 50x50x20 cm (Santarelli *et coll.*, 2003) avec une lumière indirecte rouge. Le plancher est couvert de sciure. Douze heures avant l'essai, l'alimentation est retirée de la cage. Au moment du test, une seule croquette de nourriture est placée sur une plate-forme de papier blanc placé au centre de la boîte et l'animal est placé dans un coin de la boîte de test. La latence mise pour commencer à consommer la croquette est enregistrée au cours des 3 minutes suivantes. Ce test induit un conflit motivationnel entre la motivation pour manger la nourriture et la peur de s'aventurer dans le centre de l'arène. Ce paradigme est en mesure d'évaluer les effets du traitement chronique avec ADs chez des souris stresses et non stresses. Un exemple de dispositif issu de Surget *et coll.* (2008) est présenté figure 11.

Figure 11. Dispositif du NSF test

5.2.1.4. Test de l'interaction social

Le test de l'interaction sociale qui a été introduit à l'origine par File en 1985 (File, 1985). Il consiste à quantifier le nombre de comportements sociaux entre les animaux, dans un environnement plus ou moins stressant (lumière de forte ou faible intensité, nouveauté de l'arène dans laquelle se produit la rencontre, etc.). Il s'agit d'un modèle de comportement utile pour des essais de médicaments anxiolytiques. En effet, les anxiolytiques augmentent généralement le temps passé dans les interactions sociales.

5.2.2. Modèles pour l'anxiété pathologique

5.2.2.1. Exploration dans la boite de Hughes

Ce test a été décrit initialement par Hughes (Hughes, 1968). Il est réalisé à l'aide d'un dispositif composé de deux secteurs séparés par des portes mobiles. Chaque secteur est subdivisé en trois parties connectées par des ouvertures (figure 12). Le rongeur est restreint, avec de la nourriture et de la boisson, pendant 24 heures dans l'un des deux secteurs afin de l'habituer à cette partie du dispositif. Puis le passage vers le second secteur inconnu est permis. Les comportements observés concernent les tentatives d'entrées dans le secteur nouveau, le temps passé dans cette partie, les postures étirées, les redressements dans chaque partie et le nombre de transitions entre les deux secteurs et à l'intérieur de chaque secteur. La fréquentation du secteur inconnu est donc librement permise ici ; il a été montré que contrairement à ce qui se produit lorsque l'animal est contraint dans une arène inconnue, l'exploration libre n'est pas stressante. En effet, les animaux ne présentent pas d'augmentation de la cortisolémie dans cette situation (Cigrang *et coll.*, 1986). Ainsi, ce test

permet d'évaluer les comportements anxieux dans une situation qui n'est pas stressante chez des animaux normaux. Si toutefois les animaux présentent un comportement néophobique, comme c'est le cas avec certaines lignées de souris ou après certaines mutations, on peut dire que ces animaux constituent des modèles de pathologies anxieuses, puisqu'ils présentent des comportements anxieux dans une situation non anxiogène chez le sujet normal. Il a été proposé que cette anxiété excessive corresponde aux comportements survenant dans l'anxiété généralisée chez l'Homme (Belzung et Griebel, 2001).

Figure 12. Dispositif de l'exploration libre

5.2.2.2. Exploration libre en champs ouvert

Ce test est réalisé dans une arène circulaire ; l'animal est introduit dans une cage connecté à l'arène et a la possibilité d'y entrer librement. L'arène est souvent éclairée par une lumière rouge afin de proposer à l'animal une zone grande et inconnue, mais elle ne possède pas de caractéristiques aversives, tout comme dans le test de l'exploration libre. Les variables observées sont similaires à celles enregistrées lors de la procédure classique à l'exception de la latence à entrer dans l'arène ainsi que des comportements d'évaluation du risque (Hughes et Beveridge, 1986)

5.2.2.3. Batterie de tests de défense chez la souris (MDTB)

Le paradigme de la « batterie de tests de défense chez la souris » (MDTB) utilise une vaste analyse éthologique pour générer un profil plus complet de comportement en fonction du traitement (Blanchard *et coll.*, 1997, Griebel *et coll.*, 1995). Il a été suggéré que les comportements défensifs chez les mammifères inférieurs constituent un modèle efficace pour la compréhension de troubles émotionnels (Blanchard, 1984). Les comportements défensifs se

produisent en réponse à un nombre de stimuli menaçant, y compris les prédateurs, les attaques des congénères, et les objets ou situations dangereux. De tels comportements peuvent facilement être étudiés chez les rongeurs qui montrent un large répertoire défensif face au danger. Le dispositif utilisé est MDTB est une piste ovale. Le MDTB se compose de cinq essais, basés soit sur la confrontation avec une menace potentielle (défense contextuelle) soit sur la présence réelle d'une menace proximale (un rat). Cette dernière situation induit des modifications du comportement de fuite, d'évaluation du risque et de menaces défensives et de comportements d'attaque, tandis que la première induit des tentatives d'échappement.

D'une manière générale, les composés anxiolytiques ont tendance à diminuer les comportements défensifs. Cependant, un point fort de ce modèle est de générer différentes réponses comportementales en fonction du type de composé anxiolytique utilisé (Blanchard *et coll.*, 1997). Ainsi, les BDZs diminuent l'évaluation des risques des animaux poursuivi par les rats, les menaces défensives ainsi que les comportements d'attaque, tandis que les agonistes 5-HT1A affectent principalement la défense contextuelle, les menaces défensives et des comportements d'attaque. En outre, les inhibiteurs de la recapture 5-HT et les antagonistes du récepteur de la cholécystokinine B ont un impact plus important sur les réponses de fuite que sur d'autres réactions défensives. Pris ensemble, ces observations suggèrent que l'évaluation des risques, la fuite, les menaces défensives, les comportements d'attaque et les tentatives d'échappement reflètent chacun différents aspects de comportements anxiété-like.
Etant donné le répertoire comportemental affecté par cette procédure, il a été proposé que ce paradigme modèle plus particulièrement les attaques de panique (Belzung et Griebel, 2001, Griebel *et coll.*, 1995)

5.2.2.4. Modèles PTSD : Test d'exploration libre avec exposition à un chat

Un modèle animal valable du PTSD doit être un modèle original d'une expérience traumatisante. Pour qu'un stress soit perçu comme un événement traumatisant, il doit être incontrôlable et imprévisible. En effet, la prévisibilité et la contrôlabilité déterminent l'intensité et la gravité du PTSD ainsi que la persistance voire l'aggravation des symptômes. Parmi ces symptômes, on trouve des modifications biologiques et comportementales à long terme. Bien que le PTSD ait des dimensions psychologiques hors de portée d'un modèle animal, les modifications biologiques et comportementales pourraient être induites et mesurables dans un tel modèle. Le modèle du PTSD doit être en mesure de simuler les effets du traumatisme d'une origine exogène et de traiter immédiatement une expérience aiguë ainsi

que les séquelles à long terme du traumatisme. Ce type de modèle est donc basé sur l'exposition des rongeurs à des prédateurs naturels.

Un exemple de modèle de PTSD est une version modifiée du test d'exploration libre (Griebel *et coll.*, 1993) dans lequel est évalué le comportement des souris à la suite d'une exposition à un chat ou alternativement à des fèces de chats (Belzung *et coll.*, 2001). Environ 20 h avant l'exposition au chat, la souris est placée dans une des deux moitiés d'un dispositif similaire à la boîte de Hugues. Le dispositif se compose d'une boîte de PVC (30 × 20 × 20 cm) recouvert de plexiglas et subdivisé en six carrés exploratoires de taille égale, qui sont toutes reliées entre elles par de petites entrées. Il peut être divisé en deux dans la longueur avec trois compartiments de chaque côté. De cette manière, la souris va se familiariser pendant 20h à une moitié du dispositif. Le sol de cette moitié est couvert de sciure et l'animal à un accès illimité à la nourriture et l'eau. Ensuite, la souris va être placée dans un dispositif où elle sera confrontée à un chat pendant 5 minutes. Le chat est placé dans une cage de PVC (82 × 56 × 62 cm) divisé en deux compartiments, l'un contenant le chat, dans l'autre est placée la souris. La séparation se compose d'une paroi transparente avec des trous permettant au chat de passer ses pattes de l'autre côté. Après la souris est remise dans le dispositif initial avec la possibilité de réaliser une exploration libre des compartiments non familiers, dans lesquels se trouve un stimulus rappelant le traumatisme (crotte de chat) ou un stimulus neutre (patte à modeler). Certains comportements d'évitements ne sont observés que chez les animaux pré-exposés au chat et aux fèces de chat ; ils semblent associés à des comportements ressemblant à ceux des sujets PTSD.

D'autres modèles animaux de stress post-traumatiques sont aussi utilisés comme de brefs chocs électriques avec des expositions répétées et des situations de rappel chez la souris.

Objectifs de la thèse

Comme nous l'avons vu, le BDNF est impliqué dans une multitude de fonctions essentielles pour la fonction et la survie des neurones. De ce fait, il est impliqué directement ou indirectement dans de nombreux circuits cérébraux qui sont associés à des fonctions supérieures et complexes comprenant notamment les traits psychologiques et comportementaux d'un individu. Des perturbations de l'activité du BDNF pourraient, au-delà de certains dysfonctionnements cellulaires, contribuer à la mise en place de processus néfastes à l'origine de troubles du comportement ou de troubles affectifs. Ainsi, il été suggéré que des altérations de l'activité du BDNF soient à l'origine de maladies psychiatriques et particulièrement des troubles anxio-dépressifs.

Au cours de ce travail, nous avons donc voulu préciser la relation entre le BDNF et l'apparition des troubles anxio-dépressifs ainsi que son implication dans l'efficacité des traitements antidépresseurs. Pour cela, nous avons utilisé différents modèles d'anxiété et de la dépression chez la souris. Nous avons privilégié l'utilisation de modèles éthologiques et inconditionnés puisque ces derniers présentent une plus grande validité de face et de construction. En outre, nous avons choisi d'étudier cette question en utilisant deux types d'approches différentes et complémentaires :

- une approche corrélationnelle, en étudiant l'association entre un polymorphisme du gène BDNF et le phénotype des animaux.
- une approche « lésionnelle », par la délétion d'un allèle du gène BDNF (utilisation de souris hétérozygote BDNF $^{+/-}$).

En effet, différentes approches sont nécessaires pour décrire comment des gènes ou des facteurs environnementaux contribuent à des traits psychologiques ou à des comportements complexes. Dans ce contexte, les lignées de souris consanguines fournissent un outil appréciable pour évaluer l'influence des gènes sur le fonctionnement normal et pathologique du cerveau grâce à leur isogénicité (individu génétiquement identique). Une large hétérogénéité phénotypique a été décrite parmi ces lignées pour plusieurs comportements. Il n'est donc pas impossible que certaines de ces différences soient causées par l'existence de variants alléliques du gène BDNF. Dans un premier temps, notre but a donc été de rechercher un éventuel polymorphisme fonctionnel dans la région codante du gène BDNF dans six lignées de souris consanguines (A/J, BALB/c, C3H, C57BL/6, CBA et DBA/2) parmi les plus utilisées dans la recherche. Ayant constaté un polymorphisme Leu32Met, notre objectif a été d'examiner le phénotype de ces souris en fonction des variants alléliques dans plusieurs

mesures : expression hippocampique du BDNF, poids, comportement alimentaire, activité locomotrice, anxiété, apprentissage et mémoire (article 1).

Dans un deuxième temps, notre but a été d'examiner l'implication du polymorphisme Leu32Met dans la survenue d'un syndrome dépressif-like chez des souris confrontées au modèle de dépression UCMS ainsi que son implication dans l'action des ADs. Dans cette optique, nous avons tenté d'identifier des lignées sensibles à plusieurs types d'altérations dues à l'UCMS ainsi qu'à leur réversion par un traitement antidépresseur. Des souris provenant de sept lignées différentes (A/J, BALB/c, C3H, C57BL/6, CBA, DBA/2 et FVB) ont été exposées à un régime de 9 semaines de stress chronique. Après 4 semaines, les souris ont été traitées quotidiennement soit au sérum physiologique (NaCl 0.9%) soit à l'imipramine (20mg/kg/jour, ip). Les effets de l'UCMS et du traitement ont été évalués par des mesures physiques (état du pelage, poids) comportementales (novelty-suppressed feeding test et actimètre) et neuroendocrines (taux fécaux de métabolites de la corticostérone) (article 2).

Enfin, dans un troisième temps, nous avons cherché à déterminer le rôle du niveau d'expression du BDNF dans la vulnérabilité au stress chronique et dans l'action des antidépresseurs. Pour cela, nous avons utilisé des souris hétérozygotes BDNF $^{+/-}$ qui ont une expression monoallélique du BDNF (diminution de 50% du taux d'expression de la protéine). Des souris sauvages BDNF $^{+/+}$ et des souris hétérozygotes BDNF $^{+/-}$ ont été exposées à un régime de 8 semaines de UCMS et, après les 2 premières semaines de UCMS, à un traitement de 6 semaines à l'imipramine (20mg/kg/jour, ip). L'état du pelage, le poids, la consommation de nourriture, l'activité locomotrice, l'agressivité (resident/intruder test), le niveau d'anxiété (novelty-suppressed feeding test) et de résignation (test de la queue suspendue), la concentration de corticostérone plasmatique et le niveau d'expression de BDNF dans l'hippocampe ont été évaluées (article 3).

Dans le présent manuscrit, les résultats seront donc présentés sous forme de ces 3 articles. Etant donné que la méthodologie employée est décrite soigneusement dans chacun de ces articles, nous n'avons pas rédigé de partie « Matériel et méthode » spécifique au manuscrit de thèse, pour éviter les redondances. Après les 3 articles, on trouvera une discussion générale qui fera une synthèse des conclusions que l'on peut titrer de l'ensemble de ces 3 contributions. Enfin, comme j'ai aussi participé à d'autres études en cours au laboratoire, j'ai mis les autres articles rédigé en Annexes, pensant que cela permettrait au lecteur d'avoir une idée de l'ensemble de mon travail.

Article 1 - Association of a mouse BDNF polymorphism (Leu32Met) and the behavioral phenotypes in six inbred strains

Dans cet article nous avons recherché et trouve un polymorphisme (Leu32Met) dans la région codante du gène BDNF dans six lignées consanguines de souris. En autres, nous avons examiné le phénotype et en particulaire le comportement anxieux en fonction des variants alléliques.

Association of a mouse BDNF polymorphism (Leu32Met) and the behavioral phenotypes in six inbred strains

Yadira Ibarguen-Vargas[1,2], Alexandre Surget[1,2], Patrick Vourc'h[1,2], Wissam El-Hage[1,2], Christian Andres[1,2], Anne-Marie Leguisquet[1,2], Catherine Belzung[1,2].

[1] Université François Rabelais de Tours, Tours, France.

[2] U930, FRE CNRS 2448, Inserm, Tours, France.

Abstract

Clinical correlates as well as basic research provided accumulative evidences for an involvement of the Brain-Derived Neurotrophic Factor (BDNF) in several complex behavioral traits. Inbred mouse brain surveys demonstrated obvious phenotypic differences in various measures and behaviors. Taken together, it is possible that variations in BDNF gene account for these differences. Hence, the aimed of this study was first to investigate functional polymorphisms of the mouse *bdnf* gene in six inbred mouse strains (A/J, BALB/c, C3H, C57BL/6, CBA, and DBA/2). We reported a single nucleotide polymorphism (SNP) of the mouse BDNF gene which induces an amino acid substitution (Leu32Met) at codon 32 in the prodomain of the precursor BDNF protein. We further examined whether this polymorphism could affect the behavioral phenotypes of these inbred mouse strains. While the *bdnf* Leu32Met polymorphism was not found to be associated with body weight, food intake, locomotor activity, learning or memory, the allelic distribution among strains significantly paralleled with anxiety-related behaviors. Indeed, strains carrying Met allele displayed greater propensity to elevate anxiety level. A precise analysis of the data highlights a specific contribution of this polymorphism in neophobia and trait anxiety. Finally, our findings suggest that the bdnf Leu32Met polymorphism can contribute to the behavioral inter-strain differences and support a role for BDNF in anxiety.

Introduction

Brain-derived neurotrophic factor (BDNF) is involved in a variety of neural processes and brain functions which may impact complex behavioral phenotypes. Indeed, accumulative evidences suggest that BDNF plays a role in food intake (Lebrun *et al.*, 2006, Unger *et al.*, 2007), activity (Kent *et al.*, 2005, Tapia-Arancibia *et al.*, 2004), social interactions (Taylor *et al.*, 2008), drug addiction (Tsai, 2007), learning and memory (Tyler *et al.*, 2002), as well as in mood and emotional reactivity (Martinowich *et al.*, 2007). Because alterations of these behaviors have frequently been associated with mental illnesses, current research focuses on BDNF dysfunction as a putative primary cause underlying psychiatric disorders.

Accordingly, the *bdnf* gene is attracting much interest as a putative candidate gene involved in behavioral disturbances. Studies based on targeting invalidation of the *bdnf* gene in mice brought some informative evidences suggesting an involvement of BDNF in complex behavioral traits. For example, heterozygous *bdnf* $^{+/-}$ mice which have roughly half reduced levels of BDNF display various significant behavioral disturbances including hyperphagia and obesity (Lyons *et al.*, 1999), hyperlocomotion (Kernie *et al.*, 2000), aggressiveness (Lyons *et al.*, 1999) as well as learning and memory deficits (Linnarsson *et al.*, 1997). Moreover, these results were corroborated by a clinical case report of a monoallelic expression of the *bdnf* gene which was associated with increased *ad libitum* food intake, severe early-onset obesity, hyperactivity and cognitive impairment (Gray *et al.*, 2006).

Nevertheless, other research approaches are required to describe all genetic components and environmental factors contributing to complex behavioral traits. In that context, the isogenicity of inbred mouse strains provides an invaluable tool for investigating gene-environment interactions. Large phenotypic differences have been described among inbred mouse strains in various measures and behaviors (Crawley *et al.*, 1997). These differences might also be explained by variations of the *bdnf* gene in particular strains. Hence, we analyzed the sequence of the coding region of BDNF in six inbred mouse strains (A/J, BALB/c, C3H, C57BL/6, CBA and DBA/2) and reported a unique single nucleotide polymorphism (SNP) responsible for a Leu32Met substitution in the pro-domain of BDNF (recently registered with dbSNP number rs27524348). We next examined the phenotype of these mouse strains for various physical (body weight) and behavioral measures (free exploratory paradigm, splash test, food intake, actimeter, social learning of food preference, as well as reviewed results in the elevated zero-maze). We here report several strain differences and evidences for a putative Leu32Met polymorphism association with anxiety-like behaviors.

Materials and methods

Animals

Male mice from six inbred strains: A/J obtained from Harlan (Gannat, France) and BALB/cByJ, C3H/HeJ, C57BL/6J, CBA/J and DBA/2J from Centre d'élevage Janvier (Le Genest Saint Isle, France). Different cohorts of mice were used in the different behavioral paradigms. Mice were aged 7-8 weeks at their arrival and 3-4 months when tested (except for body weight, 10-11 weeks). All animals were housed in groups of 5 under a 12/12h light/dark cycle (lights on at 20:00), 22±2°C, food and water *ad libitum*. The treatment of the animals was in accordance with the European Community Council directive 86/609/EEC.

Sequencing of BDNF coding region

DNA was extracted from the extremity of tails. PCR amplification generated two overlapping fragments on the BDNF exon VIII, containing the entire coding sequence of pro-BDNF (Liu *et al.*, 2006). PCR was performed in a final volume of 50 µL containing 100 ng DNA, 1X buffer, 1 mM MgCl2, 200 µM dNTP, 0.5 µL recombinant Taq DNA polymerase (Invitrogen) an 1 µM of each of the forward and reverse primers (5' region of exon VIII: F 5'-CTTCCTTCCCACAGTTCCAC-3', R 5'-CGGCATCCAGGTAATTTTTG-3', 399 bp, Tm 60°C) (3' region of exon VIII: F 5'-CTCTACTCTTTCTGCTGG-3', R 5'-GAATAATTTACCCTGTTATG-3', 505 bp, Tm 54°C). The amplification included an initial step at 94°C 5min, 35 cycles of 30 sec 94°C, 30 sec Tm (see before), 1 min 72°C, and a final step at 72°C 1 min. PCR products (5 µL) were separated on 1.5 % agarose gel and visualized by ethidium bromide staining. Sequencing was performed on each strand of PCR products using Big Dye Terminator chemistry (Perkin Elmer). The primers used for sequencing were the same as for amplification. Electrophoresis and analysis were done on an ABI Prism 377 sequencer (Perkin Elmer).

Hippocampus sampling and real time quantitative PCR

Mice were killed by CO_2 asphyxiation and decapitated. The hippoccampus was dissected and immediately frozen on dry ice before conservation at -80°C until analysis. This brain region was selected because it is one of the main sites of BDNF release. Total RNA was extracted using Trizol (Invitrogen, Cergy Pontoise, France) according to manufacturer's instructions and treated with DNAseI (Invitrogen, 1 µL/µg RNA). Reverse transcription reaction was

performed at 42°C 45 min in a final volume of 50 µL containing 500 ng of total RNA, 100 ng random hexamers, 1 mM dNTP, 10 mM DTT, 1X buffer, 10 U superscript II reverse transcriptase (Invitrogen). The reaction mixture for real time quantitative PCR consisted in 10 ng cDNA from reverse transcription reaction, 12.5 µL of Platinium SYBR Green qPCR SuperMix UDG (Invitrogen), 0.4 pmol/µL of both forward and reverse primers. β-actin F: 5'-gacaggatgcagaaggagattact-3', R: 5'-atctgctggaaggtggacag-3'; BDNF (Alfonso *et al.*, 2006). Threshold cycle (Ct) values were obtained and relative levels of transcription were calculated by the $2^{-\Delta\Delta Ct}$ method.

Free exploratory paradigm

The apparatus consisted of two parts (30×10×10cm each) separated by sliding-doors. Each part was divided into three sections (10×10 cm). One part was covered with fresh sawdust and contained free available food and water. Mice were introduced in this part 24h before the start of the test, the sliding-doors being closed. Testing took place during the dark phase, under red light and began with the opening of the sliding-doors. Time spent in the new part and attempts (partial entry in the new part followed by a withdrawal) were scored during 10 min (Belzung & Berton, 1997).

Splash test

This test was conducted as previously described (Santarelli *et al.*, 2003, Surget *et al.*, 2008). It consisted in squirting a 10% sucrose solution on the mouse in its home cage. The grooming frequency was recorded during five minutes after the vaporization.

Body weight and food intake

Mice were weighed two weeks after their arrival in the lab allowing them to adapt to their new housing conditions.

Twelve hours before testing food intake, food was removed from the cage. The feeding drive of each animal (3-4 months of age) was then assessed by replacing regular food pellets in the cage and measuring the amount of food consumed over a period of 15 minutes (15-min food consumption).

Actimeter

The actimeter allowed the assessment of the activity of mice in their home cage, thus excluding the possibility of biasing the results due to novelty-induced anxiety. The home cage

was placed in the centre of a device, which consisted of a 20x20cm square plane with photoelectric beams, crossing the plane from midpoint to midpoint of opposite sides, thus dividing the plane into four quadrants. The actimeter automatically detects the movement of the animal when it crossed through, allowing to quantify the number of beam breaks. The higher the score, the more the mouse moved. Testing was carried out from 10:00h and lasted 4 hours to allow a better estimation of the basal locomotor activity.

Social learning of food preference (SLFP)

This test was a modified version of the one used by Levy and colleagues (Levy *et al.*, 2003). One mouse was chosen randomly in each cage to be the demonstrator and the others were then called the observers. Cued diets consisted of powdered regular chow mixed with either 2% cocoa (COC) or 1% cinnamon (CIN). The cued diets were mixed 1 week before they were needed. The demonstrator animals were isolated at 21:00h. The next day, from 09:00h to 11:00h, the regular food was replaced by cued diet (either one COC or one CIN pellet) for 2h in the cage of the demonstrator. After this confrontation of the demonstrator with the cued diet, it was placed back into the cage with the associated observers. The demonstrators remained with the observers for 30min and further were again isolated. Experimentators ensured that contacts between demonstrators and observers occurred. At 21:00h, the observer mice were isolated. The next day, from 09:00h to 10:00h, after removal of the regular food, one COC and one CIN pellet (only one of them was thus identical to the cued diet consumed by the demonstrator) were introduced in the cage of the observer at the opposite side of the mouse. Observer animals were allowed to eat both pellets undisturbed for 1h, when CIN and COC were weighed. The proportion of the total diet intake that was the same as the cued diet consumed by the demonstrator to which a given mouse was confronted was computed for each animal (proportional intake of cued diet = cued diet intake / total food intake) and then it was expressed in percentage. As a control, no preference for either COC or CIN was found without learning session (data not shown).

Statistical analysis

When assumptions for parametric statistics were ensured (normality and homoscedasticity), ANOVAs were performed to examine the effects of strain and of polymorphism (mice with Leu allele versus mice with Met allele), followed by a Fisher *post hoc* analysis when required (i.e., $p<0.05$). Otherwise, data were analyzed by the non-parametric procedures: Kruskal–

Wallis “ANOVA by ranks” and Mann-Whitney’s U-tests. A t-test for one sample was used in the SLFP to compare the distribution of the strain sample to the theoretical distribution.

With the aim of facilitating the understanding of the results in the main test, a representation of the inter-strain differences in the main text were made as follow: when a virgule “,” separates two strains, it indicates both that no significant difference occurred between them and that the strains displayed identical profile of paired comparison; a “≤” indicates that both strains were not significantly different but their profiles of paired comparisons were distinct allowing a classification; a “< ” between two strains indicates that the result of one strain is significantly lower than the one of the other strain. All tests required a $p<0.05$ for significance. The number of mice per group is indicated in the figure legends.

Results

Analysis of bdnf gene sequence and expression in the hippocampus

A single substitution of a thymine by an adenine was observed at base 94 (94T>A) in the coding sequence of the exon 8 of the *bdnf* gene (Fig. 1a) in several mouse strains when compared with the published sequence (AY057907) from the C57BL/6 strain (Fig. 1b). This substitution changed a leucine to a methionine at position 32 (Leu32Met) in the pro-domain of the BDNF protein. This substitution occurred in A/J, BALB/c, CBA and DBA/2 strains while C3H and C57BL/6 strains exhibited the initial published sequence with a thymine at base 94 (Fig. 1c).

Using real time quantitative PCR, we next analyzed the level of *bdnf* mRNA in the hippocampus of the different strains (Fig. 1d). We did not observe significant variation in *bdnf* expression when comparing the strains (Kruskal-Wallis: $H_{5,18} = 3.74$, $p=0.587$). We could not detect any relationship between the level of *bdnf* expression and the allele of the Leu32Met polymorphism (Mann-Whitney U: $Z_U=1.26$, $p=0.21$).

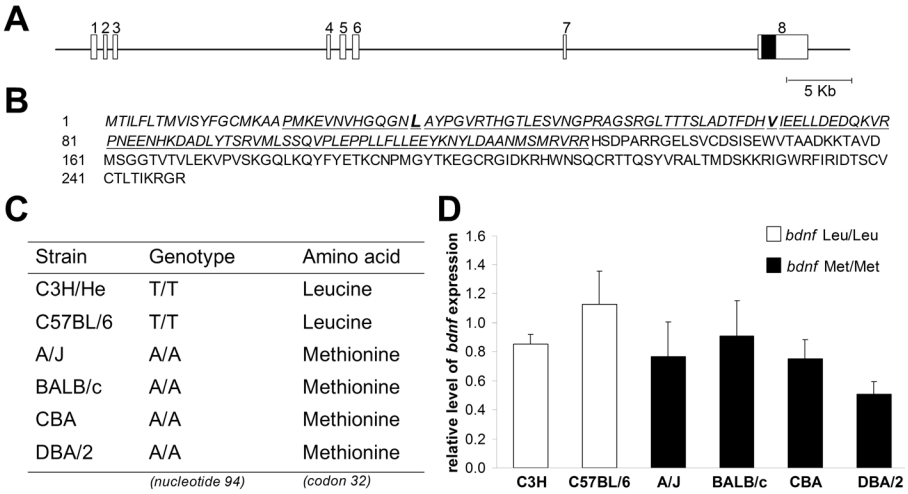

Figure 1. Mouse *bdnf* gene and hippocampal expression in six inbred strains. (A) Mouse BDNF gene structure. Open boxes indicates exons, dark box represents the coding sequence (Liu et al., 2005). (B) Deduced primary sequence of the prepro-BDNF protein of the C57BL/6 mouse strain. The prepro-domain is in italic, the pro-domain is underlined. The 94T>A polymorphism modifying a leucine to a methionine is indicated (leucine in bold and higher police size at position 32). The valine (bold in the sequence) corresponds to the Val66Met polymorphism observed in the human BDNF protein. (C) Genotypes at position 94 in the coding sequence of BDNF gene and deduced amino acids at position 32 of BDNF protein in the mouse strains analyzed. (D) Relative level of BDNF mRNA expression in the hippocampus of several inbred mouse strains (n=3-4 per group). Data represent mean ± SEM.

Association between Leu32Met polymorphism and behavioral phenotype

Free exploratory paradigm. Because involvement of BDNF in anxiety-like behaviors was suggested in mice, we firstly examined the possibility of an association of the *bdnf* Leu32Met polymorphism with anxiety-like phenotypes. Mice from the six strains were confronted simultaneously with familiar and novel compartments in the free exploratory paradigm. Low time in the novel areas along with high level of attempts to enter is usually considered to parallel greater neophobic behaviors (Belzung & Berton, 1997). Statistical analysis revealed significant strain differences in both the time in the novel compartments (ANOVA: $F_{5,54}$=72.83, $p < 0.001$) and the number of attempts ($H_{5,54}$=50.8, $p < 0.001$). *Post hoc* comparisons divided the six strains into three categories for both the time in the novel areas (lowest to highest duration: A/J < BALB/c, CBA < C3H, C57BL/6, DBA/2; Fig. 2a) and for the number of attempts (smallest to largest value: C57BL/6, DBA/2, C3H < CBA < BALB/c, A/J; Fig. 2b). It is noteworthy that C3H and C57BL/6 mice were found in the same category for both measures. Indeed, significant effects of the Leu32Met polymorphism emerged from our results with higher time in the novel compartments (Z_U=3.84, $p<0.001$) and lesser attempts (Z_U=4.64, $p<0.001$) for the Leu allele carriers than the Met allele carriers.

Splash test. In this test, the arrival of a sudden stimulus (squirting a sucrose solution) initiates a conflicting motivation between vigilance (rearing) and grooming (Ducottet *et al.*, 2004).

Kruskal-Wallis test revealed significant inter-strain differences ($H_{5,65}$=42.64, p<0.001). A/J, C57BL/6 and DBA/2 mice displayed higher frequency of grooming than BALB/c, C3H and CBA (Fig. 2c). This classification does not parallel with the *bdnf* allele distribution which is confirmed by the lack of significant effect due to the polymorphism ($F_{1,69}$=0.5, p=0.484).

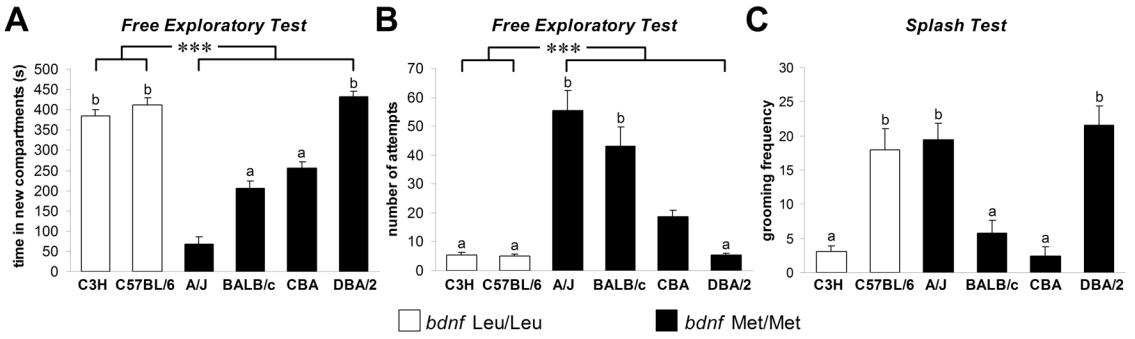

Figure 2. Effect of the *bdnf* Leu32Met polymorphism on the anxiety-like behaviors in six inbred mouse strains. (A) Time spent in the novel compartments and (B) number of attempts to enter in the novel compartments during the free exploratory test (n=10 mice/group). (C) Grooming frequency in the splash test (n=11-12 mice/group). Data represent mean ± SEM. The strains not sharing a common letter are significantly different (at least p<0.05). ***, p<0.001 indicates a significant effect of the polymorphism.

Body weight and feeding behavior. To determine whether this polymorphism could contribute to BDNF involvement in weight gain and feeding motivation, we measured body weight of each strain as well as food intake, and further analyzed if these parameters correlated with *bdnf* allele distribution. Although inbred mouse strains displayed significant differences in the body weight ($F_{5,207}$=40.15, p<0.001) as follow A/J < DBA/2 ≤ C3H, C57BL/6 ≤ BALB/c ≤ CBA (Fig. 3a), these differences are not related to the Leu32Met polymorphism (Z_U=1.41, p=0.159). Moreover, the feeding drive was estimated by 15-min home food consumption following 12h food-deprivation (Fig. 3b). Food intake was not significantly affected neither by strains ($F_{5,65}$=2.11, p=0.076) nor by Leu32Met polymorphism ($F_{1,69}$=0.014, p=0.907).

Actimeter. Locomotor activity was assessed in the home cage for four hours during the nocturnal phase. Considering that deficit in BDNF was associated with hyperactivity, it was therefore possible that the *bdnf* Leu32Met polymorphism underlie changes in locomotor activity. Although strain differences emerged from these data ($F_{5,65}$=4.03, p<0.01) as follow CBA, C3H ≤ A/J ≤ DBA/2 ≤ BALB/c, C57BL/6 (Fig. 3c), no effect due to the Leu32Met polymorphism was found ($F_{1,69}$=0.22, p=0.642).

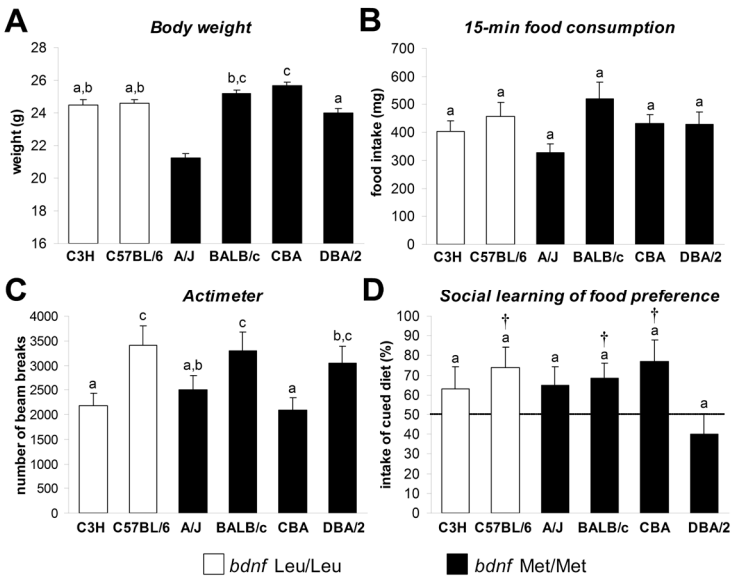

Figure 3. Effect of the *bdnf* Leu32Met polymorphism on body weight, food intake, locomotor activity, learning and memory in six inbred mouse strains. (A) Body weight (n=34-38 mice/group). (B) 15-min food consumption in the home cage after a 12h-food deprivation (n=11-12 mice/group). (C) Number of beam breaks in the actimeter (n=11-12 mice/group). (D) Percentage of cued diet intake in the social learning of food preference (n=7-8 mice/group). Data represent mean ± SEM. The strains not sharing a common letter are significantly different (at least $p<0.05$). †, $p<0.05$ indicates significant differences between observed value and non-preference threshold (50%, black line).

SLFP paradigm. Considering that learning and memory are also influenced by BDNF, we aimed to assess the impact of the Leu32Met polymorphism on these functions. Nevertheless, two (C3H and CBA) of the strains have the rld mutation which causes retinal degeneration (Wahlsten *et al.*, 2003). As a result of which, testing mice using paradigms with visuospatial tasks (such as Morris Water Maze or contextual fear conditioning) would have been unproductive. Instead, we tested strain performance in the SLFP paradigm which is based on olfactory cues. The proportion of the total diet intake that was the same as the cued diet eaten by demonstrator was computed for each animal. A lack of learning had to be characterized by a 50% intake of the cued diet. Differences were observed across strains for the acquisition of the food preference (Fig. 3d). BALB/c ($t=2.52$, $p<0.05$), C57BL/6 ($t=2.41$, $p<0.05$) and CBA ($t=2.47$, $p<0.05$) mice displayed a significant preference for the demonstrator diet, which was not observed in A/J ($t=1.53$, $p=0.171$), C3H ($t=1.15$, $p=0.289$) and DBA/2 ($t=0.95$, $p=0.374$). However, statistical analysis was unable to reveal any significant difference when comparing strains ($F_{5,40}=1.71$, $p=0.154$) or in function of the *bdnf* genotype ($F_{1,44}=0.52$, $p=0.474$).

Other data. Scientific literature abounds with inbred strains surveys investigating specific behavioral phenotypes. We found two studies employing together the same six strains used here and investigating behaviors relevant for our study. The first study was realized in our lab

and investigated strain differences in a chronic model of depression (the unpredictable chronic mild stress) and of antidepressant reversal (Ibarguen-Vargas *et al.*, 2008). Indeed, BDNF has frequently been associated with major depressive disorders and antidepressant action (Martinowich *et al.*, 2007). This mouse model of depression induced various strain-dependent alterations and antidepressant responses, but no clear association was established with the *bdnf* Leu32Met polymorphism (data not shown).

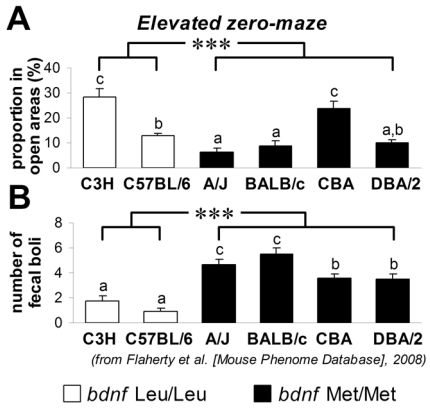

Figure 4. Effect of the Leu32Met polymorphism in the elevated zero-maze in a project of the Mouse Phenome Database (A) Time proportion spent in open areas (n=19-20 mice/group) and (B) number of fecal boli (n=17-20 mice/group) in the elevated zero-maze were tested in the same six strains used in our study, as part of the Mouse Phenome Project (http://www.jax.org/phenome), MPD accession number 118 (Flaherty et al., 2001). Data represent mean ± SEM. The strains not sharing a common letter are significantly different (at least $p<0.05$). ***, $p<0.001$ indicates a significant effect of the polymorphism.

The second study arose from available resources from the Mouse Phenome Database (http://www.jax.org/phenome). Anxiety-related behaviors were assessed in the elevated zero-maze during a 5-min test period in different inbred mouse strains, including the six used in the present study (Flaherty *et al.*, 2001). This paradigm usually correlates lower time in open versus closed quadrants and greater defecations to higher anxiety-like behaviors (Shepherd *et al.*, 1994). These data revealed significant inter-strain differences in both the time in open areas ($H_{5,112}=46.53$, $p<0.001$) and the number of fecal boli ($F_{5,109}=19.36$, $p<0.001$). *Post hoc* comparisons sorted strains in several categories for the time in open quadrants (A/J, BALB/c ≤ DBA/2 ≤ C57BL/6 < CBA, C3H; Fig. 4a) and for the number of fecal boli (C57BL/6, C3H < DBA/2, CBA ≤ A/J ≤ BALB/c; Fig. 4b). These classifications seem to parallel with *bdnf* allele distribution among the strains. Indeed, b*dnf* Met allele carriers are significantly associated with lower time in open quadrants ($F_{1,116}=13.1$, $p<0.001$) and greater number of fecal boli ($Z_U=6.71$, $p<0.001$) than *bdnf* Leu carriers.

Discussion

In this study, we reported a SNP (94T>A) in the mouse *bdnf* gene inducing an amino acid substitution (leucine to methionine) at codon 32 in the pro-domain of the precursor protein of BDNF. C3H and C57BL/6 strains exhibited a thymine at base 94 similar to the published sequence (AY057907), while A/J, BALB/c, CBA and DBA/2 strains had an adenine at base 94. Further, we examined the *bdnf* expression and screened various behavioral phenotypes relevant for BDNF function in these six inbred mouse strains. While no relationship between this SNP and the *bdnf* expression was found in the hippocampus, the *bdnf* Leu32Met polymorphism was found to contribute to strain differences in anxiety-like phenotypes but not in body weight, feeding and grooming behaviors, activity, learning and memory. *Bdnf* Met allele correlated with higher anxiety-like behaviors in the free exploratory paradigm as well as in the elevated zero-maze from the Mouse Phenome Database (Flaherty *et al.*, 2001).

We firstly aimed to examine whether the Leu32Met SNP in the *bdnf* gene could contribute to possible inter-strain differences of the basal *bdnf* expression in the hippocampus. This brain area was selected because it is known as one of the main sites of BDNF release as well as one of regions the more studied for learning, memory and anxiety (Chen *et al.*, 2006). No significant difference of *bdnf* mRNA level was found between strains suggesting that the Leu32Met polymorphism does not influence the hippocampal *bdnf* expression. Nevertheless, it cannot be excluded that this SNP affects the *bdnf* expression in stimulated conditions or in other regions. Indeed, the human *bdnf* Val66Met polymorphism has no effect on *bdnf* mRNA level but impacts cellular processing, trafficking and activity-dependant secretion of BDNF (Chen *et al.*, 2004, Egan *et al.*, 2003). Moreover, changes in complex behaviors such as contextual learning and anxiety have been observed in a transgenic mouse in which the human *bdnf* Val66Met polymorphism is modeled (Chen *et al.*, 2006). The Leu32Met polymorphism of the mouse *bdnf* gene displayed similar features to the human polymorphism (located in the prodomain of the proBDNF, a substitution from an aliphatic amino acid to a Met), and it therefore might similarly affect BDNF physiology and complex behavioral traits.

By examining the behavioral profiles of the six strains used here, our study revealed critical differences among inbred mouse strains and highlighted the *bdnf* Leu32Met polymorphism as a contributor to behavioral phenotypes, particularly to anxiety-related behaviors. Indeed, mice carrying the Met allele displayed greater propensity to develop anxiety-related behaviors. Accordingly, Leu allele would be protective while Met allele would represent a risk factor for anxiety. It is now assumed that anxiety should not be conceptualized as a continuum but as a

multidimensional construct with qualitative differences (Endler & Kocovski, 2001). From this perspective, it is important to distinguish trait anxiety from state anxiety as suggested by Spielberger (Spielberger, 1966). State anxiety can be defined as a transitory emotion to a stressful situation with physiological arousal whereas trait anxiety can be viewed as an individual's predisposition and an enduring characteristic. In rodent, it has been suggested that state and trait anxiety can be evoked distinctly depending on the experimental paradigms used (Belzung & Griebel, 2001). From this point of view, the free exploratory paradigm is thought to model trait anxiety (Belzung & Berton, 1997). In this test, mice have free access to novelty and do not display neuroendocrine changes (Misslin & Cigrang, 1986), as a consequence of which this paradigm can be considered to be devoid of stressful components. Therefore, neophobic reactions (i.e. avoidance of new compartments) in this procedure indicate a constant feature of the behavior assimilated to trait anxiety. Because *bdnf* allele distribution among strains correlated with neophobic level in the free exploratory paradigm, the contribution of the *bdnf* Leu32Met polymorphism in anxiety-related phenotype may be related to changes in trait anxiety.

On the other hand, behavioral tests based on the application of an anxiogenic stimulus are presumed to model state anxiety. Both the elevated zero-maze and the splash test belong to this category. The former is built on the forced confrontation with unfamiliar aversive place while the latter is performed in the context of the home cage but based on the arrival of a sudden stimulus (squirting sucrose solution). However, strains behaved distinctly in these paradigms: while strain performances in the splash test were unrelated to the *bdnf* Leu32Met polymorphism, this SNP seemed to contribute to their behavior in the elevated zero-maze. This result indicates that both tests underlie different aspects of state anxiety. Indeed, it can be important to take into account the multiple facets of state and trait anxiety. Endler and Kocovski (2001) suggested that the level of state anxiety is dependent on the congruence of the facet of trait anxiety and the stressful situation. In this light, considering that the elevated zero-maze is based on exposure to a new environment, the *bdnf* Leu32Met polymorphism might be involved more specifically in a neophobic aspect of anxiety. This outcome strengthened the results obtained in the free exploratory paradigm.

In any case, our findings are consistent with earlier studies underlining a role of BDNF in anxiety. Indeed, a relationship between the *bdnf* Val66Met variant and anxiety-related phenotypes in human was previously demonstrated, despite some inconsistencies(Surtees *et al.*, 2007). The Met66 allele was found alternately to be protective against neuroticism (Hunnerkopf *et al.*, 2007) and anxiety-related traits (Lang *et al.*, 2005), or to be a risk factor

for anxiety and for depression-anxiety comorbidity (Enoch *et al.*, 2007, Jiang *et al.*, 2005). Other informative reports arose from strategies based on targeting invalidation of *bdnf* gene. Conditional deletion of *bdnf* in the adult mouse forebrain led to increased anxiety-like behaviors (Rios *et al.*, 2001), although conflicting results were reported (Chan *et al.*, 2006, Monteggia *et al.*, 2007). Furthermore, mice lacking one *bdnf* allele exhibit exaggerated anxiety-like behaviors when associated with deficiency in the serotonin transporter (Ren-Patterson *et al.*, 2005), suggesting potential epistasic interactions between *bdnf* and serotonin system in anxiety.

All other traits investigated in our study were not affected by the *bdnf* Leu32Met polymorphism. Indeed, we examined body weight, feeding behaviors, activity, learning and memory but no relationship with these *bdnf* allelic variants was found. When regarding the results from a similar strain survey in the unpredictable chronic mild stress (Ibarguen-Vargas *et al.*, 2008), the occurrence of depression-like states or antidepressant responses was independent of the *bdnf* allelic variant. Taken together, the *bdnf* Leu32Met polymorphism seems constrictively related to the sole anxiety-like behaviors. However, the *bdnf* Val66Met polymorphism was more largely associated with several of these traits in human genetic studies such as learning and memory (Egan *et al.*, 2003, Hariri *et al.*, 2003), hyperactivity (Kent *et al.*, 2005), depression-related disorders (Hwang *et al.*, 2006, Post, 2007, Ribeiro *et al.*, 2007), antidepressant reversal (Choi *et al.*, 2006), despite some discrepancies (Conner *et al.*, 2008, Hong *et al.*, 2003, Tsai *et al.*, 2003). Actually, complex behaviors and psychological traits are often variable and influenced by a constellation of genes as well as environmental factors. Even if the *bdnf* Leu32Met polymorphism contributes at least partially to these traits, strain heterogeneity in genetic background might perhaps mask detectable phenotypes.

In summary, our findings demonstrated an association between the *bdnf* Leu32Met polymorphism and anxiety-related phenotypes, particularly the Met allele correlated with increases of neophobia and trait anxiety. These results support previous evidences of an involvement of BDNF in anxiety behavior but refine its role in neophobic reactions and trait anxiety level. Future studies should develop congenic mice in which both *bdnf* allelic variants would be present in the same genetic background in order to unravel more accurately the contribution of this SNP in complex behavioral traits.

Acknowledgement

We express our gratitude to Rose-Anne Thepault, Sylviane Védrine and Raymond Jegat for the beneficial helps.

References

Alfonso, J., Frick, L.R., Silberman, D.M., Palumbo, M.L., Genaro, A.M. & Frasch, A.C. (2006) Regulation of hippocampal gene expression is conserved in two species subjected to different stressors and antidepressant treatments. *Biol Psychiatry,* **59,** 244-251.

Belzung, C. & Berton, F. (1997) Further pharmacological validation of the BALB/c neophobia in the free exploratory paradigm as an animal model of trait anxiety. *Behav Pharmacol,* **8,** 541-548.

Belzung, C. & Griebel, G. (2001) Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res,* **125,** 141-149.

Chan, J.P., Unger, T.J., Byrnes, J. & Rios, M. (2006) Examination of behavioral deficits triggered by targeting Bdnf in fetal or postnatal brains of mice. *Neuroscience,* **142,** 49-58.

Chen, Z.Y., Jing, D., Bath, K.G., Ieraci, A., Khan, T., Siao, C.J., Herrera, D.G., Toth, M., Yang, C., McEwen, B.S., Hempstead, B.L. & Lee, F.S. (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science,* **314,** 140-143.

Chen, Z.Y., Patel, P.D., Sant, G., Meng, C.X., Teng, K.K., Hempstead, B.L. & Lee, F.S. (2004) Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J Neurosci,* **24,** 4401-4411.

Choi, M.J., Kang, R.H., Lim, S.W., Oh, K.S. & Lee, M.S. (2006) Brain-derived neurotrophic factor gene polymorphism (Val66Met) and citalopram response in major depressive disorder. *Brain Res,* **1118,** 176-182.

Conner, A.C., Kissling, C., Hodges, E., Hunnerkopf, R., Clement, R.M., Dudley, E., Freitag, C.M., Rosler, M., Retz, W. & Thome, J. (2008) Neurotrophic factor-related gene polymorphisms and adult attention deficit hyperactivity disorder (ADHD) score in a high-risk male population. *Am J Med Genet B Neuropsychiatr Genet.*

Crawley, J.N., Belknap, J.K., Collins, A., Crabbe, J.C., Frankel, W., Henderson, N., Hitzemann, R.J., Maxson, S.C., Miner, L.L., Silva, A.J., Wehner, J.M., Wynshaw-Boris, A. & Paylor, R. (1997) Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology (Berl),* **132,** 107-124.

Ducottet, C., Aubert, A. & Belzung, C. (2004) Susceptibility to subchronic unpredictable stress is related to individual reactivity to threat stimuli in mice. *Behav Brain Res*, **155,** 291-299.

Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B. & Weinberger, D.R. (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell,* **112,** 257-269.

Endler, N.S. & Kocovski, N.L. (2001) State and trait anxiety revisited. *J Anxiety Disord,* **15,** 231-245.

Enoch, M.A., White, K.V., Waheed, J. & Goldman, D. (2007) Neurophysiological and genetic distinctions between pure and comorbid anxiety disorders. *Depress Anxiety*.

Flaherty, L., Cook, M.N. & Williams, R.W. (2001) Anxiety-related behaviors in the elevated zero-maze. MPD:118. Mouse Phenome Database Web Site, The Jackson Laboratory, Bar Harbor, Maine USA. World Wide Web (URL: http://www.jax.org/phenome, April 2008)

Gray, J., Yeo, G.S., Cox, J.J., Morton, J., Adlam, A.L., Keogh, J.M., Yanovski, J.A., El Gharbawy, A., Han, J.C., Tung, Y.C., Hodges, J.R., Raymond, F.L., O'Rahilly, S. & Farooqi, I.S. (2006) Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. *Diabetes,* **55,** 3366-3371.

Hariri, A.R., Goldberg, T.E., Mattay, V.S., Kolachana, B.S., Callicott, J.H., Egan, M.F. & Weinberger, D.R. (2003) Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *J Neurosci,* **23,** 6690-6694.

Hong, C.J., Huo, S.J., Yen, F.C., Tung, C.L., Pan, G.M. & Tsai, S.J. (2003) Association study of a brain-derived neurotrophic-factor genetic polymorphism and mood disorders, age of onset and suicidal behavior. *Neuropsychobiology*, **48,** 186-189.

Hunnerkopf, R., Strobel, A., Gutknecht, L., Brocke, B. & Lesch, K.P. (2007) Interaction between BDNF Val66Met and dopamine transporter gene variation influences anxiety-related traits. *Neuropsychopharmacology,* **32,** 2552-2560.

Hwang, J.P., Tsai, S.J., Hong, C.J., Yang, C.H., Lirng, J.F. & Yang, Y.M. (2006) The Val66Met polymorphism of the brain-derived neurotrophic-factor gene is associated with geriatric depression. *Neurobiol Aging,* **27,** 1834-1837.

Ibarguen-Vargas, Y., Surget, A., Touma, C., Palme, R. & Belzung, C. (2008) Multifaceted strain-specific effects in a chronic mouse model of depression and of antidepressant reversal. *Psychoneuroendocrinology*.

Jiang, X., Xu, K., Hoberman, J., Tian, F., Marko, A.J., Waheed, J.F., Harris, C.R., Marini, A.M., Enoch, M.A. & Lipsky, R.H. (2005) BDNF variation and mood disorders: a novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. *Neuropsychopharmacology,* **30,** 1353-1361.

Kent, L., Green, E., Hawi, Z., Kirley, A., Dudbridge, F., Lowe, N., Raybould, R., Langley, K., Bray, N., Fitzgerald, M., Owen, M.J., O'Donovan, M.C., Gill, M., Thapar, A. & Craddock, N. (2005) Association of the paternally transmitted copy of common Valine allele of the Val66Met polymorphism of the brain-derived neurotrophic factor (BDNF) gene with susceptibility to ADHD. *Mol Psychiatry,* **10,** 939-943.

Kernie, S.G., Liebl, D.J. & Parada, L.F. (2000) BDNF regulates eating behavior and locomotor activity in mice. *Embo J,* **19,** 1290-1300.

Lang, U.E., Hellweg, R., Kalus, P., Bajbouj, M., Lenzen, K.P., Sander, T., Kunz, D. & Gallinat, J. (2005) Association of a functional BDNF polymorphism and anxiety-related personality traits. *Psychopharmacology (Berl),* **180,** 95-99.

Lebrun, B., Bariohay, B., Moyse, E. & Jean, A. (2006) Brain-derived neurotrophic factor (BDNF) and food intake regulation: a minireview. *Auton Neurosci,* **126-127,** 30-38.

Levy, F., Melo, A.I., Galef, B.G., Jr., Madden, M. & Fleming, A.S. (2003) Complete maternal deprivation affects social, but not spatial, learning in adult rats. *Dev Psychobiol,* **43,** 177-191.

Linnarsson, S., Bjorklund, A. & Ernfors, P. (1997) Learning deficit in BDNF mutant mice. *Eur J Neurosci,* **9,** 2581-2587.

Liu, Q.R., Lu, L., Zhu, X.G., Gong, J.P., Shaham, Y. & Uhl, G.R. (2006) Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. *Brain Res,* **1067,** 1-12.

Lyons, W.E., Mamounas, L.A., Ricaurte, G.A., Coppola, V., Reid, S.W., Bora, S.H., Wihler, C., Koliatsos, V.E. & Tessarollo, L. (1999) Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc Natl Acad Sci U S A,* **96,** 15239-15244.

Martinowich, K., Manji, H. & Lu, B. (2007) New insights into BDNF function in depression and anxiety. *Nat Neurosci,* **10,** 1089-1093.

Misslin, R. & Cigrang, M. (1986) Does neophobia necessarily imply fear or anxiety? *Behav Processes,* **12,** 45-50.

Monteggia, L.M., Luikart, B., Barrot, M., Theobold, D., Malkovska, I., Nef, S., Parada, L.F. & Nestler, E.J. (2007) Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. *Biol Psychiatry,* **61,** 187-197.

Post, R.M. (2007) Role of BDNF in bipolar and unipolar disorder: clinical and theoretical implications. *J Psychiatr Res,* **41,** 979-990.

Ren-Patterson, R.F., Cochran, L.W., Holmes, A., Sherrill, S., Huang, S.J., Tolliver, T., Lesch, K.P., Lu, B. & Murphy, D.L. (2005) Loss of brain-derived neurotrophic factor gene allele exacerbates brain monoamine deficiencies and increases stress abnormalities of serotonin transporter knockout mice. *J Neurosci Res,* **79,** 756-771.

Ribeiro, L., Busnello, J.V., Cantor, R.M., Whelan, F., Whittaker, P., Deloukas, P., Wong, M.L. & Licinio, J. (2007) The brain-derived neurotrophic factor rs6265 (Val66Met) polymorphism and depression in Mexican-Americans. *Neuroreport,* **18,** 1291-1293.

Rios, M., Fan, G., Fekete, C., Kelly, J., Bates, B., Kuehn, R., Lechan, R.M. & Jaenisch, R. (2001) Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Mol Endocrinol,* **15,** 1748-1757.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C. & Hen, R. (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science,* **301,** 805-809.

Shepherd, J.K., Grewal, S.S., Fletcher, A., Bill, D.J. & Dourish, C.T. (1994) Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. *Psychopharmacology (Berl),* **116,** 56-64.

Spielberger, C.D. (1966) The effects of anxiety on complex learning and academic achievement. In Spielberger, C.D. (ed), *Anxiety and behaviour.* Academic Press, New York, pp. 361-398.

Surget, A., Saxe, M., Leman, S., Ibarguen-Vargas, Y., Chalon, S., Griebel, G., Hen, R. & Belzung, C. (2008) Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. *Biol Psychiatry*. in press.

Surtees, P.G., Wainwright, N.W., Willis-Owen, S.A., Sandhu, M.S., Luben, R., Day, N.E. & Flint, J. (2007) No association between the BDNF Val66Met polymorphism and mood status in a non-clinical community sample of 7389 older adults. *J Psychiatr Res,* **41,** 404-409.

Tapia-Arancibia, L., Rage, F., Givalois, L. & Arancibia, S. (2004) Physiology of BDNF: focus on hypothalamic function. *Front Neuroendocrinol,* **25,** 77-107.

Taylor, W.D., Zuchner, S., McQuoid, D.R., Steffens, D.C., Blazer, D.G. & Krishnan, K.R. (2008) Social support in older individuals: The role of the BDNF Val66Met polymorphism. *Am J Med Genet B Neuropsychiatr Genet*.

Tsai, S.J. (2007) Increased central brain-derived neurotrophic factor activity could be a risk factor for substance abuse: Implications for treatment. *Med Hypotheses,* **68,** 410-414.

Tsai, S.J., Cheng, C.Y., Yu, Y.W., Chen, T.J. & Hong, C.J. (2003) Association study of a brain-derived neurotrophic-factor genetic polymorphism and major depressive disorders, symptomatology, and antidepressant response. *Am J Med Genet B Neuropsychiatr Genet,* **123,** 19-22.

Tyler, W.J., Alonso, M., Bramham, C.R. & Pozzo-Miller, L.D. (2002) From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. *Learn Mem,* **9,** 224-237.

Unger, T.J., Calderon, G.A., Bradley, L.C., Sena-Esteves, M. & Rios, M. (2007) Selective deletion of Bdnf in the ventromedial and dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity. *J Neurosci,* **27,** 14265-14274.

Wahlsten, D., Rustay, N.R., Metten, P. & Crabbe, J.C. (2003) In search of a better mouse test. *Trends Neurosci,* **26,** 132-136.

-

Article 2 - Multifaceted strain-specific effects in a chronic mouse model of depression and of antidepressant reversal

Dans cet article nous avons exploré la sensibilité de six lignées consanguines des souris dans le modèle du stress chronique, ainsi que leur réponse à un traitement chronique avec un antidépresseur. Dans une deuxième étape, nous avons étudié le lien entre des variantes phénotypiques et le polymorphisme Leu32Met du gène BDNF ; cette deuxième partie du travail n'est pas présentée dans l'article mais dans une discussion complémentaire (page 108)

Psychoneuroendocrinology (en revision)

Multifaceted strain-specific effects in a chronic mouse model of depression and of antidepressant reversal

Yadira Ibarguen-Vargas[a,b], Alexandre Surget[a,b], Chadi Touma[c], Rupert Palme[d], Catherine Belzung[a,b,*]

[a] U930, Inserm, Tours, France

[b] Université François Rabelais, Tours, France

[c] Department of Behavioral Neuroendocrinology, Max Planck Institute of Psychiatry, Munich, Germany

[d] Institute of Biochemistry, Department of Natural Sciences, University of Veterinary Medicine, Vienna, Austria

* Corresponding author: Faculté des Sciences et Techniques, Université François Rabelais, Bât O Parc de Grandmont, 37200 Tours, France. Phone: +33 2 47 36 69 94; Fax: +33 2 47 36 72 85. E-mail: catherine.belzung@univ-tours.fr

Running title: Strain-specific effects in a chronic model of depression and of antidepressant reversal

SUMMARY

Etiopathogenesis of depression and origin of treatment insensitivity remain poorly understood, although the contribution of the genetic makeup is established. The homozygosity of inbred mouse strains provides a useful tool to investigate linkage of genes to behaviors or drug responses. Hence, we aimed to identify inbred mouse strains (among A/J, BALB/c, C3H, C57BL/6, CBA, DBA and FVB) sensitive to a 9-week unpredictable chronic mild stress (UCMS) and, from the fifth week onward, to antidepressant (AD) reversal (imipramine, 20mg/kg/day i.p.) in different depression-related changes: physical, behavioral and neuroendocrine states. UCMS induced a significant deterioration of the coat state (in all the strains), a blunted emotional reactivity in the novelty-suppressed feeding (NSF) test (A/J, BALB/c, C57BL/6) and changes in the level of fecal corticosterone metabolites (BALB/c, C57BL/6, DBA, FVB). Imipramine treatment reversed the UCMS-induced alterations on the coat state (BALB/c, DBA), in the NSF test (A/J, BALB/c, C57BL/6) and in fecal corticosterone metabolites (BALB/c, C57BL/6). C3H, CBA and FVB mice were irresponsive to imipramine treatment. It is noteworthy that physical or behavioral changes due to UCMS did occur without hypothalamo-pituitary-adrenal (HPA) axis alterations in some strains (A/J, C3H, CBA), although the AD reversal in BALB/c and C57BL/6 was associated with HPA axis normalization. Finally, UCMS is shown here to discriminate various endophenotypes and to replicate in a strain-dependent manner diverse profiles reminiscent of the human disease subtypes. UCMS may thus enable to select strains suited for investigations of specific depression-related aspects and can be an appropriate model to dissect genetic factors associated with increased vulnerability, particular symptoms of affective disorders, and AD resistance.

KEYWORDS

inbred strains, unpredictable chronic mild stress, imipramine, corticosterone, HPA axis, novelty-suppressed feeding.

INTRODUCTION

Major depressive disorder (MDD) embodies one of the most common and serious health problems of western societies (Murray and Lopez, 1997). MDD is not a well-defined syndrome as it encloses various subtypes with different patterns of alterations. While the etiology of MDD is multifactorial and far from ideally identified, chronic stress or stressful events have been characterized as one of the major environmental factors precipitating depression (Kendler et al., 1999). This is corroborated by the frequent occurrence of neuroendocrine stress system disturbances in MDD, such as hypercortisolemia and hypothalamo-pituitary-adrenal (HPA) axis negative feedback impairments (Holsboer, 2000). However, an adverse experience does not automatically trigger depressive episodes, but an individuals' vulnerability is related to personal history of stressful life events as well as developmental and genetic factors (Caspi et al., 2003). Likewise, the large heterogeneity in the symptom patterns, the presence of HPA disturbances and the frequently observed insensitivity to antidepressants (AD) could mainly originate from the genetic makeup. The knowledge of the genetic basis of these individual differences could help to unravel the origin of vulnerability, MDD subtypes and AD resistance. Animal models are thus needed as they enable to facilitate the discovery process of candidate genes.

Studies on inbred mouse strains may provide a powerful tool to understand the influence of genes in normal and altered brain function. Interestingly, a large variability in the response of different inbred strains has been observed in mouse models of depression (Bai et al., 2001; Liu and Gershenfeld, 2001; Lucki et al., 2001; David et al., 2003; Liu and Gershenfeld, 2003; Mineur et al., 2003; Ripoll et al., 2003; Pothion et al., 2004; Crowley et al., 2005; Ducottet and Belzung, 2005; Mineur et al., 2006). Nonetheless, the possibility to sort strains according to their propensity to develop depression-like behaviors or ADs response seems vain related to the discrepancy of results among models and studies. This could be due to the large diversity of models used. These models have in common to be sensitive to ADs, but they thoroughly differ in the theoretical background with which they are constructed. The most widely used are the forced swimming test (FST) and the tail suspension test (TST). Both tests are based on the exposure to a single aversive and inescapable situation, which induces a behavioral shift from struggling to immobility, interpreted as "behavioral despair"; a single AD administration is able to decrease the duration of immobility (Cryan and Holmes, 2005; Cryan et al., 2005; Jacobson and Cryan, 2007). Even though they gained popularity as pharmacological screening assays, the utilization of these bioassays of AD-like effects are increasingly extended in studies prospecting for neurobiology and pathophysiology as well as

to identify causative genes of depression. However, the fact that MDD is a chronic disease and that ADs are clinically active only after at least 3 weeks of treatment makes the validity of such models questionable, in particular when examining the relevant mechanisms implicated in the etiology, maintenance and treatment of MDD. Validated chronic models of depression, such as the Unpredictable Chronic Mild Stress (UCMS) paradigm, could represent an alternative to avoid such drawbacks. Indeed, UCMS model is based on subjecting mice to a chronic period (generally five to nine weeks) of socio-environmental mild stressors. Such a treatment recapitulates several behavioral and physiological depression-related impairments, which can be reversed by a chronic but not acute AD treatment (Belzung and Surget, 2008): decreased sucrose consumption (interpreted as anhedonia), increased fearfulness/anxiety-related behaviors, altered weight gain, degradation of the coat state (interpreted as the loss of interest in performing customary tasks).

An inbred mouse strain survey in the UCMS paradigm can be a first step toward the discovery of additional genes involved in the vulnerability to stress exposure, in the development of different MDD-associated symptoms and in the ADs' insensitivity. The major goal of the present study was thus to identify inbred mouse strains sensitive to the UCMS procedure and to AD reversal regarding different depression-related changes. Mice from seven different strains (A/J, BALB/c, C3H, C57BL/6, CBA, DBA and FVB) were subjected to a 9-week UCMS regimen. From the fifth week onward, we initiated a treatment by administering i.p. vehicle or imipramine (20mg/kg) daily. The effects of UCMS and of imipramine treatment were assessed using physical measures (coat state, weight), behavioral tests (novelty-suppressed feeding [NSF] test and actimeter) and, to consider HPA axis functioning, the level of fecal corticosterone metabolites.

METHODS

Animals

Male mice from seven inbred strains (A/J, BALB/cJ, CBA/J, C3H/HeJ, C57BL/6J, DBA/2J, FVB/NJ) were obtained from Centre d'élevage Janvier (Le Genest Saint Isle, France) and Harlan (Gannat, France). They were aged 7 weeks at their arrival in our lab. Before the onset of the experiments, all animals were housed in groups of 5 and were maintained under standard laboratory conditions under a 12/12h light/dark cycle (lights on at 2000h), 22±2°C, food and water *ad libitum*. The treatment of the animals was in accordance with the European Community Council directive 86/609/EEC.

Drugs

Imipramine hydrochloride (Sigma-Aldrich) was used in this study. Imipramine was prepared as solutions in physiological saline (NaCl 0.9%). Concentration was adjusted to administer a final volume of 10ml/kg.

General procedure

At their arrival, mice were kept in the laboratory for 2 weeks before the onset of the experiments. Then, a 9-week UCMS procedure was conducted. UCMS-exposed mice (2/3) were maintained under the same standard laboratory conditions but were isolated in small individual cages (24x11x12cm) while non-stressed control mice (1/3) were housed in groups of 4 or 5 in standard laboratory cages (42x28x18cm). The first four weeks of UCMS regimen were drug-free and treatment began from the fifth week of UCMS to the end of behavioral testing. Vehicle (NaCl 0.9%) or imipramine (20mg/kg/day) was administered i.p. once a day. Each strain was divided in three groups of mice: control/vehicle, UCMS/vehicle and UCMS/imipramine. Each group consisted of 9-13 animals. The dose was chosen on the basis of previous experiments showing that the compound is active at this concentration (Santarelli et al., 2003; Surget et al., 2008). The body weight and the state of the coat were assessed weekly until the end of UCMS. The day after the last body weight and coat state evaluation, behavioral testing was performed according to the following schedule: the first day NSF test and the fourth day actimeter. Control animals were isolated 2 days before the actimeter and until the end of the actimeter session, while, to avoid experimental bias, the cage of stressed mice was changed at the same time. One week after the last body weight and coat state evaluation, mice were sacrificed by CO2 asphyxia (from 1000h to 1500h), and then feces

were collected. Testing and feces collection were always carried out during the dark period. The experimental design is illustrated in figure 1.

Unpredictable Chronic Mild Stress (UCMS)

The stress regimen is a variant of the UCMS procedure used in our lab (Santarelli et al., 2003; Surget et al., 2008). Mice were subjected to various and repeated unpredictable stressors for a period of nine weeks. The different stressors were: altered bedding (sawdust change, removal of sawdust, damp sawdust, substitution of sawdust with 21°C water), cage tilting (45°), predator sounds (15 min), cage exchange (mice were positioned in the empty cage of another male), altered length and time of light/dark cycle. The body weight and the state of the coat were assessed once a week until the end of the nine weeks. The total score of the state of the coat resulted from the sum of the score obtained from seven different body parts: head, neck, dorsal coat, ventral coat, tail, forepaws and hindpaws. For each body area, a score of 0 was given for a well-groomed coat and 1 for an unkempt coat. This index has been pharmacologically validated in previous studies using BALB/c mice (Griebel et al., 2002a; 2002b; Ducottet et al., 2003; Santarelli et al., 2003; Alonso et al., 2004; Yalcin et al., 2005; Surget et al., 2008).

Novelty-Suppressed Feeding (NSF) Test

The NSF Test was a similar version of the one used by Surget and colleagues (2008). The testing apparatus consisted of a wooden box, 33x33x30cm with an indirect red light. The floor was covered with 2 cm sawdust. Twelve hours before the test, food was removed from the cages. At the time of testing, a single pellet of food (regular chow) was placed on a white paper platform positioned in the center of the box. An animal was placed in a corner of the test box. The latency to start consuming the pellet was recorded within a 3-minute period. This test induced a conflicting motivation between the drive to eat the food pellet and the fear of venturing into the center of the arena. This paradigm is able to reveal the effects of chronic ADs treatment in non-stressed mice. In this study, we wanted to highlight the specific chronic ADs effect in stressed mice. So, compared with the study of Santarelli and colleagues (2003), we reduced the dimension of the apparatus by 40% and we used a red light instead a white light in order to strongly decrease the fear of venturing into the center. Moreover, antidepressants are known to have various effects on appetite. To control this potential confounding factor, the feeding drive of each animal was assessed by returning it to the

familiar environment of the home cage immediately after the test, and measuring the amount of food consumed over a period of 5 minutes (home food consumption).

Actimeter

The actimeter allowed the assessment of the activity of mice in their home cage, thus excluding the possibility of biasing the results due to novelty-induced anxiety. The home cage was placed in the centre of a device, which consisted of a 20x20cm square plane with two light beams, crossing the plane from midpoint to midpoint of opposite sides, thus dividing the plane into four quadrants. The actimeter automatically detects the movement of the animal when it crossed through, allowing to establish a score. The higher the score, the more the mouse moved. Testing was carried out from 1100h and lasted 2 hours to allow a better estimation of the basal locomotor activity.

Collection of feces

Feces were collected at the time of euthanasia between 1000h to 1500h, which is during the dark period, and further directly from the colon by dissection. Fecal boli of each mouse were immediately put into micro-centrifuge tubes and then stored at -20°C until extraction. The feces collection ensured avoiding urine contaminations.

Extraction procedure and analysis of fecal steroid metabolites

The collected fecal samples were analyzed for immunoreactive corticosterone metabolites (CM) using a 5α-pregnane-3β,11β,21-triol-20-one enzyme-immunoassay (EIA). Details regarding development, biochemical characteristics, and biological validation of this assay are described by Touma and colleagues (2003; 2004). Before EIA analysis the fecal samples were dried, homogenized and aliquots of 0.05 g were extracted with 1 ml of 80% methanol. A detailed description of the extraction procedure and the assay performance has been published elsewhere (Touma et al., 2003). The intra- and inter-assay coefficients of variation were 9.1% and 14.0%, respectively.

Statistics

One-way ANOVAs were performed for each strain (3 groups: control/vehicle, UCMS/vehicle and UCMS/imipramine) followed by a Fisher *post hoc* analysis when required.

RESULTS

Evaluation of coat state

The state of the coat was evaluated before the onset of the UCMS regimen and then once a week until the end of UCMS nine weeks later (Fig.1). The total score resulted from the sum of the score obtained from seven different body parts (see methods); the higher the score, the more the coat state deteriorated worsened (Fig.2).

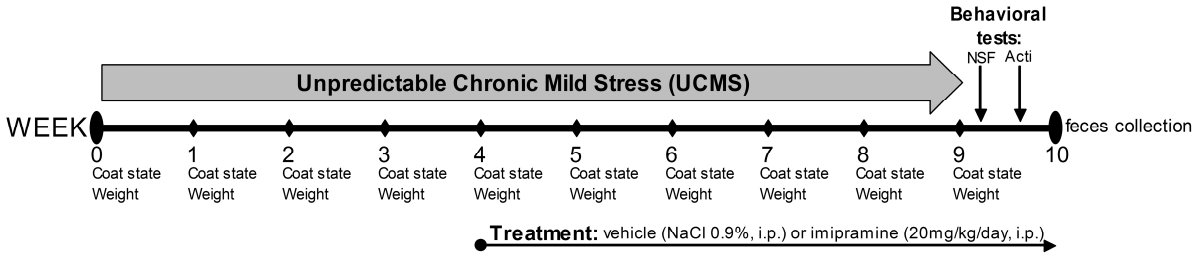

Figure 1. Experimental design. Seven strains were used (A/J, BALB/c, C3H, C57BL/6, CBA, DBA and FVB). The Unpredictable Chronic Mild Stress (UCMS) regimen lasted 9 weeks. Three groups per strains (n=9-13 mice/group) were used: Control/Vehicle, UCMS/Vehicle and UCMS/imipramine. Each week, the coat state was evaluated and the body weight measured by two experimenters blind to the treatment. The first four weeks of UCMS regimen were drug-free. Imipramine or vehicle treatments began after four weeks of UCMS and continued until the end of the experiment (week 10). Imipramine (20mg/kg/day) or vehicle was administered intraperitoneally once a day. The week following the end of the UCMS regimen, the Novelty-Suppressed Feeding (NSF) test and actimeter (acti) were carried out. At the end, feces were collected at the time of euthanasia (Sac.) in the cage and directly in the colon (4-6 mice/group). These feces samples were processed to evaluate the level of fecal corticosterone metabolites

For A/J strain, the ANOVA revealed significant differences after 3 weeks of UCMS regimen (week 0-2, $F_{2,35} \leq 3.02$, $p \geq 0.07$; week 3-9, $F_{2,35} \geq 9.93$, $p < 0.001$); the 9-week UCMS protocol provoked a worsening of the coat state so that the difference between control/vehicle and UCMS/vehicle mice reached significance after 3 weeks to the end (week 3-9, $p < 0.001$); imipramine treatment was found unable to reverse the UCMS-induced deterioration of the coat state as *post hoc* analysis revealed no significant difference between UCMS/vehicle and UCMS/imipramine groups.

Similarly, the UCMS regimen induced a degradation of the coat state in BALB/c mice but with a faster onset effect (ANOVA: week 0, $F_{2,31}=0.91$, $p=0.41$; week 1, $F_{2,31}=6.41$, $p<0.01$; week 2-9, $F_{2,31} \geq 8.74$, $p<0.001$), UCMS/vehicle mice were significantly different from control/vehicle mice already after one week of UCMS (week 1, $p<0.01$; week 2-9: $p<0.001$).

The chronic imipramine treatment counteracted the UCMS-induced deterioration of the coat state after 4-week administration ($p<0.01$) and this imipramine effect was sustained for the last coat state evaluation ($p<0.001$).

Also C3H mice were sensitive to the UCMS-induced deterioration of the coat state after 3 weeks of UCMS regimen and this effect was preserved until the end (ANOVA: week 0-2, $F_{2,33}\leq1.24$, $p\geq0.3$; week 3, $F_{2,33}=3.88$, $p<0.05$; week 4-9, $F_{2,33}\geq9.85$, $p<0.001$; Fisher *post hoc* test: control/vehicle vs. UCMS/vehicle, week 3, $p<0.05$; week 4-9, $p<0.001$). No significant reversal effect by imipramine treatment was found for this strain.

The UCMS procedure induced a relatively weak increase of the coat state scores in C57BL/6 mice, nevertheless sufficient to reach statistical significance for the last 3 coat state evaluations (ANOVA: week 0-6, $F_{2,32}\leq2.94$, $p\geq0.07$; week 7-9, $F_{2,32}\geq3.49$, $p<0.05$; *post hoc* analysis: control/vehicle vs. UCMS/vehicle, week 7-9, $p<0.05$). This deterioration was, however, not significantly improved by imipramine treatment.

The coat state of CBA mice significantly worsened after 4 weeks of UCMS application and further until the last evaluation (ANOVA: week 0-3, $F_{2,32}\leq1.95$, $p\geq0.16$; week 4, $F_{2,32}=4.21$, $p<0.05$; week 5, $F_{2,32}=6.1$, $p<0.01$; week 6-9, $F_{2,32}\geq10.31$, $p<0.001$; Fisher *post hoc* test: control/vehicle vs. UCMS/vehicle, week 4, $p<0.05$; week 5, $p<0.01$; week 6-9, $p<0.001$). The imipramine treatment failed to reverse the UCMS-induced deterioration of the coat state.

The DBA strain displayed a UCMS-induced deterioration of the coat state from the third to the last evaluation (ANOVA: week 0-1, $F_{2,32}\leq0.96$, $p\geq0.39$; week 2, $F_{2,32}=3.5$, $p<0.05$; week 3-9, $F_{2,32}\geq17.64$, $p<0.001$; Fisher *post hoc* test: control/vehicle vs. UCMS/vehicle, week 2, $p<0.05$; week 3-9, $p<0.001$). Imipramine treatment improved the coat state in a significant manner during the last two weeks ($p<0.01$).

The coat state scores of UCMS-treated FVB mice augmented along the UCMS procedure, to become significant from the fourth to the last evaluation (ANOVA: week 0-2, $F_{2,28}\leq1.86$, $p\geq0.17$; week 3, $F_{2,28}=5.66$, $p<0.01$; week 4-9, $F_{2,28}\geq12$, $p<0.001$; Fisher *post hoc* test: control/vehicle vs. UCMS/vehicle, week 3, $p<0.05$; week 4-9, $p<0.001$). No significant difference due to the treatment was found in the UCMS-treated FVB mice.

Taken together, the 9-week UCMS protocol induced a gradual deterioration of the coat state in all strains but with strain-specific differences in the kinetic of alteration as well as the amplitude. BALB/c and DBA strains were the most sensitive strains to UCMS. The C57BL/6 strain displayed the least responsiveness with a weak sensitivity to UCMS effect on coat state, while A/J, C3H, CBA and FVB exhibited intermediate profiles. Finally, chronic imipramine

treatment counteracted the UCMS effects on coat state in a statistically significant manner only in the BALB/c and DBA strain.

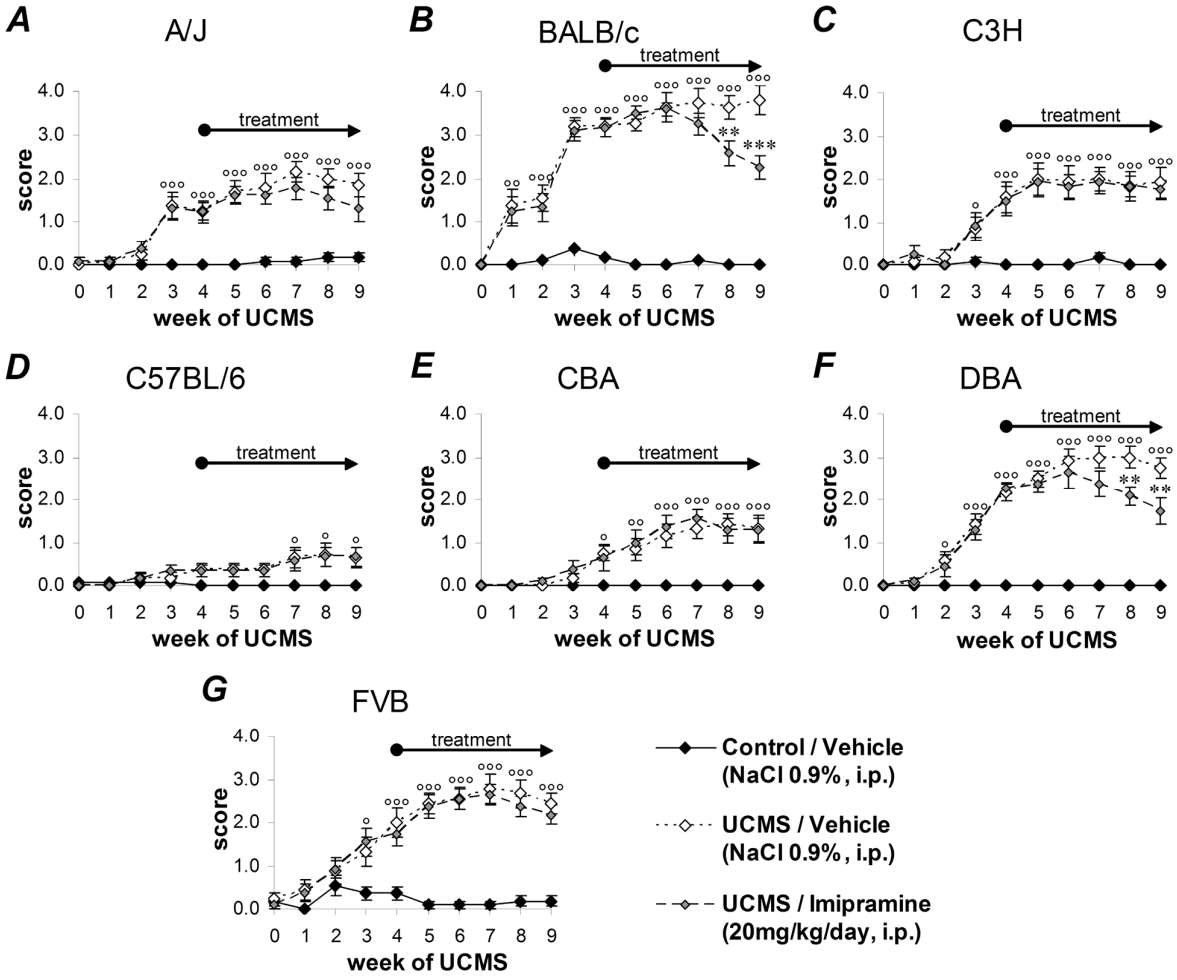

Figure 2. Effect of Unpredictable Chronic Mild Stress (UCMS) and of imipramine treatment on the coat state in A/J (A), BALB/c (B), C3H (C), C57BL/6 (D), CBA (E), DBA (F) and FVB (G) mice. N = 9-13 mice/group. Data represent mean ± SEM. °, P < 0.05, °°, P < 0.01 and °°°, P < 0.001 between control/vehicle and UCMS/vehicle. **, P < 0.01 and ***, P < 0.001 imipramine-treated versus vehicle-treated mice.

Body weight

The body weight was measured before the onset of the UCMS regimen and then once a week until the end of the UCMS procedure nine weeks later (Fig.1). ANOVA failed to establish significant modifications due to UCMS application or imipramine treatment in all strains (Fig.3; week 0-9: A/J, $F_{2,35} \leq 0.55$, $p \geq 0.56$; BALB/c, $F_{2,31} \leq 2.06$, $p \geq 0.14$; C3H, $F_{2,33} \leq 0.98$, $p \geq 0.39$; C57BL/6, $F_{2,32} \leq 0.73$, $p \geq 0.48$; CBA, $F_{2,32} \leq 0.72$, $p \geq 0.49$; DBA, $F_{2,32} \leq 1.13$, $p \geq 0.34$; FVB, $F_{2,28} \leq 0.42$, $p \geq 0.66$).

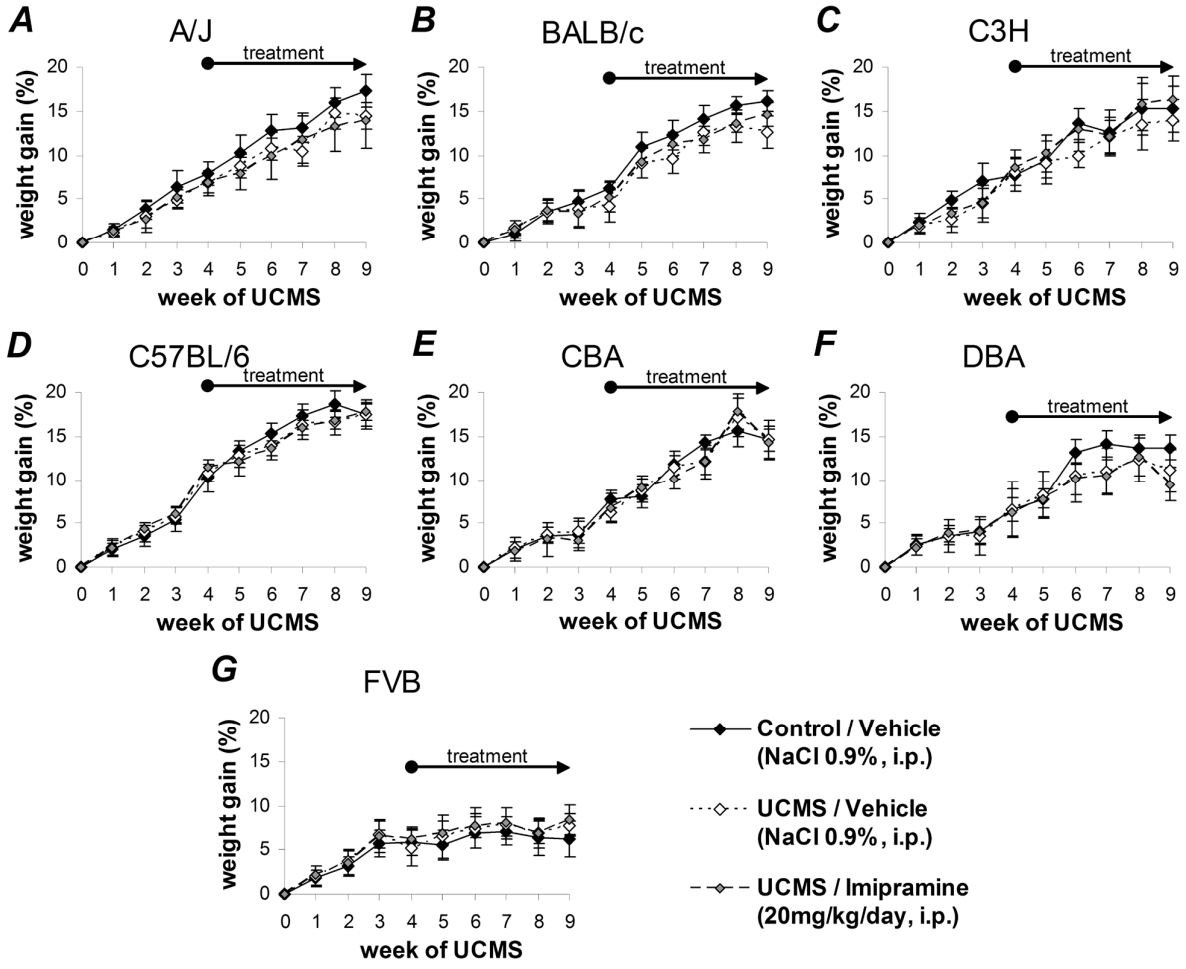

Figure 3. Effect of Unpredictable Chronic Mild Stress (UCMS) and of imipramine treatment on the body weight gain in A/J (A), BALB/c (B), C3H (C), C57BL/6 (D), CBA (E), DBA (F) and FVB (G) mice. N = 9-13 mice/group. Data represent mean ± SEM.

NSF Test

In this test, mildly food-deprived mice were exposed to a novel environment in which a food pellet is placed in the centre of the test apparatus. The NSF test is thought to assess emotional reactivity toward a new environment and induces a competition between motivational states (drive to eat the food vs. fear of venturing into the centre of the arena). It was first applied to reveal anxiolytic-like effects of drugs such as benzodiazepines or antidepressant properties specifically following chronic administration in healthy normal mice (Dulawa and Hen, 2005). This test might thus highlight an UCMS-induced increase of anxiety-related behaviors. The NSF test was performed after 9 weeks UCMS and 5 weeks of imipramine treatment, the day following the last coat state and body weight evaluation (Fig.1). The latency to start feeding on the pellet was recorded within a 3min period (Fig.4A).

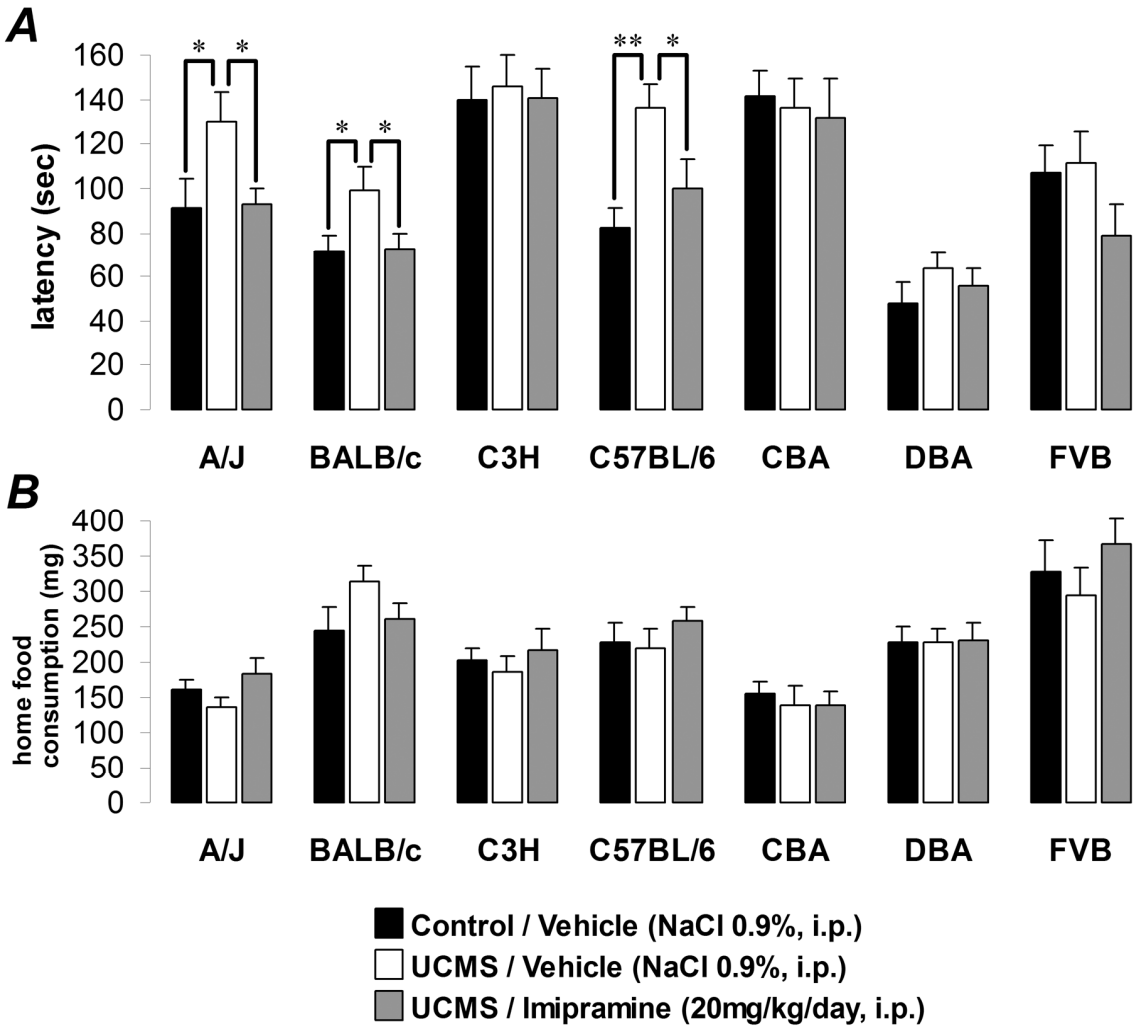

Figure 4. Effect of Unpredictable Chronic Mild Stress (UCMS) and of imipramine treatment in the Novelty-Suppressed Feeding (NSF) test with A/J, BALB/c, C3H, C57BL/6, CBA, DBA and FVB mice: (A) on the latency to chew the pellet and (B) on the home food consumption in the 5 minutes following the test. N = 9-13 mice/group. Data represent mean ± SEM. *, P < 0.05 and **, P < 0.01 between line-connected groups.

Significant differences were found in the A/J strain ($F_{2,35}$=3.49, p<0.05). Indeed, UCMS induced an increase of the latency to consume the pellet when compared with controls (p<0.05) in vehicle-treated mice. Imipramine treatment permitted to reverse this detrimental effect (p<0.05). Similar results were found in BALB/c mice ($F_{2,31}$=3.4, p<0.05) and C57BL/6 mice ($F_{2,32}$=5.94, p<0.01); the latency in the UCMS/vehicle groups was significantly higher than the one of controls (p<0.05 and p<0.01 respectively). Moreover, both strains were responsive to the AD treatment, as a reversal effect of imipramine was found in UCMS-subjected mice (p<0.05). On the contrary, no significant effects due to UCMS or treatment

were found in the four others strains, i.e. C3H ($F_{2,33}$=0.05, p=0.95), CBA ($F_{2,32}$=0.46, p=0.63), DBA ($F_{2,32}$=0.18, p=0.83) and FVB ($F_{2,28}$=1.75, p=0.19).

None of these results can be explained by changes in hunger or motivation to feed due to the UCMS or treatment as no significant differences were found for the 5-minute home food consumption assessed immediately after the test in all strains and treatment groups (Fig.4B): A/J ($F_{2,35}$=1.94, p=0.15), BALB/c ($F_{2,31}$=1.76, p=0.19), C3H ($F_{2,33}$=0.37, p=0.69), C57BL/6 ($F_{2,32}$=0.65, p=0.53), CBA ($F_{2,32}$=0.22, p=0.8), DBA ($F_{2,32}$=0.003, p=0.99), FVB ($F_{2,28}$=0.88, p=0.42).

In summary, three strains (A/J, BALB/c and C57BL/6) were both sensitive to the UCMS-induced detrimental effect and responsive to AD reversal in the NSF test, while no change due to UCMS or to imipramine treatment occurred in the four other strains (C3H, CBA, DBA and FVB). These results are not confounded by differences in feeding drive.

Actimeter

The actimeter was performed 5 days after the last coat state and body weight evaluation, i.e. after 5 weeks plus 4 days of imipramine administration (Fig.1). Actimeter allowed the assessment of the locomotor activity of mice in their home cage. The higher the score, the more the mouse moved. The ANOVA failed to reveal significant differences in any strain (Fig.5): A/J ($F_{2,35}$=0.83, p=0.44), BALB/c ($F_{2,31}$=0.07, p=0.93), C3H ($F_{2,33}$=1.17, p=0.32), C57BL/6 ($F_{2,32}$=0.68, p=0.51), CBA ($F_{2,32}$=0.23, p=0.79), DBA ($F_{2,32}$=1.13, p=0.34), FVB ($F_{2,28}$=0.99, p=0.38).

Fecal corticosterone metabolites

Feces were collected one week after the last coat state and body weight evaluation, i.e. after 6-weeks of imipramine administration (Fig.1), during the dark phase over a period of 5 h. Corticosterone metabolite concentrations are expressed in [ng/50mg feces]. Results are illustrated in Fig.6.

On the one hand, neither UCMS nor treatment was found to have an effect on fecal corticosterone metabolites in A/J ($F_{2,11}$=0.01, p=0.99), C3H ($F_{2,11}$=1.36, p=0.3) and CBA ($F_{2,12}$=0.34, p=0.72) mice. On the other hand, the ANOVA revealed significant changes in fecal corticosterone metabolites in BALB/c ($F_{2,12}$=6.8, p<0.01), C57BL/6 ($F_{2,13}$=5.99, p<0.05), DBA ($F_{2,11}$=8.1, p<0.01) and FVB ($F_{2,12}$=5.3, p<0.05). Nevertheless, these changes differed in a strain-dependent manner. In BALB/c mice, UCMS induced a significant increase of fecal corticosterone metabolites (p<0.01), an effect that could be restored by imipramine

treatment (UCMS/vehicle vs. UCMS/imipramine: $p<0.05$). An opposite pattern was observed in C57BL/6. Here the UCMS regimen significantly decreased fecal corticosterone metabolites ($p<0.01$) while ADs reversed this diminution ($p<0.05$). A significant decrease of fecal corticosterone metabolites was also found in DBA mice from the UCMS/vehicle group when compared to controls ($p<0.01$); however imipramine was unable to counteract this change. Similar to C57BL/6 mice, FVB mice showed a significant diminution of fecal corticosterone metabolites induced by the UCMS procedure ($p<0.05$), but were irresponsive to the reversal effect of imipramine.

Taken together, the UCMS model induced alterations of the concentration in fecal corticosterone metabolites in 4 strains (BALB/c, C57BL/6, DBA and FVB), while imipramine restored a control-like concentration in UCMS-subjected mice only in two of these strains (BALB/c and C57BL/6).

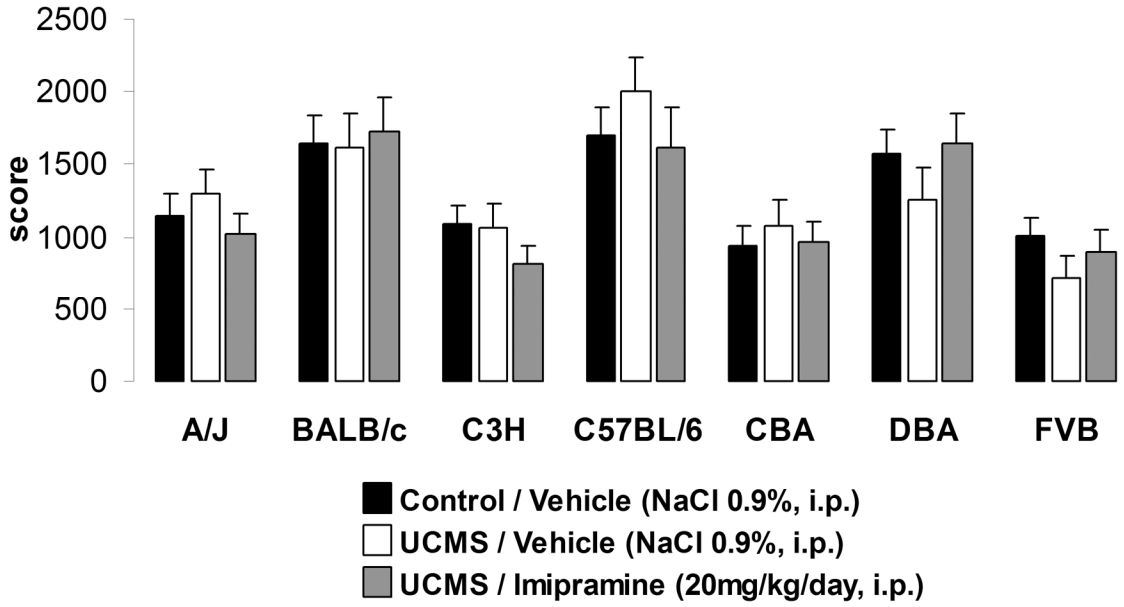

Figure 5. Effect of Unpredictable Chronic Mild Stress (UCMS) and of imipramine treatment in the actimeter with A/J, BALB/c, C3H, C57BL/6, CBA, DBA and FVB mice. N = 9-13 mice/group. Data represent mean ±SEM.

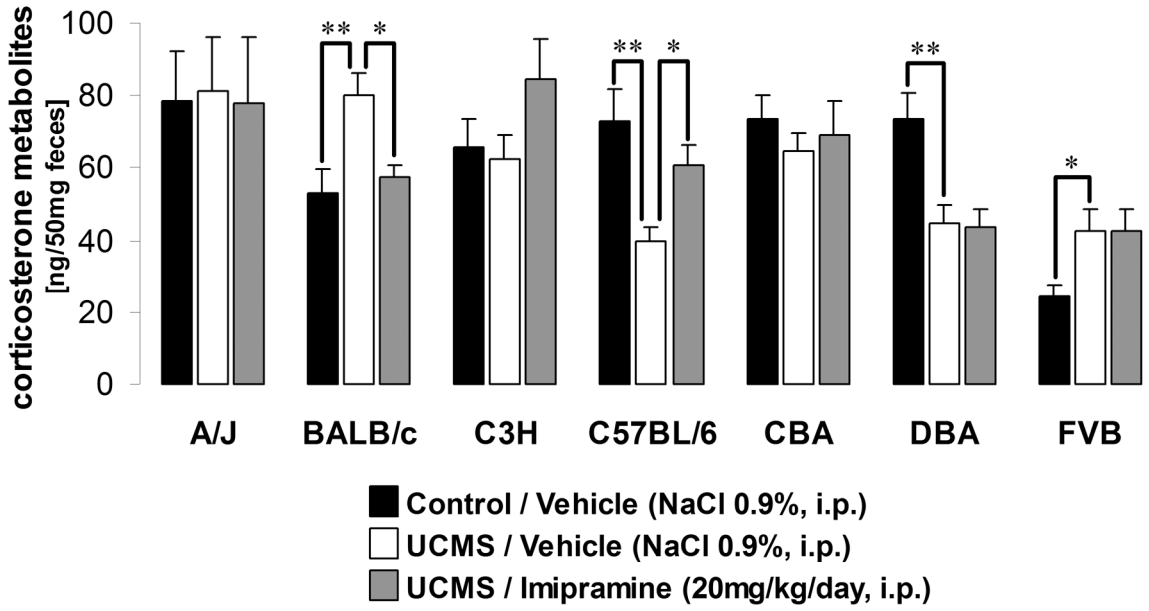

Figure 6. Effect of Unpredictable Chronic Mild Stress (UCMS) and of imipramine treatment on the level of fecal corticosterone metabolites in A/J, BALB/c, C3H, C57BL/6, CBA, DBA and FVB mice. N = 4-6 mice/group. Data represent mean ± SEM. *, $P < 0.05$ and **, $P < 0.01$ between line-connected groups.

DISCUSSION

This study reports strain differences for the sensitivity to UCMS, a chronic and naturalistic model of depression, and for the response to AD treatment. All seven strains were sensitive to at least one of the UCMS-induced changes, while four strains (A/J, BALB/c, C57BL/6 and DBA) were responsive to at least one of the AD reversal effects. Interestingly, our results revealed profiles of alterations and of imipramine action, depending upon the strain tested (summarized in Table I). Therefore, each UCMS- and imipramine-sensitive variable can be independent of the others, as it may model an endophenotype related to a particular symptom of MDD. Three fields of possible MDD-associated alterations were tested in this study: physical state (coat state, body weight), behavior (locomotor activity, anxiety-related behaviors using the NSF test) and neuroendocrine measures (fecal corticosterone metabolites). BALB/c and C57BL/6 strains were the only strains exhibiting UCMS-induced alterations in all 3 fields investigated, with significant changes seen in coat state (though weak for C57BL/6), the NSF test and the concentration of fecal corticosterone metabolites. Moreover both strains were also the most responsive to AD reversal, as imipramine restored these alterations in BALB/c mice as well as in C57BL/6 mice (except for coat state in this last strain). A/J, DBA and FVB were sensitive to the UCMS model for two parameters: the coat state and the NSF test for A/J, the coat state and the level of fecal corticosterone metabolites for DBA and FVB. A reversal effect of imipramine was found in A/J and DBA, but not in FVB, for only one parameter: the NSF test for A/J and the coat state for DBA. Lastly, C3H and CBA were the sole strains exhibiting a similar profile. They were the less sensitive to the detrimental effects of UCMS as only a significant deterioration of the coat state was found for both strains; moreover both strains were irresponsive to the imipramine treatment. Although differences in inbred mouse strains are not exclusively related to genetic background but are also influenced by epigenetic factors and parental behaviors (Calatayud et al., 2004), the existence of these six different profiles underlines the contribution of inheritable factors in the sensitivity to stress exposure and to ADs' effects and thus could provide important information for the understanding of the genetic basis associated with stress vulnerability, MDD-associated alterations and ADs-treatment resistance.

Strain-dependent changes in the physical state

Our results showed that a 9-week UCMS protocol induced a progressive deterioration of the coat state in all strains, but with a strain-specific kinetic and amplitude. BALB/c and DBA strains were the most sensitive strains to the worsening effect of UCMS on coat state, since

significant deterioration occurred faster and with higher scores in these strains. The C57BL/6 strain displayed a different profile with a weak sensitivity to UCMS effect on coat state, while A/J, C3H, CBA and FVB exhibited intermediate profiles. These results confirmed those of Mineur and colleagues (2003, 2006), who found a significant worsening of the coat state after a 4-week UCMS application in BALB/c, C57BL/6, DBA and FVB strains. Another UCMS study found significant deterioration in CBA but not in C57BL/6 and DBA (Pothion et al., 2004); however, assessment of coat deterioration in this study is questionable since no scoring was used and could be responsible for the divergence.

Table I. Summary of the effects of a nine-week Unpredictable Chronic Mild Stress (UCMS) regimen and of the imipramine treatment.

STRAIN	EFFECTS OF UCMS & IMIPRAMINE REVERSAL				
	Coat state score	Weight	NSF test latency	Loco-motor activity	[CORT]
A/J	+	0	+/R	0	0
BALB/c	+/R	0	+/R	0	+/R
C3H	+	0	0	0	0
C57BL/6	+	0	+/R	0	-/R
CBA	+	0	0	0	0
DBA	+/R	0	0	0	-
FVB	+	0	0	0	+

A "+" means a significant increase of the measure due to UCMS; a "-" indicates a significant decrease of the measure due to UCMS; a "0" indicates a lack of significant change; a "R" indicates a significant reversal by imipramine treatment. NSF, Novelty-Suppressed Feeding; [CORT], level of fecal corticosterone metabolites.

Chronic imipramine treatment counteracted the UCMS effects on coat state only in the BALB/c and DBA strain. It is noteworthy that both strains were the most affected by the UCMS for this measure; imipramine was thus able to prevent the detrimental effects of UCMS on this index when deterioration was relatively high. Thus, we cannot exclude that, rather than being ineffective in the 5 other strains, the lack of imipramine's efficacy may be explained by floor effects. Considering that the C57BL/6 strain was highly responsive to imipramine in all other UCMS-induced alterations of this study, this latter argument could apply for this strain. Alternatively, this measure might be useless to assess adequately the effect of UCMS on C57BL/6 strain due to the black coat of this strain which renders coat state difficult to assess. ADs effect in the UCMS model was never compared among various inbred mouse strains to date; only BALB/c mice were previously tested with fluoxetine, desipramine,

CRF1 antagonists or V1b antagonist (Griebel et al., 2002a; 2002b; Ducottet et al., 2003; Santarelli et al., 2003; Alonso et al., 2004; Yalcin et al., 2005; Surget et al., 2008). These compounds were able to block or to reverse the UCMS-induced deterioration of the coat state.

The body weight was measured each week from the onset of the UCMS regimen to the end. No change in body weight due to UCMS or imipramine was reported in any strain used in this experiment. This result is in accordance with previous studies using BALB/c mice in a 5-week UCMS (Santarelli et al., 2003; Yalcin et al., 2005; Surget et al., 2008). Nevertheless, recent experiments in our laboratories found significant reductions of body weight gain in BALB/c mice and recovery by fluoxetine treatment after a minimum of 5-week UCMS in more intensive protocols in terms of stressor occurrence and combination (see Belzung and Surget, 2008). Although body weight disruption was not found in any strain in this experiment, it can be induced, at least in BALB/c mice, by the UCMS model in a protocol-dependent manner.

Strain-dependent changes in the behavioral tests

The behavioral effects of UCMS and imipramine treatment were assessed in the NSF test and in the actimeter. The NSF was used to highlight an UCMS-induced increase of anxiety-related behaviors. While the behavior of C3H, CBA, DBA and FVB in the NSF test was altered neither by UCMS nor by imipramine treatment, the A/J, BALB/c and C57BL/6 strains displayed a significant augmentation of the latency in the NSF test for UCMS/vehicle mice, suggesting an UCMS-related overstatement of emotional reactivity. Furthermore, the imipramine treatment was able to restore the latency to the control level in these 3 strains. Confounding effects of feeding drive or activity can be excluded as food consumption and locomotion in the home cage remained unchanged.

Strain-dependent changes in the level of fecal corticosterone metabolites

Considering that HPA axis disturbances such as hypercortisolemia or blunted negative feedback response are one of the most consistent biological markers of MDD (Holsboer, 2000), we assessed the level of fecal corticosterone metabolites in order to investigate first if the UCMS model is able to induce increased levels of corticosterone, second if these changes are strain-dependent, and third if they are related to particular UCMS-induced symptoms and could be reversed by imipramine treatment. UCMS induced alterations of the concentration of fecal corticosterone metabolites in 4 strains (BALB/c, C57BL/6, DBA and FVB), but this effect was restored to control levels by imipramine only in two of these strains (BALB/c and

C57BL/6). Further, these changes were strongly strain-specific as we obtained four different profiles in these four strains. As expected, BALB/c and FVB mice displayed an increase of fecal corticosterone metabolites after UCMS. Imipramine treatment restored this increase of fecal corticosterone metabolites to control levels in BALB/c, but not in FVB. Considering that physical and behavioral changes due to UCMS were reversed by imipramine in BALB/c but not in FVB, the changes in corticosterone metabolites in these two strains are consistent with clinical data showing that, when MDD is associated with HPA disturbances, clinical remission parallels the normalization of HPA axis (Nemeroff, 1996). On the other hand, a significant UCMS-induced reduction of the level of fecal corticosterone metabolites was found in C57BL/6 and DBA, but imipramine reversed this diminution only in C57BL/6 mice. This result is reminiscent of the atypical subtype of MDD, which is characterized by reduced activity of HPA axis, contrasting with the HPA overdrive seen in the melancholic subtype (Gold and Chrousos, 2002; Antonijevic, 2006). Lastly, A/J, C3H and CBA showed no significant change in fecal corticosterone metabolites due to UCMS or imipramine treatment. This result shows that the physical and/or behavioral UCMS-induced symptoms, at least for the ones investigated in this study, are not necessarily associated with disturbances of corticosterone levels. This is clinically relevant since MDD can also occur without HPA axis dysfunction (Watson et al., 2002; Gervasoni et al., 2004).

It should be noted, however, that only one time point of fecal corticosterone metabolites level was analyzed in our study. Since glucocorticoids such as corticosterone display regular circadian variations as well as episodic pulsatile secretion pattern (Axelrod and Reisine, 1984; Touma et al., 2004), corticosterone levels at only one time point could be unrepresentative of the whole perturbations of the HPA dysfunction. We thus cannot exclude that A/J, C3H and CBA strains displayed significant changes in corticosterone levels at other time points and that the HPA non-reversal profiles of FVB and DBA strains were just specific to this time point but not to others.

Besides investigating other time points, in future studies the combined dexamethasone suppression/CRF stimulation test which allows assessing the HPA feedback integrity should be used to highlight more accurately HPA axis dysfunctions, since HPA axis disturbances in patients might be more associated with blunted negative feedback than hypercortisolemia (Swaab et al., 2005).

Strain differences are relevant to the clinic

Our results emphasize how the UCMS model of depression can generate several clinically-relevant profiles, which mimic subtypes of MDD-associated syndromes, using inbred mouse strains. BALB/c and C57BL/6 were sensitive to the physical and behavioral alterations of UCMS, these modifications were associated to HPA up- or down-regulation respectively and they were AD responsive. DBA mice displayed physical and neuroendocrine UCMS-induced modifications and imipramine reversal of the physical but not of the HPA changes. The A/J strain was sensitive to the physical and behavioral alterations of UCMS, AD responsive in the NSF test, but the HPA axis was not dysregulated. FVB mice were sensitive to the physical alterations of UCMS, the HPA axis was up-regulated, but modifications were AD resistant. C3H and CBA mice were sensitive to the physical alterations of UCMS, but the neuroendocrine pattern remained unaltered and the modifications were AD resistant.

The MDD encompasses two major subtypes, the melancholic and atypical depressions, which display contrasting psychological and neurovegetative symptoms, particularly HPA disturbances were found in the both subtypes but in an opposing direction: up-regulated in melancholia while down-regulated in atypical depression (Gold and Chrousos, 2002; Antonijevic, 2006). It is noteworthy that the UCMS model induced similar divergent biological alterations with HPA overdrive and hypocortisolemia, reminiscent of the melancholic and atypical features respectively. Our results show that a unique etiological factor (chronic stress) was able to induce opposing patterns of alterations according to the genetic background. Taken together, we can easily speculate that the genetic makeup, more than the environmental factors, contributes to the distinct clinical phenotypes. In any case, these findings highlight the UCMS model as a suited experimental tool to dissect genetic factors associated with particular subtypes of depression-related syndromes.

Comparison with other inbred mouse strain surveys in bio-assays

Briefly, we established that, in the seven strains investigated in this study, four strains were responsive to the imipramine treatment at least in one measure: DBA on the coat state, A/J in the NSF test, C57BL/6 in the NSF test and fecal corticosterone metabolites and BALB/c in this three measures, while C3H, CBA and FVB were insensitive (Table 1). Among all the strains tested, BALB/c and C57BL/6 were the only strains in which imipramine reversed at least two parameters, which might limit the usefulness of this model particularly for genetically-modified animals that are bred on other backgrounds. However, the heaviness of a UCMS procedure with seven strains made that the range of behavioral alterations investigated

here is quite limited (emotional reactivity and locomotor activity), it is thus possible that more strains would be sensitive to imipramine reversal in other depression-related behaviors (anhedonia, despair, sleep disturbance, appetite, social behavior). Moreover, such a shortcoming becomes an advantage to scrutinize genetic factors involved in vulnerability, pathophysiology or AD resistance.

The overwhelming majority of published studies in the field are carried out with tests of behavioral despair (FST and TST) in "normal" unstressed mice treated acutely with ADs. Although these studies are assumed to investigate disease or treatment mechanisms, both FST and TST present weaker face and construct validities than UCMS; even their predictive validity and reproducibility seem to be overestimated since discrepancies arose from recent inbred strain surveys in these bio-assays. Liu and Gershenfeld (2001) found that DBA and FVB were responsive to imipramine in the TST while A/J, BALB/c, C3H and C57BL/6 not, whereas C57BL/6 mice were shown to be sensitive to imipramine in many other studies using the TST (Cryan et al., 2005). In the FST, imipramine was found to decrease immobility in BALB/c (Schechter and Chance, 1979) and C57BL/6 (Bai et al., 2001), while David and colleagues (2003) found no effect in C57BL/6 and DBA. Other discrepancies are also found with other compounds: the tricyclic desipramine (Lucki et al., 2001; David et al., 2003; Ripoll et al., 2003), the selective serotonin reuptake inhibitor (SSRI) citalopram (David et al., 2003; Ripoll et al., 2003; Crowley et al., 2005), the SSRI paroxetine (David et al., 2003; Ripoll et al., 2003). Therefore, these bioassays detect discrepant results among laboratories and even in the same group when both tests were used with various compounds (David et al., 2003; Ripoll et al., 2003). These findings, together with the fact that UCMS induced different effects between the FST and the TST (Mineur et al., 2006), suggest that both tests correspond to distinct entities. Nevertheless, it might be possible that these differences could account for the heterogeneity of the protocols used. In any case, while considering that no correlation was found between baseline immobility, saline immobility and ADs response across strains in these mouse strain surveys (Liu and Gershenfeld, 2001; Lucki et al., 2001; Crowley et al., 2005), these results point out that is questionable to use only these acute models in studies prospecting for neurobiological processes involved in the human disease and ADs response.

Conclusion

Chronic models of depression such as the UCMS could be suitable to investigate neurobiological mechanisms of pathophysiology and treatment of MDD. Indeed, the UCMS model reproduces chronic features of depression and the clinical time-course of ADs action,

so that it exhibits higher face and construct validities than the TST and FST. Moreover, our results show that it can be possible to distinguish various UCMS-induced endophenotypes which replicate specific symptoms and even subtypes of MDD-associated syndromes, reminiscent of the melancholic or atypical features of the human disease. Indeed, UCMS may enable to choose strains accurate for specific aspects of MDD. For example, some strains may be relevant to model MDD associated with HPA dysfunction (strains sensitive to physical, behavioral and neuroendocrine alterations), others to model ADs resistance (i.e. strains sensitive to UCMS but insensitive to ADs). As a next step, other MDD-associated symptoms should be investigated to determine more accurately inbred strains profiles such as other relevant behavioral alterations (anhedonia, sleep disturbance) or physiological measures (Dex/CRF test, brain monoamines level, hippocampal neurogenesis). Given that mice were shown to display these endophenotypes in a strain-dependent manner, UCMS is an appropriate model to dissect genetic factors associated with vulnerability, particular symptoms of affective disorders, and AD resistance.

Disclosure/Conflict of Interest:

Y. Ibarguen-Vargas, A. Surget, C. Touma and R. Palme declare that, except for income received from our primary employer, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest. C. Belzung receives compensation as a consultant for Takeda.

REFERENCES

Alonso, R., Griebel, G., Pavone, G., Stemmelin, J., Le Fur, G. and Soubrie, P., 2004. Blockade of CRF(1) or V(1b) receptors reverses stress-induced suppression of neurogenesis in a mouse model of depression. Mol. Psychiatry 9, 278-286, 224.

Antonijevic, I. A., 2006. Depressive disorders -- is it time to endorse different pathophysiologies? Psychoneuroendocrinology 31, 1-15.

Axelrod, J. and Reisine, T. D., 1984. Stress hormones: their interaction and regulation. Science (New York, N.Y 224, 452-459.

Bai, F., Li, X., Clay, M., Lindstrom, T. and Skolnick, P., 2001. Intra- and interstrain differences in models of "behavioral despair". Pharmacol. Biochem. Behav. 70, 187-192.

Belzung, C. and Surget, A., 2008. Unpredictable chronic mild stress in mice. In: Kalueff, A. V. and LaPorte, J. L., (Eds), Experimental models in neurobehavioral research. Nova Science, New York, (in press).

Calatayud, F., Coubard, S. and Belzung, C., 2004. Emotional reactivity in mice may not be inherited but influenced by parents. Physiol. Behav. 80, 465-474.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A. and Poulton, R., 2003. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301, 386-389.

Crowley, J. J., Blendy, J. A. and Lucki, I., 2005. Strain-dependent antidepressant-like effects of citalopram in the mouse tail suspension test. Psychopharmacology (Berl.) 183, 257-264.

Cryan, J. F. and Holmes, A., 2005. The ascent of mouse: advances in modelling human depression and anxiety. Nat. Rev. Drug Discov. 4, 775-790.

Cryan, J. F., Mombereau, C. and Vassout, A., 2005. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neurosci. Biobehav. Rev. 29, 571-625.

David, D. J., Renard, C. E., Jolliet, P., Hascoet, M. and Bourin, M., 2003. Antidepressant-like effects in various mice strains in the forced swimming test. Psychopharmacology (Berl.) 166, 373-382.

Ducottet, C., Griebel, G. and Belzung, C., 2003. Effects of the selective nonpeptide corticotropin-releasing factor receptor 1 antagonist antalarmin in the chronic mild stress model of depression in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 27, 625-631.

Ducottet, C. and Belzung, C., 2005. Correlations between behaviours in the elevated plus-maze and sensitivity to unpredictable subchronic mild stress: evidence from inbred strains of mice. Behav. Brain Res. 156, 153-162.

Dulawa, S. C. and Hen, R., 2005. Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. Neurosci. Biobehav. Rev. 29, 771-783.

Gervasoni, N., Bertschy, G., Osiek, C., Perret, G., Denis, R., Golaz, J., Rossier, M. F., Bondolfi, G. and Aubry, J. M., 2004. Cortisol responses to combined dexamethasone/CRF test in outpatients with a major depressive episode. J. Psychiatr. Res. 38, 553-557.

Gold, P. W. and Chrousos, G. P., 2002. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRF/NE states. Mol. Psychiatry 7, 254-275.

Griebel, G., Simiand, J., Serradeil-Le Gal, C., Wagnon, J., Pascal, M., Scatton, B., Maffrand, J. P. and Soubrie, P., 2002a. Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V1b receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. Proc. Natl. Acad. Sci. U.S.A. 99, 6370-6375.

Griebel, G., Simiand, J., Steinberg, R., Jung, M., Gully, D., Roger, P., Geslin, M., Scatton, B., Maffrand, J. P. and Soubrie, P., 2002b. 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4- methylphenyl)ethyl]5-methyl-N-(2-propynyl)-1, 3-thiazol-2-amine hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing factor(1) receptor antagonist. II. Characterization in rodent models of stress-related disorders. J. Pharmacol. Exp. Ther. 301, 333-345.

Holsboer, F., 2000. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23, 477-501.

Jacobson, L. H. and Cryan, J. F., 2007. Feeling strained? Influence of genetic background on depression-related behavior in mice: a review. Behav. Genet. 37, 171-213.

Kendler, K. S., Karkowski, L. M. and Prescott, C. A., 1999. Causal relationship between stressful life events and the onset of major depression. Am. J. Psychiatry 156, 837-841.

Liu, X. and Gershenfeld, H. K., 2001. Genetic differences in the tail-suspension test and its relationship to imipramine response among 11 inbred strains of mice. Biol. Psychiatry 49, 575-581.

Liu, X. and Gershenfeld, H. K., 2003. An exploratory factor analysis of the Tail Suspension Test in 12 inbred strains of mice and an F2 intercross. Brain Res. Bull. 60, 223-231.

Lucki, I., Dalvi, A. and Mayorga, A. J., 2001. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. Psychopharmacology (Berl.) 155, 315-322.

Mineur, Y. S., Prasol, D. J., Belzung, C. and Crusio, W. E., 2003. Agonistic behavior and unpredictable chronic mild stress in mice. Behav. Genet. 33, 513-519.

Mineur, Y. S., Belzung, C. and Crusio, W. E., 2006. Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. Behav. Brain Res. 175, 43-50.

Murray, C. J. and Lopez, A. D., 1997. Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. Lancet 349, 1436-1442.

Nemeroff, C. B., 1996. The corticotropin-releasing factor (CRF) hypothesis of depression: new findings and new directions. Mol. Psychiatry 1, 336-342.

Pothion, S., Bizot, J. C., Trovero, F. and Belzung, C., 2004. Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. Behav. Brain Res. 155, 135-146.

Ripoll, N., David, D. J., Dailly, E., Hascoet, M. and Bourin, M., 2003. Antidepressant-like effects in various mice strains in the tail suspension test. Behav. Brain Res. 143, 193-200.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C. and Hen, R., 2003. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301, 805-809.

Schechter, M. D. and Chance, W. T., 1979. Non-specificity of "behavioral despair" as an animal model of depression. Eur. J. Pharmacol. 60, 139-142.

Surget, A., Saxe, M., Leman, S., Ibarguen-Vargas, Y., Chalon, S., Griebel, G., Hen, R. and Belzung, C., 2008. Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. Biol. Psychiatry (in press).

Swaab, D. F., Bao, A. M. and Lucassen, P. J., 2005. The stress system in the human brain in depression and neurodegeneration. Ageing Res. Rev. 4, 141-194.

Touma, C., Sachser, N., Mostl, E. and Palme, R., 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. Gen. Comp. Endocrinol. 130, 267-278.

Touma, C., Palme, R. and Sachser, N., 2004. Analyzing corticosterone metabolites in fecal samples of mice: a noninvasive technique to monitor stress hormones. Horm. Behav. 45, 10-22.

Watson, S., Gallagher, P., Del-Estal, D., Hearn, A., Ferrier, I. N. and Young, A. H., 2002. Hypothalamic-pituitary-adrenal axis function in patients with chronic depression. Psychol. Med. 32, 1021-1028.

Yalcin, I., Aksu, F. and Belzung, C., 2005. Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol. Eur. J. Pharmacol. 514, 165-174.

Discussion complémentaire de l'article 2

Cette étude indique donc une variabilité de la réponse des lignées de souris aux effets de l'UCMS et à ceux d'un traitement chronique aux antidépresseurs. A présent, dans cette discussion complémentaire, nous allons tenter d'examiner le rôle du polymorphisme Leu32Met du BDNF dans la survenue d'un syndrome dépressif-like dans le modèle de dépression de l'UCMS et dans l'action chronique des antidépresseurs évaluée dans ce modèle. Ce modèle, comme le démontre l'article, est capable d'induire plusieurs type d'altérations plus ou moins indépendantes ; il permet donc d'évaluer si ce polymorphisme est plus particulièrement impliqué dans l'une ou l'autre de ces altérations.

Dans cette optique, notre objectif a été d'établir le profil des altérations physiques, comportementales et neuroendocrines induites par l'UCMS dans chacune des 7 lignées testées (A/J, BALB/c, C3H, C57BL/6, CBA, DBA/2 et FVB), ainsi que leur propension à répondre à l'action « thérapeutique » d'un traitement à l'imipramine, puis d'explorer une éventuelle corrélation entre le génotype pour le gène BDNF et ces résultats (la lignée FVB a entre-temps été génotypée et nous avons montré que cette souche porte l'allèle Met).

Les résultats obtenus pour chaque lignée ont été détaillés dans l'article. Ci-dessous, nous tentons d'établir un lien entre les altérations observées avec le génotype pour le gène BDNF (table II).

Table II. Synthèse des effets du régime UCMS et du traitement à l'imipramine en fonction des variants alléliques (Leu32Met) et des lignées.

Lignées	allèle *bdnf* (aa32)	EFFETS DE L'UCMS & DE L'IMIPRAMINE				
		État du pelage	Poids	NSF test (latence)	Activité locomotrice	[CORT]
C3H	Leu/Leu	+	0	0	0	0
C57BL/6	Leu/Leu	+	0	+/R	0	-/R
A/J	*Met/Met*	+	*0*	*+/R*	*0*	*0*
BALB/c	*Met/Met*	*+/R*	*0*	*+/R*	*0*	*+/R*
CBA	*Met/Met*	+	*0*	*0*	*0*	*0*
DBA/2	*Met/Met*	*+/R*	*0*	*0*	*0*	-
FVB	*Met/Met*	+	*0*	*0*	*0*	+

Un « + » indique une augmentation significative due à l'UCMS de la mesure ; un « - » indique une diminution significative due à l'UCMS de la mesure ; un « 0 » indique un manque de changement significatif ; un « R » indique une réversion significative par le traitement à l'imipramine. Abréviations : aa, acide aminé ; [CORT], concentration des métabolites de la corticostérone dans les fecès ; NSF, novelty-suppressed feeding.

Les profils obtenus ne montre aucun point commun dans les mesures explorées entre les différentes lignées porteurs de l'allèle Leu (ie C3H et C57BL/6) d'un côté et entre celles porteurs de l'allèle Met (ie A/J, BALB/c, CBA, DBA/2 et FVB) de l'autre côté. En effet, nous avons trouvé une grande hétérogénéité de patterns d'altérations et de réversion due au traitement. Les lignées BALB/c (Met/Met) et C57BL/6 (Leu/Leu), distinctes pour le SNP, ont été sensibles aux altérations physiques et comportementales induites par l'UCMS, ces modifications ont été associées respectivement à une augmentation ou une réduction de l'activité de l'axe HPA et ces 2 lignées ont été les plus sensibles à l'effet thérapeutique de l'imipramine. Les souris DBA (Met/Met) ont présenté des altérations physiques et neuroendocrines dues à l'UCMS ainsi qu'une réversion de l'imipramine des altérations physiques alors que les A/J (Met/Met), pourtant identiques aux précédentes en ce qui concerne le polymorphisme, ont été sensibles aux seules modifications physiques et comportementales induites par l'UCMS, l'imipramine ayant contrecarré ces modifications au niveau comportemental seulement. Les FVB (Met/Met), elles aussi identiques aux deux précédentes sur le plan du polymorphisme du BDNF, ont présenté des altérations physiques et neuroendocrines à la suite de l'UCMS alors que cette lignée a été insensible aux traitements. Enfin, les lignées C3H (Leu/Leu) et CBA (Met/Met) ont été seulement sensibles à la dégradation de l'état du pelage, laquelle n'est pas contrecarrée par l'imipramine. On peut remarquer qu'il n'y a sans doute aucune relation entre le génotype BDNF des lignées et leur sensibilité à l'UCMS ou au traitement, étant donné que des lignées portant le même allèle peuvent montrer des profils opposés.

En outre, cette variabilité n'a même pas permis de dégager des tendances communes à un génotype malgré une exploration comportementale assez diverse. En effet, il n'y a pas une relation entre le polymorphisme Leu32Met du BDNF et au moins une des mesures étudiées.
Ainsi, une variété de réponses au modèle de dépression UCMS et au traitement a été établie indépendamment du SNP. On peut donc en déduire que le polymorphisme Leu32Met du BDNF n'est probablement pas impliqué dans la survenue d'un syndrome dépressif-like ou dans l'action des ADs. En fait, les traits psychologiques et les comportements complexes sont souvent très variables et sont généralement influencés par une multitude de gènes et de facteurs environnementaux. Ainsi, la variabilité entre les lignées dépend en général plus de l'arrière-fond génétique que d'un simple gène. De plus, même si ce polymorphisme avait un rôle dans les effets étudiés ici, des phénomènes de compensation peuvent émerger et empêcher la détection d'un phénotype associé à l'un de ces allèles. Des souris congéniques

dans lesquelles les deux allèles seraient présents dans un même arrière-fond génétique pourraient donc être utiles afin d'évaluer plus précisément l'influence de ce polymorphisme sur la vulnérabilité au stress chronique et sur l'action des antidépresseurs.

Article 3 - Deficit in BDNF level does not increase vulnerability to stress but dampens antidepressant-like effects in the unpredictable chronic mild stress.

Dans cet article, nous avons voulu déterminer le rôle du niveau d'expression du BDNF dans la vulnérabilité au stress chronique et dans l'action des antidépresseurs dans un approche comparatif avec des souris hétérozygotes BDNF +/- et des souris sauvages.

European Neuropsychopharmacology (soumis)

Deficit in BDNF level does not increase vulnerability to stress but dampens antidepressant-like effects in the unpredictable chronic mild stress

Yadira Ibarguen-Vargas[1-2], Alexandre Surget[1-2], Samuel Leman[1-2], Patrick Vourc'h[1-2], Christian Andres[1-2], Alain M. Gardier[3], Catherine Belzung*[1-2]

1 U930, CNRS FRE2448, Inserm, Tours, France
[2] Université François Rabelais de Tours, Tours, France
[3] Univ. Paris Sud, EA 3544, Fac. Pharmacie, Chatenay-Malabry cedex F-92296, France

*Corresponding author: Faculté des Sciences et Techniques, Université François Rabelais, Bât O Parc de Grandmont, 37200 Tours, France. Phone: +33 2 47366994; Fax: +33 2 47367285. E-mail: catherine.belzung@univ-tours.fr

Abstract

The neurotrophic hypothesis of depression is challenging by the paucity of direct links between brain-derived neurotrophic factor (BDNF) deficit and depressive-like behaviors. The unpredictable chronic mild stress (UCMS) paradigm might take our understanding a step further by examining whether depletion in *bdnf* expression can lead to enhanced vulnerability to stress and prevent antidepressant efficacy. Hence, wild-type *bdnf* $^{+/+}$ and heterozygous *bdnf* $^{+/-}$ mice were exposed to an eight-week UCMS regimen and, from the third week onward, treated with either vehicle or imipramine (20 mg/kg/day, ip). Heterozygous *bdnf* $^{+/-}$ mice displayed half BDNF level but no enhancement of the sensitivity to stress exposure. While imipramine reduced anxiety-like behaviors and increased hippocampal *bdnf* expression in both genotypes, it decreased aggressiveness and despair behaviors in wild-type but not in heterozygous mice. In summary, we demonstrate here that depletion in BDNF level dampened antidepressant-like effects but fails to increase vulnerability to chronic stress.

Keywords

Animal model, depression, imipramine, neurotrophin, novelty-suppressed feeding test, resident/intruder test, tail suspension test.

Introduction

Extensive studies over the last decade have highlighted disruptions of neural plasticity and of neurotrophins equilibrium in a growing number of psychiatric diseases, such as major depressive disorders (Duman and Monteggia, 2006; Castren et al., 2007). The "neurotrophic hypothesis of depression" emerged from these findings, based on a causal association between BDNF levels and depression-like states or antidepressant response: a decrease in BDNF synthesis and signaling is thought to generate morphological and functional brain changes precipitating depression, while its increase could underlie antidepressant action.

Clinical correlates as well as basic research support the involvement of BDNF in antidepressant response. An increase in BDNF immunoreactivity was reported in post-mortem hippocampal tissues of antidepressant-treated patients compared to the one obtained from untreated patients (Chen et al., 2001). Administration of BDNF itself or genetically-enhanced signaling of BDNF/TrkB receptor produce antidepressant-like effects in rodent models of depression (Siuciak et al., 1997; Shirayama et al., 2002; Hoshaw et al., 2005; Koponen et al., 2005). Moreover, heterozygous *bdnf* $^{+/-}$ mice, which have roughly half reduced levels of BDNF in the brain, and mice with inducible *bdnf* deletion in the forebrain exhibited blunted antidepressant-like responses in the forced swim test (FST) (Saarelainen et al., 2003; Monteggia et al., 2004).

Nevertheless, the "neurotrophic hypothesis" is challenging by inconsistent linkages between BDNF dysfunction and depressive-like behaviors. Although some observations indicate that corticosterone or stress, a factor known to precipitate depression, are associated with a decrease in BDNF levels in the rodent adult hippocampus (Barbany and Persson, 1992; Ueyama et al., 1997; Murakami et al., 2005; Tsankova et al., 2006), various strategies reducing brain BDNF levels or signaling failed to demonstrate clear-cut involvements of BDNF in depression-related symptoms. For example, *bdnf* $^{+/-}$ mice display hyperphagia, obesity, hyperactivity, aggressiveness and learning deficits, but neiher anxiety- nor depression-like behaviors (Linnarsson et al., 1997; Lyons et al., 1999; Kernie et al., 2000; Duan et al., 2003; Saarelainen et al., 2003; Chourbaji et al., 2004; Liu et al., 2004). In contrast to inducible *bdnf* knockout mice (Monteggia et al., 2004), forebrain conditional *bdnf* knockout mice was reported to exhibit depressive-like phenotypes, but these results are lacking consistency: despair-like behaviors was only obtained with females in the FST in one study (Monteggia et al., 2007), while only in the tail suspension test (TST) and with an opposite pattern in the FST in another (Chan et al., 2006). Another possibility is that deficiency in BDNF activity yields to an enhanced vulnerability to stress rather than to directly impact

depression-like behaviors. The previous studies are probably irrelevant to address this point considering that they were all performed using tests of behavioral despair (FST and TST) in "normal" rather than in "depressed" mice.

Moreover, Berton and colleagues (2006) showed that a social defeat stress paradigm increased BDNF levels in the ventral tegmental area and that a specific knockdown of *bdnf* in this brain area caused an antidepressant-like effect. Accordingly, the involvement of BDNF in depression-related states and antidepressant-like effect appears to be more complex than a single up- or down-change of the total BDNF levels in the brain. A reduction of BDNF level could thus be associated to vulnerability or resilience to chronic stress according to the brain region where it occurred. Interestingly, such an outcome could perhaps account for the lack of depressive-like phenotype of *bdnf* $^{+/-}$ mice.

In order to unravel the role of BDNF deficit in vulnerability to stress exposure, in specific depression-related alterations and in antidepressant response, we exposed heterozygous *bdnf* $^{+/-}$ mice to the unpredictable chronic mild stress (UCMS) and to imipramine, a tricyclic antidepressant. The effects of an 8-week UCMS paradigm along with a chronic imipramine treatment from the third week onward in wild-type and heterozygous *bdnf* $^{+/-}$ mice were assessed with physical measures (coat state, body weight), in feeding (24h-home food consumption), locomotor activity (home-cage actimeter), aggressiveness (resident/intruder –R/I– test), anxiety (novelty-suppressed feeding –NSF– test), despair (TST), plasma corticosterone levels and *bdnf* mRNA levels (dentate gyrus of the hippocampus). Our study shows that, although the *bdnf* $^{+/-}$ mice did not display a higher vulnerability to UCMS regimen, they failed to respond effectively to antidepressant treatment in various, but not all measures.

Experimental procedures

Animals

Production of the gene-targeting construct was previously reported (Korte et al., 1995). Wild-type *bdnf* $^{+/+}$ and littermate heterozygous mutant *bdnf* $^{+/-}$ mice were bred on a mixed S129/Sv x C57BL/6 genetic background. All the animals were genotyped by Polymerase Chain Reaction (PCR). Male mice of between 4 and 5 months of age were used in the study presented here. Before the beginning of the experiment, all animals were housed in groups of 4/5 and were maintained under standard laboratory conditions under a 12/12h light/dark cycle (lights on at 20:00h), 22±2°C, food and water *ad libitum*. The production of transgenic mice

and all of the animal experiments were done in accordance to the guidelines with the European Community Council directive 86/609/EEC.

Drugs

Imipramine hydrochloride (Sigma-Aldrich) was used in this study. Imipramine was prepared as solutions in physiological saline (0.9% NaCl). The different solutions were concentrated to administer a final volume of 10 ml/kg.

General procedure

An eight-week UCMS regimen was conducted. Mice were maintained under the same standard laboratory conditions but were isolated in small individual cages (24x11x12cm). The first two weeks of UCMS regimen were drug-free and treatment began from the third week of UCMS until the day of euthanasia. Imipramine (20 mg/kg/day) or vehicle was administered intraperitoneally (ip) once a day for six weeks. They were randomly assigned to the different treatment groups. The antidepressant dose was chosen on the basis of previous experiments showing that the compound is active at this dose in mice (Surget et al., 2008a). The body weight and the state of the coat were assessed weekly until the end of UCMS. During the eighth week of stress regimen, behavioral testing was performed according to the following sequence: 24h-home food consumption, actimeter, R/I test, NSF test and TST. Two days after the TST, mice were sacrificed by CO_2 asphyxia for brain sampling. The full experimental design is depicted in figure

UCMS regimen

The stress regimen used has been previously described (Santarelli et al., 2003; Surget et al., 2008a,b). Mice were repeatedly subjected to various stressors according to a “random” schedule for a total period of seven weeks. The different stressors were: altered bedding (sawdust change, removal or damp; substitution of sawdust with 21°C water, rat or cat feces); cage tilting (45°) or shaking (2 x 30”); cage exchange (mice positioned in the empty cage of another male); induced defensive posture (repeated slight grips on the back until the mouse showed a defensive posture) and altered length and time of light/dark cycle. Combinations of different stressors occurred sometimes. Imipramine treatment started at the beginning of the third week of UCMS exposure. The body weight and the state of the fur were assessed once a week until the end of the eight weeks. The total score of the state of the fur resulted from the sum of the score of seven different body parts: head, neck, dorsal fur, ventral fur, tail,

forepaws and the hindpaws. For each of the seven body areas, a score of 0 was given for a well-groomed fur and 1 for an unkempt fur. This index has been pharmacologically validated in previous studies (Santarelli et al., 2003; Yalcin et al., 2005; Surget et al., 2008a,b).

24h-home food consumption

The food intake of each animal was assessed by measuring the amount of food consumed over a period of 24 hours in the home cage. The food consisted in the regular chow. Food was weighted and presented to the mice at 12:00h. Twenty-four hours later, food was weighted and the amount of food consumed calculated.

Actimeter

The actimeter allowed the assessment of the activity of mice in their home cage, thus excluding the possibility of biasing the results due to novelty-induced anxiety. The home cage was placed in the centre of a device, which consisted of a 20 x 20 cm square plane with two light beams, crossing the plane from midpoint to midpoint of opposite sides, thus dividing the plane into four quadrants, automatically detecting the movement of the animal when it crossed through, allowing to establish a score. The higher the score, the more the mouse moved. Cages were placed in the device 15 minutes before the beginning of testing. Testing was carried out in the nocturnal period from 11:00h and lasted 2 hours allowing a better estimation of the basal locomotor activity.

R/I test

R/I test is a variant of the procedure previously used in our lab (Surget et al., 2008b). Isolated mice were tested against an A/J intruder. The opponent was placed into the cage of the test animal (resident) in such a way that mice were placed in opposite corners and the test started immediately, lasting for a maximum of 5 minutes. Tests were stopped two minutes after the first attack occurred. We recorded the latency of the first attack as well as the number of attacks for two minutes following the first attack.

NSF Test

The NSF Test was a modified version of the one used by Santarelli et al. (2003) and performed as previously described (Surget et al., 2008a). The testing apparatus consisted of a wooden box, 33x33x30 cm with an indirect red light. The floor was covered with 2 cm of sawdust. Six hours before the test, the food was removed from the cages. At the time of

testing, a single pellet of food (regular chow) was placed on a white paper platform positioned in the center of the box. An animal was placed in a corner of the test box. The latency to start consuming the pellet was recorded within a 3-minute period. This test induced a conflicting motivation between the drive to eat the food pellet and the fear of venturing into the center of the arena. This version was shown to be able to specifically reveal antidepressant effects only in UCMS-treated, but not in control mice through a reversal effect of the UCMS-induced increase in the latency (Surget et al., 2008b). Moreover, antidepressants are known to have various effects on appetite. To control this potential confounding factor, the feeding drive of each animal was assessed by returning it to the familiar environment of the home cage immediately after the test, and measuring the amount of food consumed over a period of 5 minutes (home food consumption).

TST

The procedure followed in this study was derived from the protocol previously described (Steru et al., 1985). Mice were suspended by the tail (approximately 1 cm from the tip of the tail) using adhesive tape to a rod at 80cm above the floor. Four animals were tested simultaneously. The trials were conducted for a period of 6 min, and were recorded by a video camera positioned directly in front of the mice while the experimenter observed the session in an adjacent room. Videotapes were subsequently scored blind by a highly trained observer. The behavioral measure scored from videotape was the duration of immobility. Mice were considered immobile only when they hung motionless.

Corticosterone radioimmunoassay

Mice were sacrificed by CO_2 asphyxiation and decapitated. While brains were removed for dissection (see below), trunk blood was collected and centrifuged at 1500g for 12min. Plasma was stored at -20°C and analyzed for total corticosterone levels using a ^{125}I-labeled corticosterone double-antibody radioimmunoassay kit (MP Biomedicals, NY). All samples were run in a single assay; the intra-assay variability was 8.26%. The percentage of cross reactivity with steroids was corticosterone 100%, desoxycorticosterone 0.34%, testosterone 0.1%, cortisol 0.05%, aldosterone 0.03%, progesterone 0.02%, androstenedione 0.01%, 5α-dihydrotestosterone 0.01%, others <0.01%.

Brain areas sampling

Mice were killed by CO_2 asphyxiation and decapitated. Brains were rapidly removed and placed in ice-cold slurry of 0.9%NaCl. Two millimeters rostro-caudal sections were quickly obtained on a brain tissue blocker. Two consecutive sections roughly from Bregma -0.6 to Bregma -4.6 were immediately transferred to wells containing RNAlater (Ambion Inc., Austin, TX). Then, each section was microdissected with the assistance of a brain atlas (Paxinos and Franklin, 2001). Dentate gyrus samples were dissected from the first and second sections. The samples were stored in RNAlater solution at -80°C until further analysis.

Extraction of RNA and Real Time PCR

Total RNA was extracted using Trizol (Invitrogen, Cergy Pontoise, France) according to manufacturer's instructions and treated with DNAseI (Invitrogen, 1 µL/µg RNA). Reverse transcription reaction was performed at 42°C 45 min in a final volume of 50 µL containing 500 ng of total RNA, 100 ng random hexamers, 1 mM dNTP, 10 mM DTT, 1X buffer, 10 U superscript II reverse transcriptase (Invitrogen). The reaction mixture for real time quantitative PCR consisted in 10 ng cDNA from reverse transcription reaction, 12.5 µL of Platinium SYBR Green qPCR SuperMix UDG (Invitrogen), 0.4 pmol/µL of both forward and reverse primers. GAPDH F: 5'-ctgcaccaccaactgcttag-3', R: 5'-gtcttctgggtggcagtgat-3'; BDNF (Alfonso et al., 2006). Threshold cycle (Ct) values were obtained and relative levels of transcription were calculated by the $2^{-\Delta\Delta Ct}$ method (GADPH).

Statistics

When the assumptions for parametric statistics were ensured (normality and homogeneity of variances), two-way ANOVAs were performed by using genotype (*bdnf* $^{+/+}$ vs. *bdnf* $^{+/-}$) or treatment (vehicle vs. imipramine) as main factors, followed by a Fisher *post hoc* analysis when required (i.e., $p<0.05$). Otherwise, data were analyzed using non-parametric procedures: an analysis was done following the Kruskal–Wallis "ANOVA by ranks" procedure, followed by a Mann-Whitney test including corrections for multiple comparisons when required (i.e., $p<0.05$). The Friedman test, a non-parametric "ANOVA by ranks" for repeated measures, was used to examine the changes of the coat state over the eight-week UCMS regimen. Moreover, a Spearman's correlation was performed to analyze the relationship between locomotor activity and body weight in heterozygous mice.

Results:

Evaluation of the coat state

The UCMS procedure induced a significant deterioration of the state of the coat in all the groups independently of the genotype or of the drug treatment (Fig.2A; Friedman test: *bdnf* $^{+/+}$-vehicle, $\chi^2_{(14,8)}$=33.42, p<0.001; *bdnf* $^{+/+}$-imipramine, $\chi^2_{(14,8)}$=29.66, p<0.001; *bdnf* $^{+/-}$-vehicle, $\chi^2_{(13,8)}$=35.04, p<0.001; *bdnf* $^{+/-}$-imipramine, $\chi^2_{(14,8)}$=29.83, p<0.001). Kruskal-Wallis test failed to establish any differences between the four groups throughout the UCMS regimen (weeks 0-8: $H_{3,55}\leq 4.6$, $p\geq 0.2$). Accordingly, heterozygous *bdnf* $^{+/-}$ mice did not show a higher sensitivity to stress exposure in this measure and chronic imipramine treatment was unable to counteract the worsening effect of UCMS on the coat state in any groups.

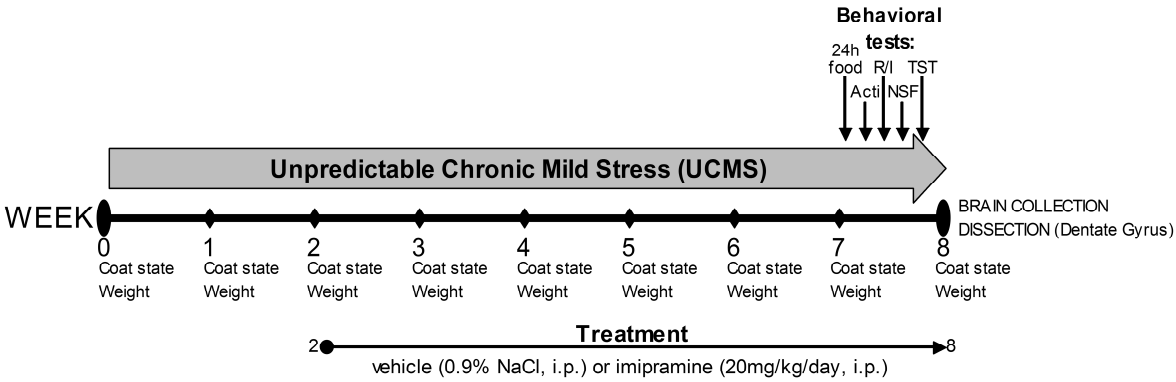

Figure 1. Experimental design. The Unpredictable Chronic Mild Stress (UCMS) regimen lasted 8 weeks. Each week, the coat state was evaluated and the body weight measured. The first two weeks of UCMS regimen were drug-free. Imipramine or vehicle treatments began after two weeks of UCMS and continued until the end of the experiment (week 8). Imipramine (20 mg/kg/day) or vehicle (0.9% NaCl) were administered intraperitoneally once a day. The week before the end of the UCMS regimen, behavioral tests were carried out once a day according to the following sequence (n=11-14 per group): 24h-home food consumption (24h food), actimeter (Acti), resident/intruder test (R/I), novelty-suppressed feeding test (NSF) and tail suspension test (TST). Towards the end of the UCMS regimen, dentate gyrus was collected for analysis of the *bdnf* expression (n=4-5).

Body weight and food intake

ANOVA revealed a significant effect of the genotype on the body weight, but not of the drug treatment or of interaction factors (Fig.2B). This genotype effect occurred intermittently (weeks 0, 2, 4, 6-8: $F_{1,51}\geq 4.16$, $p<0.05$; weeks 1, 3, 5: $F_{1,51}\leq 2.69$, $p\geq 0.11$). *Post hoc* analysis revealed frequent strong trends ($p<0.1$), but no significant difference between both genotypes for the same treatment in paired comparisons.

The 24h-home food consumption was evaluated at the beginning of the eighth week of UCMS (Fig.1). Neither genotype ($F_{1,48}$=3.07, p=0.086), nor drug treatment ($F_{1,48}$=0.03,

p=0.86) nor interaction ($F_{1,48}$=0.8, p=0.37) had a significant effect on food intake, although a trend (p<0.1) for a genotype effect on this measure was found (Fig.2C).

These results demonstrate an elevated body weight in heterozygous *bdnf* $^{+/-}$ mice compared to wild-type mice, which can perhaps be explained by increases in food intake.

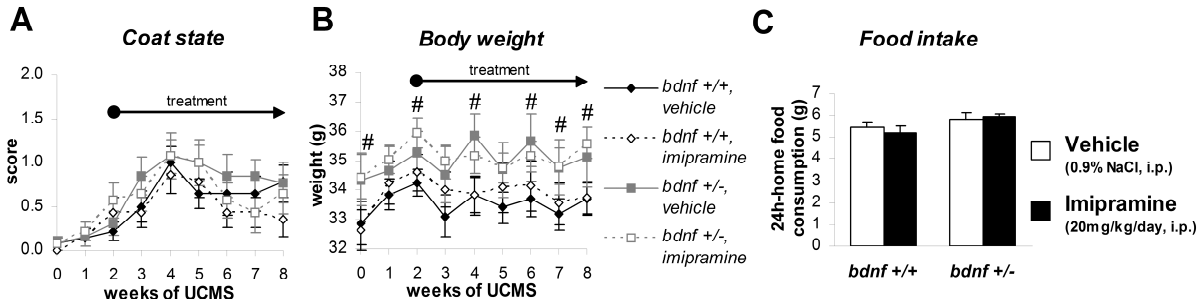

Figure 2. Effect of the heterozygous *bdnf* $^{+/-}$ system and of the imipramine treatment on the coat state, body weight and food intake in 8-week UCMS-exposed mice. (A) The UCMS model induced a significant deterioration of the coat state in all the groups, but no significant effect of genotype or of drug treatment was observed (n=13-14 per group). (B) Increase in the body weight of the *bdnf* $^{+/-}$ mice was found in several time points (p<0.05, weeks 0, 2, 4, 6, 7, 8). No effect of imipramine was shown (n=13-14 per group). (C) No significant change in the 24h-home food consumption was found (n=12-14 per group). Data represent mean ± SEM. #, p<0.05 between *bdnf* $^{+/+}$ and *bdnf* $^{+/-}$ mice.

Behavioral testing

Behavioral testing occurred on the eighth week of the UCMS regimen according to the following sequence: actimeter, R/I test, NSF test and TST (Fig.1).

In the actimeter (Fig.3A), *bdnf* $^{+/-}$ mice were more active than *bdnf* $^{+/+}$ mice (genotype: $F_{1,42}$=6.05, p<0.05), while no effect of treatment ($F_{1,42}$=0.25, p=0.62) or genotype-treatment interaction ($F_{1,42}$=0.009, p=0.93) was reported. However, *post hoc* analysis revealed no significant difference in paired comparisons, only a trend (p<0.1) for a higher activity in heterozygous *bdnf* $^{+/-}$ mice compared with the wild-type for the same treatment (i.e. vehicle or imipramine). As a previous study found that an inverse relationship between locomotor activity and body weight in heterozygous *bdnf* $^{+/-}$ mice (Kernie et al., 2000), we analyzed our data with the Spearman's correlation but no significant correlation was found between these two parameters (R=0.099), which suggests that enhanced body weight does not preclude hyperlocomotor activity in heterozygous *bdnf* $^{+/-}$ mice.

The R/I test consisted to test the aggressiveness by introducing an A/J mouse in the home cage of the tested mouse. The number of attacks for 2 min after the first attacks (Fig.3B) was recorded in a maximum of 5-min period (Kruskal-Wallis test: $H_{3,55}$=9.47, p<0.05). While the

number of attacks was similar in the vehicle-treated mice for both genotypes, imipramine was able to reduce this parameter but only in the wild-type *bdnf* $^{+/+}$ mice ($p<0.05$).

To examine anxiety-related behaviors, a 6h food-deprived mouse was exposed to the NSF test. The latency to chew the pellet was similar in wild-type *bdnf* $^{+/+}$ mice and heterozygous *bdnf* $^{+/-}$ mice for both vehicle and imipramine treatments (Fig.3C; ANOVA: genotype, $F_{1,51}=0.001$, $p=0.97$; treatment, $F_{1,51}=9.22$, $p<0.01$; interaction, $F_{1,51}=0.0001$, $p=0.99$). Imipramine treatment yielded a significant decrease in the latency to feed ($p<0.05$), indicating an antidepressant-induced decrease in anxiety-related behaviors independently of the genotype. These results cannot be explained by changes in motivation to feed as no significant differences were found for the home food consumption assessed immediately after the test (Fig.3D; ANOVA: genotype, $F_{1,51}=1.6$, $p=0.21$; treatment, $F_{1,51}=0.024$, $p=0.88$; interaction, $F_{1,51}=0.038$, $p=0.85$).

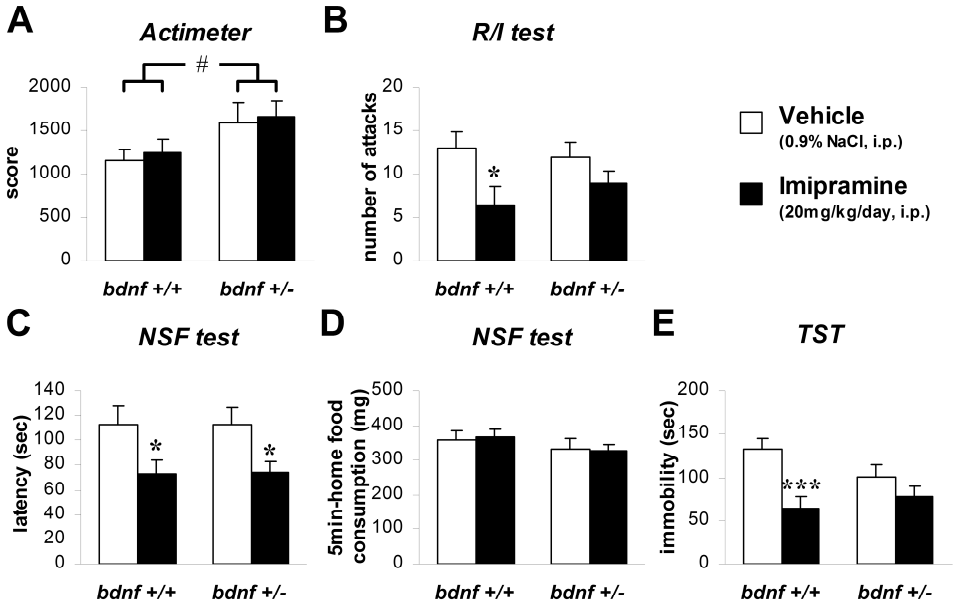

Figure 3. Effect of the heterozygous *bdnf* $^{+/-}$ system and of the imipramine treatment on the behavior in 8-week UCMS-exposed mice. (A) Heterozygous *bdnf* $^{+/-}$ mice displayed an enhancement of the locomotor activity in the actimeter ($p<0.05$). No effect of imipramine was found (n=11-12 per group). (B) In the resident/intruder (R/I) test, the treatment with imipramine was able to reduce the number of attacks in *bdnf* $^{+/+}$ ($p<0.05$) but not in *bdnf* $^{+/-}$ mice (n=13-14 per group). (C) In the novelty-suppressed feeding (NSF) test, imipramine reduced the latency to chew the food pellet in both the *bdnf* $^{+/+}$ ($p<0.05$) and the *bdnf* $^{+/-}$ mice ($p<0.05$; n=13-14 per group). (D) The latter result was shown to be independent of a confounding effect due to variations in feeding drive as no difference in the 5min-home food consumption directly measured immediately after the NSF test. (E) In the tail suspension test (TST), duration of immobility was decreased after imipramine treatment in *bdnf* $^{+/+}$ ($p<0.001$) but not in *bdnf* $^{+/-}$ mice (n=12-14 per group). Data represent mean ± SEM. #, $p<0.05$ between *bdnf* $^{+/+}$ and *bdnf* $^{+/-}$ mice. *, $p<0.05$ and ***, $p<0.001$ between vehicle- and imipramine-treated mice or between line-connected groups.

To investigate whether the deficit of BDNF level exerts an effect on the manifestation of despair behaviors following UCMS and chronic antidepressant treatment, we tested wild-type *bdnf* $^{+/+}$ and heterozygous *bdnf* $^{+/-}$ mice in the TST (Fig.3E). Mice were suspended by the tail to a rod and the time past immobile (interpreted as behavioral despair) was recorded during a unique 6-min trial (ANOVA: genotype, $F_{1,48}$=0.452, p=0.5; treatment, $F_{1,48}$=12.29, p<0.001; interaction, $F_{1,48}$=3.05, p=0.08). No significant difference was found between the vehicle-treated groups, although the wild-type *bdnf* $^{+/+}$ mice showed a trend (p<0.1) for a greater immobility time than heterozygous *bdnf* $^{+/-}$ mice. Imipramine reduced the immobility time in wild-type *bdnf* $^{+/+}$ mice (p<0.001), but not in heterozygous *bdnf* $^{+/-}$ mice.

Plasmatic corticosterone level

Since changes in hypothalamo-pituitary-adrenal axis activity is often associated to depression and antidepressant response (Holsboer, 2000), we measured by radioimmunoassay plasma corticosterone levels in mice after 8-week UCMS (Fig.4). No significant difference due to genotype ($F_{1,34}$=2.07, p=0.16), treatment ($F_{1,34}$=2.07, p=0.16) or their interaction ($F_{1,34}$=2.07, p=0.16) was found.

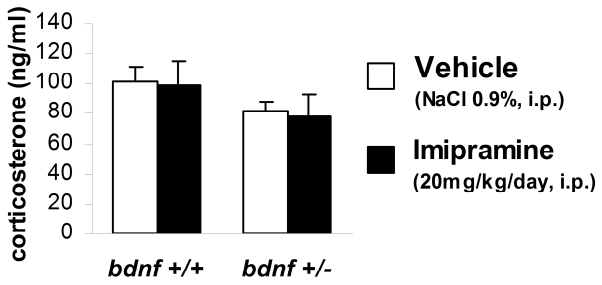

Figure 4. Effect of the heterozygous *bdnf* $^{+/-}$ system and of imipramine treatment on the plasmatic corticosterone level in 8-week UCMS-exposed mice. No significant difference in the plasmatic corticosterone level was found (n=9-10 per group). Data represent mean ± SEM.

***bdnf* mRNA level**

Because the dentate gyrus is thought to be essential in mediating central effects of BDNF on depression-related states and antidepressant-like effect (Adachi et al., 2008), we analyzed the total amount of *bdnf* mRNA in the dentate gyrus by quantitative RT-PCR (Fig.5; Kruskal-Wallis test: $H_{3,17}$=13.12, p<0.01). In vehicle-treated animals, the *bdnf* $^{+/-}$ mice exhibited roughly a 50% lower *bdnf* expression in the hippocampus than the *bdnf* $^{+/+}$ mice (p<0.05). Imipramine treatment allowed duplicating the *bdnf* mRNA level in the both genotypes

compared with vehicle-treated mice ($p<0.05$). The difference between wild-type and heterozygous was thus maintained after antidepressant treatment ($p<0.05$).

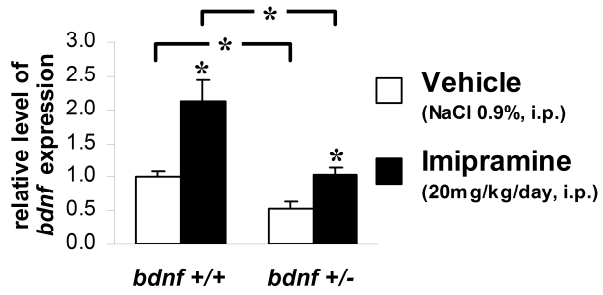

Figure 5. Effect of the heterozygous *bdnf* $^{+/-}$ system and of imipramine treatment on the total *bdnf* mRNA expression level in the dentate gyrus of 8-week UCMS-exposed mice. Imipramine induced a twofold increase of *bdnf* expression in the dentate gyrus of *bdnf* $^{+/+}$ mice ($p<0.05$) as well as of *bdnf* $^{+/-}$ mice ($p<0.05$). Compared with wild-type, *bdnf* $^{+/-}$ mice displayed roughly a half reduction of *bdnf* expression in both the vehicle- ($p<0.05$) and imipramine-treated mice ($p<0.05$; n=4-5 per group). Data represent mean ± SEM. *, $p<0.05$ between vehicle- and imipramine-treated mice or between line-connected groups.

Discussion

We investigated the consequences of the partial ablation of the *bdnf* gene in an 8-week UCMS paradigm and considered the results in the light of the "neurotrophic hypothesis of depression". Indeed, the previous reports did not investigate the propensity of BDNF to mediate depression-like behaviors or antidepressant efficacy in chronic paradigms. However, these models are very informative as they mimic the role of etiological factors leading to depressive episodes. Hence, we examined whether the heterozygous *bdnf* $^{+/-}$ mice display, first, an enhanced vulnerability to UCMS and, second, an alteration of the antidepressant efficacy. We demonstrate here that depletion in BDNF level leads to dampen the antidepressant-like effects of imipramine in several behaviors but fails to increase vulnerability to chronic stress exposure.

Findings from the present study are consistent with previous data showing an association of BDNF level with modifications of the body weight and locomotor activity. In line with a large set of studies with heterozygous *bdnf* $^{+/-}$ mice (Lyons et al., 1999; Kernie et al., 2000; Duan et al., 2003; Coppola and Tessarollo, 2004), we found an elevated body weight in heterozygous mutants compared to wild-types. Despite a trend for a genotype effect, this result arose in our study without a significant elevation of food intake. Although suggesting BDNF-related changes in metabolic regulation, the latter result must be carefully interpreted since it contrasts with previous studies describing hyperphagia as the primary cause of obesity in

these mice (Duan et al., 2003; Coppola and Tessarollo, 2004). In addition, we have to consider that we presented just one snapshot at a precise moment which could be unrepresentative of the overall food intake. In any case, it has been proposed that the elevation of body weight could originate from the direct action of BDNF in ventromedial and dorsomedial hypothalamus (Unger et al., 2007), and from the intricate relationship between BDNF and the serotoninergic brain system (Koizumi et al., 2006; Daws et al., 2007; Guiard et al., 2008) which is involved in the neural regulation of appetite and body weight (Halford et al., 2007). Moreover, we found an association between the deficit in BDNF levels and an increase in locomotor activity which is convergent with many other studies (Kernie et al., 2000; Rios et al., 2001; Duan et al., 2003). While Kernie et al. (2000) reported that hyperactivity was inversely correlated to obesity in heterozygous *bdnf* $^{+/-}$ mice, this correlation is contradicted by our data and others (Chan et al., 2006) which demonstrated that mice with increase in body weight can also be hyperactive.

Because the UCMS paradigm recapitulates the role of socio-environmental stressors precipitating depressive episodes and induces a syndrome reminiscent of depressive symptoms (for a review see Belzung and Surget, 2008), this model is particularly well-suited to investigate causal factors involved in vulnerability to stress or in development of distinct alterations. Considering measures particularly relevant for this study, UCMS was previously shown to deteriorate the coat state, to decrease the body weight gain, to elicit anxiety-like behaviors in the NSF test (increased latency), disturbances in the R/I test (increased aggressiveness), behavioral despair in the TST (increased immobility) and modification of plasma corticosterone levels. All of these alterations were shown to be reversed by chronic exposure to antidepressant treatments in a time course which parallels the clinical feature of these drugs. Regarding the paucity of a direct involvement of the loss of BDNF in the emergence of depression-like behaviors, the first goal of this study was to examine whether a depletion of BDNF makes these mutant mice more vulnerable to chronic stress exposure, in respect to the “neurotrophic hypothesis of depression”. If heterozygous *bdnf* $^{+/-}$ mice are more susceptible to UCMS, we expected a worsening effect in term of amplitude and/or kinetic of the depression-like syndrome in these mutants compared with wild-type controls. However, no evidence for an association between deficit in BDNF levels and enhanced vulnerability to UCMS arose from our data. Vehicle-treated mice were indistinguishable whether they are *bdnf* $^{+/+}$ or *bdnf* $^{+/-}$. Indeed, the deterioration of the coat state, the number of attacks in the R/I test, the latency to eat in the NSF test and the levels of plasma corticosterone were similar in both genotypes. Differences in the body weight at several time points were revealed; however,

since it occurred before the onset of the UCMS regimen, together with the fact that similar differences were previously found in non-stressed mice (see above), these differences cannot be attributed to a superior susceptibility for the UCMS procedure. Our results even demonstrated a trend for a decrease of immobility in the TST in mice lacking one *bdnf* $^{+}$ allele, which could be *a priori* interpreted as a resilient phenotype to despair behavior. Nevertheless, this latter result is mitigated by the fact that these mice were hyperactive in the actimeter, which can account for the decrease in immobility. Lastly, we found no evidence for an anxiety-like phenotype of heterozygous *bdnf* $^{+/-}$ mice, which is consistent with previous reports (Montkowski and Holsboer, 1997; MacQueen et al., 2001; Chourbaji et al., 2004; Ren-Patterson et al., 2006) despite one contradictory result (Chen et al., 2006).

We recently provided evidences from a large-scale gene expression survey that antidepressant effects vary greatly between control and UCMS-treated animals (Surget et al., 2008b). This result challenges the assumption on which the models of behavioral despair are constructed and which postulates that the clinically-relevant antidepressant effects occur independently whether animals are in “normal” or “depression-like” states. These outcomes promote the utilization of the UCMS model when examining the neurobiological mechanisms of the disease and the association between genes and antidepressant responses. Hence, the second purpose of our study was to evaluate whether BDNF mediates antidepressant efficacy in the measures sensitive to the UCMS model. In accordance with previous studies (Belzung and Surget, 2008), imipramine significantly decreased the number of attacks in the R/I test, the latency to eat in the NSF test and the duration of immobility in the TST in wild-type mice. Imipramine was able in heterozygous *bdnf* $^{+/-}$ mice to diminish the latency to eat in the NSF test, but was ineffective to reduce the number of attacks in the R/I test and the immobility in the TST. Together, our results highlighted a lessened antidepressant efficacy in the heterozygous *bdnf* $^{+/-}$ mice in contrast to wild-type *bdnf* $^{+/+}$ mice; particularly antidepressant is ineffective in the heterozygous mutants to decrease aggressiveness during social encounter and despair behavior in the TST. These results are consistent with earlier studies indicating a requirement of BDNF in the behavioral effects of the antidepressants although limited to models of behavioral despair in normal mice (Saarelainen et al., 2003; Monteggia et al., 2004, 2007); we provide here further evidences extended to social disturbances and to “depressed” UCMS-treated mice.

In contrast, we reported no change in the coat state and in the plasma corticosterone levels due to genotype or to drug treatment. We previously found that the antidepressant-induced recovery of the coat state and the change in corticosterone occurs in a strain-dependent

manner (Ibarguen-Vargas et al., 2008). Therefore, these results may well be linked, at least partly, to the genetic background of these mice which could be less sensitive to these measures (Belzung and Surget, 2008).

Since it has been hypothesized that the level of *bdnf* expression in the dentate gyrus of the hippocampus might be related to a depressive-like state or to the antidepressant effect, we examined the total *bdnf* mRNA in the dentate gyrus. As expected, heterozygous *bdnf* $^{+/-}$ mice exhibited roughly half level when compared with the wild-type *bdnf* $^{+/+}$ mice. Consistent with previous findings, treatment with imipramine induced more than a twofold increase in the *bdnf* expression in the dentate gyrus in wild-type mice. Interestingly, a similar augmentation was also observed in the *bdnf* $^{+/-}$ mice. It is noteworthy that imipramine-treated *bdnf* $^{+/-}$ mice display a similar *bdnf* expression than the vehicle-treated *bdnf* $^{+/+}$ mice, while they thoroughly differ in term of behaviors and states. This result is interesting as it shows that the level of *bdnf* expression in the dentate gyrus could not be the driver of the animals' state and performances, but only contributes to particular antidepressant-like effects.

Finally, all these data combined can be extrapolated to the studies investigating the human *bdnf* Val_{66}Met polymorphism. The replacement of Val_{66} by Met_{66} produces no effect on *bdnf* expression *per se*, but it disrupts cellular processing, trafficking and activity-dependant secretion of BDNF which might result in punctual deficits in available BDNF (Egan et al., 2003; Chen et al., 2004). The presence of these $BDNF_{Met}$ defects has attracted much interest whether this polymorphism may underlie risk factor for depression or altered antidepressant response. Although the *bdnf* Val_{66}Met polymorphism was not shown to be reliably related with occurrence of depression (Hong et al., 2003; Tsai et al., 2003; Willis-Owen et al., 2005; Hwang et al., 2006), it seems more consistently associated with modification of antidepressant efficacy (Tsai et al., 2003; Choi et al., 2006; Anttila et al., 2007), which is reminiscent of the outcomes from heterozygous *bdnf* $^{+/-}$ mice (Tsai SJ 2003, Choi MJ 2006, Antilla S 2007).

In summary, we have demonstrated that brain depletion in *bdnf* expression fails to increase vulnerability to a chronic stress procedure which mimics etiological factors for depression; however this deficit dampens some aspects of the antidepressant efficacy. This result suggests that a low BDNF level does not contribute to vulnerability for depression or depressive-like phenotypes while BDNF might be essential for specific antidepressant action. Future studies should unravel whether the involvement of BDNF in these phenotypes is due to its role in the development or in the adult brain as well as the possibility of compensatory mechanisms evolving in heterozygous mutant mice by comparing heterozygous *bdnf* $^{+/-}$ mice with conditional or inducible knockout mice in chronic models of depression.

Acknowledgement

We express our gratitude to Lucette Garreau and Sylvie Chalon (U930, inserm, Tours, France) for assistance in radioimmunoassay.

Y Ibarguen-Vargas, A Surget, S Leman, P Vourc'h, C. Andres and A.M. Gardier declare that, except for income received from our primary employer, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest. C Belzung receives compensation as a consultant for Takeda.

References

Adachi, M., Barrot, M., Autry, A. E., Theobald, D. and Monteggia, L. M. (2008). Selective loss of brain-derived neurotrophic factor in the dentate gyrus attenuates antidepressant efficacy. Biol. Psychiatry 63, 642-649.

Alfonso, J., Frick, L. R., Silberman, D. M., Palumbo, M. L., Genaro, A. M. and Frasch, A. C. (2006). Regulation of hippocampal gene expression is conserved in two species subjected to different stressors and antidepressant treatments. Biol. Psychiatry 59, 244-251.

Anttila, S., Huuhka, K., Huuhka, M., Rontu, R., Hurme, M., Leinonen, E. and Lehtimaki, T. (2007). Interaction between 5-HT1A and BDNF genotypes increases the risk of treatment-resistant depression. J. Neural Transm. 114, 1065-1068.

Barbany, G. and Persson, H. (1992). Regulation of Neurotrophin mRNA Expression in the Rat Brain by Glucocorticoids. Eur. J. Neurosci. 4, 396-403.

Belzung, C. and Surget, A. (2008). Unpredictable chronic mild stress in mice. In: Kalueff, A. V. and LaPorte, J. L., (Eds), Experimental models in neurobehavioral research. Nova Science, New York.

Berton, O., McClung, C. A., Dileone, R. J., Krishnan, V., Renthal, W., Russo, S. J., Graham, D., Tsankova, N. M., Bolanos, C. A., Rios, M., Monteggia, L. M., Self, D. W. and Nestler, E. J. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311, 864-868.

Castren, E., Voikar, V. and Rantamaki, T. (2007). Role of neurotrophic factors in depression. Curr. Opin. Pharmacol. 7, 18-21.

Chan, J. P., Unger, T. J., Byrnes, J. and Rios, M. (2006). Examination of behavioral deficits triggered by targeting Bdnf in fetal or postnatal brains of mice. Neuroscience 142, 49-58.

Chen, B., Dowlatshahi, D., MacQueen, G. M., Wang, J. F. and Young, L. T. (2001). Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol. Psychiatry 50, 260-265.

Chen, Z. Y., Patel, P. D., Sant, G., Meng, C. X., Teng, K. K., Hempstead, B. L. and Lee, F. S. (2004). Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. J. Neurosci. 24, 4401-4411.

Chen, Z. Y., Jing, D., Bath, K. G., Ieraci, A., Khan, T., Siao, C. J., Herrera, D. G., Toth, M., Yang, C., McEwen, B. S., Hempstead, B. L. and Lee, F. S. (2006). Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 314, 140-143.

Choi, M. J., Kang, R. H., Lim, S. W., Oh, K. S. and Lee, M. S. (2006). Brain-derived neurotrophic factor gene polymorphism (Val66Met) and citalopram response in major depressive disorder. Brain Res. 1118, 176-182.

Chourbaji, S., Hellweg, R., Brandis, D., Zorner, B., Zacher, C., Lang, U. E., Henn, F. A., Hortnagl, H. and Gass, P. (2004). Mice with reduced brain-derived neurotrophic factor expression show decreased choline acetyltransferase activity, but regular brain monoamine levels and unaltered emotional behavior. Brain Res. Mol. Brain Res. 121, 28-36.

Coppola, V. and Tessarollo, L. (2004). Control of hyperphagia prevents obesity in BDNF heterozygous mice. Neuroreport 15, 2665-2668.

Daws, L. C., Munn, J. L., Valdez, M. F., Frosto-Burke, T. and Hensler, J. G. (2007). Serotonin transporter function, but not expression, is dependent on brain-derived neurotrophic factor (BDNF): in vivo studies in BDNF-deficient mice. J. Neurochem. 101, 641-651.

Duan, W., Guo, Z., Jiang, H., Ware, M. and Mattson, M. P. (2003). Reversal of behavioral and metabolic abnormalities, and insulin resistance syndrome, by dietary restriction in mice deficient in brain-derived neurotrophic factor. Endocrinology 144, 2446-2453.

Duman, R. S. and Monteggia, L. M. (2006). A neurotrophic model for stress-related mood disorders. Biol. Psychiatry 59, 1116-1127.

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B. and Weinberger, D. R. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112, 257-269.

Guiard, B. P., David, D. J., Deltheil, T., Chenu, F., Le Maitre, E., Renoir, T., Leroux-Nicollet, I., Sokoloff, P., Lanfumey, L., Hamon, M., Andrews, A. M., Hen, R. and Gardier, A. M. (2008). Brain-derived neurotrophic factor-deficient mice exhibit a hippocampal hyperserotonergic phenotype. Int. J. Neuropsychopharmacol. 11, 79-92.

Halford, J. C., Harrold, J. A., Boyland, E. J., Lawton, C. L. and Blundell, J. E. (2007). Serotonergic drugs : effects on appetite expression and use for the treatment of obesity. Drugs 67, 27-55.

Holsboer, F. (2000). The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23, 477-501.

Hong, C. J., Huo, S. J., Yen, F. C., Tung, C. L., Pan, G. M. and Tsai, S. J. (2003). Association study of a brain-derived neurotrophic-factor genetic polymorphism and mood disorders, age of onset and suicidal behavior. Neuropsychobiology 48, 186-189.

Hoshaw, B. A., Malberg, J. E. and Lucki, I. (2005). Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects. Brain Res. 1037, 204-208.

Hwang, J. P., Tsai, S. J., Hong, C. J., Yang, C. H., Lirng, J. F. and Yang, Y. M. (2006). The Val66Met polymorphism of the brain-derived neurotrophic-factor gene is associated with geriatric depression. Neurobiol. Aging 27, 1834-1837.

Ibarguen-Vargas, Y., Surget, A., Touma, C., Palme, R. and Belzung, C. (2008). Multifaceted strain-specific effects in a chronic mouse model of depression and of antidepressant reversal. Psychoneuroendocrinology.

Kernie, S. G., Liebl, D. J. and Parada, L. F. (2000). BDNF regulates eating behavior and locomotor activity in mice. Embo. J. 19, 1290-1300.

Koizumi, H., Hashimoto, K. and Iyo, M. (2006). Dietary restriction changes behaviours in brain-derived neurotrophic factor heterozygous mice: role of serotonergic system. Eur. J. Neurosci. 24, 2335-2344.

Koponen, E., Rantamaki, T., Voikar, V., Saarelainen, T., MacDonald, E. and Castren, E. (2005). Enhanced BDNF signaling is associated with an antidepressant-like behavioral response and changes in brain monoamines. Cell. Mol. Neurobiol. 25, 973-980.

Korte, M., Carroll, P., Wolf, E., Brem, G., Thoenen, H. and Bonhoeffer, T. (1995). Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc. Natl. Acad. Sci. U. S. A. 92, 8856-8860.

Linnarsson, S., Bjorklund, A. and Ernfors, P. (1997). Learning deficit in BDNF mutant mice. Eur. J. Neurosci. 9, 2581-2587.

Liu, I. Y., Lyons, W. E., Mamounas, L. A. and Thompson, R. F. (2004). Brain-derived neurotrophic factor plays a critical role in contextual fear conditioning. J. Neurosci. 24, 7958-7963.

Lyons, W. E., Mamounas, L. A., Ricaurte, G. A., Coppola, V., Reid, S. W., Bora, S. H., Wihler, C., Koliatsos, V. E. and Tessarollo, L. (1999). Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. Proc. Natl. Acad. Sci. U. S. A. 96, 15239-15244.

MacQueen, G. M., Ramakrishnan, K., Croll, S. D., Siuciak, J. A., Yu, G., Young, L. T. and Fahnestock, M. (2001). Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. Behav. Neurosci. 115, 1145-1153.

Monteggia, L. M., Barrot, M., Powell, C. M., Berton, O., Galanis, V., Gemelli, T., Meuth, S., Nagy, A., Greene, R. W. and Nestler, E. J. (2004). Essential role of brain-derived neurotrophic factor in adult hippocampal function. Proc. Natl. Acad. Sci. U. S. A. 101, 10827-10832.

Monteggia, L. M., Luikart, B., Barrot, M., Theobold, D., Malkovska, I., Nef, S., Parada, L. F. and Nestler, E. J. (2007). Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. Biol. Psychiatry 61, 187-197.

Montkowski, A. and Holsboer, F. (1997). Intact spatial learning and memory in transgenic mice with reduced BDNF. Neuroreport 8, 779-782.

Murakami, S., Imbe, H., Morikawa, Y., Kubo, C. and Senba, E. (2005). Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. Neurosci. Res. 53, 129-139.

Paxinos, G. and Franklin, K. B. J. (2001). The mouse brain in stereotaxic coordinates. Academic Press, San Diego, CA.

Ren-Patterson, R. F., Cochran, L. W., Holmes, A., Lesch, K. P., Lu, B. and Murphy, D. L. (2006). Gender-dependent modulation of brain monoamines and anxiety-like behaviors in mice with genetic serotonin transporter and BDNF deficiencies. Cell. Mol. Neurobiol. 26, 755-780.

Rios, M., Fan, G., Fekete, C., Kelly, J., Bates, B., Kuehn, R., Lechan, R. M. and Jaenisch, R. (2001). Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Mol. Endocrinol. 15, 1748-1757.

Saarelainen, T., Hendolin, P., Lucas, G., Koponen, E., Sairanen, M., MacDonald, E., Agerman, K., Haapasalo, A., Nawa, H., Aloyz, R., Ernfors, P. and Castren, E. (2003). Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. J. Neurosci. 23, 349-357.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C. and Hen, R. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301, 805-809.

Shirayama, Y., Chen, A. C., Nakagawa, S., Russell, D. S. and Duman, R. S. (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J. Neurosci. 22, 3251-3261.

Siuciak, J. A., Lewis, D. R., Wiegand, S. J. and Lindsay, R. M. (1997). Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). Pharmacol. Biochem. Behav. 56, 131-137.

Steru, L., Chermat, R., Thierry, B. and Simon, P. (1985). The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berl) 85, 367-370.

Surget, A., Saxe, M., Leman, S., Ibarguen-Vargas, Y., Chalon, S., Griebel, G., Hen, R. and Belzung, C. (2008a). Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. Biol. Psychiatry (in press).

Surget, A., Wang, Y., Leman, S., Ibarguen-Vargas, Y., Edgar, N., Griebel, G., Belzung, C. and Sibille, E. (2008b). Corticolimbic transcriptome changes are state-dependent and region-specific in a rodent model of depression and of antidepressant reversal. Neuropsychopharmacology.

Tsai, S. J., Cheng, C. Y., Yu, Y. W., Chen, T. J. and Hong, C. J. (2003). Association study of a brain-derived neurotrophic-factor genetic polymorphism and major depressive disorders, symptomatology, and antidepressant response. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 123, 19-22.

Tsankova, N. M., Berton, O., Renthal, W., Kumar, A., Neve, R. L. and Nestler, E. J. (2006). Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat. Neurosci. 9, 519-525.

Ueyama, T., Kawai, Y., Nemoto, K., Sekimoto, M., Tone, S. and Senba, E. (1997). Immobilization stress reduced the expression of neurotrophins and their receptors in the rat brain. Neurosci. Res. 28, 103-110.

Unger, T. J., Calderon, G. A., Bradley, L. C., Sena-Esteves, M. and Rios, M. (2007). Selective deletion of Bdnf in the ventromedial and dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity. J. Neurosci. 27, 14265-14274.

Willis-Owen, S. A., Fullerton, J., Surtees, P. G., Wainwright, N. W., Miller, S. and Flint, J. (2005). The Val66Met coding variant of the brain-derived neurotrophic factor (BDNF) gene does not contribute toward variation in the personality trait neuroticism. Biol. Psychiatry 58, 738-742.

Yalcin, I., Aksu, F. and Belzung, C. (2005). Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol. Eur. J. Pharmacol. 514, 165-174.

DISCUSSION GENERALE

Au cours de ce travail, nous voulions déterminer l'implication du BDNF dans l'étiopathogenèse et le traitement des troubles anxio-dépressifs à travers l'utilisation de modèles murins. Nous avons choisi pour cela une double approche : corrélationnelle (étude du lien entre un polymorphisme fonctionnel du gène BDNF et le phénotype anxio-dépressif des souris) et « lésionnelle » (expression monoallélique du BDNF). Une autre originalité de ce travail a aussi été de confronter ces approches à des modèles éthologiques des troubles anxio-dépressifs et particulièrement à un modèle chronique de la dépression chez la souris, l'UCMS, qui reproduit le rôle des stresseurs socio-environnementaux dans la survenue d'épisodes dépressifs. En effet, l'hypothèse neurotrophique de la dépression n'avait jusqu'à présent jamais été testée dans l'UCMS.

Toutes nos expériences nous ont permis de mettre en évidence plusieurs résultats significatifs. Tout d'abord, nous avons pu montrer l'existence d'un polymorphisme fonctionnel du gène BDNF chez la souris. Ce polymorphisme induit la substitution d'une leucine (C3H et C57BL/6) par une méthionine (A/J, BALB/c, CBA, DBA/2 et FVB) en position 32 du proBDNF (polymorphisme Leu32Met du BDNF). À l'issue d'un large screening comportemental de ces lignées, nous avons établi que ce polymorphisme contribuait au phénotype anxiété-like des souris. En utilisant différents paradigmes comportementaux de l'anxiété, nous avons pu distinguer plus spécifiquement une contribution de ce SNP à l'expression des réactions néophobiques et l'anxiété de trait. Par contre, d'autres traits ou comportements précédemment associés au BDNF n'ont pas permis d'établir un lien de ce SNP avec l'apprentissage et la mémoire, le comportement alimentaire et la prise de poids, l'activité, le comportement dépression-like et la réponse aux antidépresseurs. Ensuite, par l'ablation partielle de l'expression du BDNF (souris BDNF $^{+/-}$), nous avons examiné l'hypothèse neurotrophique de la dépression qui suggère que le niveau de BDNF est corrélé avec l'état dépression-like. Alors que des études précédentes ont indiqué une absence de lien entre la diminution du BDNF et le comportement dépression-like, nous avons tiré profit du modèle chronique de la dépression, l'UCMS, pour démontrer que cette réduction n'induit pas non plus une plus grande vulnérabilité au stress. Par contre, nous avons montré que cette perte de BDNF diminuait l'aptitude des antidépresseurs à inverser les effets de l'UCMS, du moins pour certains traits phénotypiques.

1. Le polymorphisme Leu32Met du BDNF

Nous avons au cours de cette thèse pu mettre en évidence un polymorphisme du gène BDNF de la souris. Ce polymorphisme a récemment été enregistré (dbSNP number rs27524348) bien qu'il n'indique pas le génotype de la lignée CBA.

Ce polymorphisme consiste en une simple substitution nucléotidique dans le prodomaine du BDNF. Les lignées C3H et C57BL/6 possèdent une thymine en position 94 alors que les lignées A/J, BALB/c, CBA, DBA/2 et FVB possèdent une adénine. Cette substitution induit un changement d'acide aminé en position 32 dans la séquence de la protéine-précurseur du BDNF : une leucine (C3H et C57BL/6) est remplacée par une méthionine. Nous avons voulu évaluer si ce polymorphisme pouvait avoir un effet sur le taux d'expression du BDNF dans l'hippocampe (cette zone étant choisie car elle est l'un des principaux sites de libération du BDNF). Aucune différence dans le niveau d'ARNm du BDNF n'a été relevée dans les 6 lignées testées et par conséquent on peut affirmer qu'il n'y a pas d'effet significatif du polymorphisme sur l'expression du BDNF. Il est donc possible que ce SNP n'ait aucun effet à la fois sur l'expression du BDNF mais aussi sur l'activité intracellulaire (maturation, transport et sécrétion) et extracellulaire (liaison aux récepteurs et signalisation) de la protéine BDNF, d'autant plus que ces variants génétiques sont situés dans le prodomaine du précurseur du BDNF.

Néanmoins, alors que les pro-formes des neurotrophines ont longtemps été considérées comme inactives, il existe maintenant un nombre d'évidences en faveur d'un rôle fonctionnel de ces proformes y compris du proBDNF (Chao et Bothwell, 2002, Lee *et coll.*, 2001). Il est à noter que les séquences de pro-domaines des proneurotrophines sont très conservées entre les espèces, ce qui suggère qu'ils ont des fonctions importantes (Heinrich et Lum, 2000). Tout d'abord, les pro-domaines des neurotrophines peuvent promouvoir la maturation de protéines et donc être impliqués dans certains processus comme le repliement des neurotrophines (Kolbeck *et coll.*, 1994, Rattenholl *et coll.*, 2001, Suter *et coll.*, 1991) ; en outre, elles participent au processus de sélection vers une voie de sécrétion particulière : constitutive ou régulée (Farhadi *et coll.*, 2000). De plus, les pro-neurotrophines peuvent présenter une interaction minimale avec les récepteurs TrK alors qu'ils se lient au p75NTR avec une plus grande affinité que la protéine mature (Lee et al 2001). Le récepteur p75NTR est connu pour se lier de façon non sélective à toutes les neurotrophines adultes de faible affinité, alors que les TrK sont des récepteurs sélectifs à haute affinité. D'autre part pour la signalisation neurotrophine / TrK, l'activation de p75NTR peut susciter des événements pro-apoptotique (Bamji *et coll.*, 1998, Barrett, 2000, Tapia-Arancibia *et coll.*, 2004, Yoon *et coll.*, 1998).

Toutefois, lorsque il y a une co-expression avec les récepteurs TrK, les récepteurs p75NTR peuvent interagir avec les récepteurs TrK et augmenter l'affinité aux neurotrophines matures. De cette façon, p75NTR peut également contribuer à la survie des cellules, au prolongement de neurites et à la plasticité synaptique (Dechant et Barde, 2002). Il a été démontré que les neurotrophines, y compris le BDNF, peuvent être sécrété dans la forme de pro-neurotrophines par une variété de cellules (Lee *et coll.*, 2001, Mowla *et coll.*, 2001). Lee et al (2001) ont constaté que les deux proNGF et proBDNF peuvent être clivés extracellulairement par plusieurs protéases et notamment la plasmine et les metalloproteinases qui présentent des modes d'expression compatible avec l'action neurotrophique y compris dans les synapses (Poo, 2001). Par conséquent, une régulation bidirectionnelle de la survie des cellules et de la plasticité cellulaire par les neurotrophines semble émerger de ces données. En plus de la quantité des neurotrophines et de la densité des récepteurs p75NTR et TrK exprimés, l'équilibre entre les actions apoptotiques, la plasticité ou la survie pourrait aussi être déterminée par le ratio de Pro-Forme disponibles et neurotrophines matures selon les activités et l'expression de plusieurs protéases (Lu *et coll.*, 2005). Ainsi, est possible qu'il existe un rôle important du proBDNF dans ces fonctions. En effet, Woo et coll (Woo *et coll.*, 2005) ont montré que l'activation des p75NTR par le proBDNF facilite la dépression à long terme dans l'hippocampe. Par conséquent, le polymorphisme Leu32Met dans la séquence du prodomaine BDNF, bien que n'altérant pas le niveau d'expression du BDNF, pourrait avoir une incidence sur l'activité neuronale à de nombreux niveaux: (1) le repliement du BDNF mature, (2) la transformation, le trafic et la sécrétion cellulaire (3) l'interaction avec les protéases extracellulaires, (4) l'affinité de liaison avec p75NTR. L'un ou l'autre de ces processus peut ensuite modifier les comportements dans lesquels on pense que le BDNF peut être impliqué.

En effet, le polymorphisme Val66Met du gène BDNF chez l'homme, qui présente des caractéristiques similaires au polymorphisme Leu32Met du gène BDNF chez la souris (situé dans le prodomaine du proBDNF, une substitution d'un acide aminé aliphatique par une Met), peut avoir une influence dans l'activité et la fonction du BDNF. Alors que le remplacement de l'allèle Val66 par un allèle Met66 ne produit aucun effet sur le ratio proBDNF / BDNF mature et sur la sécrétion constitutive, il perturbe fortement l'activité intracellulaire, le transport et l'adressage, notamment vers les synapses et la sécrétion régulée du BDNF (dépendante de l'activité) (Egan *et coll.*, 2003). Ces perturbations biologiques pourraient avoir une incidence sur des fonctions plus complexes, comme le comportement ainsi que à des états pathologiques. En effet, le polymorphisme Val66Met a été également associé à la mémoire humaine ainsi qu'à la fonction hippocampique et au volume hippocampique (Szeszko *et coll.*,

2005), à la dépression (Hong *et coll.*, 2003a, Szeszko *et coll.*, 2005, Tsai *et coll.*, 2003), au trouble bipolaire (Kunugi *et coll.*, 2004, Lohoff *et coll.*, 2005), à l'anxiété (Enoch *et coll.*, 2007, Hunnerkopf *et coll.*, 2007, Jiang *et coll.*, 2005, Lang *et coll.*, 2005), au trouble d'hyperactivité avec déficit d'attention (Conner *et coll.*, 2008, Kent *et coll.*, 2005), à la schizophrénie (Hall *et coll.*, 2003a, Kanazawa *et coll.*, 2007, Neves-Pereira *et coll.*, 2005), au trouble obsessionnel-compulsif (Hall *et coll.*, 2003a), aux troubles de l'alimentation (Mercader *et coll.*, 2007, Ribases *et coll.*, 2003), à l'abus de substances (Gratacos *et coll.*, 2007, Tsai, 2007), à la maladie de Alzeihmer (Kunugi *et coll.*, 2001, Tsai *et coll.*, 2004) et à la maladie de Parkinson (Hakansson *et coll.*, 2003, Karamohamed *et coll.*, 2005). L'ensemble de ces observations indique qu'il n'est pas inutile d'étudier l'association de ce polymorphisme « silencieux » avec les comportements anxieux, les états dépressifs-like ou les effets des antidépresseurs.

2. BDNF et anxiété

Comme nous venons de le voir, des variations dans le gène BDNF peuvent affecter un nombre de traits normaux ou pathologiques assez divers. De ce fait, à la suite de la découverte chez la souris du polymorphisme Leu32Met du BDNF, notre objectif été de caractériser les profils comportementaux des six lignées que nous avions initialement génotypées dans différents traits pour lesquels il a été proposé que le BDNF joue un rôle : la prise de poids, le comportement alimentaire, l'apprentissage et la mémoire, l'activité locomotrice, les comportements anxieux.

Au cours de ce travail, nous avons finalement trouvé parmi toutes ces mesures un lien entre ce polymorphisme et un phénotype anxiété-like. En examinant les profils comportementaux de six lignées de souris, nous avons constaté que les souris qui possédaient l'allèle Met (A/J, BALB/c, CBA, DBA/2) montraient une réactivité émotionnelle et des comportements anxiété-like significativement plus importants que les souris portant l'allèle Leu (C3H et C57BL/6). En effet, la distribution des allèles Leu et Met parmi les lignées a été corrélée avec le temps passé dans les nouveaux compartiments et le nombre de tentatives d'entrée dans le test de l'exploration libre ainsi qu'avec le temps passé dans les zones ouvertes et le nombre de défécation dans le labyrinthe en zéro surélevé. Ces variables sont considérées comme estimant le phénotype anxiété-like. Ainsi, l'allèle Met pourrait être un facteur de risque dans le développement de comportements anxiété-like.

Une implication du BDNF dans le développement de comportements anxieux est en accord avec plusieurs publications étudiant l'association du polymorphisme Val66Met du BDNF avec des traits phénotypiques chez l'Homme, même si des divergences notables existent. Tout d'abord, une étude récente a montré une corrélation négative entre le niveau de stress psychologique au travail et la concentration de BDNF sérique, suggérant que le niveau de BDNF dans le sang soit un marqueur biologique de stress et d'anxiété (Mitoma *et coll.*, 2008). Les autres études cliniques ont surtout recherché un lien entre le polymorphisme Val66Met du BDNF et l'anxiété avec des résultats assez contradictoires. Certaines études ont trouvé un effet protecteur de l'allele Met66 contre l'anxiété de trait (Lang *et coll.*, 2005) et contre le neuroticisme (Hunnerkopf *et coll.*, 2007), trait psychologique marqué par une tendance à ressentir des émotions négatives et considéré comme un facteur de risque pour l'anxiété (Brandes et Bienvenu, 2006) et la dépression (Enns et Cox, 1997). D'autres, au contraire, ont présenté l'allèle Met66 comme un facteur de risque pour les comorbidités anxio-dépressives (Enoch *et coll.*, 2007, Jiang *et coll.*, 2005).

Les études chez l'animal ont également cherché un lien entre BDNF et anxiété. Chen et collègues (Chen *et coll.*, 2006) ont récemment modélisé le polymorphisme Val66Met du BDNF en générant des souris transgéniques exprimant l'un ou l'autre des variants (knock-in). Ces souris reproduisent les principales caractéristiques observées chez l'homme comme une diminution du volume de l'hippocampe chez les porteurs de l'allèle Met66 (Pezawas *et coll.*, 2004, Szeszko *et coll.*, 2005). En particulier, leur étude révèle une augmentation du comportement anxiété-like dans le labyrinthe en croix surélevée, l'open-field et le NSF test. De plus, ils ont trouvé un phénotype identique chez des souris hétérozygotes BDNF $^{+/-}$. Ces souris ont une expression monoallélique du BDNF ce qui induit une diminution d'environ 50% des niveaux d'ARNm du BDNF. Néanmoins, ces résultats avec les souris BDNF $^{+/-}$ contredisent tous les autres résultats réalisés avec des mutants hétérozygotes. En effet, Montkowski et Holsboer (Montkowski et Holsboer, 1997), MacQueen et coll (Macqueen *et coll.*, 2001), Chourbaji et coll (Chourbaji *et coll.*, 2004) et Ren-Patterson et coll (Ren-Patterson *et coll.*, 2006) n'ont trouvé aucune évidence indiquant un effet d'une diminution de l'expression du BDNF sur le phénotype anxieté-like dans plusieurs paradigmes différents. Nous aussi, lors de notre étude avec les souris hétérozygotes BDNF $^{+/-}$, nous n'avons trouvé aucune augmentation des comportements anxiété-like dans le NSF test chez les souris BDNF $^{+/-}$, bien qu'il faut prendre en compte le fait que dans notre étude les souris venaient de subir un procédure d'UCMS alors que dans les autres travaux mentionnés il s'agit de souris non stressées. Il est en tout cas frappant de constater que dans les 2 cas le phénotype anxiété-like

n'est pas altéré par la réduction de BDNF alors qu'il l'est par le polymorphisme Met32Leu du BDNF murin et le polymorphisme Val66Leu du BDNF humain. La différence principale entre l'expression monoallélique du BDNF et les effets du polymorphisme Val66Met est que dans le premier cas les effets concernent une diminution globale du BDNF alors que dans le deuxième cas le niveau d'expression du BDNF est normal, tout comme la sécrétion constitutive, alors que le transport du BDNF vers les synapses et la sécrétion régulée sont altérés (du moins chez l'Homme). Il est donc possible que l'intégrité des processus de sécrétion régulée du BDNF soit nécessaire à la diminution d'un comportement anxiété-like tandis que la conservation d'à peu près 50% du niveau de BDNF suffise à maintenir la capacité du système BDNF/TrkB à réagir efficacement lors de situations anxiogéniques. Cette hypothèse est confortée par le fait que l'absence totale de BDNF chez des souris KO conditionnelles, au contraire de la diminution partielle des souris BDNF $^{+/-}$, induit une augmentation des comportements anxiété-like (Rios *et coll.*, 2001). Il serait bien évidemment nécessaire d'évaluer le lien entre le polymorphisme du BDNF murin et le transport du BDNF vers les synapses et la sécrétion régulée.

Par rapport aux résultats précédents, nos travaux ont également permis un éclairage nouveau sur le rôle du BDNF dans le phénotype anxiété-like. Une analyse plus précise de nos résultats montre en effet que le BDNF n'est pas simplement impliqué dans un changement quantitatif du niveau d'anxiété mais qu'il pouvait aussi être plus spécifiquement engagé dans des types particuliers d'anxiété. En effet, il a été suggéré que l'anxiété ne doit pas être conceptualisée comme un continuum mais comme une construction multidimensionnelle (Endler et Kocovski, 2001). Dans cette optique, il est important de distinguer l'anxiété d'état de l'anxiété de trait (Spielberger et Smith, 1966). L'anxiété d'état peut être définie comme une réactivité émotionnelle transitoire à une situation potentiellement stressante associée à une activation physiologique, tandis que l'anxiété de trait peut être vue comme un prédisposition individuelle, une caractéristique durable voire un facteur de risque pour les troubles anxio-dépressifs (Belzung et Griebel, 2001). Il peut être possible de discerner l'anxiété d'état et l'anxiété de trait à partir de paradigmes différents chez les rongeurs (Belzung et Griebel, 2001). Dans le paradigme de l'exploration libre, les souris ont librement accès à la nouveauté et ne présentent pas de modifications neuroendocriniennes (Cigrang *et coll.*, 1986) ; cette situation expérimentale peut par conséquent être considérée comme dépourvue de composantes stressants. Par conséquent, les réactions néophobiques (comme éviter les compartiments nouveaux) indiquent une caractéristique constante du comportement assimilé à l'anxiété de trait. D'autre part, du comportement des tests basés sur l'application d'un stimulus

anxiogénique sont perçus comme modèle d'anxiété état. Le labyrinthe en zéro appartient à cette catégorie. Il est base sur la confrontation avec un lieu aversif inconnu. Le splash test rentre lui aussi dans cette définition. Bien que la validité prédictive du splash test comme test d'anxiété n'ait encore jamais été évalué, il est lui aussi basé sur l'irruption d'un stimulus anxiogénique : l'aspersion soudaine d'une solution de sucrose. Cette situation va entraîner un conflit entre les comportements de vigilance (redressements) et les comportements de toilettage (Ducottet et Belzung, 2005). Néanmoins, alors qu'un phénotype anxiété-like a été associé avec l'allèle Met dans le labyrinthe en zéro surélevé, les différences de comportements anxiété-like ne corrèlent pas dans le splash test avec la répartition allélique parmi les lignées. Ce résultat indique que les 2 tests pourraient mesurer différents aspects de l'anxiété d'état. Il est en effet important de prendre en compte les multiples facettes de l'anxiété d'état et de trait. Endler et Kocovski (Endler et Kocovski, 2001) suggèrent que le niveau de l'anxiété d'état à un moment donné est dépendant de la coïncidence du type d'anxiété de trait mise en jeu et de la situation stressante. Étant donné que le labyrinthe en zéro surélevé est basé sur l'exposition à un nouvel environnement, le polymorphisme Leu32Met du BDNF peut être impliqué dans un aspect néophobique de l'anxiété, ce qui conforte les résultats obtenus dans le test d'exploration libre.

Finalement, en plus de montrer un lien entre un nouveau polymorphisme murin du BDNF et le phénotype anxiété-like des différentes lignées, nos travaux ont permis de préciser le rôle du BDNF dans les comportements anxiété-like, particulièrement dans les réactions néophobiques et l'anxiété de trait.

3. BDNF et dépression

L'hypothèse neurotrophique de la dépression n'avait jusqu'à présent jamais été testée dans le modèle de l'UCMS. Les études précédentes ont surtout utilisé des bio-essais (test de la nage forcée et test de la queue suspendue) qui ont de plus faibles validités de face et de construction (Chan *et coll.*, 2006, Chourbaji *et coll.*, 2004, Macqueen *et coll.*, 2001, Monteggia *et coll.*, 2004, Monteggia *et coll.*, 2007, Saarelainen *et coll.*, 2003). Il y a bien quelques études qui ont utilisé un modèle chronique comme le modèle de stress psychosocial, mais l'effet de ce paradigme était mesuré seulement par une variable, l'évitement d'un congénère (Berton *et coll.*, 2006, Tsankova *et coll.*, 2006). Or, un autre atout de l'UCMS est qu'il induit un syndrome comprenant plusieurs types d'altérations indépendants les uns des autres mais analogues à différents symptômes de la dépression. Sachant que sous l'appellation

générale « dépression » se retrouvent souvent des troubles assez disparates, la précédente caractéristique de l'UCMS représente également un avantage en ce que ce modèle permet de différencier un effet spécifique d'un rôle global. En outre, étant donné qu'il est basé sur une exposition chronique des souris à des stresseurs socio-environnementaux, l'UCMS est aussi un modèle approprié pour étudier la vulnérabilité au stress, qui est un facteur étiologique majeur de la dépression. Enfin, sur le plan de la validation pharmacologique, il reproduit le délai nécessaire de quelques semaines (en général 3-4) avant l'apparition des premiers effets bénéfiques des antidépresseurs.

Le modèle UCMS a été l'assise sur laquelle ont été élaborées nos deux dernières études qui avaient pour but de tester l'implication du BDNF dans la vulnérabilité au stress, la pathophysiologie et le traitement de la dépression. Nous avons donc confronté nos deux approches, l'étude du polymorphisme Leu32Met et l'ablation partielle de l'expression du BDNF, à ce modèle. Nos résultats montrent qu'aucune de nos deux approches n'apporte d'éléments en faveur d'une implication du BDNF dans la vulnérabilité au stress ou l'apparition de certains types d'altérations ; par contre une diminution du BDNF réduit l'efficacité des antidépresseurs à contrecarrer certains effets de l'UCMS.

3.1. BDNF et état dépression-like

Afin d'examiner la relation entre le BDNF et l'apparition d'un état dépression-like, nous avons tout d'abord comparé, par rapport au polymorphisme Leu32Met du BDNF, les résultats de 7 lignées de souris consanguines exposées à l'UCMS sur une série de variables physiques (état du pelage, poids), comportementales (NSF, activité locomotrice) et neuroendocrines (concentration des métabolites de la corticostérone dans les fèces). En dépit de différences remarquables entre les 7 lignées dans le pattern d'altérations induites par l'UCMS (cf article 2), aucun lien n'a été trouvé entre l'allèle porté par les souris et leur phénotype. On retrouve en effet à la fois des lignées portant l'allèle Leu et des lignées portant l'allèle Met parmi celles qui ont été les plus sensibles à l'UCMS comme la BALB/c (allèle Met) et la C57BL/6 (allèle Leu) et parmi celles qui l'ont été le moins comme la C3H (allèle Leu) et la CBA (allèle Met). De plus, il aurait aussi été possible que ce polymorphisme affecte seulement un type d'altérations particulières, néanmoins aucune association de ce type n'est mise en évidence dans nos résultats.

Ensuite, nous avons examiné si la perte d'un allèle du BDNF, et par conséquent de la moitié de l'expression du BDNF, pouvait conduire à une vulnérabilité accrue dans l'UCMS. Nous

avons donc regardé si, après une exposition à l'UCMS, les souris hétérozygotes BDNF $^{+/-}$ présentaient des altérations plus importantes que les souris sauvages BDNF $^{+/+}$ sur plusieurs variables physiques (état du pelage, poids), comportementales (prise alimentaire, activité locomotrice, R/I test, NSF test, TST) et physiologique (concentration plasmatique de la corticostérone, expression du BDNF dans l'hippocampe). Finalement, aucune différence significative due au génotype n'a été trouvé chez ces souris pour toutes les variables mesurées sauf en ce qui concerne le niveau d'ARNm du BDNF qui était, sans surprise, moitié moindre chez les mutants hétérozygotes.

En résumé, nos deux stratégies ont été incapables de mettre en évidence une implication du BDNF dans la vulnérabilité au stress chronique et dans la mise en place d'altérations particulière. Ces travaux confortent les précédentes études qui ont montré qu'une perte d'expression du BDNF n'induit pas l'apparition de phénotypes dépression-like dans des bio-essais, comme les tests de la nage forcée et de la queue suspendue, en utilisant différentes stratégies : souris hétérozygote BDNF $^{+/-}$ (Chourbaji *et coll.*, 2004, Macqueen *et coll.*, 2001, Saarelainen *et coll.*, 2003), souris knock-out inductibles dans les régions antérieures du cerveau (Monteggia *et coll.*, 2004) et souris knock-down (par un vecteur viral) dans le gyrus denté et dans la corne d'Ammon (Adachi *et coll.*, 2008). Seule une stratégie basée sur des souris knock-out conditionnelles dans les régions antérieures du cerveau a montré l'apparition de phénotypes dépression-like mais les effets sont faibles et surtout contradictoires : dans le TST mais pas dans le FST dans une étude (Chan *et coll.*, 2006), dans le FST mais seulement chez les femelles pour une autre (Monteggia *et coll.*, 2007). Finalement, nos résultats apportent l'information supplémentaire que, en plus de ne pas entraîner directement l'apparition de phénotypes dépression-like, une diminution de BDNF ne favorise pas non plus leur induction par le stress.

Tous ces résultats montrent qu'il est temps de reconsidérer l'hypothèse neurotrophique de la dépression (Groves, 2007). En effet, les premières études corrélationnelles ont montré une diminution du niveau de BDNF en préclinique chez les animaux stressés (Altar *et coll.*, 2003, Barrientos *et coll.*, 2003, Nibuya *et coll.*, 1995, Pizarro *et coll.*, 2004, Roceri *et coll.*, 2004, Smith *et coll.*, 1995, Tsankova *et coll.*, 2006, Ueyama *et coll.*, 1997, Vaidya *et coll.*, 1997) et en clinique chez les patients dépressifs (Aydemir *et coll.*, 2005, Chen *et coll.*, 2001, Dwivedi *et coll.*, 2003, Karege *et coll.*, 2002, Karege *et coll.*, 2005, Marvanova *et coll.*, 2001). Néanmoins, la grande majorité de ces travaux ont jusqu'à présent démontré l'absence de lien causal entre une diminution du niveau de BDNF et l'apparition des états dépression-like, ce qui incite à considérer la réduction du BDNF dans la dépression comme un épiphénomène ou,

au mieux, comme une conséquence des processus pathologiques mis en jeu par le stress chronique et la dépression.

3.2. BDNF et antidépresseurs

L'implication du BDNF dans l'action des antidépresseurs a également été évaluée dans nos deux études avec l'UCMS. Lors de la comparaison des lignées consanguines, certaines lignées ont été sensibles à l'action « thérapeutique » du traitement à l'imipramine (BALB/c, C57BL/6 et partiellement A/J et DBA/2) tandis que d'autres ont été résistantes (FVB voire C3H et CBA bien que ces deux dernières sont aussi moins sensibles à l'UCMS). Néanmoins, aucune relation entre le polymorphisme Leu32Met et la réponse antidépressive n'a été observée. Par contre, la diminution du BDNF induit par l'expression monoallélique du BDNF a perturbé l'action de l'imipramine dans certains tests comportementaux. En effet, alors que le traitement à l'imipramine a permis de diminuer chez les souris sauvages BDNF $^{+/+}$ le nombre d'attaque dans le R/I test, la durée d'immobilité dans le TST et la latence pour manger dans le NSF test, le même traitement a été incapable d'induire les même effets chez les souris hétérozygotes BDNF $^{+/-}$ dans le R/I test et le TST. Étant donné qu'une ablation partielle de l'expression du BDNF induit une altération de l'efficacité des antidépresseurs à réduire la résignation dans le TST et l'agressivité dans le R/I test, ces résultats suggèrent que le BDNF contribue à l'effet antidépresseur-like de l'imipramine. Nos résultats sont en accord avec les précédentes études réalisées avec des souris hétérozygotes BDNF $^{+/-}$ et des souris knockout (inductibles et conditionnelles dans les régions cérébrales antérieures) indiquant que le BDNF est nécessaire aux effets comportementaux des antidépresseurs dans des modèles de résignation (Monteggia *et coll.*, 2004, Saarelainen *et coll.*, 2003). Par rapport à ces travaux, nous fournissons des évidences supplémentaires et étendues à des souris « dépressives » et aux comportements d'agression. D'autres travaux de recherche ont également conforté nos résultats en montrant que les antidépresseurs augmentaient le niveau de BDNF dans l'hippocampe et le cortex préfrontal des rongeurs (Duman et Monteggia, 2006) mais aussi chez l'homme à la suite d'études *post mortem* ou de dosage plasmatique (Aydemir *et coll.*, 2005, Karege *et coll.*, 2002, Karege *et coll.*, 2005, Lauterborn *et coll.*, 2003, Shimizu *et coll.*, 2003). Le BDNF lui-même, quand il est injecté directement dans les ventricules ou dans l'hippocampe, est capable de produire un effet antidépresseur-like (Roceri *et coll.*, 2004, Siuciak *et coll.*, 1997). Ainsi, l'augmentation du niveau de BDNF dans les réseaux corticolimbiques (notamment hippocampe-amygdale-cortex préfrontal) impliqués dans la

régulation de l'humeur (Seminowicz *et coll.*, 2004) pourrait être un prérequis à la réponse antidépressive.

Au final, toutes ces publications ainsi que nos propres résultats indiquent que le BDNF est une cible principale des antidépresseurs et qu'il pourrait même être le levier par lequel ils induisent leurs effets thérapeutiques. Mais par quels mécanismes le BDNF permettrait-il l'action des antidépresseurs ? Les actions neurobiologiques du BDNF sont liées à l'activation, via son récepteur TrkB, de différents processus de transduction du signal. La signalisation du BDNF/TrkB est associée aux voies des MAP kinases et de la phospholipase Cγ qui peuvent activer des processus favorisant la plasticité et la survie de la cellule (Castren *et coll.*, 2007). Ainsi, les antidépresseurs en élevant le niveau de BDNF stimuleraient l'activation de ces voies et *in fine* la plasticité cellulaire et neurale (LTP, synaptogenèse, neurogenèse, survie neuronale). Il a donc été proposé que l'activation de la voie BDNF/TrkB, en contribuant à renforcer les processus de plasticité, permette la restauration de réseaux corticolimbiques préalablement endommagés par la pathophysiologie dépressive, laquelle pourrait par contre être indépendante de la diminution du BDNF (Castren *et coll.*, 1998).

Toutefois, il semblerait que l'association positive entre l'élévation du BDNF et l'action antidépressive dépende du circuit cérébral impliqué. En effet, un pattern inverse à ce qui est rapporté dans les réseaux portant sur l'implication du BDNF dans les aires corticolimbiques est décrit dans la voie mésolimbique dopaminergique (aire tegmentale ventrale – noyau accumbens), reconnue pour être impliquée dans les phénomènes de récompense, de plaisir et d'addiction (Nestler et Carlezon, 2006). L'injection de BDNF dans cette voie induit un effet dépression-like (Eisch *et coll.*, 2003) tandis que la délétion locale du BDNF grâce à un vecteur viral produit un effet antidepresseur-like (Berton *et coll.*, 2006). Ces études démontrent donc que le BDNF est un facteur clé, nécessaire aux modifications structurelles de réseaux neuronaux, et que ce sont ensuite les fonctions de ces réseaux qui déterminent si la stimulation de la plasticité favorise ou enraye la réponse antidépressive.

D'autre part, la dépression et l'anxiété sont souvent comorbides et les traitements aux antidépresseurs forment la majorité des pharmacothérapies pour les deux troubles (Zohar et Westenberg, 2000). Toutefois, ils engagent des étiologies différentes bien que basées en partie sur des mécanismes en partie chevauchants. Il est donc important de caractériser quels sont les mécanismes neurobiologiques que ces deux troubles partagent et lesquels sont spécifiques à l'un des deux. Particulièrement, il est intéressant d'examiner le rôle précis du BDNF dans la dépression et l'anxiété. Nous avons vu précédemment à travers nos résultats que l'implication du BDNF dans l'émergence de comportements anxiété-like et dépression-like est très

différente. Il est donc envisageable que le rôle du BDNF lors d'un traitement varie selon qu'il s'agit d'effets antidépresseurs ou d'effets anxiolytiques. Étant donné les liens étroits entre dépression et anxiété, il est difficile de dissocier clairement dans des paradigmes comportementaux la réponse dépression-like de la réponse anxiété-like. Néanmoins, nous avons entrepris de tester les souris dans le NSF test afin de tenter d'extraire la composante anxiolytique des effets du traitement. Le NSF test est un test d'anxiété sensible à une injection unique de molécules anxiolytiques (Poschel, 1971, Soubrie *et coll.*, 1975) mais aussi capable de révéler les effets anxiolytiques d'un traitement chronique, et non aigu, aux antidépresseurs (Santarelli *et coll.*, 2003). Nos résultats ont montré que, à la suite de l'UCMS, le traitement à l'imipramine était parvenu à agir dans le NSF test chez les souris sauvages mais aussi chez les hétérozygotes au contraire de ce qui est observé lors du R/I test et du TST. Ainsi, l'ablation partielle de l'expression du BDNF altère l'effet antidépresseur-like mais n'empêche pas l'effet anxiolytique-like de l'imipramine. Ce résultat est à mettre en parallèle avec la capacité du système BDNF/TrkB (discutée précédemment) à réagir efficacement lors de situations anxiogéniques même lorsque le niveau de BDNF est réduit de 50%. Dans ce cas, l'efficacité de l'action anxiolytique d'un traitement antidépresseur nécessiterait le renforcement de la réactivité immédiate du système BDNF/TrkB sans recourir à une élévation de l'expression du BDNF. Un certain niveau de base comme dans le cas des souris hétérozygotes BDNF $^{+/-}$ serait alors suffisant pour permettre cette action.

Conclusion

Au cours de ce travail, nous avons pu démontrer un rôle du BDNF dans les comportements de type anxieux et dans l'action des antidépresseurs. Par contre, il semblerait que la mise en place d'un état dépression-like se fasse indépendamment de changements dans le système BDNF/TrkB. Notre double approche s'est révélée utile et complémentaire. En effet, c'est à travers l'étude du polymorphisme Leu32Met que nous avons pu mettre en évidence une implication du BDNF dans les phénotypes anxiété-like alors que c'est grâce à l'ablation partielle de l'expression du BDNF que nous avons démontré le lien entre le niveau de BDNF et les effets antidépresseurs-like. Ce travail de thèse suggère, à la lecture de ces dernières observations, une différence de nature entre l'implication du BDNF dans les comportements anxiété-like et dans les comportements antidépresseurs-like. Les premiers pourraient dépendre de la réactivité rapide du système BDNF/TrkB sans forcément solliciter des changements importants du niveau de BDNF alors que les seconds seraient beaucoup plus tributaires d'une élévation du niveau d'expression du BDNF. Ainsi, les augmentations de BDNF pourraient stimuler lors d'un état dépressif-like les processus de plasticité et permettre la restauration fonctionnelle de réseaux corticolimbiques impliqués dans la régulation de l'humeur. Ces réseaux avaient pu être préalablement endommagés par la pathophysiologie dépressive ou dans le cas de nos modèles animaux par les effets de l'UCMS. Nos résultats indiquent par contre que la cause de ces altérations n'est probablement pas liée à des changements dans le système BDNF/TrkB.

Finalement, l'hypothèse principale qui ressort de ces données propose que l'état d'intégrité plastique des réseaux neuronaux dans l'hippocampe n'est pas le substrat majeur des troubles de l'humeur, mais que son rétablissement induit l'effet antidépresseur. Les facteurs neurotrophiques eux-mêmes ne contrôlent donc pas l'humeur, mais agissent comme des outils nécessaires à l'action des antidépresseurs.

Des travaux futurs devront préciser quel est l'impact réel du polymorphisme Leu32Met du BDNF au niveau moléculaire (repliement de la protéine, clivage de la proforme, affinité de liaison de la proforme au p75 et TrkB) et cellulaire (adressage, transport, sécrétion) par des études d'expression *in vitro*. Afin d'éliminer les biais possibles dus à l'arrière fond génétique de chaque lignée consanguine, il sera utile de développer une ou plusieurs lignées congéniques dans lesquelles le polymorphisme Leu32Met sera exprimé dans des individus isogéniques ou, alternativement, au moins utiliser une génération F2 entre quatre lignées parentales distinctes pour ce polymorphisme. Notre hypothèse sur la dissociation des

processus impliquant le BDNF dans l'état anxiété-like et dans la réponse antidépressive pourra être testé en examinant les effets d'une inhibition de la réactivité du système BDNF/TrkB lors de situations anxiogéniques en antagonisant d'une manière aigüe le récepteur TrkB, soit par des molécules antagonistes soit par des anticorps dirigés contre le récepteur, à la suite ou non d'un traitement chronique antidépresseur.

BIBLIOGRAPHIE

Abelson, J.L., Glitz, D., Cameron, O.G., Lee, M.A., Bronzo, M. & Curtis, G.C. (1992) Endocrine, cardiovascular, and behavioral responses to clonidine in patients with panic disorder. *Biol Psychiatry,* **32,** 18-25.

Acheson, A., Barker, P.A., Alderson, R.F., Miller, F.D. & Murphy, R.A. (1991) Detection of brain-derived neurotrophic factor-like activity in fibroblasts and Schwann cells: inhibition by antibodies to NGF. *Neuron,* **7,** 265-275.

Adachi, M., Barrot, M., Autry, A.E., Theobald, D. & Monteggia, L.M. (2008) Selective loss of brain-derived neurotrophic factor in the dentate gyrus attenuates antidepressant efficacy. *Biol Psychiatry,* **63,** 642-649.

Akaneya, Y., Tsumoto, T. & Hatanaka, H. (1996) Brain-derived neurotrophic factor blocks long-term depression in rat visual cortex. *J Neurophysiol,* **76,** 4198-4201.

Alonso, M., Vianna, M.R., Depino, A.M., Mello e Souza, T., Pereira, P., Szapiro, G., Viola, H., Pitossi, F., Izquierdo, I. & Medina, J.H. (2002) BDNF-triggered events in the rat hippocampus are required for both short- and long-term memory formation. *Hippocampus,* **12,** 551-560.

Altar, C.A. & DiStefano, P.S. (1998) Neurotrophin trafficking by anterograde transport. *Trends Neurosci,* **21,** 433-437.

Altar, C.A., Whitehead, R.E., Chen, R., Wortwein, G. & Madsen, T.M. (2003) Effects of electroconvulsive seizures and antidepressant drugs on brain-derived neurotrophic factor protein in rat brain. *Biol Psychiatry,* **54,** 703-709.

American Psychiatric Association (2000) *Diagnostic and Statistical Manual of Mental Disorders,* APA, Washington DC.

Andrews, J.M., Ninan, P.T. & Nemeroff, C.B. (1996) Venlafaxine: a novel antidepressant that has a dual mechanism of action. *Depression,* **4,** 48-56.

Angst, J., Merikangas, K.R. & Preisig, M. (1997) Subthreshold syndromes of depression and anxiety in the community. *J Clin Psychiatry,* **58 Suppl 8,** 6-10.

Artigas, F., Nutt, D.J. & Shelton, R. (2002) Mechanism of action of antidepressants. *Psychopharmacol Bull,* **36 Suppl 2,** 123-132.

Association des psychiatres du Canada (2006) Guide de pratique clinique; traitement des troubles anxieux. *Can J Psychiatry.* pp. 1S-96S.

Aydemir, O., Deveci, A. & Taneli, F. (2005) The effect of chronic antidepressant treatment on serum brain-derived neurotrophic factor levels in depressed patients: a preliminary study. *Prog Neuropsychopharmacol Biol Psychiatry,* **29,** 261-265.

Bamji, S.X., Majdan, M., Pozniak, C.D., Belliveau, D.J., Aloyz, R., Kohn, J., Causing, C.G. & Miller, F.D. (1998) The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. *J Cell Biol,* **140,** 911-923.

Ban, T.A. (2001) Pharmacotherapy of mental illness--a historical analysis. *Prog Neuropsychopharmacol Biol Psychiatry,* **25,** 709-727.

Barde, Y.A., Edgar, D. & Thoenen, H. (1982) Purification of a new neurotrophic factor from mammalian brain. *Embo J,* **1,** 549-553.

Barrett, G.L. (2000) The p75 neurotrophin receptor and neuronal apoptosis. *Prog Neurobiol,* **61,** 205-229.

Barrientos, R.M., Sprunger, D.B., Campeau, S., Higgins, E.A., Watkins, L.R., Rudy, J.W. & Maier, S.F. (2003) Brain-derived neurotrophic factor mRNA downregulation produced by social isolation is blocked by intrahippocampal interleukin-1 receptor antagonist. *Neuroscience,* **121,** 847-853.

Bartoletti, A., Cancedda, L., Reid, S.W., Tessarollo, L., Porciatti, V., Pizzorusso, T. & Maffei, L. (2002) Heterozygous knock-out mice for brain-derived neurotrophic factor show a pathway-specific impairment of long-term potentiation but normal critical period for monocular deprivation. *J Neurosci,* **22,** 10072-10077.

Belzung, C., El Hage, W., Moindrot, N. & Griebel, G. (2001) Behavioral and neurochemical changes following predatory stress in mice. *Neuropharmacology,* **41,** 400-408.

Belzung, C. & Griebel, G. (2001) Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res,* **125,** 141-149.

Belzung, C., Misslin, R., Vogel, E., Dodd, R.H. & Chapouthier, G. (1987) Anxiogenic effects of methyl-beta-carboline-3-carboxylate in a light/dark choice situation. *Pharmacol Biochem Behav,* **28,** 29-33.

Berrios, G.E. & Olivares, J.M. (1995) The anhedonias: a conceptual history. *Hist Psychiatry,* **6,** 453-470.

Berton, O., McClung, C.A., Dileone, R.J., Krishnan, V., Renthal, W., Russo, S.J., Graham, D., Tsankova, N.M., Bolanos, C.A., Rios, M., Monteggia, L.M., Self, D.W. & Nestler, E.J. (2006) Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science,* **311,** 864-868.

Bishop, J.F., Mueller, G.P. & Mouradian, M.M. (1994) Alternate 5' exons in the rat brain-derived neurotrophic factor gene: differential patterns of expression across brain regions. *Brain Res Mol Brain Res,* **26,** 225-232.

Blanchard, D.C. (1984) Applicability of animal models to human aggression. *Prog Clin Biol Res,* **169,** 49-74.

Blanchard, D.C. & Blanchard, R.J. (1990) Behavioral correlates of chronic dominance-subordination relationships of male rats in a seminatural situation. *Neurosci Biobehav Rev,* **14,** 455-462.

Blanchard, R.J., Griebel, G., Henrie, J.A. & Blanchard, D.C. (1997) Differentiation of anxiolytic and panicolytic drugs by effects on rat and mouse defense test batteries. *Neurosci Biobehav Rev,* **21,** 783-789.

Bolanos, C.A. & Nestler, E.J. (2004) Neurotrophic mechanisms in drug addiction. *Neuromolecular Med,* **5,** 69-83.

Brambilla, F., Perna, G., Garberi, A., Nobile, P. & Bellodi, L. (1995) Alpha 2-adrenergic receptor sensitivity in panic disorder: I. GH response to GHRH and clonidine stimulation in panic disorder. *Psychoneuroendocrinology,* **20,** 1-9.

Bramham, C.R. & Messaoudi, E. (2005) BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. *Prog Neurobiol,* **76,** 99-125.

Brandes, M. & Bienvenu, O.J. (2006) Personality and anxiety disorders. *Curr Psychiatry Rep,* **8,** 263-269.

Bremner, J.D., Elzinga, B., Schmahl, C. & Vermetten, E. (2008) Structural and functional plasticity of the human brain in posttraumatic stress disorder. *Prog Brain Res,* **167,** 171-186.

Bremner, J.D., Innis, R.B., Southwick, S.M., Staib, L., Zoghbi, S. & Charney, D.S. (2000) Decreased benzodiazepine receptor binding in prefrontal cortex in combat-related posttraumatic stress disorder. *Am J Psychiatry,* **157,** 1120-1126.

Brown, E.S., Rush, A.J. & McEwen, B.S. (1999) Hippocampal remodeling and damage by corticosteroids: implications for mood disorders. *Neuropsychopharmacology,* **21,** 474-484.

Carney, R.M., Freedland, K.E., Miller, G.E. & Jaffe, A.S. (2002) Depression as a risk factor for cardiac mortality and morbidity: a review of potential mechanisms. *J Psychosom Res,* **53,** 897-902.

Carrasco, J.L. & Sandner, C. (2005) Clinical effects of pharmacological variations in selective serotonin reuptake inhibitors: an overview. *Int J Clin Pract,* **59,** 1428-1434.

Castren, E. (2004a) Neurotrophic effects of antidepressant drugs. *Curr Opin Pharmacol,* **4,** 58-64.

Castren, E. (2004b) Neurotrophins as mediators of drug effects on mood, addiction, and neuroprotection. *Mol Neurobiol,* **29,** 289-302.

Castren, E., Berninger, B., Leingartner, A. & Lindholm, D. (1998) Regulation of brain-derived neurotrophic factor mRNA levels in hippocampus by neuronal activity. *Prog Brain Res,* **117,** 57-64.

Castren, E., Voikar, V. & Rantamaki, T. (2007) Role of neurotrophic factors in depression. *Curr Opin Pharmacol,* **7,** 18-21.

Chan, J.P., Unger, T.J., Byrnes, J. & Rios, M. (2006) Examination of behavioral deficits triggered by targeting Bdnf in fetal or postnatal brains of mice. *Neuroscience,* **142,** 49-58.

Chao, M.V. (2003) Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nat Rev Neurosci,* **4,** 299-309.

Chao, M.V. & Bothwell, M. (2002) Neurotrophins: to cleave or not to cleave. *Neuron,* **33,** 9-12.

Chaperon, F. & Thiebot, M.H. (1999) Behavioral effects of cannabinoid agents in animals. *Crit Rev Neurobiol,* **13,** 243-281.

Charney, D.S., Woods, S.W., Krystal, J.H., Nagy, L.M. & Heninger, G.R. (1992) Noradrenergic neuronal dysregulation in panic disorder: the effects of intravenous yohimbine and clonidine in panic disorder patients. *Acta Psychiatr Scand,* **86,** 273-282.

Charrier, D., Dangoumau, L., Puech, A.J., Hamon, M. & Thiebot, M.H. (1995) Failure of CCK receptor ligands to modify anxiety-related behavioural suppression in an operant conflict paradigm in rats. *Psychopharmacology (Berl),* **121,** 127-134.

Chen, B., Dowlatshahi, D., MacQueen, G.M., Wang, J.F. & Young, L.T. (2001) Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol Psychiatry,* **50,** 260-265.

Chen, Z.Y., Jing, D., Bath, K.G., Ieraci, A., Khan, T., Siao, C.J., Herrera, D.G., Toth, M., Yang, C., McEwen, B.S., Hempstead, B.L. & Lee, F.S. (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science,* **314,** 140-143.

Chiu, S., Singh, A.N., Chiu, P. & Mishra, R.K. (2001) Inverse agonist binding of peripheral benzodiazepine receptors in anxiety disorder. *Eur Arch Psychiatry Clin Neurosci,* **251,** 136-140.

Chourbaji, S., Hellweg, R., Brandis, D., Zorner, B., Zacher, C., Lang, U.E., Henn, F.A., Hortnagl, H. & Gass, P. (2004) Mice with reduced brain-derived neurotrophic factor

expression show decreased choline acetyltransferase activity, but regular brain monoamine levels and unaltered emotional behavior. *Brain Res Mol Brain Res,* **121,** 28-36.

Cigrang, M., Vogel, E. & Misslin, R. (1986) Reduction of neophobia in mice following lesions of the caudate-putamen. *Physiol Behav,* **36,** 25-28.

Conner, A.C., Kissling, C., Hodges, E., Hunnerkopf, R., Clement, R.M., Dudley, E., Freitag, C.M., Rosler, M., Retz, W. & Thome, J. (2008) Neurotrophic factor-related gene polymorphisms and adult attention deficit hyperactivity disorder (ADHD) score in a high-risk male population. *Am J Med Genet B Neuropsychiatr Genet.*

Connor, B. & Dragunow, M. (1998) The role of neuronal growth factors in neurodegenerative disorders of the human brain. *Brain Res Brain Res Rev,* **27,** 1-39.

Cotter, D., Mackay, D., Chana, G., Beasley, C., Landau, S. & Everall, I.P. (2002) Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder. *Cereb Cortex,* **12,** 386-394.

Crawley, J. & Goodwin, F.K. (1980) Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol Biochem Behav,* **13,** 167-170.

Cryan, J.F., Markou, A. & Lucki, I. (2002) Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci,* **23,** 238-245.

Cryan, J.F. & Mombereau, C. (2004) In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. *Mol Psychiatry,* **9,** 326-357.

D'Sa, C. & Duman, R.S. (2002) Antidepressants and neuroplasticity. *Bipolar Disord,* **4,** 183-194.

De Bellis, M.D., Casey, B.J., Dahl, R.E., Birmaher, B., Williamson, D.E., Thomas, K.M., Axelson, D.A., Frustaci, K., Boring, A.M., Hall, J. & Ryan, N.D. (2000) A pilot study of amygdala volumes in pediatric generalized anxiety disorder. *Biol Psychiatry,* **48,** 51-57.

Dechant, G. & Barde, Y.A. (2002) The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system. *Nat Neurosci,* **5,** 1131-1136.

Delagrange, P. & Boutin, J.A. (2006) Therapeutic potential of melatonin ligands. *Chronobiol Int,* **23,** 413-418.

Deltheil, T., Guiard, B.P., Guilloux, J.P., Nicolas, L., Delomenie, C., Reperant, C., Maitre, E.L., Leroux-Nicollet, I., Benmansour, S., Coudore, F., David, D.J. & Gardier, A.M. (2007) Consequences of changes in BDNF levels on serotonin neurotransmission, 5-

HT transporter expression and function: Studies in adult mice hippocampus. *Pharmacol Biochem Behav*.

Dluzen, D.E., Story, G.M., Xu, K., Kucera, J. & Walro, J.M. (1999) Alterations in nigrostriatal dopaminergic function within BDNF mutant mice. *Exp Neurol,* **160,** 500-507.

Dougherty, D.D., Rauch, S.L., Deckersbach, T., Marci, C., Loh, R., Shin, L.M., Alpert, N.M., Fischman, A.J. & Fava, M. (2004) Ventromedial prefrontal cortex and amygdala dysfunction during an anger induction positron emission tomography study in patients with major depressive disorder with anger attacks. *Arch Gen Psychiatry,* **61,** 795-804.

Drevets, W.C. (1999) Prefrontal cortical-amygdalar metabolism in major depression. *Ann N Y Acad Sci,* **877,** 614-637.

Drevets, W.C., Price, J.L., Simpson, J.R., Jr., Todd, R.D., Reich, T., Vannier, M. & Raichle, M.E. (1997) Subgenual prefrontal cortex abnormalities in mood disorders. *Nature,* **386,** 824-827.

Driessen, M., Herrmann, J., Stahl, K., Zwaan, M., Meier, S., Hill, A., Osterheider, M. & Petersen, D. (2000) Magnetic resonance imaging volumes of the hippocampus and the amygdala in women with borderline personality disorder and early traumatization. *Arch Gen Psychiatry,* **57,** 1115-1122.

Duan, W., Guo, Z., Jiang, H., Ware, M., Li, X.J. & Mattson, M.P. (2003) Dietary restriction normalizes glucose metabolism and BDNF levels, slows disease progression, and increases survival in huntingtin mutant mice. *Proc Natl Acad Sci U S A,* **100,** 2911-2916.

Ducottet, C., Aubert, A. & Belzung, C. (2004) Susceptibility to subchronic unpredictable stress is related to individual reactivity to threat stimuli in mice. *Behav Brain Res,* **155,** 291-299.

Ducottet, C. & Belzung, C. (2005) Correlations between behaviours in the elevated plus-maze and sensitivity to unpredictable subchronic mild stress: evidence from inbred strains of mice. *Behav Brain Res,* **156,** 153-162.

Duman, R.S. & Monteggia, L.M. (2006) A neurotrophic model for stress-related mood disorders. *Biol Psychiatry,* **59,** 1116-1127.

Dwivedi, Y., Rizavi, H.S., Conley, R.R., Roberts, R.C., Tamminga, C.A. & Pandey, G.N. (2003) Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. *Arch Gen Psychiatry,* **60,** 804-815.

Ebner, K. & Singewald, N. (2006) The role of substance P in stress and anxiety responses. *Amino Acids,* **31,** 251-272.

Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B. & Weinberger, D.R. (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell,* **112,** 257-269.

Eisch, A.J., Bolanos, C.A., de Wit, J., Simonak, R.D., Pudiak, C.M., Barrot, M., Verhaagen, J. & Nestler, E.J. (2003) Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression. *Biol Psychiatry,* **54,** 994-1005.

Endler, N.S. & Kocovski, N.L. (2001) State and trait anxiety revisited. *J Anxiety Disord,* **15,** 231-245.

Enns, M.W. & Cox, B.J. (1997) Personality dimensions and depression: review and commentary. *Can J Psychiatry,* **42,** 274-284.

Enoch, M.A., White, K.V., Waheed, J. & Goldman, D. (2007) Neurophysiological and genetic distinctions between pure and comorbid anxiety disorders. *Depress Anxiety*.

Ernfors, P., Ibanez, C.F., Ebendal, T., Olson, L. & Persson, H. (1990a) Molecular cloning and neurotrophic activities of a protein with structural similarities to nerve growth factor: developmental and topographical expression in the brain. *Proc Natl Acad Sci U S A,* **87,** 5454-5458.

Ernfors, P., Lee, K.F. & Jaenisch, R. (1994) Mice lacking brain-derived neurotrophic factor develop with sensory deficits. *Nature,* **368,** 147-150.

Ernfors, P., Wetmore, C., Olson, L. & Persson, H. (1990b) Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. *Neuron,* **5,** 511-526.

Farhadi, H.F., Mowla, S.J., Petrecca, K., Morris, S.J., Seidah, N.G. & Murphy, R.A. (2000) Neurotrophin-3 sorts to the constitutive secretory pathway of hippocampal neurons and is diverted to the regulated secretory pathway by coexpression with brain-derived neurotrophic factor. *J Neurosci,* **20,** 4059-4068.

Fava, M., Rankin, M.A., Wright, E.C., Alpert, J.E., Nierenberg, A.A., Pava, J. & Rosenbaum, J.F. (2000) Anxiety disorders in major depression. *Compr Psychiatry,* **41,** 97-102.

File, S.E. (1985) Animal models for predicting clinical efficacy of anxiolytic drugs: social behaviour. *Neuropsychobiology,* **13,** 55-62.

Forbes, N.F., Stewart, C.A., Matthews, K. & Reid, I.C. (1996) Chronic mild stress and sucrose consumption: validity as a model of depression. *Physiol Behav,* **60,** 1481-1484.

Fuchs, E., Czeh, B., Kole, M.H., Michaelis, T. & Lucassen, P.J. (2004) Alterations of neuroplasticity in depression: the hippocampus and beyond. *Eur Neuropsychopharmacol,* **14 Suppl 5,** S481-490.

Fuchs, E. & Fliugge, G. (2006) Experimental animal models for the simulation of depression and anxiety. *Dialogues Clin Neurosci,* **8,** 323-333.

Fuchs, E. & Flugge, G. (2002) Social stress in tree shrews: effects on physiology, brain function, and behavior of subordinate individuals. *Pharmacol Biochem Behav,* **73,** 247-258.

Gammans, R.E., Stringfellow, J.C., Hvizdos, A.J., Seidehamel, R.J., Cohn, J.B., Wilcox, C.S., Fabre, L.F., Pecknold, J.C., Smith, W.T. & Rickels, K. (1992) Use of buspirone in patients with generalized anxiety disorder and coexisting depressive symptoms. A meta-analysis of eight randomized, controlled studies. *Neuropsychobiology,* **25,** 193-201.

Gilbertson, M.W., Shenton, M.E., Ciszewski, A., Kasai, K., Lasko, N.B., Orr, S.P. & Pitman, R.K. (2002) Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nat Neurosci,* **5,** 1242-1247.

Goodman, W.K. (2004) Selecting pharmacotherapy for generalized anxiety disorder. *J Clin Psychiatry,* **65 Suppl 13,** 8-13.

Gratacos, M., Gonzalez, J.R., Mercader, J.M., de Cid, R., Urretavizcaya, M. & Estivill, X. (2007) Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. *Biol Psychiatry,* **61,** 911-922.

Griebel, G., Belzung, C., Misslin, R. & Vogel, E. (1993) The free-exploratory paradigm: an effective method for measuring neophobic behaviour in mice and testing potential neophobia-reducing drugs. *Behav Pharmacol,* **4,** 637-644.

Griebel, G., Blanchard, D.C., Jung, A. & Blanchard, R.J. (1995) A model of 'antipredator' defense in Swiss-Webster mice: effects of benzodiazepine receptor ligands with different intrinsic activities. *Behav Pharmacol,* **6,** 732-745.

Griebel, G., Simiand, J., Serradeil-Le Gal, C., Wagnon, J., Pascal, M., Scatton, B., Maffrand, J.P. & Soubrie, P. (2002a) Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V1b receptor antagonist, SSR149415, suggest an innovative

approach for the treatment of stress-related disorders. *Proc Natl Acad Sci U S A,* **99,** 6370-6375.

Griebel, G., Simiand, J., Steinberg, R., Jung, M., Gully, D., Roger, P., Geslin, M., Scatton, B., Maffrand, J.P. & Soubrie, P. (2002b) 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4- methylphenyl)ethyl]5-methyl-N-(2-propynyl)-1, 3-thiazol-2-amine hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing factor(1) receptor antagonist. II. Characterization in rodent models of stress-related disorders. *J Pharmacol Exp Ther,* **301,** 333-345.

Grimm, J.W., Lu, L., Hayashi, T., Hope, B.T., Su, T.P. & Shaham, Y. (2003) Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. *J Neurosci,* **23,** 742-747.

Grimsley, S. (2005) Anxiety Disorders. In Koda-Kimble MA., Young, L. & Kradjan, W. (eds), *Applied therapeutics.* Philadelphie, pp. 30-32.

Groves, J.O. (2007) Is it time to reassess the BDNF hypothesis of depression? *Mol Psychiatry,* **12,** 1079-1088.

Guiard, B.P., David, D.J., Deltheil, T., Chenu, F., Le Maitre, E., Renoir, T., Leroux-Nicollet, I., Sokoloff, P., Lanfumey, L., Hamon, M., Andrews, A.M., Hen, R. & Gardier, A.M. (2008) Brain-derived neurotrophic factor-deficient mice exhibit a hippocampal hyperserotonergic phenotype. *Int J Neuropsychopharmacol,* **11,** 79-92.

Hajszan, T., MacLusky, N.J. & Leranth, C. (2005) Short-term treatment with the antidepressant fluoxetine triggers pyramidal dendritic spine synapse formation in rat hippocampus. *Eur J Neurosci,* **21,** 1299-1303.

Hakansson, A., Melke, J., Westberg, L., Shahabi, H.N., Buervenich, S., Carmine, A., Klingborg, K., Grundell, M.B., Schulhof, B., Holmberg, B., Ahlberg, J., Eriksson, E., Sydow, O., Olson, L., Johnels, B. & Nissbrandt, H. (2003) Lack of association between the BDNF Val66Met polymorphism and Parkinson's disease in a Swedish population. *Ann Neurol,* **53,** 823.

Hall, D., Dhilla, A., Charalambous, A., Gogos, J.A. & Karayiorgou, M. (2003a) Sequence variants of the brain-derived neurotrophic factor (BDNF) gene are strongly associated with obsessive-compulsive disorder. *Am J Hum Genet,* **73,** 370-376.

Hall, F.S., Drgonova, J., Goeb, M. & Uhl, G.R. (2003b) Reduced behavioral effects of cocaine in heterozygous brain-derived neurotrophic factor (BDNF) knockout mice. *Neuropsychopharmacology,* **28,** 1485-1490.

Hariri, A.R., Goldberg, T.E., Mattay, V.S., Kolachana, B.S., Callicott, J.H., Egan, M.F. & Weinberger, D.R. (2003) Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *J Neurosci,* **23,** 6690-6694.

Harro, J. (2006) CCK and NPY as anti-anxiety treatment targets: promises, pitfalls, and strategies. *Amino Acids,* **31,** 215-230.

Heilig, M. (2004) The NPY system in stress, anxiety and depression. *Neuropeptides,* **38,** 213-224.

Heinrich, G. & Lum, T. (2000) Fish neurotrophins and Trk receptors. *Int J Dev Neurosci,* **18,** 1-27.

Hemmings, S.M., Kinnear, C.J., Van Der Merwe, L., Lochner, C., Corfield, V.A., Moolman-Smook, J.C. & Stein, D.J. (2007) Investigating the role of the brain-derived neurotrophic factor (BDNF) val66met variant in obsessive-compulsive disorder (OCD). *World J Biol Psychiatry***,** 1-9.

Hill, M.N. & Gorzalka, B.B. (2005) Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? *Behav Pharmacol,* **16,** 333-352.

Hirayasu, Y., Shenton, M.E., Salisbury, D.F., Kwon, J.S., Wible, C.G., Fischer, I.A., Yurgelun-Todd, D., Zarate, C., Kikinis, R., Jolesz, F.A. & McCarley, R.W. (1999) Subgenual cingulate cortex volume in first-episode psychosis. *Am J Psychiatry,* **156,** 1091-1093.

Hofer, M., Pagliusi, S.R., Hohn, A., Leibrock, J. & Barde, Y.A. (1990) Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. *Embo J,* **9,** 2459-2464.

Holsboer, F. (2000) The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology,* **23,** 477-501.

Holsboer, F. (2001a) Antidepressant drug discovery in the postgenomic era. *World J Biol Psychiatry,* **2,** 165-177.

Holsboer, F. (2001b) Prospects for antidepressant drug discovery. *Biol Psychol,* **57,** 47-65.

Holsboer, F. (2001c) Stress, hypercortisolism and corticosteroid receptors in depression: implications for therapy. *J Affect Disord,* **62,** 77-91.

Hong, C.J., Huo, S.J., Yen, F.C., Tung, C.L., Pan, G.M. & Tsai, S.J. (2003a) Association study of a brain-derived neurotrophic-factor genetic polymorphism and mood disorders, age of onset and suicidal behavior. *Neuropsychobiology,* **48,** 186-189.

Hong, C.J., Liu, H.C., Liu, T.Y., Lin, C.H., Cheng, C.Y. & Tsai, S.J. (2003b) Brain-derived neurotrophic factor (BDNF) Val66Met polymorphisms in Parkinson's disease and age of onset. *Neurosci Lett,* **353,** 75-77.

Hong, C.J., Yu, Y.W., Lin, C.H. & Tsai, S.J. (2003c) An association study of a brain-derived neurotrophic factor Val66Met polymorphism and clozapine response of schizophrenic patients. *Neurosci Lett,* **349,** 206-208.

Horger, B.A., Iyasere, C.A., Berhow, M.T., Messer, C.J., Nestler, E.J. & Taylor, J.R. (1999) Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. *J Neurosci,* **19,** 4110-4122.

Hughes, R.N. (1968) Behaviour of male and female rats with free choice of two environments differing in novelty. *Anim Behav,* **16,** 92-96.

Hughes, R.N. & Beveridge, I.J. (1986) Behavioral effects of prenatal exposure to caffeine in rats. *Life Sci,* **38,** 861-868.

Hunkeler, E.M., Spector, W.D., Fireman, B., Rice, D.P. & Weisner, C. (2003) Psychiatric symptoms, impaired function, and medical care costs in an HMO setting. *Gen Hosp Psychiatry,* **25,** 178-184.

Hunnerkopf, R., Strobel, A., Gutknecht, L., Brocke, B. & Lesch, K.P. (2007) Interaction between BDNF Val66Met and dopamine transporter gene variation influences anxiety-related traits. *Neuropsychopharmacology,* **32,** 2552-2560.

Hwang, J.P., Tsai, S.J., Hong, C.J., Yang, C.H., Lirng, J.F. & Yang, Y.M. (2006) The Val66Met polymorphism of the brain-derived neurotrophic-factor gene is associated with geriatric depression. *Neurobiol Aging,* **27,** 1834-1837.

Hyman, C., Hofer, M., Barde, Y.A., Juhasz, M., Yancopoulos, G.D., Squinto, S.P. & Lindsay, R.M. (1991) BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature,* **350,** 230-232.

Invernizzi, R.W. & Garattini, S. (2004) Role of presynaptic alpha2-adrenoceptors in antidepressant action: recent findings from microdialysis studies. *Prog Neuropsychopharmacol Biol Psychiatry,* **28,** 819-827.

Jesberger, J.A. & Richardson, J.S. (1985) Animal models of depression: parallels and correlates to severe depression in humans. *Biol Psychiatry,* **20,** 764-784.

Jiang, X., Xu, K., Hoberman, J., Tian, F., Marko, A.J., Waheed, J.F., Harris, C.R., Marini, A.M., Enoch, M.A. & Lipsky, R.H. (2005) BDNF variation and mood disorders: a novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. *Neuropsychopharmacology,* **30,** 1353-1361.

Jones, K.R., Farinas, I., Backus, C. & Reichardt, L.F. (1994) Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. *Cell,* **76,** 989-999.

Kalueff, A.V. (2007) Neurobiology of memory and anxiety: from genes to behavior. *Neural Plast,* **2007,** 78171.

Kanazawa, T., Glatt, S.J., Kia-Keating, B., Yoneda, H. & Tsuang, M.T. (2007) Meta-analysis reveals no association of the Val66Met polymorphism of brain-derived neurotrophic factor with either schizophrenia or bipolar disorder. *Psychiatr Genet,* **17,** 165-170.

Karamohamed, S., Latourelle, J.C., Racette, B.A., Perlmutter, J.S., Wooten, G.F., Lew, M., Klein, C., Shill, H., Golbe, L.I., Mark, M.H., Guttman, M., Nicholson, G., Wilk, J.B., Saint-Hilaire, M., DeStefano, A.L., Prakash, R., Tobin, S., Williamson, J., Suchowersky, O., Labell, N., Growdon, B.N., Singer, C., Watts, R., Goldwurm, S., Pezzoli, G., Baker, K.B., Giroux, M.L., Pramstaller, P.P., Burn, D.J., Chinnery, P., Sherman, S., Vieregge, P., Litvan, I., Gusella, J.F., Myers, R.H. & Parsian, A. (2005) BDNF genetic variants are associated with onset age of familial Parkinson disease: GenePD Study. *Neurology,* **65,** 1823-1825.

Karege, F., Perret, G., Bondolfi, G., Schwald, M., Bertschy, G. & Aubry, J.M. (2002) Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res,* **109,** 143-148.

Karege, F., Vaudan, G., Schwald, M., Perroud, N. & La Harpe, R. (2005) Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. *Brain Res Mol Brain Res,* **136,** 29-37.

Kaschka, W., Feistel, H. & Ebert, D. (1995) Reduced benzodiazepine receptor binding in panic disorders measured by iomazenil SPECT. *J Psychiatr Res,* **29,** 427-434.

Kaufman, J. & Charney, D. (2000) Comorbidity of mood and anxiety disorders. *Depress Anxiety,* **12 Suppl 1,** 69-76.

Kempermann, G. & Kronenberg, G. (2003) Depressed new neurons--adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. *Biol Psychiatry,* **54,** 499-503.

Kendler, K.S., Karkowski, L.M. & Prescott, C.A. (1999) Causal relationship between stressful life events and the onset of major depression. *Am J Psychiatry,* **156,** 837-841.

Kent, L., Green, E., Hawi, Z., Kirley, A., Dudbridge, F., Lowe, N., Raybould, R., Langley, K., Bray, N., Fitzgerald, M., Owen, M.J., O'Donovan, M.C., Gill, M., Thapar, A. & Craddock, N. (2005) Association of the paternally transmitted copy of common Valine

allele of the Val66Met polymorphism of the brain-derived neurotrophic factor (BDNF) gene with susceptibility to ADHD. *Mol Psychiatry,* **10,** 939-943.

Kernie, S.G., Liebl, D.J. & Parada, L.F. (2000) BDNF regulates eating behavior and locomotor activity in mice. *Embo J,* **19,** 1290-1300.

Kessler, R.C. (1997) The effects of stressful life events on depression. *Annu Rev Psychol,* **48,** 191-214.

Kobayashi, T., Hayashi, E., Shimamura, M., Kinoshita, M. & Murphy, N.P. (2008) Neurochemical responses to antidepressants in the prefrontal cortex of mice and their efficacy in preclinical models of anxiety-like and depression-like behavior: a comparative and correlational study. *Psychopharmacology (Berl),* **197,** 567-580.

Kolbeck, R., Jungbluth, S. & Barde, Y.A. (1994) Characterisation of neurotrophin dimers and monomers. *Eur J Biochem,* **225,** 995-1003.

Kontkanen, O. & Castren, E. (1999) Trophic effects of selegiline on cultured dopaminergic neurons. *Brain Res,* **829,** 190-192.

Koponen, E., Rantamaki, T., Voikar, V., Saarelainen, T., MacDonald, E. & Castren, E. (2005) Enhanced BDNF signaling is associated with an antidepressant-like behavioral response and changes in brain monoamines. *Cell Mol Neurobiol,* **25,** 973-980.

Kunugi, H., Iijima, Y., Tatsumi, M., Yoshida, M., Hashimoto, R., Kato, T., Sakamoto, K., Fukunaga, T., Inada, T., Suzuki, T., Iwata, N., Ozaki, N., Yamada, K. & Yoshikawa, T. (2004) No association between the Val66Met polymorphism of the brain-derived neurotrophic factor gene and bipolar disorder in a Japanese population: a multicenter study. *Biol Psychiatry,* **56,** 376-378.

Kunugi, H., Ueki, A., Otsuka, M., Isse, K., Hirasawa, H., Kato, N., Nabika, T., Kobayashi, S. & Nanko, S. (2001) A novel polymorphism of the brain-derived neurotrophic factor (BDNF) gene associated with late-onset Alzheimer's disease. *Mol Psychiatry,* **6,** 83-86.

Lader, M. (1991) History of benzodiazepine dependence. *J Subst Abuse Treat,* **8,** 53-59.

Lang, U.E., Hellweg, R., Kalus, P., Bajbouj, M., Lenzen, K.P., Sander, T., Kunz, D. & Gallinat, J. (2005) Association of a functional BDNF polymorphism and anxiety-related personality traits. *Psychopharmacology (Berl),* **180,** 95-99.

Lauterborn, J.C., Truong, G.S., Baudry, M., Bi, X., Lynch, G. & Gall, C.M. (2003) Chronic elevation of brain-derived neurotrophic factor by ampakines. *J Pharmacol Exp Ther,* **307,** 297-305.

Lebrun, B., Bariohay, B., Moyse, E. & Jean, A. (2006) Brain-derived neurotrophic factor (BDNF) and food intake regulation: a minireview. *Auton Neurosci,* **126-127,** 30-38.

Lee, B.H., Kim, H., Park, S.H. & Kim, Y.K. (2007) Decreased plasma BDNF level in depressive patients. *J Affect Disord,* **101,** 239-244.

Lee, R., Kermani, P., Teng, K.K. & Hempstead, B.L. (2001) Regulation of cell survival by secreted proneurotrophins. *Science,* **294,** 1945-1948.

Leibrock, J., Lottspeich, F., Hohn, A., Hofer, M., Hengerer, B., Masiakowski, P., Thoenen, H. & Barde, Y.A. (1989) Molecular cloning and expression of brain-derived neurotrophic factor. *Nature,* **341,** 149-152.

Lein, E.S. & Shatz, C.J. (2000) Rapid regulation of brain-derived neurotrophic factor mRNA within eye-specific circuits during ocular dominance column formation. *J Neurosci,* **20,** 1470-1483.

Leonard, B.E. (2001a) Changes in the immune system in depression and dementia: causal or co-incidental effects? *Int J Dev Neurosci,* **19,** 305-312.

Leonard, B.E. (2001b) The immune system, depression and the action of antidepressants. *Prog Neuropsychopharmacol Biol Psychiatry,* **25,** 767-780.

Lessmann, V. (1998) Neurotrophin-dependent modulation of glutamatergic synaptic transmission in the mammalian CNS. *Gen Pharmacol,* **31,** 667-674.

Lessmann, V., Gottmann, K. & Malcangio, M. (2003) Neurotrophin secretion: current facts and future prospects. *Prog Neurobiol,* **69,** 341-374.

Liberzon, I. & Phan, K.L. (2003) Brain-imaging studies of posttraumatic stress disorder. *CNS Spectr,* **8,** 641-650.

Liebowitz, M.R. (1993) Depression with anxiety and atypical depression. *J Clin Psychiatry,* **54 Suppl,** 10-14; discussion 15.

Linnarsson, S., Bjorklund, A. & Ernfors, P. (1997) Learning deficit in BDNF mutant mice. *Eur J Neurosci,* **9,** 2581-2587.

Lister, R.G. (1987) The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology (Berl),* **92,** 180-185.

Liu, I.Y., Lyons, W.E., Mamounas, L.A. & Thompson, R.F. (2004a) Brain-derived neurotrophic factor plays a critical role in contextual fear conditioning. *J Neurosci,* **24,** 7958-7963.

Liu, J., Yu, B., Orozco-Cabal, L., Grigoriadis, D.E., Rivier, J., Vale, W.W., Shinnick-Gallagher, P. & Gallagher, J.P. (2005a) Chronic cocaine administration switches

corticotropin-releasing factor2 receptor-mediated depression to facilitation of glutamatergic transmission in the lateral septum. *J Neurosci,* **25,** 577-583.

Liu, Q.R., Lu, L., Zhu, X.G., Gong, J.P., Shaham, Y. & Uhl, G.R. (2006) Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. *Brain Res,* **1067,** 1-12.

Liu, Q.R., Walther, D., Drgon, T., Polesskaya, O., Lesnick, T.G., Strain, K.J., de Andrade, M., Bower, J.H., Maraganore, D.M. & Uhl, G.R. (2005b) Human brain derived neurotrophic factor (BDNF) genes, splicing patterns, and assessments of associations with substance abuse and Parkinson's Disease. *Am J Med Genet B Neuropsychiatr Genet,* **134,** 93-103.

Liu, Y., Ishida, Y., Shinoda, K., Furukawa, S. & Nakamura, S. (2004b) Opposite morphological responses of partially denervated cortical serotonergic and noradrenergic axons to repeated stress in adult rats. *Brain Res Bull,* **64,** 67-74.

Lohoff, F.W., Sander, T., Ferraro, T.N., Dahl, J.P., Gallinat, J. & Berrettini, W.H. (2005) Confirmation of association between the Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) gene and bipolar I disorder. *Am J Med Genet B Neuropsychiatr Genet,* **139,** 51-53.

Loiseau, F., Le Bihan, C., Hamon, M. & Thiebot, M.H. (2005) Antidepressant-like effects of agomelatine, melatonin and the NK1 receptor antagonist GR205171 in impulsive-related behaviour in rats. *Psychopharmacology (Berl),* **182,** 24-32.

Loiseau, F., Le Bihan, C., Hamon, M. & Thiebot, M.H. (2006) Effects of melatonin and agomelatine in anxiety-related procedures in rats: interaction with diazepam. *Eur Neuropsychopharmacol,* **16,** 417-428.

Lu, B. (2003) BDNF and activity-dependent synaptic modulation. *Learn Mem,* **10,** 86-98.

Lu, B. & Chow, A. (1999) Neurotrophins and hippocampal synaptic transmission and plasticity. *J Neurosci Res,* **58,** 76-87.

Lu, B., Pang, P.T. & Woo, N.H. (2005) The yin and yang of neurotrophin action. *Nat Rev Neurosci,* **6,** 603-614.

Lu, L., Grimm, J.W., Dempsey, J. & Shaham, Y. (2004) Cocaine seeking over extended withdrawal periods in rats: different time courses of responding induced by cocaine cues versus cocaine priming over the first 6 months. *Psychopharmacology (Berl),* **176,** 101-108.

Luikart, B.W. & Parada, L.F. (2006) Receptor tyrosine kinase B-mediated excitatory synaptogenesis. *Prog Brain Res,* **157,** 15-24.

Lyons, W.E., Mamounas, L.A., Ricaurte, G.A., Coppola, V., Reid, S.W., Bora, S.H., Wihler, C., Koliatsos, V.E. & Tessarollo, L. (1999) Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc Natl Acad Sci U S A,* **96,** 15239-15244.

MacQueen, G.M., Ramakrishnan, K., Croll, S.D., Siuciak, J.A., Yu, G., Young, L.T. & Fahnestock, M. (2001) Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. *Behav Neurosci,* **115,** 1145-1153.

Maes, M. (1995) Evidence for an immune response in major depression: a review and hypothesis. *Prog Neuropsychopharmacol Biol Psychiatry,* **19,** 11-38.

Magarinos, A.M., McEwen, B.S., Flugge, G. & Fuchs, E. (1996) Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. *J Neurosci,* **16,** 3534-3540.

Malberg, J.E., Eisch, A.J., Nestler, E.J. & Duman, R.S. (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci,* **20,** 9104-9110.

Malberg, J.E. & Schechter, L.E. (2005) Increasing hippocampal neurogenesis: a novel mechanism for antidepressant drugs. *Curr Pharm Des,* **11,** 145-155.

Manji, H.K. & Lenox, R.H. (2000) Signaling: cellular insights into the pathophysiology of bipolar disorder. *Biol Psychiatry,* **48,** 518-530.

Manji, H.K., Moore, G.J., Rajkowska, G. & Chen, G. (2000) Neuroplasticity and cellular resilience in mood disorders. *Mol Psychiatry,* **5,** 578-593.

Martinowich, K., Manji, H. & Lu, B. (2007) New insights into BDNF function in depression and anxiety. *Nat Neurosci,* **10,** 1089-1093.

Martis, B., Malizia, A. & Rauch, S.L. (2002) Functional neuroanatomy of anxiety disorders. In D'Haennen, H., den Boer, J.A., Westernberg, H. & Willner, P. (eds), *Texbook in Biological Psychiatry.* Wiley & Sons Limited, pp. 989-1002.

Marvanova, M., Lakso, M., Pirhonen, J., Nawa, H., Wong, G. & Castren, E. (2001) The neuroprotective agent memantine induces brain-derived neurotrophic factor and trkB receptor expression in rat brain. *Mol Cell Neurosci,* **18,** 247-258.

McEwen, B.S. (2000a) Allostasis and allostatic load: implications for neuropsychopharmacology. *Neuropsychopharmacology,* **22,** 108-124.

McEwen, B.S. (2000b) The neurobiology of stress: from serendipity to clinical relevance. *Brain Res,* **886,** 172-189.

McKinney, W.T. (1984) Animal models of depression: an overview. *Psychiatr Dev,* **2,** 77-96.

McLean, S. (2005) Do substance P and the NK1 receptor have a role in depression and anxiety? *Curr Pharm Des*, **11,** 1529-1547.

McNaughton, N. & Gray, J.A. (2000) Anxiolytic action on the behavioural inhibition system implies multiple types of arousal contribute to anxiety. *J Affect Disord*, **61,** 161-176.

Mercader, J.M., Ribases, M., Gratacos, M., Gonzalez, J.R., Bayes, M., de Cid, R., Badia, A., Fernandez-Aranda, F. & Estivill, X. (2007) Altered brain-derived neurotrophic factor blood levels and gene variability are associated with anorexia and bulimia. *Genes Brain Behav*, **6,** 706-716.

Mineur, Y.S., Prasol, D.J., Belzung, C. & Crusio, W.E. (2003) Agonistic behavior and unpredictable chronic mild stress in mice. *Behav Genet*, **33,** 513-519.

Minichiello, L., Korte, M., Wolfer, D., Kuhn, R., Unsicker, K., Cestari, V., Rossi-Arnaud, C., Lipp, H.P., Bonhoeffer, T. & Klein, R. (1999) Essential role for TrkB receptors in hippocampus-mediated learning. *Neuron*, **24,** 401-414.

Mitoma, M., Yoshimura, R., Sugita, A., Umene, W., Hori, H., Nakano, H., Ueda, N. & Nakamura, J. (2008) Stress at work alters serum brain-derived neurotrophic factor (BDNF) levels and plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) levels in healthy volunteers: BDNF and MHPG as possible biological markers of mental stress? *Prog Neuropsychopharmacol Biol Psychiatry*, **32,** 679-685.

Mizuno, M., Yamada, K., Olariu, A., Nawa, H. & Nabeshima, T. (2000) Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. *J Neurosci*, **20,** 7116-7121.

Mocaer, E., Delalleau, B., Boyer, P.A. & de Bodinat, C. (2005) [Development of a new antidepressant : agomelatine]. *Med Sci (Paris)*, **21,** 888-893.

Moncrieff, J. (2008) The creation of the concept of an antidepressant: An historical analysis. *Soc Sci Med*.

Monleon, S., D'Aquila, P., Parra, A., Simon, V.M., Brain, P.F. & Willner, P. (1995) Attenuation of sucrose consumption in mice by chronic mild stress and its restoration by imipramine. *Psychopharmacology (Berl)*, **117,** 453-457.

Monteggia, L.M., Barrot, M., Powell, C.M., Berton, O., Galanis, V., Gemelli, T., Meuth, S., Nagy, A., Greene, R.W. & Nestler, E.J. (2004) Essential role of brain-derived neurotrophic factor in adult hippocampal function. *Proc Natl Acad Sci U S A*, **101,** 10827-10832.

Monteggia, L.M., Luikart, B., Barrot, M., Theobold, D., Malkovska, I., Nef, S., Parada, L.F. & Nestler, E.J. (2007) Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. *Biol Psychiatry,* **61,** 187-197.

Montgomery, S.A., Baldwin, D.S., Blier, P., Fineberg, N.A., Kasper, S., Lader, M., Lam, R.W., Lepine, J.P., Moller, H.J., Nutt, D.J., Rouillon, F., Schatzberg, A.F. & Thase, M.E. (2007) Which antidepressants have demonstrated superior efficacy? A review of the evidence. *Int Clin Psychopharmacol,* **22,** 323-329.

Montkowski, A. & Holsboer, F. (1997) Intact spatial learning and memory in transgenic mice with reduced BDNF. *Neuroreport,* **8,** 779-782.

Mowla, S.J., Farhadi, H.F., Pareek, S., Atwal, J.K., Morris, S.J., Seidah, N.G. & Murphy, R.A. (2001) Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. *J Biol Chem,* **276,** 12660-12666.

Mu, J.S., Li, W.P., Yao, Z.B. & Zhou, X.F. (1999) Deprivation of endogenous brain-derived neurotrophic factor results in impairment of spatial learning and memory in adult rats. *Brain Res,* **835,** 259-265.

Mufson, E.J., Kroin, J.S., Sendera, T.J. & Sobreviela, T. (1999) Distribution and retrograde transport of trophic factors in the central nervous system: functional implications for the treatment of neurodegenerative diseases. *Prog Neurobiol,* **57,** 451-484.

Murakami, S., Imbe, H., Morikawa, Y., Kubo, C. & Senba, E. (2005) Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. *Neurosci Res,* **53,** 129-139.

Murer, M.G., Yan, Q. & Raisman-Vozari, R. (2001) Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. *Prog Neurobiol,* **63,** 71-124.

Murphy, R.A., Acheson, A., Hodges, R., Haskins, J., Richards, C., Reklow, E., Chlumecky, V., Barker, P.A., Alderson, R.F. & Lindsay, R.M. (1993) Immunological relationships of NGF, BDNF, and NT-3: recognition and functional inhibition by antibodies to NGF. *J Neurosci,* **13,** 2853-2862.

Narita, M., Aoki, K., Takagi, M., Yajima, Y. & Suzuki, T. (2003) Implication of brain-derived neurotrophic factor in the release of dopamine and dopamine-related behaviors induced by methamphetamine. *Neuroscience,* **119,** 767-775.

Nelson, J.C. (1999) A review of the efficacy of serotonergic and noradrenergic reuptake inhibitors for treatment of major depression. *Biol Psychiatry,* **46,** 1301-1308.

Nemeroff, C.B. (2002) Comorbidity of mood and anxiety disorders: the rule, not the exception? *Am J Psychiatry,* **159,** 3-4.

Nestler, E.J., Barrot, M., DiLeone, R.J., Eisch, A.J., Gold, S.J. & Monteggia, L.M. (2002a) Neurobiology of depression. *Neuron,* **34,** 13-25.

Nestler, E.J. & Carlezon, W.A., Jr. (2006) The mesolimbic dopamine reward circuit in depression. *Biol Psychiatry,* **59,** 1151-1159.

Nestler, E.J., Gould, E., Manji, H., Buncan, M., Duman, R.S., Greshenfeld, H.K., Hen, R., Koester, S., Lederhendler, I., Meaney, M., Robbins, T., Winsky, L. & Zalcman, S. (2002b) Preclinical models: status of basic research in depression. *Biol Psychiatry,* **52,** 503-528.

Neves-Pereira, M., Cheung, J.K., Pasdar, A., Zhang, F., Breen, G., Yates, P., Sinclair, M., Crombie, C., Walker, N. & St Clair, D.M. (2005) BDNF gene is a risk factor for schizophrenia in a Scottish population. *Mol Psychiatry,* **10,** 208-212.

Nibuya, M., Morinobu, S. & Duman, R.S. (1995) Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci,* **15,** 7539-7547.

Nilsson, A.S., Fainzilber, M., Falck, P. & Ibanez, C.F. (1998) Neurotrophin-7: a novel member of the neurotrophin family from the zebrafish. *FEBS Lett,* **424,** 285-290.

Nutt, D.J., Glue, P., Lawson, C. & Wilson, S. (1990) Flumazenil provocation of panic attacks. Evidence for altered benzodiazepine receptor sensitivity in panic disorder. *Arch Gen Psychiatry,* **47,** 917-925.

Nutt, D.J. & Malizia, A.L. (2001) New insights into the role of the GABA(A)-benzodiazepine receptor in psychiatric disorder. *Br J Psychiatry,* **179,** 390-396.

O'Neil, M.F. & Moore, N.A. (2003) Animal models of depression: are there any? *Hum Psychopharmacol,* **18,** 239-254.

Ozcelik, T., Rosenthal, A. & Francke, U. (1991) Chromosomal mapping of brain-derived neurotrophic factor and neurotrophin-3 genes in man and mouse. *Genomics,* **10,** 569-575.

Park, L.T., Jefferson, J.W. & Greist, J.H. (2000) Obsessive-compulsive disorder. Treatment options. In Palmer, K.J. (ed), *Pharmacotherapy of anxiety disorder.* Acids International, pp. 43-59.

Paykel, E.S. (2001) Stress and affective disorders in humans. *Semin Clin Neuropsychiatry,* **6,** 4-11.

Pezawas, L., Verchinski, B.A., Mattay, V.S., Callicott, J.H., Kolachana, B.S., Straub, R.E., Egan, M.F., Meyer-Lindenberg, A. & Weinberger, D.R. (2004) The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. *J Neurosci,* **24,** 10099-10102.

Pizarro, J.M., Lumley, L.A., Medina, W., Robison, C.L., Chang, W.E., Alagappan, A., Bah, M.J., Dawood, M.Y., Shah, J.D., Mark, B., Kendall, N., Smith, M.A., Saviolakis, G.A. & Meyerhoff, J.L. (2004) Acute social defeat reduces neurotrophin expression in brain cortical and subcortical areas in mice. *Brain Res,* **1025,** 10-20.

Pohl, R.B., Feltner, D.E., Fieve, R.R. & Pande, A.C. (2005) Efficacy of pregabalin in the treatment of generalized anxiety disorder: double-blind, placebo-controlled comparison of BID versus TID dosing. *J Clin Psychopharmacol,* **25,** 151-158.

Pollak, Y. & Yirmiya, R. (2002) Cytokine-induced changes in mood and behaviour: implications for 'depression due to a general medical condition', immunotherapy and antidepressive treatment. *Int J Neuropsychopharmacol,* **5,** 389-399.

Poo, M.M. (2001) Neurotrophins as synaptic modulators. *Nat Rev Neurosci,* **2,** 24-32.

Porsolt, R.D. (1997) Historical perspective on CMS model. *Psychopharmacology (Berl),* **134,** 363-364; discussion 371-367.

Porsolt, R.D., Bertin, A. & Jalfre, M. (1977a) Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther,* **229,** 327-336.

Porsolt, R.D., Le Pichon, M. & Jalfre, M. (1977b) Depression: a new animal model sensitive to antidepressant treatments. *Nature,* **266,** 730-732.

Poschel, B.P. (1971) A simple and specific screen for benzodiazepine-like drugs. *Psychopharmacologia,* **19,** 193-198.

Quitkin, F.M., Taylor, B.P. & Kremer, C. (2001) Does mirtazapine have a more rapid onset than SSRIs? *J Clin Psychiatry,* **62,** 358-361.

Raison, C.L. & Miller, A.H. (2001) The neuroimmunology of stress and depression. *Semin Clin Neuropsychiatry,* **6,** 277-294.

Rantamaki, T., Knuuttila, J.E., Hokkanen, M.E. & Castren, E. (2006) The effects of acute and long-term lithium treatments on trkB neurotrophin receptor activation in the mouse hippocampus and anterior cingulate cortex. *Neuropharmacology,* **50,** 421-427.

Rattenholl, A., Ruoppolo, M., Flagiello, A., Monti, M., Vinci, F., Marino, G., Lilie, H., Schwarz, E. & Rudolph, R. (2001) Pro-sequence assisted folding and disulfide bond formation of human nerve growth factor. *J Mol Biol,* **305,** 523-533.

Ren-Patterson, R.F., Cochran, L.W., Holmes, A., Lesch, K.P., Lu, B. & Murphy, D.L. (2006) Gender-Dependent Modulation of Brain Monoamines and Anxiety-Like Behaviors in Mice with Genetic Serotonin Transporter and BDNF Deficiencies. *Cell Mol Neurobiol*.

Ribases, M., Gratacos, M., Armengol, L., de Cid, R., Badia, A., Jimenez, L., Solano, R., Vallejo, J., Fernandez, F. & Estivill, X. (2003) Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. *Mol Psychiatry,* **8,** 745-751.

Rickels, K., DeMartinis, N. & Aufdembrinke, B. (2000) A double-blind, placebo-controlled trial of abecarnil and diazepam in the treatment of patients with generalized anxiety disorder. *J Clin Psychopharmacol,* **20,** 12-18.

Rickels, K., DeMartinis, N., Rynn, M. & Mandos, L. (1999) Pharmacologic strategies for discontinuing benzodiazepine treatment. *J Clin Psychopharmacol,* **19,** 12S-16S.

Rickels, K., Pollack, M.H., Feltner, D.E., Lydiard, R.B., Zimbroff, D.L., Bielski, R.J., Tobias, K., Brock, J.D., Zornberg, G.L. & Pande, A.C. (2005) Pregabalin for treatment of generalized anxiety disorder: a 4-week, multicenter, double-blind, placebo-controlled trial of pregabalin and alprazolam. *Arch Gen Psychiatry,* **62,** 1022-1030.

Rickels, K. & Rynn, M. (2002) Pharmacotherapy of generalized anxiety disorder. *J Clin Psychiatry,* **63 Suppl 14,** 9-16.

Rios, M., Fan, G., Fekete, C., Kelly, J., Bates, B., Kuehn, R., Lechan, R.M. & Jaenisch, R. (2001) Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Mol Endocrinol,* **15,** 1748-1757.

Roceri, M., Cirulli, F., Pessina, C., Peretto, P., Racagni, G. & Riva, M.A. (2004) Postnatal repeated maternal deprivation produces age-dependent changes of brain-derived neurotrophic factor expression in selected rat brain regions. *Biol Psychiatry,* **55,** 708-714.

Rodgers, R.J., Cao, B.J., Dalvi, A. & Holmes, A. (1997) Animal models of anxiety: an ethological perspective. *Braz J Med Biol Res,* **30,** 289-304.

Rodgers, R.J. & Johnson, N.J. (1995) Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. *Pharmacol Biochem Behav,* **52,** 297-303.

Romach, M., Busto, U., Somer, G., Kaplan, H.L. & Sellers, E. (1995) Clinical aspects of chronic use of alprazolam and lorazepam. *Am J Psychiatry,* **152,** 1161-1167.

Rouillon, F. (1999) Anxiety with depression: a treatment need. *Eur Neuropsychopharmacol,* **9 Suppl 3,** S87-92.

Rudolph, R.L. (2002) Achieving remission from depression with venlafaxine and venlafaxine extended release: a literature review of comparative studies with selective serotonin reuptake inhibitors. *Acta Psychiatr Scand Suppl***,** 24-30.

Russo-Neustadt, A., Beard, R.C. & Cotman, C.W. (1999) Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. *Neuropsychopharmacology,* **21,** 679-682.

Saarelainen, T., Hendolin, P., Lucas, G., Koponen, E., Sairanen, M., MacDonald, E., Agerman, K., Haapasalo, A., Nawa, H., Aloyz, R., Ernfors, P. & Castren, E. (2003) Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. *J Neurosci,* **23,** 349-357.

Saarelainen, T., Lukkarinen, J.A., Koponen, S., Grohn, O.H., Jolkkonen, J., Koponen, E., Haapasalo, A., Alhonen, L., Wong, G., Koistinaho, J., Kauppinen, R.A. & Castren, E. (2000) Transgenic mice overexpressing truncated trkB neurotrophin receptors in neurons show increased susceptibility to cortical injury after focal cerebral ischemia. *Mol Cell Neurosci,* **16,** 87-96.

Sairanen, M., Lucas, G., Ernfors, P., Castren, M. & Castren, E. (2005) Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *J Neurosci,* **25,** 1089-1094.

Sairanen, M., O'Leary, O.F., Knuuttila, J.E. & Castren, E. (2007) Chronic antidepressant treatment selectively increases expression of plasticity-related proteins in the hippocampus and medial prefrontal cortex of the rat. *Neuroscience,* **144,** 368-374.

Sanchez, M.M., Ladd, C.O. & Plotsky, P.M. (2001) Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. *Dev Psychopathol,* **13,** 419-449.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C. & Hen, R. (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science,* **301,** 805-809.

Sapolsky, R.M. (2001) Depression, antidepressants, and the shrinking hippocampus. *Proc Natl Acad Sci U S A*, **98,** 12320-12322.

Sapolsky, R.M., Krey, L.C. & McEwen, B.S. (1985) Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. *J Neurosci,* **5,** 1222-1227.

Sapolsky, R.M., Romero, L.M. & Munck, A.U. (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev,* **21,** 55-89.

Saylor, A.J., Meredith, G.E., Vercillo, M.S., Zahm, D.S. & McGinty, J.F. (2006) BDNF heterozygous mice demonstrate age-related changes in striatal and nigral gene expression. *Exp Neurol,* **199,** 362-372.

Scarisbrick, I.A., Jones, E.G. & Isackson, P.J. (1993) Coexpression of mRNAs for NGF, BDNF, and NT-3 in the cardiovascular system of the pre- and postnatal rat. *J Neurosci,* **13,** 875-893.

Schinder, A.F. & Poo, M. (2000) The neurotrophin hypothesis for synaptic plasticity. *Trends Neurosci,* **23,** 639-645.

Schlegel, S., Steinert, H., Bockisch, A., Hahn, K., Schloesser, R. & Benkert, O. (1994) Decreased benzodiazepine receptor binding in panic disorder measured by IOMAZENIL-SPECT. A preliminary report. *Eur Arch Psychiatry Clin Neurosci,* **244,** 49-51.

Schumacher, J., Jamra, R.A., Becker, T., Ohlraun, S., Klopp, N., Binder, E.B., Schulze, T.G., Deschner, M., Schmal, C., Hofels, S., Zobel, A., Illig, T., Propping, P., Holsboer, F., Rietschel, M., Nothen, M.M. & Cichon, S. (2005) Evidence for a relationship between genetic variants at the brain-derived neurotrophic factor (BDNF) locus and major depression. *Biol Psychiatry,* **58,** 307-314.

Seil, F.J. (2003) TrkB receptor signaling and activity-dependent inhibitory synaptogenesis. *Histol Histopathol,* **18,** 635-646.

Seligman, M.E. & Maier, S.F. (1967) Failure to escape traumatic shock. *J Exp Psychol,* **74,** 1-9.

Seminowicz, D.A., Mayberg, H.S., McIntosh, A.R., Goldapple, K., Kennedy, S., Segal, Z. & Rafi-Tari, S. (2004) Limbic-frontal circuitry in major depression: a path modeling metanalysis. *Neuroimage,* **22,** 409-418.

Sen, S., Nesse, R.M., Stoltenberg, S.F., Li, S., Gleiberman, L., Chakravarti, A., Weder, A.B. & Burmeister, M. (2003) A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. *Neuropsychopharmacology,* **28,** 397-401.

Shader, R.I. & Greenblatt, D.J. (1993) Use of benzodiazepines in anxiety disorders. *N Engl J Med,* **328,** 1398-1405.

Shekhar, A., McCann, U.D., Meaney, M.J., Blanchard, D.C., Davis, M., Frey, K.A., Liberzon, I., Overall, K.L., Shear, M.K., Tecott, L.H. & Winsky, L. (2001) Summary of a National Institute of Mental Health workshop: developing animal models of anxiety disorders. *Psychopharmacology (Berl),* **157,** 327-339.

Sheline, Y.I. (1996) Hippocampal atrophy in major depression: a result of depression-induced neurotoxicity? *Mol Psychiatry,* **1,** 298-299.

Shen, R.Y., Altar, C.A. & Chiodo, L.A. (1994) Brain-derived neurotrophic factor increases the electrical activity of pars compacta dopamine neurons in vivo. *Proc Natl Acad Sci U S A,* **91,** 8920-8924.

Shepherd, J.K., Grewal, S.S., Fletcher, A., Bill, D.J. & Dourish, C.T. (1994) Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. *Psychopharmacology (Berl),* **116,** 56-64.

Shimizu, E., Hashimoto, K. & Iyo, M. (2004) [Major depressive disorders and BDNF (brain-derived neurotrophic factor)]. *Nihon Shinkei Seishin Yakurigaku Zasshi,* **24,** 147-150.

Shimizu, E., Hashimoto, K., Okamura, N., Koike, K., Komatsu, N., Kumakiri, C., Nakazato, M., Watanabe, H., Shinoda, N., Okada, S. & Iyo, M. (2003) Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biol Psychiatry,* **54,** 70-75.

Shirayama, Y., Chen, A.C., Nakagawa, S., Russell, D.S. & Duman, R.S. (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci,* **22,** 3251-3261.

Silverstone, P.H. (1991) Is anhedonia a good measure of depression? *Acta Psychiatr Scand,* **83,** 249-250.

Siuciak, J.A., Lewis, D.R., Wiegand, S.J. & Lindsay, R.M. (1997) Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). *Pharmacol Biochem Behav,* **56,** 131-137.

Slattery, D.A., Hudson, A.L. & Nutt, D.J. (2004) Invited review: the evolution of antidepressant mechanisms. *Fundam Clin Pharmacol,* **18,** 1-21.

Smith, M.A., Makino, S., Kvetnansky, R. & Post, R.M. (1995) Effects of stress on neurotrophic factor expression in the rat brain. *Ann N Y Acad Sci,* **771,** 234-239.

Soubrie, P., Kulkarni, S., Simon, P. & Boissier, J.R. (1975) [Effects of antianxiety drugs on the food intake in trained and untrained rats and mice (author's transl)]. *Psychopharmacologia,* **45,** 203-210.

Spedding, M. & Lestage, P. (2005) [Synaptic plasticity and neuropathology: new approaches in drug discovery]. *Med Sci (Paris),* **21,** 104-109.

Spedding, M., Neau, I. & Harsing, L. (2003) Brain plasticity and pathology in psychiatric disease: sites of action for potential therapy. *Curr Opin Pharmacol,* **3,** 33-40.

Spielberger, C.D. & Smith, L.H. (1966) Anxiety (drive), stress, and serial-position effects in serial-verbal learning. *J Exp Psychol,* **72,** 589-595.

Sramek, J.J., Zarotsky, V. & Cutler, N.R. (2002) Generalised anxiety disorder: treatment options. *Drugs,* **62,** 1635-1648.

Stahl, S.M., Entsuah, R. & Rudolph, R.L. (2002) Comparative efficacy between venlafaxine and SSRIs: a pooled analysis of patients with depression. *Biol Psychiatry,* **52,** 1166-1174.

Steru, L., Chermat, R., Thierry, B. & Simon, P. (1985) The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl),* **85,** 367-370.

Surget, A., Saxe, M., Leman, S., Ibarguen-Vargas, Y., Chalon, S., Griebel, G., Hen, R. & Belzung, C. (2008) Drug-Dependent Requirement of Hippocampal Neurogenesis in a Model of Depression and of Antidepressant Reversal. *Biol Psychiatry.*

Suter, U., Heymach, J.V., Jr. & Shooter, E.M. (1991) Two conserved domains in the NGF propeptide are necessary and sufficient for the biosynthesis of correctly processed and biologically active NGF. *Embo J,* **10,** 2395-2400.

Szeszko, P.R., Lipsky, R., Mentschel, C., Robinson, D., Gunduz-Bruce, H., Sevy, S., Ashtari, M., Napolitano, B., Bilder, R.M., Kane, J.M., Goldman, D. & Malhotra, A.K. (2005) Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. *Mol Psychiatry,* **10,** 631-636.

Tamminga, C.A., Nemeroff, C.B., Blakely, R.D., Brady, L., Carter, C.S., Davis, K.L., Dingledine, R., Gorman, J.M., Grigoriadis, D.E., Henderson, D.C., RB, B.I., Killen, J., Laughren, T.P., McDonald, W.M., M. Murphy GM, J., Paul, S.M., Rudorfer, M.V., Sausville, E., Schatzberg, A.F., Scolnick, E.M. & Suppes, T. (2002) Developing novel treatments for mood disorders: accelerating discovery. *Biol Psychiatry,* **52,** 589-609.

Tapia-Arancibia, L., Rage, F., Givalois, L. & Arancibia, S. (2004) Physiology of BDNF: focus on hypothalamic function. *Front Neuroendocrinol,* **25,** 77-107.

Taylor, S., McCracken, C.F., Wilson, K.C. & Copeland, J.R. (1998) Extent and appropriateness of benzodiazepine use. Results from an elderly urban community. *Br J Psychiatry,* **173,** 433-438.

Taylor, S.F. & Liberzon, I. (2007) Neural correlates of emotion regulation in psychopathology. *Trends Cogn Sci,* **11,** 413-418.

Thomas, K.M., Drevets, W.C., Dahl, R.E., Ryan, N.D., Birmaher, B., Eccard, C.H., Axelson, D., Whalen, P.J. & Casey, B.J. (2001) Amygdala response to fearful faces in anxious and depressed children. *Arch Gen Psychiatry,* **58,** 1057-1063.

Tiihonen, J., Kuikka, J., Rasanen, P., Lepola, U., Koponen, H., Liuska, A., Lehmusvaara, A., Vainio, P., Kononen, M., Bergstrom, K., Yu, M., Kinnunen, I., Akerman, K. & Karhu, J. (1997) Cerebral benzodiazepine receptor binding and distribution in generalized anxiety disorder: a fractal analysis. *Mol Psychiatry,* **2,** 463-471.

Timmusk, T., Palm, K., Metsis, M., Reintam, T., Paalme, V., Saarma, M. & Persson, H. (1993) Multiple promoters direct tissue-specific expression of the rat BDNF gene. *Neuron,* **10,** 475-489.

Tsai, S.J. (2007) Increased central brain-derived neurotrophic factor activity could be a risk factor for substance abuse: Implications for treatment. *Med Hypotheses,* **68,** 410-414.

Tsai, S.J., Cheng, C.Y., Yu, Y.W., Chen, T.J. & Hong, C.J. (2003) Association study of a brain-derived neurotrophic-factor genetic polymorphism and major depressive disorders, symptomatology, and antidepressant response. *Am J Med Genet B Neuropsychiatr Genet,* **123,** 19-22.

Tsai, S.J., Hong, C.J., Liu, H.C., Liu, T.Y., Hsu, L.E. & Lin, C.H. (2004) Association analysis of brain-derived neurotrophic factor Val66Met polymorphisms with Alzheimer's disease and age of onset. *Neuropsychobiology,* **49,** 10-12.

Tsankova, N.M., Berton, O., Renthal, W., Kumar, A., Neve, R.L. & Nestler, E.J. (2006) Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nat Neurosci,* **9,** 519-525.

Ueyama, T., Kawai, Y., Nemoto, K., Sekimoto, M., Tone, S. & Senba, E. (1997) Immobilization stress reduced the expression of neurotrophins and their receptors in the rat brain. *Neurosci Res,* **28,** 103-110.

Uhl, G.R., Liu, Q.R., Walther, D., Hess, J. & Naiman, D. (2001) Polysubstance abuse-vulnerability genes: genome scans for association, using 1,004 subjects and 1,494 single-nucleotide polymorphisms. *Am J Hum Genet,* **69,** 1290-1300.

Vaidya, V.A., Marek, G.J., Aghajanian, G.K. & Duman, R.S. (1997) 5-HT2A receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex. *J Neurosci,* **17,** 2785-2795.

Vaidya, V.A., Terwilliger, R.M. & Duman, R.S. (1999) Role of 5-HT2A receptors in the stress-induced down-regulation of brain-derived neurotrophic factor expression in rat hippocampus. *Neurosci Lett,* **262,** 1-4.

Valle Fernandez, J. (2002) *Depresion con ansiedad.Salud Global.*

Van den Hoofdakker, R.H. (1994) Chronobiological theories of nonseasonal affective disorders and their implications for treatment. *J Biol Rhythms,* **9,** 157-183.

Vinod, K.Y. & Hungund, B.L. (2006) Role of the endocannabinoid system in depression and suicide. *Trends Pharmacol Sci,* **27,** 539-545.

Vollmayr, B. & Henn, F.A. (2001) Learned helplessness in the rat: improvements in validity and reliability. *Brain Res Brain Res Protoc,* **8,** 1-7.

Warner-Schmidt, J.L. & Duman, R.S. (2006) Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. *Hippocampus,* **16,** 239-249.

Weiss, J.M. (1997) Does decreased sucrose intake indicate loss of preference in CMS model? *Psychopharmacology (Berl),* **134,** 368-370; discussion 371-367.

Weiss, M., Blier, P. & de Montigny, C. (2007) Effect of long-term administration of the antidepressant drug milnacipran on serotonergic and noradrenergic neurotransmission in the rat hippocampus. *Life Sci,* **81,** 166-176.

Weizman, R. & Weizman, A. (2002) Neurogenetics of anxiety. In D'Haennen, H., den Boer, J.A., Westberg, H. & Willner, P. (eds), *Texbook in biological Psychiatry.* Wiley & sons Limited, pp. 1011-1024.

Wetmore, C., Ernfors, P., Persson, H. & Olson, L. (1990) Localization of brain-derived neurotrophic factor mRNA to neurons in the brain by in situ hybridization. *Exp Neurol,* **109,** 141-152.

Whishaw, I.Q. & Tomie, J.A. (1996) Of mice and mazes: similarities between mice and rats on dry land but not water mazes. *Physiol Behav,* **60,** 1191-1197.

Willner, P. (1986) Validation criteria for animal models of human mental disorders: learned helplessness as a paradigm case. *Prog Neuropsychopharmacol Biol Psychiatry,* **10,** 677-690.

Willner, P. (1991) Animal models as simulations of depression. *Trends Pharmacol Sci,* **12,** 131-136.

Willner, P., Moreau, J.L., Nielsen, C.K., Papp, M. & Sluzewska, A. (1996) Decreased hedonic responsiveness following chronic mild stress is not secondary to loss of body weight. *Physiol Behav,* **60,** 129-134.

Willner, P., Muscat, R. & Papp, M. (1992) Chronic mild stress-induced anhedonia: a realistic animal model of depression. *Neurosci Biobehav Rev,* **16,** 525-534.

Willner, P., Towell, A., Sampson, D., Sophokleous, S. & Muscat, R. (1987) Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology (Berl),* **93,** 358-364.

Woo, N.H., Teng, H.K., Siao, C.J., Chiaruttini, C., Pang, P.T., Milner, T.A., Hempstead, B.L. & Lu, B. (2005) Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. *Nat Neurosci,* **8,** 1069-1077.

Yadid, G., Nakash, R., Deri, I., Tamar, G., Kinor, N., Gispan, I. & Zangen, A. (2000) Elucidation of the neurobiology of depression: insights from a novel genetic animal model. *Prog Neurobiol,* **62,** 353-378.

Yamada, K., Mizuno, M. & Nabeshima, T. (2002) Role for brain-derived neurotrophic factor in learning and memory. *Life Sci,* **70,** 735-744.

Yamada, K. & Nabeshima, T. (2003) Brain-derived neurotrophic factor/TrkB signaling in memory processes. *J Pharmacol Sci,* **91,** 267-270.

Yamamoto, H. & Gurney, M.E. (1990) Human platelets contain brain-derived neurotrophic factor. *J Neurosci,* **10,** 3469-3478.

Yoon, S.O., Casaccia-Bonnefil, P., Carter, B. & Chao, M.V. (1998) Competitive signaling between TrkA and p75 nerve growth factor receptors determines cell survival. *J Neurosci,* **18,** 3273-3281.

Youdim, M.B. (2006) The path from anti Parkinson drug selegiline and rasagiline to multifunctional neuroprotective anti Alzheimer drugs ladostigil and m30. *Curr Alzheimer Res,* **3,** 541-550.

Zandstra, S.M., Furer, J.W., van de Lisdonk, E.H., van't, H.M., Bor, J.H., van Weel, C. & Zitman, F.G. (2002) Different study criteria affect the prevalence of benzodiazepine use. *Soc Psychiatry Psychiatr Epidemiol,* **37,** 139-144.

Zandstra, S.M., Van Rijswijk, E., Rijnders, C.A., Van De Lisdonk, E.H., Bor, J.H., Van Weel, C. & Zitman, F.G. (2004) Long-term benzodiazepine users in family practice: differences from short-term users in mental health, coping behaviour and psychological characteristics. *Fam Pract,* **21,** 266-269.

Zhou, J.N., Riemersma, R.F., Unmehopa, U.A., Hoogendijk, W.J., van Heerikhuize, J.J., Hofman, M.A. & Swaab, D.F. (2001) Alterations in arginine vasopressin neurons in the suprachiasmatic nucleus in depression. *Arch Gen Psychiatry*, **58,** 655-662.

Zohar, J. & Westenberg, H.G. (2000) Anxiety disorders: a review of tricyclic antidepressants and selective serotonin reuptake inhibitors. *Acta Psychiatr Scand Suppl*, **403,** 39-49.

ANNEXES

Dans les annexes, se trouvent trois articles déjà publiés ou en cours de publication qui sont plus ou moins en relation avec le travail de cette thèse et auxquels j'ai eu la possibilité de participer.

Le premier article en cours de publication dans *Biological Psychiatry* rapporte une série d'expériences sur le rôle de la neurogenèse hippocampique dans l'établissement d'un état dépression-like et dans l'action des antidépresseurs. Cette étude est dans la droite ligne de nos expériences sur l'implication du BDNF et de la neuroplasticité dans ce phénomène, et les résultats reflètent ceux obtenus avec le BDNF. J'ai ici contribué à la réalisation de l'UCMS et au recueil de données des tests comportementaux.

Le second article est en cours de publication dans *Neuropsychopharmacology*. Il s'agit d'une analyse par des micropuces ADN des modifications d'expression des gènes causées par l'UCMS et les antidépresseurs dans différentes régions cérébrales. J'ai également contribué dans cette étude à la réalisation de l'UCMS et au recueil de données des tests comportementaux.

Enfin, le troisième article est publié dans *Molecular Psychiatry*. Il s'agit d'une étude des modifications de l'expression génétique lors du vieillissement et des effets de l'invalidation du récepteur 5-HT_{1B}.qui montre que certains processus participent au vieillissement sont indépendant du BDNF. J'ai participé à l'analyse statistique d'une partie des données.

Article 4 - Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal.
Surget et coll., *Biol Psychiatry,* 2008, sous presse

ARCHIVAL REPORT

Drug-Dependent Requirement of Hippocampal Neurogenesis in a Model of Depression and of Antidepressant Reversal

Alexandre Surget, Michael Saxe, Samuel Leman, Yadira Ibarguen-Vargas, Sylvie Chalon, Guy Griebel, René Hen, and Catherine Belzung

Background: Depression and anxiety disorders have been linked to dysfunction of the hypothalamo-pituitary-adrenal (HPA) axis and structural changes within the hippocampus. Unpredictable chronic mild stress (UCMS) can recapitulate these effects in a mouse model, and UCMS-induced changes, including downregulation of hippocampal neurogenesis, can be reversed by antidepressant (AD) treatment. We investigated causality between changes in hippocampal neurogenesis and the effects of both chronic stress and chronic ADs.

Methods: Mice were treated with either a sham procedure or focal hippocampal irradiation to disrupt cell proliferation before being confronted with 5 weeks of UCMS. From the third week onward, we administered monoaminergic ADs (imipramine, fluoxetine), the corticotropin-releasing factor 1 (CRF_1) antagonist SSR125543, or the vasopressin 1b (V_{1b}) antagonist SSR149415 daily. The effects of UCMS regimen, AD treatments, and irradiation were assessed by physical measures (coat state, weight), behavioral testing (Splash test, Novelty-Suppressed feeding test, locomotor activity), and hippocampal BrdU labeling.

Results: Our results show that elimination of hippocampal neurogenesis has no effect on animals' sensitivity to UCMS in several behavioral assays, suggesting that reduced neurogenesis is not a cause of stress-related behavioral deficits. Second, we present evidence for both neurogenesis-dependent and -independent mechanisms for the reversal of stress-induced behaviors by AD drugs. Specifically, loss of neurogenesis completely blocked the effects of monoaminergic ADs (imipramine, fluoxetine) but did not prevent most effects of the CRF_1 and the V_{1b} antagonists.

Conclusions: Hippocampal neurogenesis might thus be used by the monoaminergic ADs to counteract the effects of stress, whereas similar effects could be achieved by directly targeting the HPA axis and related neuropeptides.

Key Words: Corticotrophin-releasing factor, depression, fluoxetine, unpredictable chronic mild stress, vasopressin, x-irradiation

Stress is a key etiological factor in anxiety and major depressive disorders (1). Most patients exhibit abnormalities of the hypothalamo-pituitary-adrenal (HPA) axis (2), which coordinates the stress response through glucocorticoid (GC) release. The hippocampus is known to negatively regulate the HPA axis and this inhibitory feedback is altered by chronic stress (3,4). The involvement of the hippocampus in depression is suggested by brain neuroimaging studies and postmortem data showing a reduction of its volume that parallels the duration of depression (5–9) as well as atrophy or neuronal loss (8,10). These alterations could involve disproportionate GC levels, which can cause structural damage in the brain including atrophy, apoptosis, and reduction of cell proliferation (11). Decreased dentate gyrus (DG) cell proliferation and neurogenesis have been observed after exposure to stressors in different species (12–18) and are related to elevated stress hormones such as GCs (19,20). Accordingly, all these data combined have led to the assumption that chronic stress could precipitate depression by altering neuroplasticity and particularly hippocampal neurogenesis.

Chronic antidepressant (AD) treatment increases cell proliferation and granule cell survival (16,21,22) and is able to reverse the stress-induced decrease of hippocampal neurogenesis (14,16,17,23). The link between hippocampal neurogenesis and AD action seems to be causal, because suppression of hippocampal neurogenesis by irradiation prevents the effects of fluoxetine and imipramine (22) as well as a putative AD with a non-monoaminergic target (24). Interestingly, the ability of AD drugs to increase hippocampal neurogenesis is a common feature of both classical ADs such as selective serotonin reuptake inhibitors (SSRI) and tricyclic drugs and atypical or potential ADs (24–27), including antagonists of corticotropin-releasing factor 1 (CRF_1) and vasopressin 1b (V_{1b}) receptors (16). For these reasons, it has been suggested that hippocampal neurogenesis might be a key factor in the action of AD drugs.

Here we have investigated the involvement of hippocampal neurogenesis in two distinct and opposing behavioral effects: the response to unpredictable chronic mild stress (UCMS) and the reversal of UCMS-induced deficits by different classes of AD drugs, focusing particularly on comparing the action of monoaminergic drugs with known clinical efficacy and compounds acting on the HPA axis (CRF_1 and V_{1b} antagonists) that possess a potent AD-like action in preclinical models (16,28–30) and in clinical studies (31–33). A targeted irradiation procedure was used to disrupt progenitor cell proliferation only in the subgranular zone (SGZ) of the hippocampus (22). Mice were then subjected to a naturalistic model of depression, the UCMS paradigm, which is known to detect the effects of chronic but not acute treatment with ADs (34,35). The effects of ablating hippocampal neurogenesis on the response to both UCMS and

From U930, inserm, Université François Rabelais de Tours (AS, SL, YI-V, SC, CB), Tours, France; CNS Research Department (GG), Sanofi-Aventis, Bagneux, France; and the Center for Neurobiology and Behavior, Columbia University (MS, RH), New York.

Address reprint requests to Catherine Belzung, Ph.D., U930, inserm, Faculté des Sciences et Techniques, Université François Rabelais de Tours, Bât O, Parc de Grandmont, 37200 Tours, France; E-mail: catherine.belzung@univ-tours.fr.

Received August 5, 2007; revised January 25, 2008; accepted February 25, 2008.

0006-3223/08/$34.00
doi:10.1016/j.biopsych.2008.02.022

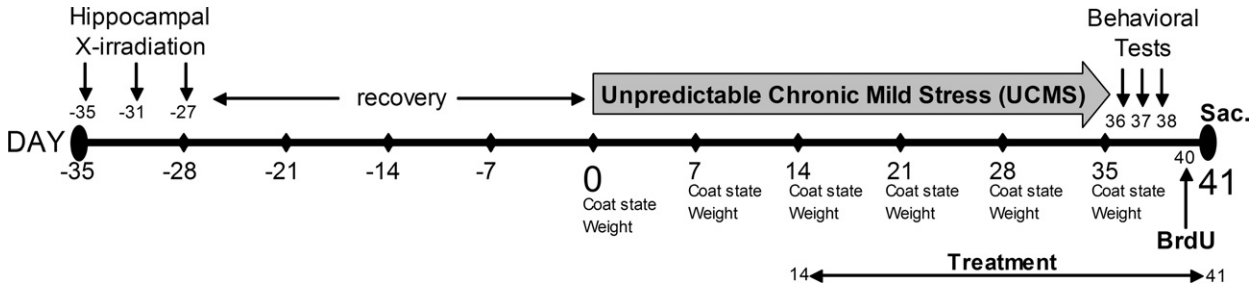

Figure 1. Schematic representation of experimental design. Five weeks before the onset of the UCMS, three focal x-irradiations were performed specifically above the hippocampus over the course of 1 week (day −35, −31, and −27). After recovery, mice were subjected to a 5-week UCMS regimen followed by behavioral tests at day 36, 37, and 38. Finally, BrdU (4 × 75 mg/kg IP, every 2 hours) was administered at day 40, mice were killed (Sac.) 24 hours after the last BrdU injection, and brains were immediately collected.

chronic AD treatment were assessed with physical measures (coat state and body weight), behavioral tests (splash test, novelty-suppressed feeding [NSF] test, and locomotor activity in an actimeter), and immunohistochemical detection of cell proliferation in the two neurogenic brain regions: the SGZ and the subventricular zone (SVZ).

Methods and Materials

Animals

Two-month-old male BALB/c mice were obtained from Taconic (Germantown, New York). All animals were housed in groups of four or five and were maintained under standard laboratory conditions (12/12-hour light/dark cycle: lights on at 8:00 PM, 22 ± 2°C, food and water ad libitum). The treatment of the animals was in accordance with the European Community Council directive 86/609/EEC and with the Guide for Care and Use of Laboratory Animals established by the National Institutes of Health of the United States of America.

Drugs

Fluoxetine (Eli Lilly, Indianapolis, Indiana), imipramine (Sigma-Aldrich, St. Louis, Missouri), SSR125543, and SSR149415 (Sanofi-Aventis, Bagneux, France) were prepared in saline (.9% sodium chloride) containing .1% Tween 80 (fluoxetine, imipramine) or 5% dimethylsulphoxide and 5% Cremophor (SSR125543, SSR149415). The solutions were administered at a volume of 10 mL/kg.

General Procedure

For each experiment, x-ray treatment was carried out on one-half of the mice. Non-irradiated mice were anesthetized and placed in the stereotaxic frame as irradiated mice but without exposure to cranial irradiation. Five weeks after the first exposure to x-irradiation, a 5-week UCMS was conducted (Figure 1). The UCMS-exposed mice were isolated in small cages (24 × 11 × 12 cm) while nonstressed mice were housed in groups of four or five in standard cages (42 × 28 × 18 cm). The first 2 weeks of UCMS were drug-free, and treatment began from the third week until the day after BrdU injection (28 days). Fluoxetine (10 mg/kg/day), imipramine (20 mg/kg/day), SSR149415 (20 mg/kg/day), SSR125543 (20 mg/kg/day) or vehicle were administered IP once/day (including for the nonstressed experiment). The body weight and the coat state were assessed weekly until the end of UCMS. The day after the last body weight and coat state measures, behavioral testing was performed: first day splash test, second day actimeter, third day NSF test. Two days after, BrdU injections were carried out. In experiments with nonstressed mice, animals were isolated 3 hours before the splash test, and the cage of stressed mice was changed at the same time. Five to seven mice from each group were randomly chosen for immunohistochemistry.

UCMS

Mice were subjected to various unpredictable stressors for 5 weeks. Alterations of the bedding (repeated sawdust changing, removal of sawdust, damp sawdust, substitution of sawdust with 21°C water), cage-tilting (45°), predator sounds (15 min), cage shift (mice were positioned in the empty cage of another male), and alterations of the light/dark cycle were used as stressors. The total score of the coat state resulted from the sum of scores from seven different body parts: head, neck, dorsal and ventral coat, tail, forepaws, and hindpaws. For each area, a score of 0 was given for well-groomed coat and 1 for an unkempt coat. This index has been pharmacologically validated in previous studies (16,22,29,30,35).

Splash Test

This test was conducted as previously described (22,36,37). It consisted of spraying a 10% sucrose solution on the mouse in its home cage. The sucrose solution dirtied the coat and induced a grooming behavior. The grooming frequency was recorded for 5 min.

NSF Test

The NSF test was a modified version of our previous study (22). The testing apparatus consisted of a 33 × 33 × 30 cm box. The floor was covered with 2 cm sawdust. Twelve hours before the test, food was removed from the cages. At the time of testing, a single pellet of food (regular chow) was placed on a white paper positioned in the box center. An animal was placed in the corner. The latency to manifestly chew the pellet was recorded within a 3-min period. This test induced a conflicting motivation between the drive to eat the food pellet and the fear of venturing into the arena. This paradigm was able to reveal the effects of chronic AD treatment in nonstressed mice (22). In this study, we reduced the dimension of the apparatus by 40% and used a red light instead of white. In this way, a 4-week treatment of fluoxetine in nonstressed mice had no effect (data not shown). In addition, ADs are known to have various effects on appetite; the feeding drive of each animal was thus assessed by returning it to the home cage immediately after the test and measuring the amount of food consumed over 5 min. No difference was observed (data not shown).

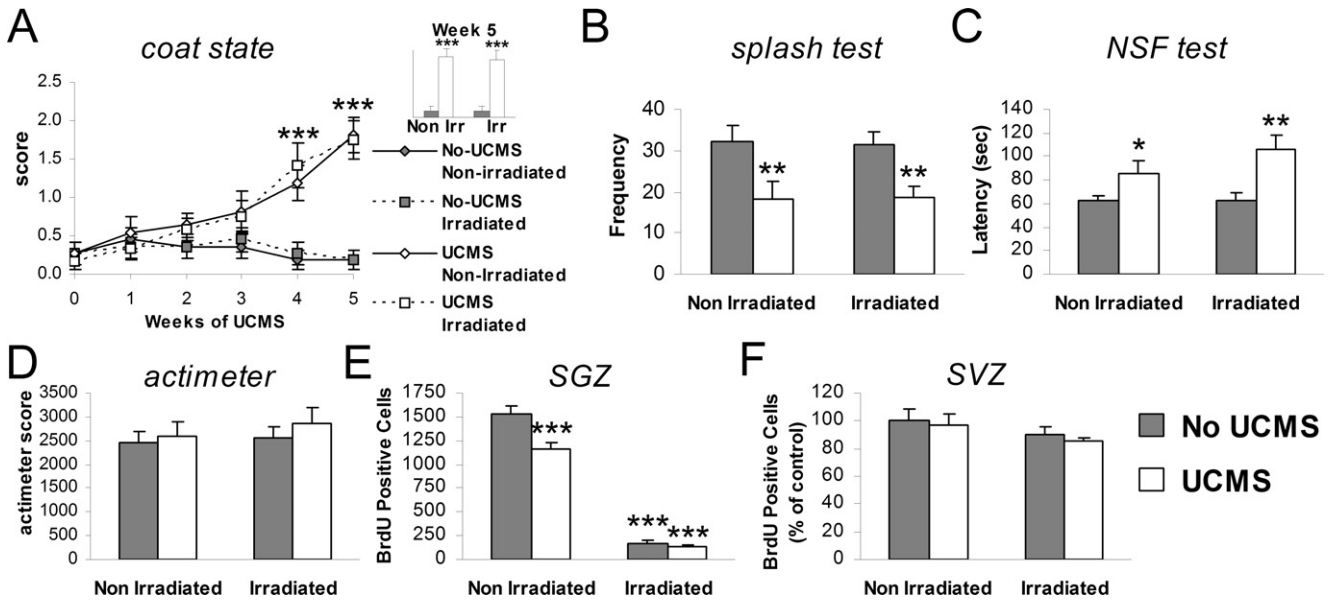

Figure 2. Effects of unpredictable chronic mild stress (UCMS) and hippocampal x-irradiation on behavior and cell proliferation in the subgranular zone (SGZ) and the subventricular zone (SVZ). **(A)** The UCMS induced a deterioration of the coat state as demonstrated by increasing coat state scores, which became significant after 4 weeks of UCMS protocol. Irradiation had no effect on this measure, because the irradiated mice showed no difference with the non-irradiated mice. Detail of the results after 5-week UCMS is illustrated above legend, $n = 11$–12 mice/group. **(B)** The UCMS induced a reduction of grooming frequency during the splash test in non-irradiated and irradiated mice. Irradiation had no effect, $n = 11$–12 mice/group. **(C)** The latency to chew the pellet was increased by UCMS regimen in the novelty-suppressed feeding (NSF) test. Irradiation had no effect, $n = 11$–12 mice/group. **(D)** Locomotor activity in the actimeter was not affected by the UCMS regimen or irradiation, $n = 11$–12 mice/group. **(E)** The UCMS reduced the number of BrdU positive cells in the SGZ of the hippocampus. X-ray procedure induced a strong disruption of cell proliferation in this area, $n = 5$–7 mice/group. **(F)** No change in cell proliferation in the SVZ due to UCMS or irradiation was found, $n = 5$–7 mice/group. Data represent mean ± SEM. ANOVA following by Fisher post hoc: $^{***}p < .001$; $^{**}p < .01$ and $^{*}p < .05$ for both UCMS group versus their respective control no-UCMS group, excepted for **(E)**: $^{***}p < .001$ versus no UCMS/non-irradiated mice.

Actimeter

The actimeter assessed the activity of mice in their home cage. The cage was placed in the center of the device, which consisted of a 20 × 20 cm square plane with photobeam detectors crossing the plane. The movement of the animal was automatically detected when it crossed through, allowing a score to be established. The higher the score was, the more the mouse moved. Testing duration was 4 hours.

Irradiation

Irradiation was performed as previously described (22). All mice were anesthetized with ketamine/xylazine (100 mg/kg and 7 mg/kg, respectively), placed in a stereotaxic frame, and then, only for irradiated mice, exposed to cranial irradiation with a Siemens Stabilopan x-ray system (Hamburg, Germany). Animals were protected with a lead shield that covered the body but left unshielded for a 3.22 × 11 mm treatment field above the hippocampus (interaural 3.00 to .00). The corrected dose rate was approximately 1.8 Gy/min at a source to skin distance of 30 cm. The procedure lasted 2 min 47 sec, delivering a total of 5 Gy. Three 5 Gy doses were given over the course of 1 week (day 0, 4, and 8).

BrdU Labeling

Mice were administered BrdU (Sigma-Aldrich, 4 × 75 mg/kg IP, every 2 hours) and killed 24 hours after the last BrdU injection. After anesthesia with ketamine/xylazine, mice were transcardially perfused: saline for 2 min, 4% paraformaldehyde (PFA)/.1 mol/L phosphate-buffered saline (PBS; pH = 7.4) for 5 min, and brains were collected, post-fixed overnight in 4% PFA at 4°C, and then cryoprotected in 30% sucrose and stored at 4°C. Serial coronal sections through the rostro-caudal brain extent were cut (45 μm), and every third section from each brain was collected and stored in PBS. The BrdU immunohistochemistry was performed on free-floating sections as described (16). Sections were treated with 3% hydrogen peroxide/50% ethanol for 20 min, rinsed in PBS, treated with 2 nmol/L hydrochloric acid (30 min), rinsed in borate buffer for 5 min (.1 mol/L, pH = 8.4), and then rinsed in PBS and incubated with a monoclonal rat anti-BrdU antibody (1:500, Oxford Biotechnology, Oxford, United Kingdom). Forty hours later, sections were rinsed in PBS, incubated 2.5 hours with a rabbit anti-rat biotinylated antibody (1:200, Vector Laboratories, Burlingame, California), and followed by amplification with an avidin-biotin complex (Elite ABC kit, Vector Laboratories). The staining was visualized with DAB (Sigma-Aldrich). The BrdU positive cell quantification was performed as described (16).

Statistics

Analyses of variance were performed by using environment (no UCMS/UCMS), irradiation (non-irradiated/irradiated), or treatment (vehicle/treated) as main factors, followed by a Fisher post hoc analysis when required.

Results

Disruption of Hippocampal Neurogenesis Has No Effect on Sensitivity to Stress

To determine whether loss of neurogenesis alters sensitivity to stress, we compared the responses of irradiated and non-irradiated mice to UCMS. In vehicle-treated mice, UCMS induced a gradual deterioration of coat state that reached significance by 4 weeks after beginning the stress ($p < .001$) and worsened until the end of the stress procedure ($p < .001$; Figure 2A). This

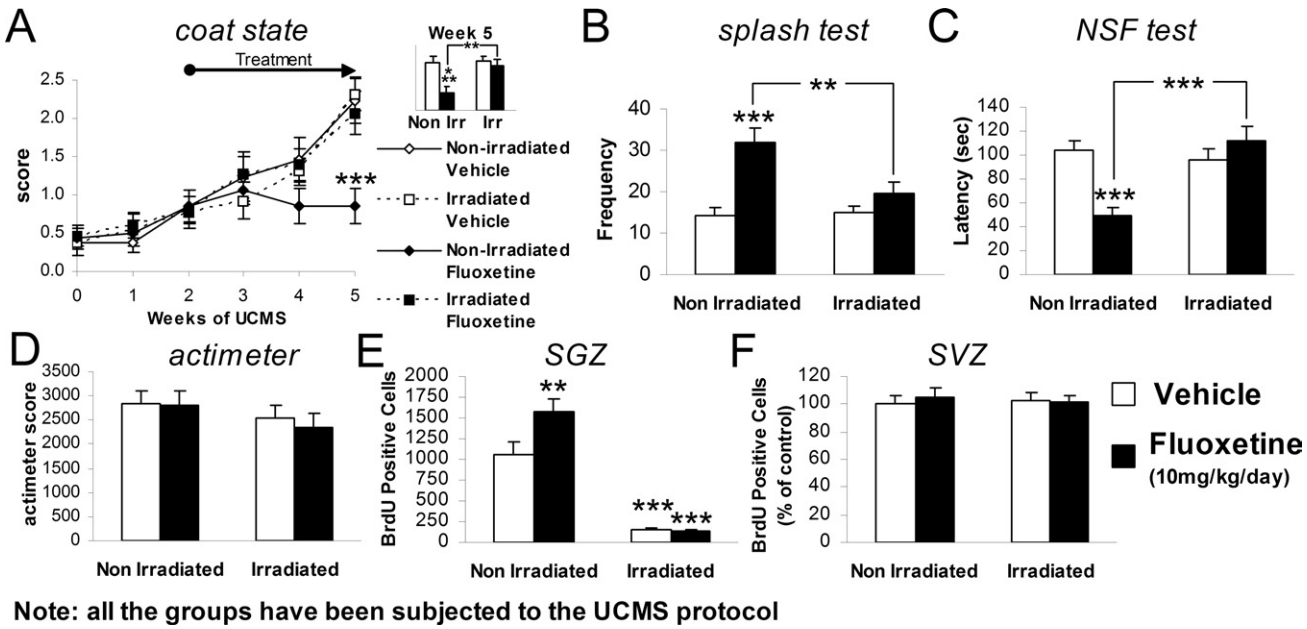

Figure 3. Hippocampal x-irradiation prevented the effects of fluoxetine (10 mg/kg/day, IP) on behavior and cell proliferation after the UCMS regimen. **(A)** Fluoxetine treatment significantly reduced the UCMS-induced deterioration of the coat state in non-irradiated mice. This fluoxetine effect was abolished in irradiated mice. Detail of the results after 5-week UCMS is illustrated above legend, $n = 13–15$ mice/group. **(B)** In the splash test, grooming behavior was increased by fluoxetine treatment only in the non-irradiated mice, $n = 13–14$ mice/group. **(C)** Fluoxetine treatment reduced the latency in the NSF test. Irradiation prevented this effect, $n = 13–14$ mice/group. **(D)** Locomotor activity in the actimeter was not affected by fluoxetine or the x-ray procedure, $n = 13–14$ mice/group. **(E)** Treatment with fluoxetine elicited a significant increase of the number of BrdU positive cells in the SGZ of the hippocampus. Irradiation potently reduced cell proliferation in the SGZ, $n = 5–7$ mice/group. **(F)** No change in cell proliferation in the SVZ due to the fluoxetine treatment or the irradiation was found, $n = 5–7$ mice/group. Data represent mean ± SEM. ANOVA following by Fisher post hoc: $^{***}p < .001$ and $^{**}p < .01$ between line-connected groups or versus control/vehicle group; except for **(E)**: $^{***}p < .001$ and $^{**}p < .01$ versus non-irradiated/vehicle mice. Abbreviations as in Figure 2.

effect was accompanied by reduced grooming in the splash test ($p < .01$; Figure 2B) and increased latency to eat in the NSF test ($p < .05$; Figure 2C), and none of these effects were due to changes in locomotor activity (Figure 2D) or body weight (Figure 1A in Supplement 1). The UCMS decreased cell proliferation in the SGZ of non-irradiated mice ($p < .001$), whereas irradiation almost completely abolished neurogenesis in this region ($p < .001$; Figure 2E). Moreover, we found that neither stress nor irradiation impacted neurogenesis in the SVZ (Figure 2F). Ablation of hippocampal cell proliferation did not elicit any intrinsic effect in nonstressed mice and did not change the sensitivity to stress as measured by coat deterioration, grooming behavior, or latency to eat in the NSF test (Figure 2; Figure 1A in Supplement 1). Together these data indicate that, although chronic stress reduces hippocampal neurogenesis, this effect does not cause or contribute to the changes in behavior observed in UCMS-subjected mice. Additionally, complete loss of hippocampal neurogenesis did not accelerate or amplify the behavioral modifications induced by the UCMS procedure.

Disruption of Hippocampal Neurogenesis Prevents the Action of Monoaminergic ADs

The idea that hippocampal neurogenesis might not be essential to the pathogenesis of depression does not exclude that it is involved in the action of ADs. We have previously shown that ablation of hippocampal neurogenesis prevented the AD activity of fluoxetine (22). Here, we have sought to determine whether neurogenesis is a conserved feature in the efficacy of ADs by comparing, in irradiated or sham mice, the effects of drugs with distinct pharmacological targets: two compounds with monoaminergic mechanisms (the SSRI fluoxetine and the tricyclic imipramine) and two compounds targeting the HPA axis (a CRF_1 and a V_{1b} antagonist).

We first aimed at confirming the involvement of neurogenesis in the effects of fluoxetine (22). Sham and irradiated mice were all exposed to a 5-week UCMS and, starting the third week, were administered either fluoxetine (10 mg/kg/day) or vehicle for 4 weeks. In non-irradiated mice, fluoxetine counteracted stress-induced effects on coat state ($p < .001$; Figure 3A), grooming frequency in the splash test ($p < .001$; Figure 3B), and latency to eat in the NSF ($p < .001$; Figure 3C). These effects were completely abolished by hippocampal irradiation, because no effect of fluoxetine was seen in irradiated mice. These results cannot be explained by a change in body weight (Figure 1B in Supplement 1) or locomotor activity (Figure 3D). Furthermore, fluoxetine induced an increase of cell proliferation in the SGZ in non-irradiated mice ($p < .01$), whereas irradiation caused a profound loss of cell proliferation in the SGZ of both vehicle and fluoxetine groups ($p < .001$; Figure 3E). These effects were restricted to the hippocampus, because neither irradiation nor fluoxetine treatment altered cell proliferation in the SVZ (Figure 3F). Thus, hippocampal irradiation prevented the effects of the SSRI fluoxetine, confirming the suggestion that hippocampal neurogenesis is required for its efficacy.

To determine whether hippocampal neurogenesis is similarly required for the efficacy of other monoaminergic ADs, we repeated the aforementioned series of experiments with the tricyclic AD imipramine (20 mg/kg/day). Chronic imipramine treatment reduced the UCMS-induced deterioration of the coat state ($p < .01$) and increased grooming frequency in non-

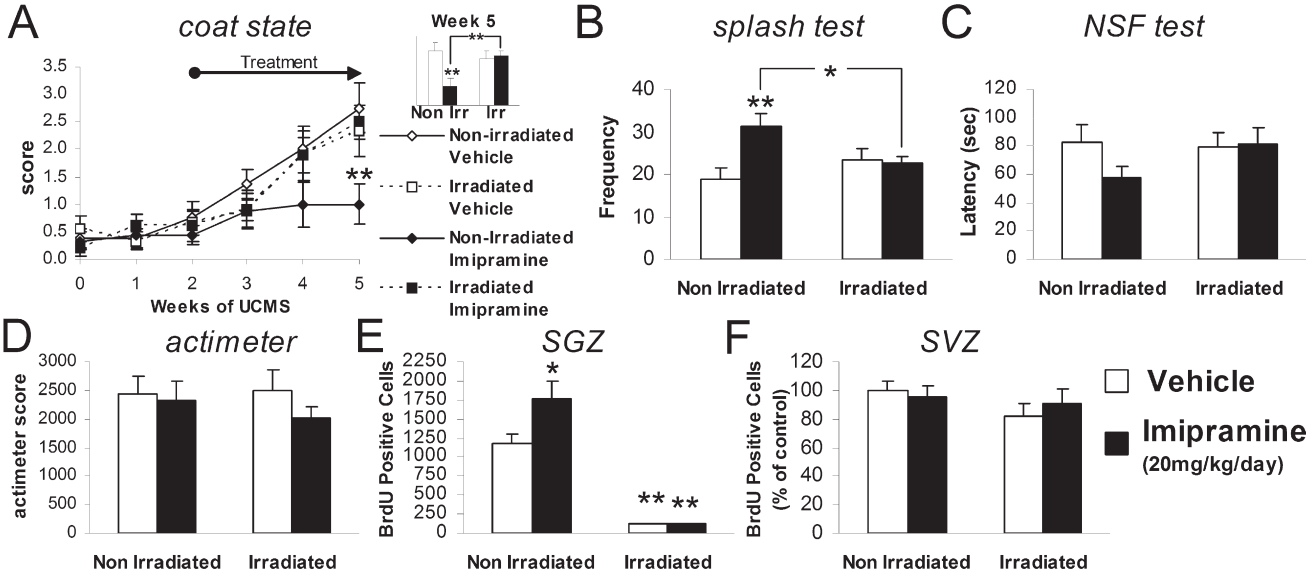

Figure 4. Hippocampal x-irradiation prevented the effects of imipramine (20 mg/kg/day, IP) on behavior and cell proliferation after the UCMS regimen. (**A**) Imipramine treatment significantly reduced in non-irradiated mice the deterioration of the coat state. This imipramine effect was abolished in irradiated mice. Detail of the results after 5-week UCMS is illustrated above legend, $n = 8–10$ mice/group. (**B**) In the splash test, imipramine treatment improved grooming behavior only in the non-irradiated mice, $n = 8–10$ mice/group. (**C**) No significant difference was found in the NSF test. $n = 8–10$ mice/group. (**D**) Locomotor activity in the actimeter was not affected by imipramine or the x-ray procedure, $n = 8–10$ mice/group. (**E**) Treatment with imipramine permitted a significant recovery in the number of BrdU positive cells in the SGZ of the hippocampus. Irradiation potently reduced cell proliferation in the SGZ, $n = 5–6$ mice/group. (**F**) No change in cell proliferation in the SVZ due to imipramine or irradiation was found, $n = 5–6$ mice/group. Data represent mean ± SEM. ANOVA following by Fisher post hoc: $^{**}p < .01$ and $^{*}p < .05$ between line-connected groups or versus control/vehicle group; except for (**E**): $^{**}p < .01$ and $^{*}p < .05$ versus non-irradiated/vehicle mice. Abbreviations as in Figure 2.

irradiated mice ($p < .05$) but not in irradiated mice (Figures 4A and 4B). There was no significant reduction of latency to eat in the NSF test (Figure 4C). Finally, imipramine increased cell proliferation in the SGZ ($p < .05$), an effect that was absent in irradiated mice (Figure 4E). Again, no effect of drug treatment was found on body weight, activity, or cell proliferation in the SVZ (Figures 4D–4F; and Figure 1C in Supplement 1). Because both monoaminergic ADs reversed the UCMS-induced effects on coat deterioration and behavioral alterations, and these effects were completely abolished by hippocampal irradiation, our results suggest that hippocampal neurogenesis is necessary for the AD action of monoaminergic compounds.

Disruption of Hippocampal Neurogenesis Does Not Prevent AD-Like Actions of CRF_1 and V_{1b} Antagonists

Because CRF_1 and V_{1b} antagonists also cause an increase in hippocampal neurogenesis (16), we investigated whether neurogenesis is required for the effects of the CRF_1 antagonist SSR125543 (20 mg/kg/day) and the V_{1b} antagonist SSR149415 (20 mg/kg/day). In non-irradiated mice, we found that SSR125543 (Figure 5) and SSR149415 (Figure 6) counteracted the deleterious effects of UCMS on coat state ($p < .05$ and $p < .01$, respectively), in the splash test ($p < .05$ and $p < .01$, respectively), and in the NSF test for the SSR125543 ($p < .05$), although a strong trend for a treatment effect was found with the SSR149415 [$F(1,37) = 3.86$, $p = 0.057$]. The effects of these compounds could not be explained by a change in locomotion (Figures 5D and 6D) or body weight (Figures 1D and 1E in Supplement 1). Both SSR125543 and SSR149415 increased hippocampal cell proliferation ($p < .001$; Figures 5E and 6E) without eliciting any such effect in the SVZ (Figures 5F and 6F). Although irradiation abolished cell proliferation in the SGZ in all mice ($p < .001$; Figures 5D and 6D), the effects of both compounds on the coat state and the splash test were intact ($p < .05$; Figures 5A and 6A) and no different from their effect in non-irradiated mice, suggesting that these effects are independent of hippocampal neurogenesis. Only one aspect in the reversal of UCMS-elicited modifications was disrupted by irradiation: the SSR125543 effect in the NSF test (Figure 5C). This could suggest that the coat state and the splash test on the one hand and the NSF test on the other reflect two different features of the depressive-like state induced by UCMS. Contrary to the coat state score and the splash test, the NSF test, which was firstly developed to characterize anxiolytic properties (38), might detect UCMS-induced anxiety-like rather than depressive-like behaviors and, on the basis of the novel environment exposure, be more sensitive to hippocampal dysfunction. Nevertheless, there was a significant main effect of drug treatment [$F(1,39) = 4.8$, $p < .05$] without an interaction in the NSF test, and irradiated/SSR125543-treated mice displayed no significant difference compared with non-irradiated/SSR125543-treated mice (Figure 5C). Finally, most AD-like effects of CRF_1 and V_{1b} antagonists arose despite the suppression of hippocampal neurogenesis.

Discussion

The UCMS-induced behavioral changes were reversed by several compounds endowed with AD-like properties, such as fluoxetine and imipramine, as well as a CRF_1 antagonist (SSR125543) and a V_{1b} antagonist (SSR149415). The x-irradiation of the hippocampus had no effect per se in the UCMS procedure, suggesting that a loss of hippocampal neurogenesis does not

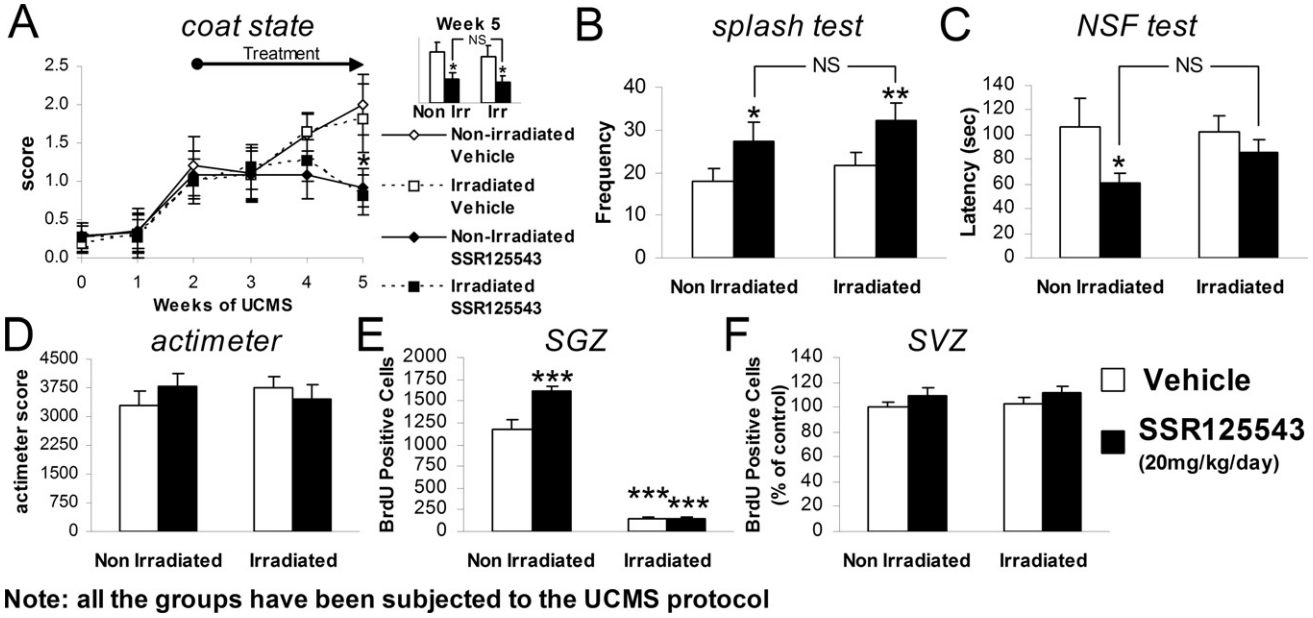

Figure 5. Hippocampal x-iradiation did not prevent several antidepressant-like effects of the corticotropin-releasing factor 1 (CRF_1) receptor antagonist, SSR125543 (20 mg/kg/day, IP), on behavior and cell proliferation after the UCMS regimen. (**A**) The SSR125543 treatment elicited a significant decrease in the deterioration of the state of the coat in non-irradiated and irradiated mice. The effect of SSR125543 was not blocked by the x-ray procedure. Detail of the results after 5-week UCMS is illustrated above legend, $n = 10–11$ mice/group. (**B**) After 5-week UCMS, the frequency of grooming was significantly increased by SSR125543 treatment in non-irradiated mice as well as in irradiated mice, $n = 10–11$ mice/group. (**C**) In the NSF test, the latency to chew the pellet was reduced by SSR125543 treatment in non-irradiated mice but not in irradiated mice. Nevertheless, the latency was not different when comparing non-irradiated/ SSR125543 mice versus irradiated/SSR125543 mice, $n = 10–11$ mice/group. (**D**) Locomotor activity in the actimeter was not affected by SSR125543 or the x-ray procedure, $n = 8–9$ mice/group. (**E**) The SSR125543 treatment induced a significant recovery in the number of BrdU positive cells in the SGZ of the hippocampus. Irradiation strongly reduced cell proliferation in the SGZ, $n = 6$ mice/group. (**F**) No change in cell proliferation in the SVZ due to SSR125543 or irradiation was found, $n = 6$ mice/group. Data represent mean ± SEM. ANOVA following by Fisher post hoc: $^{**}p < .01$; $^{*}p < .05$ and p = non-significant, between line-connected groups or versus control/vehicle group; except for (**E**): $^{***}p < .001$ versus non-irradiated/vehicle mice. Abbreviations as in Figure 2.

induce a depressive-like behavior and does not worsen the deteriorations induced by the UCMS. Nevertheless, irradiation completely abolished the AD-like effects of drugs that act via a monoaminergic mechanism (fluoxetine and imipramine). In contrast, several AD-like effects of SSR125543 and SSR149415 were maintained despite irradiation. Therefore, our results support that, although hippocampal neurogenesis is required for the AD efficacy of monoaminergic drugs, it is nonessential for some AD-like effects of compounds that modulate the HPA axis. Because several AD-like effects were seen in irradiated mice, hippocampal neurogenesis might not be seen as the final neurobiological process by which ADs reverse detrimental effects of stress.

Nevertheless, some concerns need to be considered for further interpretations of the results. First, it cannot be excluded that the coat state evaluation and splash test, unlike the NSF test, are measures that are not linked to the human disease; however, this argument is questionable, because coat state evaluation is the most prevalent, reliable, and well-validated measure used in this mouse model of depression (16,22,29,30,35–37), whereas the NSF test pre-eminently examines anxiety-related behaviors (38,39). Second, because brain penetration was demonstrated for both SSR125543 and SSR149415 (40–42), these compounds could induce their AD-like effects centrally and the HPA axis could be unaffected in our model. However, we recently found that UCMS induces changes in plasmatic corticosterone that are reversed by fluoxetine and SSR125543, highlighting the implication of the HPA axis (35). Third, another confounding point might arise concerning whether irradiation induces HPA alterations, but we previously dismissed this possibility by showing control-like HPA axis function after irradiation in both basal and stress conditions (22). Fourth, we also found that irradiation induces no modification in several other behavioral- and brain-related functions (22), although more subtle effects of irradiation could exist. Moreover, whereas irradiation induces transient inflammation, mice were allowed to recover for 5 weeks before initiating the UCMS regimen and 10 weeks before testing, a period after which the inflammatory effects of hippocampal irradiation are largely completed (43). Fifth, in contrast to our results, two studies (44,45) recently reported that fluoxetine's effects in bio-assays are independent of hippocampal neurogenesis in BALB/c mice, and that fluoxetine does not increase cell proliferation or neurogenesis in the dentate gyrus. Nevertheless, they tested fluoxetine's action in normal "non-depressed" mice, whereas we used a chronic and naturalistic model of depression in which fluoxetine's effects are examined in pathologic-like rather than baseline conditions. Considering that ADs are devoid of mood-changing effects in normal individuals, paradigms elaborated to test the action of ADs in normal "non-depressed" mice could engage different neurobiological mechanisms that are irrelevant to clinical remission. Moreover, their results are also inconsistent with several other studies using mouse models based on chronic application of stressors (16,22,46). Indeed, we have previously shown that hippocampal x-irradiation prevented fluoxetine-induced AD effects in 129SvEv as well as BALB/c strains (22), and the UCMS procedure was shown to decrease hippocampal neurogenesis in BALB/c mice (16,46). Finally, Alonso *et al.* (16) previously demonstrated that chronic fluoxetine was able to increase SGZ cell proliferation in UCMS-treated as well as control BALB/c mice.

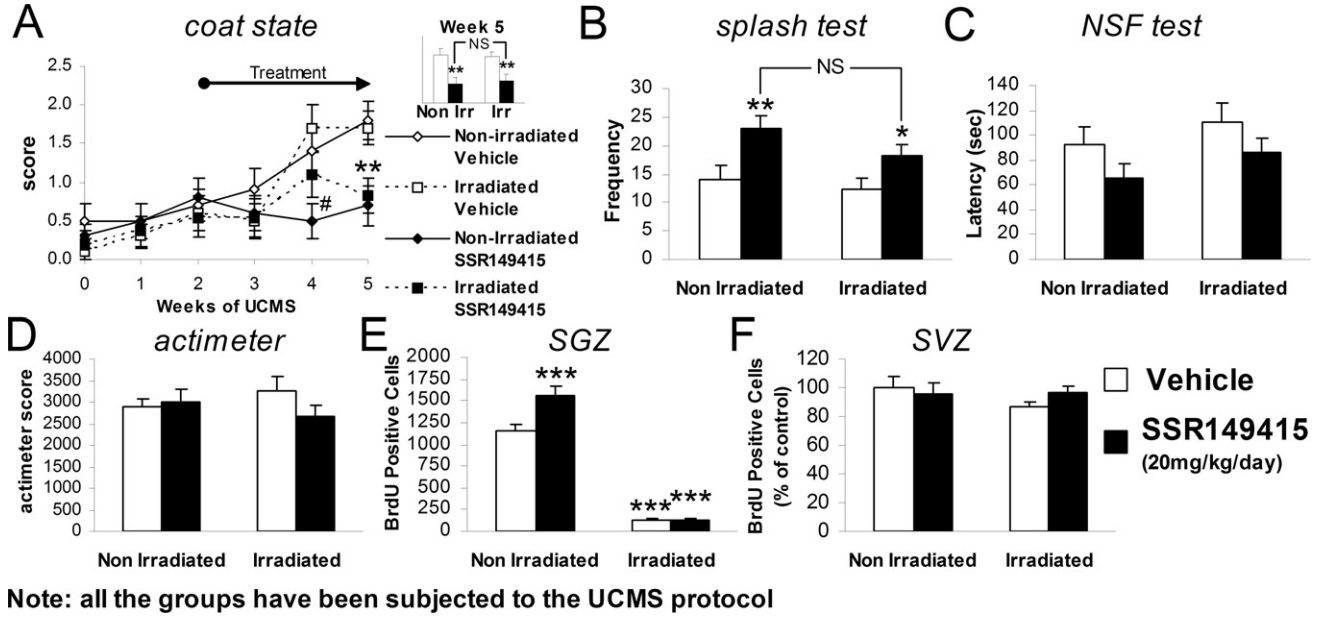

Figure 6. Hippocampal x-irradiation did not prevent the antidepressant-like effects of the vasopressin 1b (V_{1b}) receptor antagonist, SSR149415 (20 mg/kg/day, IP), on behavior and cell proliferation after the UCMS regimen. (**A**) The SSR149415 treatment significantly reduced the deterioration of the state of the coat in non-irradiated and irradiated mice. The effect of SSR149415 was not blocked by the x-ray procedure. The effect of SSR125543 was not blocked by the x-ray procedure. Detail of the results after 5-week UCMS is illustrated above legend, $n = 10–11$ mice/group. (**B**) Mice treated with SSR149415 displayed more grooming behaviors than vehicle mice in the splash test. This effect was not abolished by irradiation, $n = 10–11$ mice/group. (**C**) No significant difference was found in the NSF test. $n = 10–11$ mice/group. (**D**) Locomotor activity in the actimeter was not affected by SSR149415 or the x-ray procedure, $n = 9–10$ mice/group. (**E**) The SSR149415 treatment induced a significant recovery of BrdU positive cells in the SGZ of the hippocampus. Irradiation caused a profound loss of cell proliferation in the SGZ, $n = 6–7$ mice/group. (**F**) No change in cell proliferation in the SVZ due to SSR149415 or irradiation was found, $n = 6–7$ mice/group. Data represent mean ± SEM. ANOVA following by Fisher post hoc: $^{\#}p < .05$ for the non-irradiated/SSR149415 mice versus the control non-irradiated/vehicle mice. $^{**}p < .01$; $^{*}p < .05$ and p = non-significant between line-connected groups or versus control/vehicle group; excepted for (**E**): $^{***}p < .001$ versus non-irradiated/vehicle mice. Abbreviations as in Figure 2.

The fact that x-ray did not induce any "depressive-like" effects in nonstressed mice, as did a 5-week UCMS regimen, suggests that a decrease of hippocampal neurogenesis might not be causal in the pathogenesis of depression. A previous study showed that reduction of cell proliferation in the DG does not correlate with the development of learned helplessness (13); however, the limitation of this study is that the paradigm used had a short duration (approximately 10 days). Because new neurons take weeks to connect to their appropriate targets, 10 days of learned helplessness might be insufficient to elicit neurogenesis-related changes in hippocampal function, such as those thought to occur during depression. Not only did the stress procedure in our study last for 5 weeks but we also found that neurogenesis-deficient mice responded normally to the UCMS procedure. These findings are in accordance with the notion that the hippocampus is not the driver of affective disorders in humans (47) but do not exclude its contribution to AD-related effects that can restore normal brain functioning.

Initial studies showing that chronic AD treatments increase hippocampal neurogenesis have described two different patterns of action: compounds such as fluoxetine increased neurogenesis in both stressed and nonstressed mice, although conflicting results occurred with nonstressed mice (16,44,45); and CRF_1 and V_{1b} antagonists counteracted stress-induced deficits in hippocampal neurogenesis without stimulating this process per se (16). The decrease in hippocampal neurogenesis induced by stress is mediated by the release of adrenal hormones such as GCs, because removal of the adrenal glands abolishes stress-induced decrease of cell proliferation (48). The CRF_1 antagonists are known to cause a reduction in CRF-mediated activation of the HPA axis in response to stress, thus reducing the release of GCs (40,49,50). Similarly, the V_{1b} antagonist SSR149415 has been shown to inhibit the elevation in plasma corticotropin observed after restraint stress (51), suggesting that this drug might also be able to block stress-induced increase of GCs. Thus, it is possible that SSR125543 and SSR149415 increase hippocampal neurogenesis under stressful conditions by preventing a GC-induced decrease rather than stimulating neurogenesis per se.

A potential explanation for our results would be that, whereas monoaminergic drugs act via a hippocampal-dependent mechanism, another mechanism might underlie the action of CRF_1 and V_{1b} antagonists. Notably, depression is associated with abnormalities in HPA axis function, and AD effects have been suggested to occur via a normalization of these abnormalities (2,52–54). For example, monoaminergic ADs inhibit HPA axis activity by increasing GC receptor levels, thereby leading to an enhancement in GC receptor-mediated feedback inhibition (55) that might involve the hippocampus. Thus, one can propose that deficits observed in depressed patients or in stressed rodents might be related to GC-induced structural damage in the brain. Because the hippocampus exerts a negative feedback on the HPA axis that can be altered by chronic stress (4), it is conceivable that during cases of excess stress, hippocampal dysfunction might reduce this negative feedback, thus exacerbating GC release and accentuating brain damage and hippocampal atrophy. Because monoaminergic ADs do not directly or initially target the HPA axis, they might interrupt this cycle by stimulating neurogenesis; indeed, this might compensate for atrophy or neuronal loss and allow for restoration of normal hippocampal function, including inhibition of the HPA axis. By contrast, CRF_1

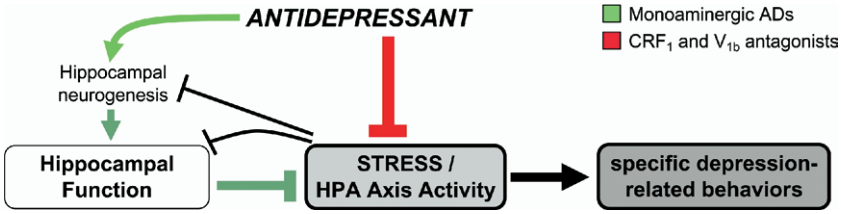

Figure 7. Antidepressant (AD) drug effects can be achieved by neurogenesis-dependent and -independent mechanisms. Stress and persistent hypothalamo-pituitary-adrenal (HPA)-axis activity can elicit changes in behavior and reduce hippocampal neurogenesis, possibly via increased glucocorticoid release. Although the hippocampus participates in the negative regulation of stress responses, the negative effects of stress on behavior and neurogenesis are not causally related. Therefore, the antidepressant effect of drugs that act directly on the HPA-axis could not require hippocampal neurogenesis. However, monoaminergic drugs might use neurogenesis to counteract the effects of stress by enhancing negative feedback provided by the hippocampus. Symbols: arrows = stimulation; bared line = inhibition. V_{1b}, vasopressin 1b; CRF_1, corticotropin-releasing factor 1.

and V_{1b} receptor antagonists might act directly on the HPA axis to restore normal GC levels, thereby counteracting such detrimental effects as hippocampal atrophy. According to this interpretation, without functional hippocampal neurogenesis, classical ADs might be either ineffective or would require a longer time to be effective, whereas the action of HPA-acting drugs would persist. This hypothesis, as summarized in Figure 7, suggests the involvement of new hippocampal neurons in the inhibitory control of the hippocampus on the HPA axis. Although this model is largely a theoretical interpretation, it was previously shown that CRF_1 and V_{1b} antagonists do not elicit significant changes in serotonin or norepinephrine levels or bind to monoaminergic receptors (unpublished results); therefore, they clearly act via different mechanisms than SSRIs and tricyclics.

In conclusion, our data suggest that hippocampal neurogenesis might not be a key factor in the pathophysiology of depression, because disruption of hippocampal neurogenesis does not induce depressive-like behaviors or alter sensitivity to chronic stress. In addition, we confirm that hippocampal neurogenesis is required for the action of monoaminergic ADs in a rodent model of depression. Finally, we show that a "therapeutic" effect can be achieved, even with ablation of hippocampal neurogenesis, by directly targeting the HPA axis and related neuropeptides.

We thank Ipek Yalcin (CNRS, Strasbourg, France) and Berardo J. Rodriguez (University of Antioquia, Medellin, Colombia) for their beneficial help.

The authors A. Surget, M. Saxe, S. Leman, Y. Ibarguen-Vargas, and S. Chalon report no biomedical financial interests or potential conflicts of interests. Dr. R. Hen receives compensation as a consultant for BrainCells, PsychoGenics, Memory Pharmaceuticals, Servier, Astra Zeneca, and Lundbeck in relation to the generation of novel antidepressants. Dr. C. Belzung receives compensation as a consultant for Takeda. Dr. G. Griebel is an employee of Sanofi-Aventis company.

Supplementary material cited in this article is available online.

1. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, *et al.* (2003): Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science* 301:386–389.
2. Heuser I (1998): Anna-Monika-Prize paper. The hypothalamic-pituitary-adrenal system in depression. *Pharmacopsychiatry* 31:10–13.
3. Herman JP, Schafer MK, Young EA, Thompson R, Douglass J, Akil H, *et al.* (1989): Evidence for hippocampal regulation of neuroendocrine neurons of the hypothalamo-pituitary-adrenocortical axis. *J Neurosci* 9:3072–3082.
4. Mizoguchi K, Ishige A, Aburada M, Tabira T (2003): Chronic stress attenuates glucocorticoid negative feedback: Involvement of the prefrontal cortex and hippocampus. *Neuroscience* 119:887–897.
5. Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS (2000): Hippocampal volume reduction in major depression. *Am J Psychiatry* 157:115–118.
6. Frodl T, Meisenzahl EM, Zetzsche T, Hohne T, Banac S, Schorr C, *et al.* (2004): Hippocampal and amygdala changes in patients with major depressive disorder and healthy controls during a 1-year follow-up. *J Clin Psychiatry* 65:492–499.
7. MacQueen GM, Campbell S, McEwen BS, Macdonald K, Amano S, Joffe RT, *et al.* (2003): Course of illness, hippocampal function, and hippocampal volume in major depression. *Proc Natl Acad Sci U S A* 100:1387–1392.
8. Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW (1996): Hippocampal atrophy in recurrent major depression. *Proc Natl Acad Sci U S A* 93:3908–3913.
9. Campbell S, MacQueen G (2006): An update on regional brain volume differences associated with mood disorders. *Curr Opin Psychiatry* 19: 25–33.
10. Duman RS, Malberg J, Thome J (1999): Neural plasticity to stress and antidepressant treatment. *Biol Psychiatry* 46:1181–1191.
11. Sapolsky RM (2000): Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch Gen Psychiatry* 57:925–935.
12. Gould E, McEwen BS, Tanapat P, Galea LA, Fuchs E (1997): Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *J Neurosci* 17:2492–2498.
13. Vollmayr B, Simonis C, Weber S, Gass P, Henn F (2003): Reduced cell proliferation in the dentate gyrus is not correlated with the development of learned helplessness. *Biol Psychiatry* 54:1035–1040.
14. Malberg JE, Duman RS (2003): Cell proliferation in adult hippocampus is decreased by inescapable stress: Reversal by fluoxetine treatment. *Neuropsychopharmacology* 28:1562–1571.
15. Pham K, Nacher J, Hof PR, McEwen BS (2003): Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. *Eur J Neurosci* 17:879–886.
16. Alonso R, Griebel G, Pavone G, Stemmelin J, Le Fur G, Soubrie P (2004): Blockade of CRF(1) or V(1b) receptors reverses stress-induced suppression of neurogenesis in a mouse model of depression. *Mol Psychiatry* 9:278–286.
17. Czeh B, Michaelis T, Watanabe T, Frahm J, de Biurrun G, van Kampen M, *et al.* (2001): Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. *Proc Natl Acad Sci U S A* 98:12796–12801.
18. Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E (1998): Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. *Proc Natl Acad Sci U S A* 95:3168–3171.
19. McEwen BS (2001): Plasticity of the hippocampus: Adaptation to chronic stress and allostatic load. *Ann N Y Acad Sci* 933:265–277.
20. Sapolsky RM (2003): Stress and plasticity in the limbic system. *Neurochem Res* 28:1735–1742.
21. Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000): Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci* 20:9104–9110.
22. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, *et al.* (2003): Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 301:805–809.

23. van der Hart MG, Czeh B, de Biurrun G, Michaelis T, Watanabe T, Natt O, *et al.* (2002): Substance P receptor antagonist and clomipramine prevent stress-induced alterations in cerebral metabolites, cytogenesis in the dentate gyrus and hippocampal volume. *Mol Psychiatry* 7:933–941.
24. Jiang W, Zhang Y, Xiao L, Van Cleemput J, Ji SP, Bai G, *et al.* (2005): Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. *J Clin Invest* 115:3104–3116.
25. Rupniak NM (2002): Elucidating the antidepressant actions of substance P (NK1 receptor) antagonists. *Curr Opin Investig Drugs* 3:257–261.
26. Yoshimizu T, Chaki S (2004): Increased cell proliferation in the adult mouse hippocampus following chronic administration of group II metabotropic glutamate receptor antagonist, MGS0039. *Biochem Biophys Res Commun* 315:493–496.
27. McEwen BS, Magarinos AM, Reagan LP (2002): Structural plasticity and tianeptine: Cellular and molecular targets. *Eur Psychiatry* 17(suppl 3): 318–330.
28. Louis C, Cohen C, Depoortere R, Griebel G (2006): Antidepressant-like effects of the corticotropin-releasing factor 1 receptor antagonist, SSR125543, and the vasopressin 1b receptor antagonist, SSR149415, in a DRL-72 s schedule in the rat. *Neuropsychopharmacology* 31:2180–2187.
29. Griebel G, Simiand J, Serradeil-Le Gal C, Wagnon J, Pascal M, Scatton B, *et al.* (2002): Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V1b receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. *Proc Natl Acad Sci U S A* 99:6370–6375.
30. Griebel G, Simiand J, Steinberg R, Jung M, Gully D, Roger P, *et al.* (2002): 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4- methylphenyl)ethyl]5-methyl-N-(2-propynyl)-1, 3-thiazol-2-amine hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing factor(1) receptor antagonist. II. Characterization in rodent models of stress-related disorders. *J Pharmacol Exp Ther* 301: 333–345.
31. Zobel AW, Nickel T, Kunzel HE, Ackl N, Sonntag A, Ising M, *et al.* (2000): Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: The first 20 patients treated. *J Psychiatr Res* 34:171–181.
32. Kunzel HE, Zobel AW, Nickel T, Ackl N, Uhr M, Sonntag A, *et al.* (2003): Treatment of depression with the CRH-1-receptor antagonist R121919: Endocrine changes and side effects. *J Psychiatr Res* 37:525–533.
33. Ising M, Zimmermann US, Kunzel HE, Uhr M, Foster AC, Learned-Coughlin SM, *et al.* (2007): High-affinity CRF1 receptor antagonist NBI-34041: Preclinical and clinical data suggest safety and efficacy in attenuating elevated stress response. *Neuropsychopharmacology* 32:1941–1949.
34. Willner P, Muscat R, Papp M (1992): Chronic mild stress-induced anhedonia: A realistic animal model of depression. *Neurosci Biobehav Rev* 16:525–534.
35. Belzung C, Surget A (2008): Unpredictable chronic mild stress in mice. In: Kalueff AV, LaPorte JL, editors. *Experimental Models in Neurobehavioral Research*. New York: Nova Science.
36. Ducottet C, Belzung C (2005): Correlations between behaviours in the elevated plus-maze and sensitivity to unpredictable subchronic mild stress: Evidence from inbred strains of mice. *Behav Brain Res* 156:153–162.
37. Yalcin I, Aksu F, Belzung C (2005): Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol. *Eur J Pharmacol* 514:165–174.
38. Soubrie P, Kulkarni S, Simon P, Boissier JR (1975): [Effects of antianxiety drugs on the food intake in trained and untrained rats and mice (author's transl)]. *Psychopharmacologia* 45:203–210.
39. Dulawa SC, Hen R (2005): Recent advances in animal models of chronic antidepressant effects: The novelty-induced hypophagia test. *Neurosci Biobehav Rev* 29:771–783.
40. Gully D, Geslin M, Serva L, Fontaine E, Roger P, Lair C, *et al.* (2002): 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4- methylphenyl)ethyl]5-methyl-N-(2-propynyl)-1,3-thiazol-2-amine hydrochloride (SSR125543A): A potent and selective corticotrophin-releasing factor(1) receptor antagonist. I. Biochemical and pharmacological characterization. *J Pharmacol Exp Ther* 301:322–332.
41. Griebel G, Simiand J, Stemmelin J, Serradeil-Le Gal C, Wagnon J, Steinberg R, *et al.* (2004): Anxiolytic- and antidepressant-like effects of non-peptide vasopressin V1b receptor antagonists. *Act Chim Ther* 30:37–54.
42. Hodgson RA, Higgins GA, Guthrie DH, Lu SX, Pond AJ, Mullins DE, *et al.* (2007): Comparison of the V1b antagonist, SSR149415, and the CRF1 antagonist, CP-154,526, in rodent models of anxiety and depression. *Pharmacol Biochem Behav* 86:431–440.
43. Meshi D, Drew MR, Saxe M, Ansorge MS, David D, Santarelli L, *et al.* (2006): Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. *Nat Neurosci* 9:729–731.
44. Holick KA, Lee DC, Hen R, Dulawa SC (2008): Behavioral effects of chronic fluoxetine in BALB/cJ mice do not require adult hippocampal neurogenesis or the serotonin 1A receptor. *Neuropsychopharmacology* 33:406–417.
45. Huang GJ, Bannerman D, Flint J (2008): Chronic fluoxetine treatment alters behavior, but not adult hippocampal neurogenesis, in BALB/cJ mice. *Mol Psychiatry* 13:119–121.
46. Mineur YS, Belzung C, Crusio WE (2007): Functional implications of decreases in neurogenesis following chronic mild stress in mice. *Neuroscience* 150:251–259.
47. Carlson PJ, Singh JB, Zarate CA Jr., Drevets WC, Manji HK (2006): Neural circuitry and neuroplasticity in mood disorders: Insights for novel therapeutic targets. *NeuroRx* 3:22–41.
48. Tanapat P, Hastings NB, Rydel TA, Galea LA, Gould E (2001): Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via an adrenal hormone-dependent mechanism. *J Comp Neurol* 437:496–504.
49. Lelas S, Zeller KL, Ward KA, McElroy JF (2003): The anxiolytic CRF(1) antagonist DMP696 fails to function as a discriminative stimulus and does not substitute for chlordiazepoxide in rats. *Psychopharmacology (Berl)* 166:408–415.
50. McElroy JF, Ward KA, Zeller KL, Jones KW, Gilligan PJ, He L, *et al.* (2002): The CRF(1) receptor antagonist DMP696 produces anxiolytic effects and inhibits the stress-induced hypothalamic-pituitary-adrenal axis activation without sedation or ataxia in rats. *Psychopharmacology (Berl)* 165: 86–92.
51. Serradeil-Le Gal C, Wagnon J, Simiand J, Griebel G, Lacour C, Guillon G, *et al.* (2002): Characterization of (2S,4R)-1-[5-chloro-1-[(2,4-dimethoxyphenyl)sulfonyl]-3-(2-methoxy-phenyl) −2-oxo-2,3-dihydro-1H-indol-3-yl]-4-hydroxy-N,N-dimethyl-2-pyrrolidine carboxamide (SSR149415), a selective and orally active vasopressin V1b receptor antagonist. *J Pharmacol Exp Ther* 300:1122–1130.
52. Aihara M, Ida I, Yuuki N, Oshima A, Kumano H, Takahashi K, *et al.* (2007): HPA axis dysfunction in unmedicated major depressive disorder and its normalization by pharmacotherapy correlates with alteration of neural activity in prefrontal cortex and limbic/paralimbic regions. *Psychiatry Res* 155:245–256.
53. Linkowski P, Mendlewicz J, Kerkhofs M, Leclercq R, Golstein J, Brasseur M, *et al.* (1987): 24-hour profiles of adrenocorticotropin, cortisol, and growth hormone in major depressive illness: Effect of antidepressant treatment. *J Clin Endocrinol Metab* 65:141–152.
54. Nemeroff CB, Owens MJ (2004): Pharmacologic differences among the SSRIs: Focus on monoamine transporters and the HPA axis. *CNS Spectr* 9:23–31.
55. Pariante CM, Miller AH (2001): Glucocorticoid receptors in major depression: Relevance to pathophysiology and treatment. *Biol Psychiatry* 49: 391–404.

Article 5 - Corticolimbic transcriptome changes are state-dependent and region-specific in a rodent model of depression and of antidepressant reversal.

Surget et coll., *Neuropsychopharmacology*, 2008, sous presse

Neuropsychopharmacology (2008), 1–18

www.neuropsychopharmacology.org

Corticolimbic Transcriptome Changes are State-Dependent and Region-Specific in a Rodent Model of Depression and of Antidepressant Reversal

Alexandre Surget[1], Yingjie Wang[2], Samuel Leman[1], Yadira Ibarguen-Vargas[1], Nicole Edgar[2,3], Guy Griebel[4], Catherine Belzung[1] and Etienne Sibille*[,2,3]

[1]U930 FRE CNRS 2448, INSERM and Université François Rabelais, Tours, France; [2]Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA; [3]Center For Neuroscience, University of Pittsburgh, Pittsburgh, PA, USA; [4]CNS Research Department, Sanofi-Aventis, Bagneux, France

Gene microarrays may enable the elucidation of neurobiological changes underlying the pathophysiology and treatment of major depression. However, previous studies of antidepressant treatments were performed in healthy normal rather than 'depressed' animals. Since antidepressants are devoid of mood-changing effects in normal individuals, the clinically relevant rodent transcriptional changes could remain undetected. We investigated antidepressant-related transcriptome changes in a corticolimbic network of mood regulation in the context of the unpredictable chronic mild stress (UCMS), a naturalistic model of depression based on socio-environmental stressors. Mice subjected to a 7-week UCMS displayed a progressive coat state deterioration, reduced weight gain, and increased agonistic and emotion-related behaviors. Chronic administration of an effective (fluoxetine) or putative antidepressant (corticotropin-releasing factor-1 (CRF_1) antagonist, SSR125543) reversed all physical and behavioral effects. Changes in gene expression differed among cingulate cortex (CC), amygdala (AMY) and dentate gyrus (DG) and were extensively reversed by both drugs in CC and AMY, and to a lesser extent in DG. Fluoxetine and SSR125543 also induced additional and very similar molecular profiles in UCMS-treated mice, but the effects of the same drug differed considerably between control and UCMS states. These studies established on a large-scale that the molecular impacts of antidepressants are region-specific and state-dependent, revealed common transcriptional changes downstream from different antidepressant treatments and supported CRF_1 targeting as an effective therapeutic strategy. Correlations between UCMS, drug treatments, and gene expression suggest distinct AMY neuronal and oligodendrocyte molecular phenotypes as candidate systems for mood regulation and therapeutic interventions.
Neuropsychopharmacology advance online publication, 4 June 2008; doi:10.1038/npp.2008.76

Keywords: stress; serotonin; CRF; transcriptome; amygdala; cingulate

INTRODUCTION

Although depression is a leading cause of disability worldwide and a serious health problem, mechanisms underlying its pathophysiology remain poorly characterized (Wong and Licinio, 2001; Nestler *et al*, 2002; Belmaker and Agam, 2008). Monoaminergic imbalances participate in the pathogenesis of depression, and response to antidepressant treatment is associated with increased monoaminergic neurotransmission (Duman *et al*, 1997; Manji *et al*, 2001; Nestler *et al*, 2002). However, the rapid antidepressant-induced neurochemical changes do not parallel the 2–4 weeks of drug exposure required for therapeutic effects, indicating that downstream events must occur (Duman *et al*, 1997, 1999; Manji *et al*, 2001; Nestler *et al*, 2002). Indeed, the pathophysiology and treatment of depression induce biological events of greater complexity than changes in monoamine levels, including (1) structural and ultra-structural changes, altered synaptic, glial, or neuronal density, as reported in frontal/cingulate cortex (CC), amygdala (AMY), and hippocampus (Drevets *et al*, 1997; Sheline *et al*, 1998; Rajkowska *et al*, 1999; Bowley *et al*, 2002), (2) variations in neurotrophic factors and neurotransmitters systems (Duman *et al*, 1997; Castren, 2004), and (3) altered signal-transduction pathways (Manji *et al*, 2001; Shelton, 2007), which together may affect gene regulation through transcription factors or chromatin modifications (Tsankova *et al*, 2006).

Several gene microarray studies have attempted to characterize the molecular correlates of antidepressant treatment in rodents (Landgrebe *et al*, 2002; Rausch *et al*, 2002; Newton *et al*, 2003; Drigues *et al*, 2003; Palotas *et al*,

*Correspondence: Dr E Sibille, Department of Psychiatry, University of Pittsburgh, 3811 O'Hara Street, BST W 1643, Pittsburgh, PA 15213-2593, USA, Tel: + 412 624 0804, E-mail: sibilleel@upmc.edu
Received 19 February 2008; accepted 25 April 2008

2004; Altar *et al*, 2004; Wong *et al*, 2004; Ploski *et al*, 2006; Takahashi *et al*, 2006); however, differences in treatments, exposure time, and brain regions investigated have yielded no consensus. Moreover, Conti *et al* (2007) recently reported that changes in gene transcripts after three different antidepressant modalities in normal control rats were mostly brain region-specific. Importantly, these previous array studies were performed in healthy normal rather than 'depressed' animals, with the underlying assumption that antidepressant effects may be similar to those occurring in depressive-like states.

The unpredictable chronic mild stress (UCMS) is an informative model to study depression in animals (Willner, 2005), as it mimics in a naturalistic way the role of socio-environmental stressors in precipitating a depressive pathology and the time frame of therapeutic responses to antidepressants. Specifically, the random application of various environmental and social mild stressors for several weeks results in a syndrome in mice that is reminiscent of symptoms of depression, including increased fearfulness/anxiety-like behavior, decreased consumption of palatable food, and physiological changes (Santarelli *et al*, 2003; Pothion *et al*, 2004; Mineur *et al*, 2006). Here, we investigated transcriptome changes in UCMS-treated mice, and after reversal by chronic exposure to a selective serotonin reuptake inhibitor (fluoxetine) or a corticotropin-releasing factor-1 (CRF_1) antagonist (SSR125543). CRF_1 antagonists target a non-monoaminergic system that directly affects the stress pathway and display promising antidepressant profiles in preclinical tests and clinical trials (Zobel *et al*, 2000; Griebel *et al*, 2002; Kunzel *et al*, 2003; Ising *et al*, 2007; Surget *et al*, 2008). Currently, no single brain area has been identified as a primary region affected in depression; however, meta-analyses of altered brain function point toward a corticolimbic circuitry of mood regulation that is affected in depression (Mayberg, 1997; Seminowicz *et al*, 2004). This circuitry includes areas of the prefrontal cortex, the ACC, the hippocampus, anterior thalamic nuclei and the AMY. Thus, we focused on three rodent equivalent brain areas within this corticolimbic network: CC, AMY, and dentate gyrus (DG).

Here, we confirm that the physical and behavioral effects of UCMS are effectively reversed by chronic exposure to both drugs, and demonstrate that the molecular correlates of UCMS and antidepressant treatments are state-dependent and brain region-specific, thus confirming our hypothesis that antidepressant effects in control animals do not extrapolate to 'depressive states.' Our studies revealed different sets of putative therapeutic targets for each brain area, which may reflect functional differences between areas within a neural network of mood regulation.

MATERIALS AND METHODS

Animals

Male BALB/c mice (8 weeks old) (Centre d'élevage Janvier, Le Genest Saint Isle, France) were group-housed ($n = 4$–5 per cage) under standard conditions (12/12 h light–dark cycle, $22 \pm 1^{\circ}C$, food and water *ad libitum*) for 3 weeks prior to the experiments. To avoid possible bias due to acute effect of behavioral testing on gene expression, the experiment required two sets of mice: the first one ($n = 18$–19 per group) supplied physical data and tissue samples for microarray and PCR analyses, while the second one ($n = 11$–12 per group) provided behavioral data. All animal care and treatment were in accordance with the European Community Council directive 86/609/EEC and with the Guide for Care and Use of Laboratory Animals established by the US National Institute of Health.

Drugs

The selective serotonin reuptake inhibitor fluoxetine (Eli Lilly, Indianapolis, IN, USA) and the CRF_1 antagonist SSR125543 (Sanofi-Aventis, Bagneux, France) were prepared in saline (NaCl 0.9%) containing 5% dimethyl sulfoxide and 5% cremophor EL. Vehicle, fluoxetine (20 mg/kg/day) and SSR125543 (20 mg/kg/day) were administered intraperitoneally (i.p.), based on previous experiments (Griebel *et al*, 2002; Alonso *et al*, 2004; Kulkarni and Dhir, 2007). Concentrations were adjusted to administer 10 ml/kg.

UCMS

Mice were subjected to various stressors according to a 'random' schedule for 7 weeks (Figure 1). UCMS-exposed mice were maintained under standard laboratory conditions but were isolated in small individual cages (24 cm × 11 cm × 12 cm), while non-stressed controls were group-housed in standard laboratory cages (42 cm × 28 cm × 18 cm). Drug or vehicle treatments started on day 14 and stopped the day after the end of UCMS (day 50). The stressors were: altered bedding (sawdust change, removal, or damp; substitution of sawdust with 21°C water, rat, or cat feces); cage tilting (45°) or shaking (2 × 30 sec); cage exchange (mice positioned in the empty cage of another male); induced defensive posture (repeated slight grips on the back until the mouse showed a defensive posture) and altered length and time of light–dark cycle. Body weight and coat state were assessed weekly, as markers of the progression of the UCMS-evoked syndrome. The total score resulted from the sum of scores obtained from the head, neck, dorsal coat, ventral coat, tail, forepaws, and hindpaws (0 = well-groomed, 1 = unkempt). This index has been pharmacologically validated (Griebel *et al*, 2002; Santarelli *et al*, 2003; Ducottet *et al*, 2003; Surget *et al*, 2008).

Novelty-Suppressed Feeding Test

The novelty-suppressed feeding (NSF) test was modified from Santarelli *et al* (2003) and performed after 45 days of UCMS. The testing apparatus consisted of a wooden box (33 cm × 33 cm × 30 cm) with an indirect red light. The floor was covered with 2 cm sawdust. At 12 h before the test, food was removed from the cages. At the time of testing, a single pellet of regular chow was placed on a white paper in the center of the box. An animal was placed in a corner of the box. The latency to manifestly chew the pellet was recorded for 3 min. This test induces a conflict between the drive to eat and the fear of venturing into the open center. To control for potential antidepressant effects on appetite, we

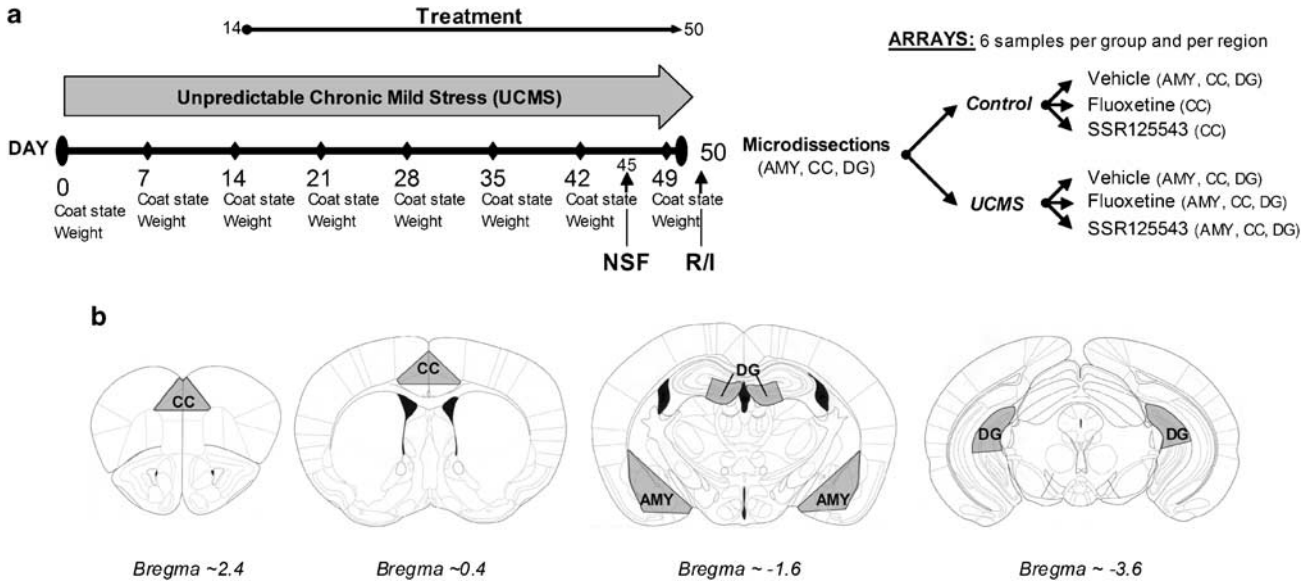

Figure 1 Experimental design. (a) Six groups of mice ($n = 18–19$ mice per group) were used depending on the environment (control/UCMS) and the treatment (vehicle/fluoxetine/SSR125543). The UCMS regimen lasted 7 weeks. The coat state was evaluated and the body weight was measured weekly by two experimenters blind to the treatment. Drug or vehicle treatments started on day 14 and continued until the end of UCMS on day 50. Fluoxetine (20 mg/kg/day), SSR125543 (20 mg/kg/day), or vehicle were administered intraperitoneally once a day to UCMS-exposed or control mice. Toward the end of the UCMS regimen, six mice per group were chosen in such a way that the mice were representative of their group in terms of physical state and behavior. Amygdala (AMY), dentate gyrus (DG), and cingulate cortex (CC) were collected for microarray analysis from these six mice per group the day after the end of UCMS. Since behavioral testing would interfere with the 'control' state of control groups, acute behavioral testing was performed on independent groups ($n = 11–12$ mice per group) subjected to the same UCMS protocol. Tests included the novelty suppressed feeding (NSF) test at day 45 and the resident/intruder (R/I) test at day 50. NSF and R/I tests were only performed once, to avoid interferences of short inter-test time periods. (b) Representative figures of the brain regions dissected (see Materials and methods). Figures are adapted from Paxinos and Franklin (2001).

measured food consumption over 5 min after mice returned to their home cage.

Resident/Intruder Test

The resident/intruder (R/I) test was modified from previously described protocols (Guillot *et al*, 1994; Mineur *et al*, 2003) and was performed after 50 days of UCMS. Control mice were single-housed 2 days before testing. UCMS-treated mice were also placed in new cages 2 days prior to testing. All mice were tested against a 6-month-old intruder. The opponent was placed into the home cage of the test animal (resident) so that mice were in opposite corners. Latencies of the first attack and number of attacks were recorded for 10 min. Attacking intruder mice were excluded.

Behavioral Data Statistical Analysis

The effects on physical and behavioral states of 7-week UCMS (environment), 5-week antidepressant treatments, and their interactions were evaluated by ANOVAs. Significant main effects or interaction were followed up with *post hoc* Tukey test (HSD for *n* different), where appropriate.

Brain Area Sampling

Brain areas were collected at the time of maximum UCMS and antidepressant effects (7 weeks of UCMS and 5 weeks of treatment) and 5 h after the last injection. To avoid experimenter-dependent bias, brain were microdissected by a single investigator. Brains were rapidly removed from CO_2-killed mice and placed in ice-cold slurry of 0.9% NaCl. Rostro-caudal sections (2 mm) were quickly obtained on a brain tissue blocker. Four consecutive sections from Bregma +2.4 to −3.6 (Paxinos and Franklin, 2001) were transferred to RNAlater (Ambion Inc., Austin, TX) and microdissected. CC was dissected from the first two sections and included part of the prelimbic cortex. AMY was obtained from the third section and DG from the third and the fourth sections. Corpus callosum and anterior commissure were collected as a combined white matter (WM) sample. Samples were stored in RNAlater at −80°C.

Microarray Samples

Total RNA was extracted with Trizol (Invitrogen, Carlsbad, CA) and assessed by chromatography (Agilent Bioanalyzer, Santa Clara, CA). Average expert scoring number (RIN) was 8.48 ± 0.03 (mean ± SEM), consistent with excellent RNA quality. Microarray samples were prepared according to the manufacturer's protocol (Affymetrix, Santa Clara, CA). In brief, 3 μg of total RNA were reverse-transcribed and converted into double-stranded cDNA. A biotinylated complementary RNA (cRNA) was transcribed *in vitro*, using an RNA polymerase T7 promoter introduced during the reverse transcription. Fragmented labeled cRNA sample (20 μg) was hybridized onto MOE430-2.0 microarrays. A high-resolution image of the hybridization pattern was obtained by laser scanning and stored in a raw file. Samples were randomly distributed at all experimental steps to avoid simultaneous processing of related samples. Probeset signal

intensities were extracted with the Affymetrix Microarray GCOS software for assessment of quality control, and with the Robust Multi-array Average algorithm (Irizarry *et al*, 2003) for data analysis. Six arrays were retained per experimental group (Figure 1), representing a total of 84 arrays. Microarray quality control parameters (Mean ± SEM) were as follows: noise (RawQ) <5 (1.83 ± 0.05), background signal <100 (250 targeted intensity for array scaling; 52.5 ± 1.3), consistent number of genes detected as present (57.2 ± 0.1), consistent scaling factors (1.29 ± 0.06), actin and GAPDH 3′/5′ signal ratios <3 (ACT, 1.27 ± 0.01, GAPDH, 0.85 ± 0.01), and consistent detection of BioB/C spiked controls. Probesets with average signal intensity below 10 in all groups were considered at background level and were removed, leaving 25 859 probesets for analysis.

Microarray Data Analysis

The goal of the analysis was to use profiles of expression over large groups of genes as an 'experimental assay' to investigate molecular effects. Therefore, thresholds for gene selection were kept at moderate stringencies ($P<0.05$, changes $>20\%$) and no correction for multiple testing were applied. This approach potentially carries a high rate of false positives at the single gene level (see discussion), but is helpful when investigating broader effects over larger sets of genes, as previously applied by us and others to the characterization of brain function (Berton *et al*, 2006; Sibille *et al*, 2007b). Here, converging results across different treatments and concordance among treatments, behaviors, and gene expression were applied to identify UCMS and drug effects. For UCMS and UCMS + drug effects, ANOVA models with UCMS exposure and drug treatments as cofactors were fitted to all transcripts in each brain region, followed by two-group analyses based on the relevant questions (ie, UCMS effect, antidepressant effect, unpaired *t*-statistics). To assess antidepressant reversal of UCMS effect, we measured the extent by which drug treatments brought transcripts back to non-stressed levels, with '0% reversal' meaning that antidepressant had no effect on reversing the UCMS effects on that gene, and '100% reversal' meaning that gene transcript levels were back to control non-stressed control levels in UCMS-/drug-treated animals. Reversal was capped at 100% for genes whose antidepressant-related changes were in opposite directions from UCMS effects (Figure 5). Pearson correlation coefficients were used to measure similarities in expression profiles between areas and/or treatments using average $\log_2$-based ratio of changes.

WM/GM ratios and Glial *vs* Neuronal Enrichment of Genes

Ratios of transcript levels between gray matter (GM) and adjacent WM samples were calculated as estimates of relative glial to neuronal origins of transcripts for every gene *within* GM samples, as previously described (Sibille *et al*, 2008). The three brain areas were compared to a unique set of seven WM samples. On the basis of $\sim 25\,000$ expressed genes, the percentage of genes displaying neuronal enrichment, glial enrichment, or expressed in both cellular populations were assessed using a 1.5-fold level of enrichment. Results were used as reference values to compare 'expected' *vs* observed distributions in groups of identified genes (see Table 1).

Functional Classification of Genes

Biological functions for lists of genes were annotated using the Database for Annotation, Visualization and Integrated Discovery (DAVID) tool (Dennis *et al*, 2003) that is freely available at http://david.abcc.ncifcrf.gov/. Over-representation of gene ontology (GO) terms was calculated using the default parameters of the 'functional annotation clustering' tool under the 'high' classification stringency set up. To reduce the redundancy of annotations, this tool groups together results in clusters of GO groups with similar annotations. *P*-values associated with each annotation are calculated with the Fisher's exact test.

Real-Time Quantitative PCR

cDNAs were obtained from the original RNA pool used for array analysis. PCR products were amplified in quadruplets on an Opticon real-time PCR machine (Bio-Rad, Hercules, CA), as previously described (Galfalvy *et al*, 2003). Primer-dimers were assessed by amplifying primers without cDNA. Primers were retained if they produced no primer-dimers or non-specific signal after 35 cycles. Results were calculated as relative intensity compared to actin.

RESULTS

UCMS-Induced Changes in Physical State are Reversed by Chronic Exposure to Fluoxetine and SSR125543

Mice were submitted to a 7-week UCMS regimen or maintained under normal non-stressful control conditions and were administered daily from the third week with the vehicle, an effective (fluoxetine), or a putative antidepressant drug (CRF_1 antagonist SSR125543) (Figure 1). UCMS exposure resulted in a progressive deterioration of the coat state (Figure 2a), which became significant across groups at the beginning of the third week ($P<0.05$), reached a plateau at the beginning of the fifth week and remained significantly elevated compared to control mice until the end of the experiment ($P<0.001$). Fluoxetine-treated UCMS mice began to show a reversal of the coat state deterioration after 3 weeks of treatment (corresponding to the fifth week of UCMS). This improvement in coat state became significant at the beginning of the fourth week of treatment ($P<0.01$, compared to UCMS vehicle mice), while fur scores of fluoxetine-treated mice were not different from control mice after 5 weeks of treatment ($P<0.001$, compared to UCMS/vehicle mice; $P>0.05$, compared to control mice).

Similarly, treatment with SSR125543 reversed the UCMS-induced deterioration of the coat state, but with a faster onset of action. The coat state of UCMS/SSR125543-treated mice did not further degrade during the first week of treatment and was significantly different from UCMS/vehicle mice after only 2 weeks of SSR125543 exposure ($P<0.001$). This improvement persisted until the end of the experiment ($P<0.001$, after 3, 4, and 5 weeks of treatment). The coat state of UCMS and SSR125543-treated mice had returned to

normal condition after 3 weeks of treatment ($P>0.05$, compared to control mice). No changes in fur coat state were observed in control mice treated with vehicle, fluoxetine, or SSR125543.

UCMS treatment also induced significant differences in body weight changes (Figure 2b). Normal weight gain was reduced by UCMS starting in the fifth week of treatment ($P<0.05$). The statistical significance of the reduced weight gain was strengthened in the last 2 weeks of the experiment ($P<0.001$). Both fluoxetine and SSR125543 treatments partially blocked the UCMS-induced reduction of body weight gain in a significant manner in the last week of the experiment, corresponding to 5 weeks of drug treatment ($P<0.05$, compared to UCMS/vehicle mice). However, contrary to the effect on coat degradation, the effects of fluoxetine and SSR125543 on weight gain followed overlapping trajectories. Both compounds had no effect on body weight gain in non-stressed control mice.

Taken together, these results demonstrated a progressive effect of UCMS on physical aspects (fur coat degradation and reduced weight gain) that were reversed by chronic exposure to an effective (fluoxetine) or putative (SSR125543) antidepressant drug. Trajectories of drug reversals of UCMS effects demonstrated delayed onsets that paralleled the clinical time frame of the therapeutic effects of antidepressant treatments, although SSR125543 displayed a shorter delay of onset on the fur index compared to fluoxetine.

UCMS-Induced Behavioral Changes are Reversed by Chronic Exposure to Fluoxetine and SSR125543

UCMS exposure has been previously shown to induce a series of behavioral changes, including increased agonistic and anxiety-/depression-like (ie, 'emotion-related') behaviors that are reminiscent of symptoms of depression in human subjects (Mineur *et al*, 2003; Santarelli *et al*, 2003). Mice used in the investigation of UCMS-induced physical changes were not submitted to behavioral tests to preserve a non-stressed state in control mice for the microarray analyses and the real-time quantitative PCR (qPCR), thus results presented here were obtained in parallel cohorts of the same age and exposed to the same UCMS protocol.

An NSF test was performed after a 45 days of UCMS to assess putative alterations in emotion-related behavior due to UCMS application. A 12-h food deprived mouse was introduced in an arena in which a pellet of regular food is placed in the center. This test induces a conflicting motivation between the drive to eat the food pellet and the fear of venturing into the center of the arena. The latency to

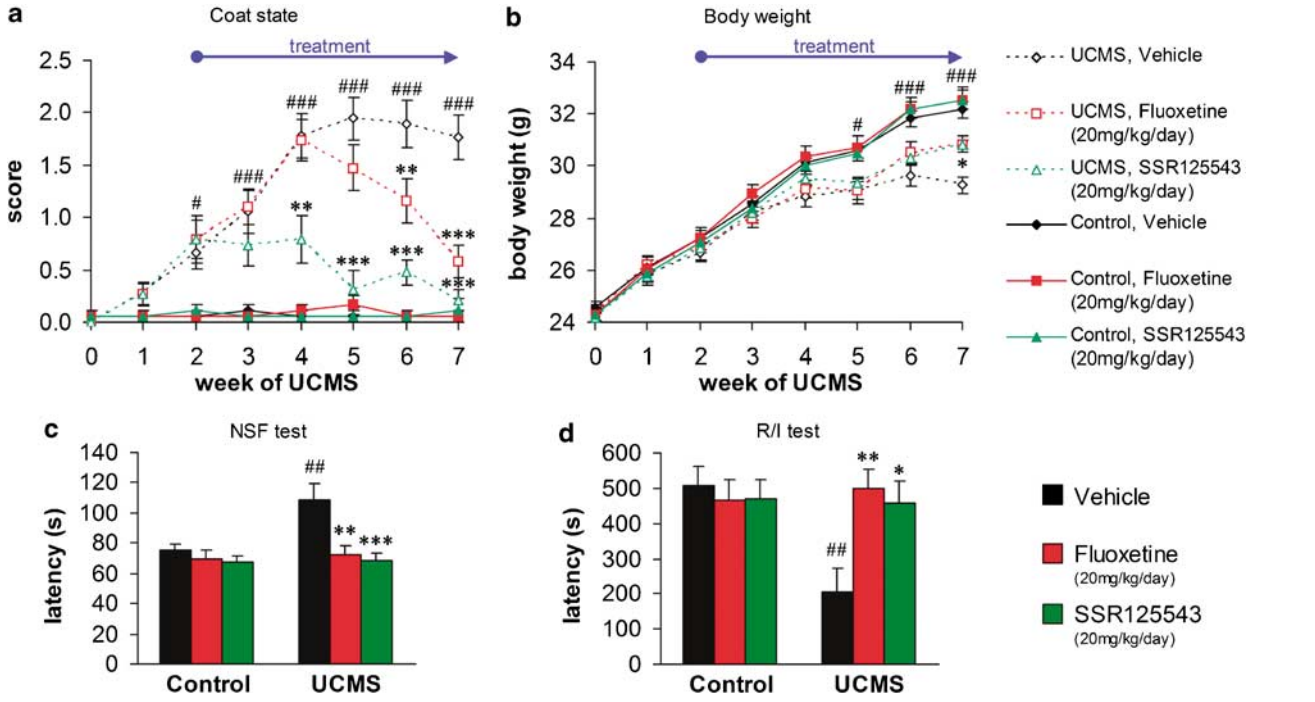

Figure 2 UCMS-induced changes in physical state and behavior are reversed by chronic treatment with fluoxetine or SSR125543. (a) UCMS induced a significant deterioration of the coat state, as demonstrated by increasing coat state scores (see Materials and methods). Drug treatments initiated in the third week of UCMS exposure reversed this deterioration after 2 weeks of SSR125543 treatment and after 4 weeks of fluoxetine treatment (ANOVA: environment, week 1, $F_{1,104}=9.34$, $P<0.01$; weeks 2–7 $F_{1,104}>35.06$, $P<0.001$; treatment, week 4, $F_{2,104}=6.39$, $P<0.01$, weeks 5–7, $F_{2,104}>15.8$, $P<0.001$; environment × treatment: weeks 4–7, $F_{2,104}>6.39$, $P<0.01$). (b) UCMS significantly disrupted the normal gain in body weight, starting at the fifth week of UCMS regimen. The progressive reversal of UCMS effect on weight gain by fluoxetine and SSR125543 exposure became significant in the last week of UCMS (ANOVA: environment, weeks 4–7, $F_{1,104}>10$, $P<0.001$; treatment, week 7, $F_{2,104}=5.34$, $P<0.01$). $n=18$–19 mice per group. *Post hoc* Tukey test: $^{\#}P<0.05$ and $^{\#\#\#}P<0.001$ for UCMS/vehicle mice *vs* control/vehicle mice; $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ for UCMS/treated group *vs* UCMS/vehicle group. (c) The latency to begin eating in the NSF test was increased by UCMS. This effect was reversed by both fluoxetine and SSR125543 treatments (ANOVA: environment, $F_{1,65}=5.79$, $P<0.05$; treatment, $F_{2,65}=8.49$, $P<0.001$; environment × treatment, $F_{2,65}=3.89$, $P<0.05$). (d) UCMS induced a significant decrease in the latency to attack the intruder in the R/I test, while both fluoxetine and SSR125543 treatment reversed this effect. (ANOVA: environment × treatment, $F_{2,65}=4.75$, $P<0.05$). $n=11$–12 mice per group. *Post hoc* Tukey test: $^{\#\#}P<0.01$ for UCMS/vehicle mice *vs* control/vehicle mice; $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ for UCMS/drug-treated group *vs* UCMS/vehicle group. Data represent mean ± SEM.

chew the pellet was significantly altered (Figure 2c). UCMS-exposed mice showed an increased latency compared to control mice ($P<0.01$). Fluoxetine as well as SSR125543 significantly counteracted this UCMS-induced behavioral alteration ($P<0.01$ and 0.001, respectively). Moreover, neither the UCMS procedure nor the drugs tested produced a significant change in the home food consumption during the 5 min following the NSF test (data not shown), suggesting that the feeding drive subsequent to a 12-h deprivation was not different between experimental groups.

To evaluate changes in agonistic behavior, a R/I test was performed after a 50-day UCMS procedure. An intruder mouse was placed in the home cage of UCMS or control mice chronically treated with vehicle, fluoxetine, or SSR125543. The latency of the first attack and the number of attacks by the resident mouse were recorded. Significant differences were found for both measures (Figure 2d). UCMS exposure significantly reduced the latency ($P<0.01$) and increased the number of attacks (data not shown; $P<0.05$). Fluoxetine and SSR125543 treatments completely reversed the UCMS-induced disruption in social encounter in latency (fluoxetine, $P<0.01$ and SSR125543, $P<0.05$) and in number of attacks (fluoxetine, $P<0.01$ and SSR125543, $P<0.05$). Antidepressant treatments had no effect in control mice.

Taken together, these results demonstrated that in addition to inducing physical changes, UCMS induced a pattern of increased agonistic and emotion-related behaviors that were significantly reversed by chronic exposure to an effective (fluoxetine) or putative (SSR125543) antidepressant drug, thus corresponding to 'depressed' (UCMS syndrome) and 'recovered' (antidepressant-reversed) states in the UCMS model.

The Gene Expression Correlates of a 'Depressive-Like' State Vary Across Corticolimbic Brain Regions

To identify changes in gene expression correlating with UCMS and antidepressant treatments, we performed a microarray analysis at the 7-week time point, corresponding to the period of (1) established increase in fur coat index, (2) increased agonistic and emotion-related behaviors, and (3) complete reversal of both effects by fluoxetine or SSR125543 (Figure 1). Samples were obtained from the CC, AMY, and DG ($n=6$ arrays per treatment and per brain area), as brain areas for which the human homologous areas participate in a corticolimbic network of mood regulation that is affected in depression (Mayberg, 1997; Seminowicz *et al*, 2004). RNA samples were processed on MOE430 2.0 arrays (Affymetrix Inc.), which interrogate the levels of $\sim$40 000 gene transcripts (see Materials and methods). As the analytical goal was to use profiles of expression over large groups of genes as an 'experimental assay' to measure UCMS and drug effects, thresholds for gene selection were kept at moderate stringencies for group comparisons ($P<0.05$ and changes greater than 20%; see Materials and methods and Discussion). We have previously demonstrated the validity of this approach at characterizing robust and significant biological events in brain tissue (Sibille *et al*, 2007). Accordingly, the levels of 254 gene transcripts were significantly affected by UCMS in CC, 299 in AMY, and 166 in DG (Figure 3; Supplementary Tables S1–S3).

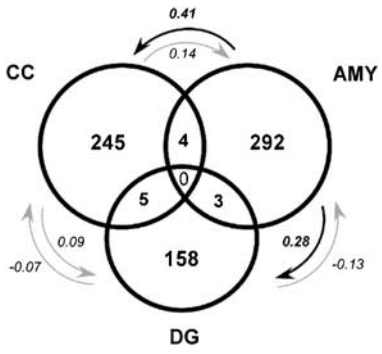

Figure 3 Corticolimbic brain region specificity of UCMS-induced changes in gene transcript levels. Venn diagram of transcript levels with significant UCMS effect in CC, AMY, and DG. Changes were mostly restricted to each of the three brain areas investigated, as little overlap was observed across areas. Arrows indicate directional correlations between changes in transcript levels for genes identified in one area (origin of arrow) and changes for the same genes in the other area (end of arrow). Italic values are Pearson coefficient factors (r) of corresponding correlations. Bold italic values indicate significance r values ($P<5e^{-5}$).

Very little overlap was observed across the three areas investigated, although transcript changes in AMY moderately, but significantly, predicted similar trends for the same transcripts in CC and DG (bold arrows in Figure 3, Pearson correlation $=0.41$ and 0.28, respectively, P-values $<5\times e^{-5}$). On the other hand, UCMS-induced transcript changes in CC or DG did not predict corresponding changes or trends for the same transcripts in any other areas (gray arrows in Figure 3, Pearson correlations P-values >0.05). Independent real-time qPCR assessments of gene transcript levels significantly correlated with array results (Figure 4; $n=10$ genes, $r=0.72$, $P<0.01$), thus confirming the validity of the array measurements.

Taken together, these results suggested that the gene expression correlates of the UCMS-induced 'depressive-like' state were mostly brain region-specific within a corticolimbic network of mood regulation, although AMY changes moderately predicted similar trends for the same transcripts in CC and DG.

Fluoxetine and SSR125543 Reverse the Effect of UCMS on Gene Expression

We next investigated whether the reversal of the UCMS physical and behavioral phenotypes by chronic antidepressant treatments also correlated with a reversal of the UCMS molecular phenotype. For each gene affected by UCMS, the percentage of reversal of UCMS effect by chronic fluoxetine or SSR125543 was calculated and gene-wise values were averaged for all UCMS-affected genes in each brain area (see Materials and methods). For instance, in CC, the chronic fluoxetine treatment reversed the effect of UCMS on gene expression by $\sim$75%, meaning that the residual changes in transcript levels in the UCMS- and fluoxetine-treated group represented on average only $\sim$25% of the full UCMS effect (Figure 5a, middle panel). SSR125543 reversed only $\sim$44% of the UCMS molecular profile in CC (Figure 5a, lower panel). In AMY, both drug treatments reversed the UCMS effect to a large extent ($>$70%; Figure 5b, middle and lower

panels). In contrast, these effects were weaker in DG, reaching only 28 and 39% reversal for fluoxetine and SSR125543, respectively (Figure 5c, middle and lower panels) Tables S1–S3.

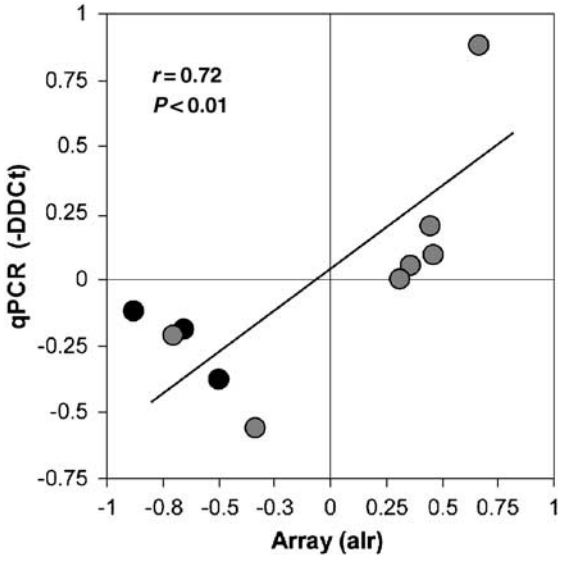

Figure 4 Validation of array results by independent qPCR measurements. Ten genes with significant differences in AMY were independently assessed by qPCR (*Mobp*, *Cnp1*, *Enpp2*, *Cpne9*, *Pitpnc1*, *Rph3a*, *Dgkg*, *Gabraa2*, *Kctd12*, and *Arhgap6*). Black dots represent oligodendrocyte-related genes (*Mobp*, *Cnp1*, and *Enpp2*) (see Table 2). *r*, Pearson correlation factor; Alr, average $\log_2$ of (UCMS/control) expression ratio; –DDCt represent differences in PCR cycle thresholds between UCMS and control samples, which are equivalent to $\log_2$ values of ratios.

Thus, antidepressant treatments reversed the effect of UCMS on altered gene expression, although the extent of reversal varied across areas and treatments according to the following order: $(AMY_{fluoxetine} = AMY_{SSR125543} = CC_{fluoxetine}) > CC_{SSR125543} \geqslant DG_{SSR125543} > DG_{fluoxetine}$. Overall, AMY displayed highest and most consistent antidepressant reversals of UCMS effects.

Fluoxetine and SSR125543 Treatments Induce Additional and Very Similar Transcriptome Effects in a Brain Region-Specific Manner

Beyond reversing the molecular correlates of UCMS, fluoxetine and SSR125543 treatments affected the expression levels of very large numbers of additional genes (fluoxetine: 2540 genes in CC, 640 genes in AMY, and 294 genes in DG; SSR125543, 562 genes in CC, 507 genes in AMY, and 122 genes in DG; Table S4), yielding a pool of ~3600 genes affected by antidepressant treatments in any of the three brain areas investigated. Interestingly, very few genes were affected by the same drug across brain areas (<10% of the fluoxetine-related gene pools and <2% of the SSR125543 pools), while much larger number of genes were similarly affected by the two different drug treatments within each brain area (48% in CC, 25% in AMY, and 18% in DG) (see Supplementary Table S4). Accordingly, transcript changes displayed high correlations between fluoxetine and SSR125543 effects *within* each brain area (Figure 6a), but low correlations for the effects of the same treatment *across* two different brain areas (Figure 6b).

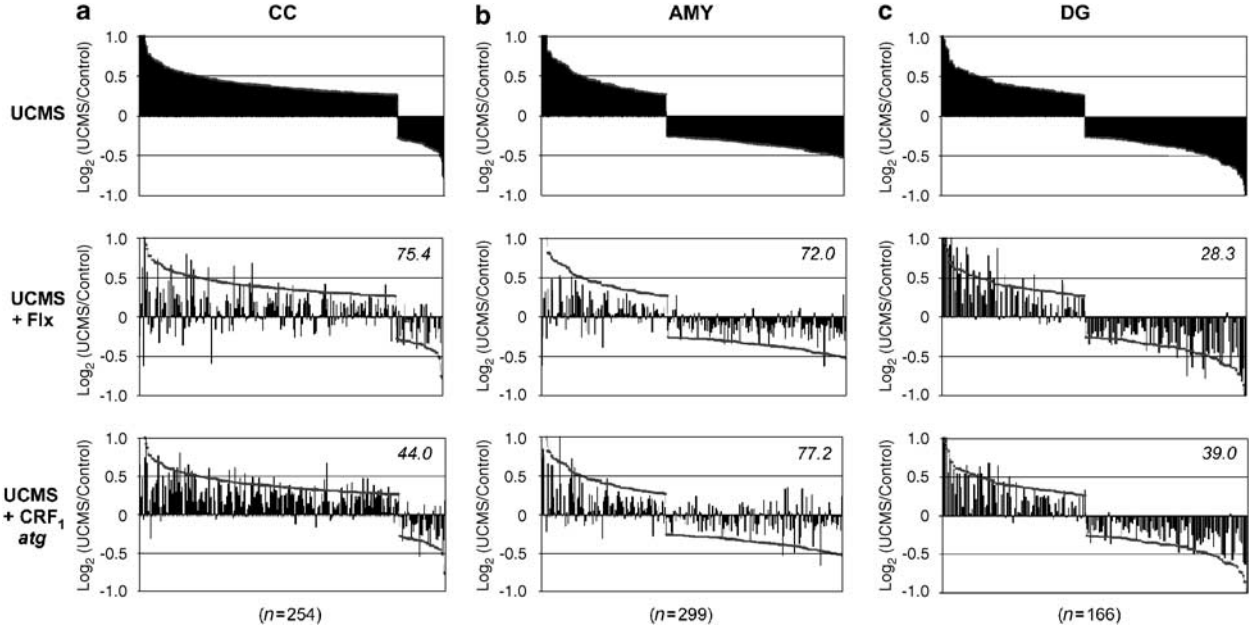

Figure 5 Differential reversal of the UCMS gene expression profiles by antidepressant treatments. Profiles of changes in gene transcript levels for UCMS-affected genes (top panels) and after reversal by antidepressant treatments (middle and low panels) in CC (a), AMY (b), and DG (c). Selected gene groups are from Figure 3. Top panels: UCMS-affected genes are organized by the magnitude of their changes in transcript levels along the *x* axis and form a continuous profile with a pre-determined 20% effect cutoff value (see Materials and methods). Vertical bars indicate the amplitude of changes ($\log_2$ of UCMS/control expression ratio). Middle and low panels: vertical bars indicate the relative transcript levels for the same UCMS-affected genes after chronic fluoxetine or SSR125543 treatment. Note that the profiles have shifted away from UCMS levels (black contour line), back toward control 'no change' levels ($\log_2 r \sim 0$). The average percentage of drug reversal of the UCMS molecular profile is indicated per brain area and drug treatment. Flx, fluoxetine; CRF_1*atg*, SSR125543.

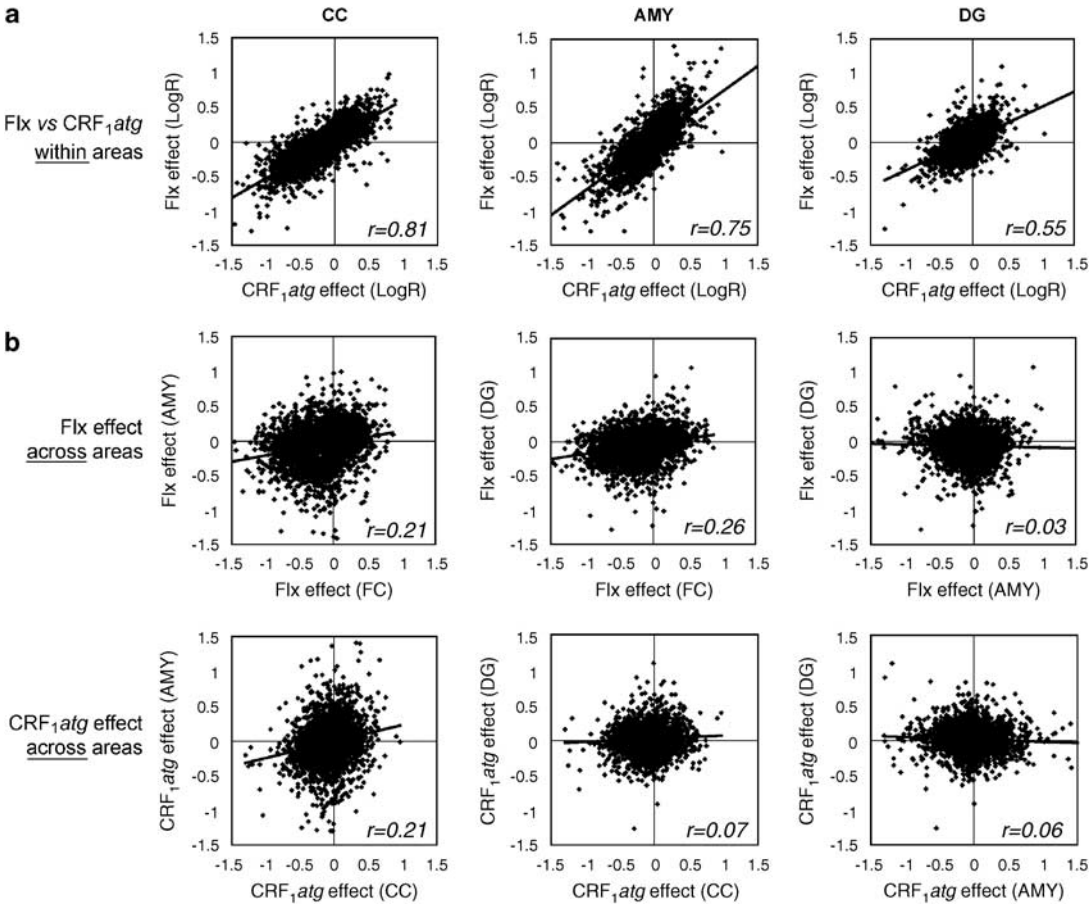

Figure 6 The transcriptome effects of two different drug treatments are more similar *within* brain areas, compared to the effect of each individual treatment *across* areas. For a comprehensive overview, the effects of antidepressant treatments on gene transcript levels are depicted for the pool of ~3600 genes that were affected by either treatment in any of the three areas (see text). Similar results were observed with more restricted pools of genes based on single drug effect or within a single brain area (not shown). (a) High correlations (0.55–0.81) and high graph slopes for linear fit (0.46–0.72, black bars) were observed between the effects of two antidepressant treatments on gene transcript levels within a brain region, indicating high similarity of molecular impacts for the two different drug treatments. (b) Low correlations (−0.03 to 0.26) combined with low graph slopes (−0.03 to 0.17) reveal poor similarities in the molecular impact of a same drug treatment across any two brain areas investigated. *x*–*y* axes values are $\log_2$(UCMS antidepressant-treated/control) for the respective drug treatment. Solid bars indicate trend lines. *r*, Pearson correlation coefficients; Flx, fluoxetine; CRF$_1$*atg*, SSR125543.

These results revealed that, in addition to reversing the effect of UCMS, antidepressant treatments affected the transcript levels of large numbers of genes, and that the transcriptional profiles of the two different drug treatments were more similar *within* a brain area, compared to the effect of the same treatment *across* areas, thus demonstrating that the molecular impacts of fluoxetine and SSR125543 treatments were mostly determined by the brain area, rather than by their molecular targets (see Discussion).

Transcriptome Effects of Fluoxetine and SSR125543 Treatments are State-Dependent in CC

The large-scale effects of antidepressant treatments have been mostly characterized in normal or control animals and it is not known whether results apply to depressive-like states. Here, we directly addressed this question by comparing the effects of chronic fluoxetine and SSR125543 exposure on CC gene expression in control and in UCMS-treated mice. Results indicated that larger number of transcript changes was induced by both drug treatments in control mice (3221 genes for fluoxetine and 3004 genes for SSR125543; Table S5) compared to UCMS-treated mice (2540 genes for fluoxetine and 562 genes for SSR125543). Similar to the results in UCMS-treated mice, the molecular impacts of the two different drug treatments were strikingly similar in control mice (Figure 7a, $r = 0.94$, slope $= 0.90$). On the other hand, comparing the effects of fluoxetine or SSR125543 between control and UCMS-treated groups revealed much lower correlations ($r = 0.22$ and 0.46) and greatly reduced amplitudes of altered transcript levels (Figure 7b, slopes = 0.11–0.19), denoting a poor conservation of drug effect between control and UCMS states. The converse analysis of identifying genes that were modulated by drug treatments in UCMS-treated mice and comparing the extent of transcript changes for those genes in drug-treated control mice yielded very comparable results ($r = 0.24$–0.30, slope = 0.26–29; not shown), thus confirming the low conservation of drug treatment effects between control and UCMS states.

Another notable difference was the magnitude of effects on gene transcript levels across states and treatments.

Fluoxetine and SSR125543 each affected ~12% of all genes with detectable expression in control animals, but only 9.8 and 2.2% in UCMS-treated states for fluoxetine and SSR125543, respectively. In comparison, less than 1% of the genes were affected by UCMS alone.

Taken together, these results revealed that, in addition to being brain area-dependent, the molecular correlates of antidepressant treatments were state-dependent in CC, as demonstrated by low similarities of drug-induced transcript profiles between UCMS and control states. Our results also revealed differences in drug-induced gene expression plasticity, with fewer genes affected by UCMS alone, and according to the following order of magnitude: UCMS < drugs in UCMS < drugs in controls.

Correlations between Gene Expression, UCMS Behavior and Antidepressant Treatments Identify Candidate Genes for Mood Regulation and/or Genes with Therapeutic Potential

On the basis of patterns of altered transcript levels, genes were classified according to their potential contribution to the expression of the UCMS phenotype and/or to the reversal of that phenotype (Figure 8). Specifically, we focused on two categories of genes of interest: (1) 'mood-dependent and therapeutic' genes, as affected in opposite directions by UCMS and antidepressant treatments, and thus matching the behavioral phenotype, and (2) 'therapeutic-only' genes that were not affected by UCMS, but that displayed significant transcript changes after both antidepressant exposures in UCMS-treated mice (Figure 8). The assumption of 'therapeutic-only' potential was that a reversal of the UCMS phenotype may occur through biological pathways that are independent of UCMS effects. For both categories, a positive antidepressant effect had to correspond to significant and similar effects after both drug treatments, reflecting the similar reversal of physical and behavioral UCMS effects by fluoxetine and SSR125543.

A total of 768 putative 'mood-dependent and therapeutic' or 'therapeutic-only' genes were identified (Table 2 and Supplementary Tables S6–S11). Consistent with the previously described region-specific effects, gene selections displayed very limited overlap across brain areas. In AMY, genes of interest (Figure 8) were for the most part in the 'mood-dependent and therapeutic' gene category ($n = 241$) compared to 'therapeutic-only' genes ($n = 82$), reflecting the extensive and uniform antidepressant reversal of UCMS effects by both treatments (see Figure 5b; ie, gene transcripts mostly tracked both UCMS and antidepressant effects). In CC, the discrepancy in the molecular impact of antidepressants (Figure 5a; 75.4% fluoxetine and 44.0% SSR125543 reversals of UCMS effects) translated in fewer genes with 'mood-dependent and therapeutic' profiles ($n = 146$) and more genes with potential 'therapeutic-only' involvement ($n = 238$). In contrast, much fewer genes of interest were identified in DG (62 and 19, respectively), due to the combined low UCMS effect, moderate antidepressant reversal, and overall lower molecular impact of antidepressant treatments (see next, Table 2, and Supplementary Tables S6–S11).

In summary, correlations between altered gene expression, UCMS behavior, and response to antidepressant treatments identified brain region-specific candidate genes for mood regulation and/or genes with therapeutic potential, with AMY displaying the highest content of genes with potential dual contribution to the UCMS phenotype and therapeutic antidepressant reversal.

Identified Gene Transcripts Suggest Different Oligodendroglial and Neuronal Molecular Phenotypes in Correlation with UCMS and Antidepressant Treatment in AMY

We have previously shown that array data from adjacent WM samples can be used to generate WM/GM ratios that are specific for each gene (Erraji-BenChekroun *et al*, 2005; Sibille *et al*, 2008). WM/GM ratios can be used as estimates of gene transcript enrichment either in glia (WM/GM > 1.5), neurons (WM/GM < −1.5), or both cellular population (−1.5 < WM/GM < 1.5) and provide a wider view of overall patterns relating to glial and neuronal functions (Sibille *et al*, 2008). For instance, displaying UCMS-affected genes according to the extent of their transcript changes (up and

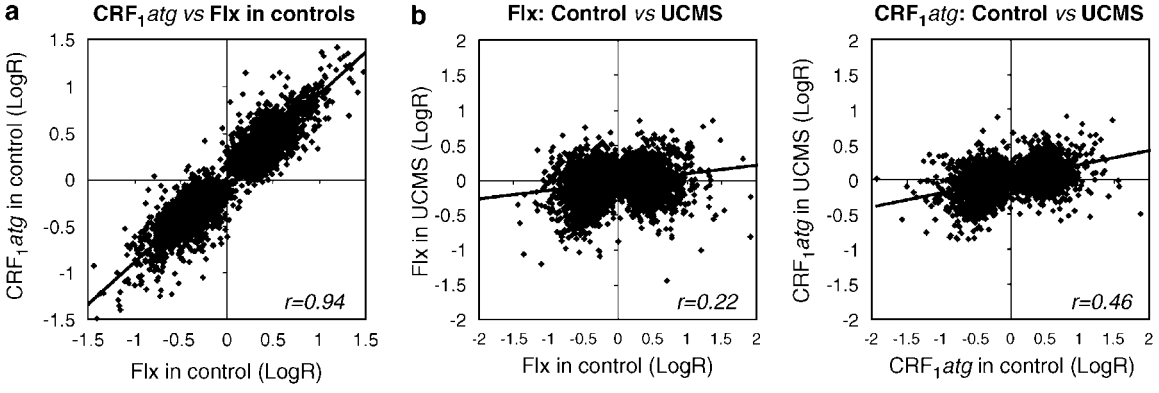

Figure 7 State-dependent effects of fluoxetine and SSR125543 treatments in CC. (a) Chronic drug treatments induced extensive and highly similar transcriptome changes in control non-stressed mice. In total, 3221 genes were significantly affected by fluoxetine and 3004 genes for fluoxetine or SSR125543 with ~60% of genes significantly affected by both treatments (see Supplementary Information). The correlation graph includes a total of 4305 antidepressant-affected genes ($r = 0.94$, slope = 0.90). (b) Lower correlations ($r = 0.22$–0.46) and much reduced graph slopes (0.11–0.19) suggest that the same drug has different effects in control and UCMS-treated mice. *x*–*y* axes values are $\log_2$(UCMS antidepressant-treated/control) for the respective drug treatment; *r*, Pearson correlation coefficients; Flx, fluoxetine; CRF_1atg, SSR125543.

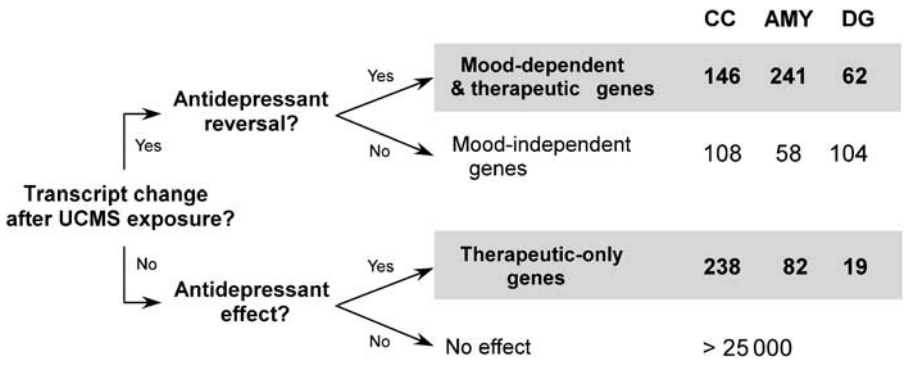

Figure 8 Patterns of UCMS behavior/gene expression/antidepressant effect identified candidate genes for involvement in mood regulation or with potential for therapeutic applications. For positive antidepressant reversal or for antidepressant effect, genes had to be significantly and similarly affected by fluoxetine and SSR125543 treatments.

Table 1 Proportional representations (%) of transcripts with enriched glial or neuronal origin within groups of identified genes

	Cellular enrichment of gene transcripts			
	# Genes	~ Neuronal (%)	Both (%)	~ Glial (%)
CC		*24**	*56**	*20**
'Mood and therapeutic'	146	19	53	**28**
'Therapeutic only'	238	21	59	20
AMY		*23**	*57**	*20**
'Mood and therapeutic'	241	23	38	**39**
'Therapeutic only'	82	**46**	37	17
DG		*23**	*53**	*24**
'Mood and therapeutic'	62	23	48	29
'Therapeutic only'	19	11	36	53

'# genes' indicates the number of genes identified in the respective category. Italic values with (*) indicate expected proportions of glial- and neuronal-enriched genes (see Materials and methods). Gene categories are described in the text. Bold numbers represent values that are significantly different from expected proportions (χ^2 tests, $P<0.05$ adjusted for multiple comparisons). Differences from expected proportions in 'therapeutic only' genes were not significant in DG, due to the low number of genes within that category.

down arrows in Figure 9) and based on their estimated cellular origin of transcripts (yellow, gray, or black bars in Figure 9) clearly identified predominantly downregulated glial-enriched genes in AMY (Figure 9a), as visually represented by the overall yellow color in the lower portion of the central panel. Within our categories of genes of interest (Figure 8), statistical assessments confirmed that AMY glial-enriched genes were significantly over-represented in the 'mood and therapeutic' gene category, while neuronal-enriched genes were more numerous in the 'therapeutic-only' gene categories (Table 1). The CC and DG 'mood and therapeutic' and 'therapeutic-only' gene pools displayed proportions of glial- or neuronal-enriched gene transcripts that did not differ greatly from their expected proportions (Table 1), with the exception of a slight over-representation of glial-enriched genes in correlation with mood levels in CC.

While caution should be applied for identification of single genes in array studies under medium statistical stringency (see Discussion), looking at the convergence of single genes (Table 2) and at over-representation of biological functions within the lists of identified genes (Table 3) revealed complex sets of changes involving numerous cellular functions (receptors, signaling, transcription, metabolism, structural components, etc.). Summarizing this information, three important themes emerged. First, consistent with brain region-specific effects of UCMS and antidepressant treatments, none of the selected genes were identified across regions. Second, genes coding for several components of neurotransmitter systems (GABA, glutamate, and peptides), signal-transduction pathways (PKC, PLC, and MAPK) and second messenger systems (cAMP) previously associated with altered mood and/or antidepressant treatments were identified here (underlined in Table 2), were over-represented in AMY (20 genes out of 31, $P=0.005$). Third, numerous oligodendrocyte markers were exclusively downregulated within the 'mood and therapeutic' gene category in AMY (*Mobp*, *Edg2*, *Gsn*, *Cnp1*, *Gpr37*, *Pllp*, *Enpp2*, *Pmp22*, *Mpzl1*, and *Plp1*; bold in Table 2; 'myelin' functional cluster in Table 3; black dots in Figure 4), thus strongly suggesting an antidepressant-sensitive oligodendrocyte-related phenotype in the AMY of UCMS-treated mice (see Discussion).

Taken together, these results revealed different and complex molecular phenotypes in correlation with UCMS and antidepressant treatments in the three brain regions investigated, but also identified the AMY as a crucial area of interest, with a neuronal molecular pathology affecting previously identified candidate neurotransmitter systems, and a glial phenotype focused on UCMS-downregulated markers of oligodendrocyte structure and function, in reminiscence of prior reports in the AMY (Hamidi *et al*, 2004) and in nearby cortical regions (Aston *et al*, 2005) of human depressed subjects. It is important to note that while our analytical approach relied on the cumulative effects across large group of genes to identify robust region- and state-dependent effects of UCMS and antidepressants, results for single genes should be considered in the context

Table 2 Identified genes (selected) corresponding to 'mood' and/or 'therapeutic' gene categories in the three brain regions investigated

Gene category	Direction of transcript changes	# Genes	Neuronal-enriched (# genes) gene codes	Neuronal and glial expressed (# genes) gene codes	Glial-enriched (# genes) gene codes
CC					
Mood and therapeutic	UCMS ↑ AD ↓	121	(18) Nr1d2, Ankd13c, Nanos1, Smoc2, Phf17, Pik3cd, Pclo, Acvr2a, Atpif1, Rragd, Thtpa, Zhx3 …	(66) AR, Gspt2, Vps13a, Agpat6, Commd2, Yipf5, ccdc84, Slc24a4, Pcdh17, Tbc1d8, Lyst, Toak3, Spen, Dnajb4, Auts2, Ints8, Stk4, Atp10a, Txnrd1, Dhodh, Cry1, Makpb1, Exosc1, Lix1l, Mtrf1l, Hexim1, Fzd1, Stx18, Bcl9, Ccnd2, Suclg2, Siah2, Ift80, Ube2z, Evl, P4ha2 …	(37) Ubie, Asph, Cth, Slc31a2, Wdfy1, E2f5, CCnd2, Dbt, Plch1, Osbpl9, Scn7a, Cklf, Rps6ka5, Irf2bp2, Hus1, Fcho1, Oprm1, Gpsm2, Rbm3, Hist2h3c1, Hspa1b, Tsnax, Pdgfc, Tenc1 …
	UCMS ↓ AD ↑	25	(5) Ddit4l, Nptx2, Add2, Cast …	(13) Bcl11a, Cstad, Ptpra, Hlcs, Sema5b, Grin2c, Ktcd15 …	(7) Ktcd15, Cald1, Rcn3, Filip1 …
Therapeutic-only	UCMS ↔ AD ↓	194	(45) Ovol2, Exph5, Npas4, Itga8, Cbln4, Tbr1, Cxxc4, Cxadr, Ints8, St6gal2, Neurod6, Ube3a, Grm8, Mmp16, ENtp7, Mapk10, Slc19a2, Runx1t1, Synj2, Per2, Ube1l2, Klf4, Ccnc, Hmgcr …	(118) Minpp1, Sema4a, Ppm1a, Atp5g1, Crim1, Lats1, Arl8b, Gtpbp4, Lig4, Plekha3, Pdss1, Cnot4, Sbk1, Hspa8, Matn2, Cttnbp2, Fubp1, Gpatc2, Mib1, Rad21, Inoc1, Copa, Top2b, Syncrip, Mas1, Surf4, Btg2, Atr, DNAjc3, Spsb4, Cpeb2, Pfkfb3, Taf4a, Ppp1r8, Akap10, Idi1, Ddx5, Mtm2, Pip5k1a, Jak2, Sirt1, Rbbp9, Rapgef4, Eif2s2, Acsl3 …	(31) Tob2, Nr2c2, Sfrs1, Sp3, Sptcl2, Idh1, Gprk5, Gpt2, Dlg5, Map4k5, Elf2, Maml1, Qser1, Gabrg1, Insm1, Lrch1, Mettl7a, Hspa1b, Pkp2, Cacnb2, Prkcd …
	UCMS ↔ AD ↑	44	(11) Pvalb, Nell2, Rab6b, Ntrk3, Usp7, Fndc5, Rtn1 …	(23) Plekha5, Acp1, Arhgef4, Commd5, Endog, Gcap14, Scn4b, Slc25a5, Gdpd1, Gpr172b, Dag1, Jarid1a, Psma4, Nfib, Fance, Ntng1 …	(10) Pcdh9, Slc26a11, Endod1, Ppr1r16b, Ednrb, Wnk1, S100a16, Dbndd2 …
AMY					
Mood and therapeutic	UCMS ↑ AD ↓	82	(40) Igfbp3, Per2, Alkbh8, Nrip1, Grin2b, Akap5, Dgkg, Atp11c, Kitl, Scn3a, Map2k6, Cys1, Socs2, Srpk2, Ankrd43, Arhgap6, Npy1r, Npy2r, Cacnb2, Gda, Mchr1, Camk2d, Grin3a, Kcnd3, Gpr178 …	(39) Katna1, Igf1r, Nfib, Ccdc43, Chsy1, Sec24d, Nfix, Josd3, Jun, Matn2, Cttnbp2, Mgrn1, Peli1, Nptx1, Kctd12, Taok3, Gabra2, Ttc26, Nrg1 …	(3) Trp53bp2, Mettl7a
	UCMS ↓ AD ↑	195	(15) Mpped1, Accn4, Prss12, Elmo1, Npy, Sorcs3, Cckbr, Cutl2 …	(51) Nupr1, Pdia5, Gpc1, Cxcl4, Itm2a, Klf7, Adcy2, Aif1, Incenp, Diras2, Chrdl1, Hapln1, Gnb4, Scotin, Egr1, Lynx1, Igsf21, Gal3st3, Rgs6, Rph3aPpp1r16a, Rbbp4, Scube1 …	(144) Litaf, **Mobp**, **Edg2**, Adamts4, Ptprd, Arhgap25, Trf, **Gsn**, **Gpr37**, Cdc42ep2, Efhd1, Rhog, **Cnp1**, Itfg3, Apod, Gng11, **Pllp**, Bcas1, **Enpp2**, Dusp16, Cryab, **Pmp22**, Qdpr, **Mpzl1**, Rab31, **Plp1**, Aga, Rgs3, Car2, Rorb, Lamp1, Lgals1, Prom1, Rtkn, Cpne9, Grm3, Ifit2, P2rx4, Hip1r, Plcd1, Slco2b1, Bok, Dusp3, Stard10, Pgm1, Tinf2, Zyx …
Therapeutic-only	UCMS ↔ AD ↓	62	(36) Rpa2, Ap1g1, Slk, Pcdh20, Efnb2, Phip, Pde7a, Rgmb, Arl5a, Ryr2, Timp2, Grin2a, Smad3, Kcnj9, Apaf1, Nptxr …	(22) Trib1, Usp1, Tiparp, GPR172b, Tor1b, Bzw2, Itpkc, Kpnb1, Pigh, Cpd, Htr4, Sgpl1, Mycl1 …	(4) Spa17, Ss18, Slco3a1
	UCMS ↔ AD ↑	20	(2) Cpne5	(8) Gfpt2, Fxn, Dxd42, Trio, Adam23, Xrcc2, Car7	(10)Gja12, Tmcc1, Vangl1, Rela, Slc22a8

Table 2 Continued

Gene category	Direction of transcript changes	# Genes	Neuronal-enriched (# genes) gene codes	Neuronal and glial expressed (# genes) gene codes	Glial-enriched (# genes) gene codes
DG					
Mood and therapeutic	UCMS ↑ AD ↓	31	(4) Mef2c, Lin7b, Fndc1, EST	(19) Sdc4, Mgst3, Ses6l a1, Man2b1, Bcat1, Sh2d3c, Wipi2, Nono, Numb, Cacna1a, gpatc2, Tiam2, Per2, Pvr, Hdac8	(8) Pcolce2, Tox, Bgn, Serpinh1, Syt2, Agpat4 …
	UCMS ↓ AD ↑	31	(10) Nol5, Bsn, Nfkbiz, Dio2, Dazl, Zmynd19, Letm1, Pip5k1b …	(11) Mest, Pura, Cistn3, Wdr22, Prpf40a, Mapk1, Suz12, Pdhb …	(10) Rgs3, Fbxo32, Pex2, Egln3, Slc12a3, Ripk1, Nadsyn1, Als40a1 …
Therapeutic-only	UCMS ↔ AD ↓	15	(0)	(6) Prickle1, Gltscr1, Ephb2, Gria4, RIK	(9) Nlk, Pomt2, Nrip1, Pcdhgc4, Smad7, Fos, Ripk4 …
	UCMS ↔ AD ↑	4	(2) Ncl …	(1)	(1) Gm10602

Underline indicates genes within biological systems previously associated with altered mood and/or antidepressant treatment. Bold indicates oligodendrocyte-specific genes. EST and Riken cDNAs were omitted. AD, antidepressant treatment. See Supplementary tables for complete lists and annotations.

of the moderate statistical stringencies applied (see Discussion). Specifically, we make available tables of results as supplements so that individual results can be compared and validated across studies.

DISCUSSION

To gain insight into brain function during mood regulation, we investigated correlations between behavioral and molecular changes in the UCMS rodent model of depression and of antidepressant reversal. We confirm the validity of UCMS at inducing robust physical and behavioral changes that are reminiscent of a depressive-like state in mice, and show that both symptom dimensions are reversed by chronic exposure to an effective (fluoxetine) or a putative (SSR125543, CRF_1 antagonist) antidepressant drug. Measuring changes in large-scale gene expression as an indirect assessment of brain function, we report that the molecular correlates of UCMS and antidepressant treatments differ across areas of a corticolimbic network of mood regulation (CC, AMY, and DG), and that two antidepressant treatments induced very similar transcriptome changes within areas, despite targeting different biological systems (brain region-specific antidepressant effects). We also demonstrate that the effects of antidepressant treatments vary greatly depending on whether treated animals are in a control or 'depressed' state (state-dependent changes).

Physical and Behavioral Changes Affected by the UCMS Model of Depression and Antidepressant Treatment

UCMS exposure elicits a syndrome with a range of phenotypes that are analogous to symptoms of depression, including low stress coping and anxiety-/depression-like behaviors (Santarelli *et al*, 2003; Ducottet *et al*, 2004; Yalcin *et al*, 2005), decreased reward function (Pothion *et al*, 2004), increased glucocorticoid levels (Ayensu *et al*, 1995; Banasr *et al*, 2007; Li *et al*, 2007) and decreased hippocampal cell proliferation and neurogenesis (Alonso *et al*, 2004). Here, mice subjected to a 7-week UCMS paradigm developed a progressive deterioration of their coat state, a decline in weight gain, an exaggerated emotional reactivity in the NSF test, and social disturbances in the R/I test (Figure 2). Chronic administration of fluoxetine reversed these UCMS-induced deficits after 4 (coat state, NSF test) to 5 weeks (body weight, R/I test) of treatment. Likewise, SSR125543 showed antidepressant-like properties, as this compound counteracted all UCMS-related effects, including with a faster onset of improvement in the quality of the coat state (after 2 weeks of treatment) compared to fluoxetine (3–4 weeks of treatment). Overall, antidepressant reversals of UCMS effects occurred in a time course that paralleled therapeutic improvements in depressed subjects, thus emphasizing UCMS as a valid model for investigating depressive pathophysiology and mechanisms of antidepressant reversal. Indeed, UCMS fulfills several criteria for a valid model of depression, including: (1) good face validity (close ethological counterpart for emotion-related and

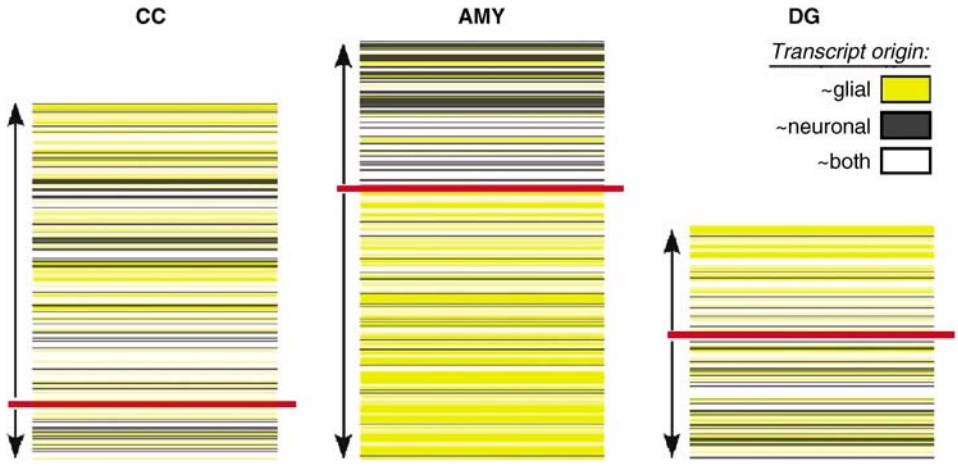

Figure 9 Glial/neuronal enrichment of altered gene expression in three brain areas in the UCMS model. Transcripts in CC ($n = 254$), AMY ($n = 299$), and DG ($n = 166$) are organized vertically according to the extent of altered gene expression. Up and down arrows indicate up- and downregulation of gene transcripts in the UCMS model. Color overlay indicates the cellular origin of the transcript: yellow ~glial origin (WM/GM > 1.5), gray ~ neuronal origin (−1.5 < WM/GM), and white ~both cellular populations (−1.5 < WM/GM < 1.5) (Sibille *et al*, 2008). Note the apparent increased representation of glial expression in downregulated genes in AMY. Red bar separates up- from downregulated genes.

Table 3 Functional annotation of identified gene sets

Area	Gene category	Glial/neuronal origin	Functional cluster	# Groups/cluster
CC	Mood and therapeutic	Neuronal	Cation binding; metal ion binding; ion binding	3
		Glial	Intracellular organelle	4
			Nucleosome; chromatin assembly	6
			Chromosome organization and biogenesis	2
	Therapeutic only	Neuronal	Zinc; zinc-finger; zinc ion binding	3
			Intracellular membrane-bound organelle	2
			Proteolysis during cellular protein catabolism	10
			Regulation of transcription, DNA dependent	7
		Glial	Metabolism	2
			Serine/threonine-protein kinase	8
AMY	Mood and therapeutic	Neuronal	Ion channel activity	10 *P* < *e*−*04*
			Neuropeptide and neurotransmitter receptor activity	7
		Glial	Myelin; ionic insulation of neurons by glial cells	7 *P* < *e*−*05*
	Therapeutic only	Neuronal	Ion channel activity	6
			Transmembrane	2
		Glial	Transmembrane	1
DG	Mood and therapeutic	Neuronal	None	
		Glial	None	
	Therapeutic only	Neuronal	None	
		Glial	Regulation of transcription, DNA dependent	12
			Protein amino-acid phosphorylation	8
			Cellular metabolism	3

To reduce the redundancy of gene ontology (GO) annotations, clusters of GO groups with *P*-values less than 0.05 and with similar annotations were regrouped (ie, # groups/clusters; see Materials and methods). Results in DG were either borderline or not significant due to the small number of genes. The only two functional clusters that displayed very low *P*-values were identified in AMY.

anhedonia-like behaviors), (2) good construct validity (unpredictable 'psycho-social' stress mimics real-life stress etiology and recruits equivalent neuroendocrine systems, (3) good predictive validity (pharmacological reversal by antidepressant treatments), and (4) respect of time courses for mechanisms of disease and drug reversal. On the other hand, alternate paradigms frequently used to either induce or characterize depressive-like states (social defeat, learned

helpnessness), or to predict of antidepressant activities (forced swim and tail suspension tests), follow only limited numbers of these criteria in modeling depression (Cryan and Holmes, 2005).

Region-Specific and State-Dependent Corticolimbic Transcriptome Changes in a Validated Rodent Model of Depression and of Antidepressant Reversal

Results from our large-scale gene expression studies suggest that molecular changes following UCMS and/or antidepressant exposure reflect changes in the activity of the respective brain areas, rather than the direct recruitment (by UCMS and/or antidepressant) of common molecular or cellular mechanisms. In other words, although the brain areas investigated participate in a corticolimbic network of mood regulation (Seminowicz *et al*, 2004; Pezawas *et al*, 2005), the observed differences in transcriptome changes may reflect brain area differences in terms of intrinsic cellular networks, activation states, information processing, and adaptive mechanisms. Brain area-specific effects of BDNF have been reported in the social defeat stress paradigm (Berton *et al*, 2006), and area-specific regulation of ERK MAP kinase in UCMS-treated rats (Gourley *et al*, 2007), but to our knowledge, this has not been investigated on a large-scale. Conti *et al* (2007) have reported that electro-convulsive therapy, sleep deprivation, and fluoxetine induced transcriptome profiles that mostly differed across brain areas and treatment modalities, although the interpretation of these findings were limited by a lack of statistical validation and by the use of normal control rats.

Accordingly, the present results confirm our hypothesis that antidepressant effects in control animals do not extrapolate to 'depressive states.' Indeed, considering that antidepressants are devoid of mood-changing effects in non-depressed healthy humans, and in view of the poor correlation of transcriptome effects of antidepressant treatments between control and UCMS states (Figure 7), the critical antidepressant-induced modifications in gene regulation appear to be conditional on the presence of a depression-related neuropathology. Here, correlating behavior and physical changes with response to antidepressant treatments and with changes in gene expression in a validated rodent model of depression, we report sets of genes with potential roles in mood regulation and/or therapeutic treatment, and confirm the AMY as a key brain area for investigating the molecular pathology of depression.

Fluoxetine and CRF_1 Antagonist Treatments of UCMS-Exposed Mice Induced Similar Physical, Behavioral, and Genomic Profiles

The uniform reversal of the physical, behavioral, and transcriptome effects of UCMS by two different antidepressants is consistent with clinical data, as antidepressants typically treat the depressive syndrome as a whole, but is surprising from a mechanistic point of view. Indeed, the striking similarities in transcriptome profiles after the two treatments (Figures 6 and 7) strongly suggest that SSR125543 recapitulates in a brain region-specific manner the overall effects of increased serotonin signaling, and that similar cellular circuitries are ultimately targeted by increased serotonin availability and CRF_1 blockade, at least in the areas investigated. The serotonin and CRF systems interact on midbrain serotonergic neurons (Price *et al*, 1998; Jankowski and Sesack, 2004; Waselus *et al*, 2005) and CRF_1 antagonism could mimic the effect of fluoxetine on serotonin levels (Kirby *et al*, 2000; Lukkes *et al*, 2007). Alternatively, fluoxetine and SSR125543 could regulate a common hormonal system, such as glucocorticoids and the hypothalamic–pituitary–adrenal (HPA) axis, to produce a similar global effect on the brain. HPA abnormalities are common in depression (Holsboer, 2000; Pariante and Miller, 2001) and normalization of HPA function is required for clinical remission in these subjects (Linkowski *et al*, 1987; Wodarz *et al*, 1992). Although CRF_1 antagonists are generally thought to act directly in various brain regions (Arzt and Holsboer, 2006), CRF_1 receptors are in fact the main glucocorticoid secretagogues. Thus, HPA modulation by antagonism of CRF_1 receptor or by fluoxetine could represent a common downstream mechanism for both drugs. The direct impact of CRF_1 receptor antagonism on the HPA axis could also explain the shorter delay of onset of SSR125543 on the fur index, although whether this early CRF_1 antagonist effect also correlated with early reversals of UCMS-induced behavioral or molecular changes is not known.

Low Gene Expression Plasticity in UCMS Compared to Antidepressant Treatments

By testing transcriptome changes under several experimental conditions, our studies revealed critical differences in the scope of the transcriptome effects of UCMS and antidepressant treatments, and further suggested differences in drug-induced gene expression plasticity between UCMS or control states. Indeed, UCMS resulted in fewer transcriptional changes in all three brain areas, compared to antidepressant treatments. A potential explanation is that UCMS recruits limited numbers of endogenous neural networks that are involved in the stress response, while drug treatments target indiscriminately much wider systems. Interestingly, antidepressant treatments affected even larger number of genes in the absence of UCMS (Figure 7). This effect is reported in CC, as array data in antidepressant-exposed control mice was only generated in CC, thus we can only speculate that similar contrasts between effects of antidepressant in control and 'depressed' mice would be present elsewhere. Taken together, our results suggest that UCMS exposure reduced the overall gene expression-related plasticity, with the following effect sizes: UCMS < (antidepressant in UCMS) < (antidepressant in control).

From the experimental point of view, these large differences in genes affected by UCMS and antidepressant treatments, combined with the poor conservations of antidepressant effects across brain areas and 'emotional' states, suggest three notable implications for investigating disease mechanisms: first, brain areas and neural networks appear less plastic under UCMS or 'depressed' state compared to a control non-stressed state; second, the effects of antidepressant treatments are much larger than the correlates of UCMS, encompassing much more than a reversal of UCMS effects, and thus do not represent the opposite of a 'depressive' effect for the vast majority of

genes; third, results from antidepressant treatment in control animals are unlikely to extrapolate to UCMS or 'depressed' states, thus highlighting the relevance of investigating neurobiological mechanisms in the context of a depression-related neuropathology. Finally, characterizing such large-scale effects and interactions between the environment (ie, UCMS) and drug treatments will be a necessary first step in comparing results across studies, for realistic meta-analysis of large-scale gene array studies.

Enriched UCMS- and Antidepressant-Related Molecular Pathology in AMY

Results presented here suggest a crucial role for AMY in the molecular correlates of UCMS and antidepressant treatments, as AMY changes (1) predicted similar trends for transcripts in CC and DG (Figure 3), (2) displayed highest and most consistent antidepressant reversals of UCMS effects (Figure 5), and (3) suggested distinct glial and neuronal phenotypes consistent with previous reports in depression (Tables 1–3). Relevant biological systems included the GABA (*Gabra2* and *Gabrg1*), glutamate (*Grin2a*, *Grin2b*, *Grin2c*, *Grin3a*, *Gria4*, and *Grm3-8*), serotonin (*Htr4*), peptide (*Igf1r*, *Igfbp3*, *Npy*, *Npy1r*, *NPY2r*, and *Mchr1*), and other (*P2rx4* and *Ntrk3*) neurotransmitter systems, and the phospholipase C (*Plch1*, *Dgkg*, and *Plcd1*), protein kinase C (*Akap5-10* and *Prkcd*), mitogen-activated protein kinase (*MAKbp1*, *Map2k6*, *Map4k5*, *Mapk1*, and *Mapk10*), and cyclic adenosine monophosphate (*Adcy2* and *Pde7a*)-related pathways. AMY glial-related changes suggested an antidepressant-sensitive downregulation of oligodendrocyte structure and function (*Mobp*, *Edg2*, *Gsn*, *Cnp1*, *Gpr37*, *Pllp*, *Enpp2*, *Pmp22*, *Mpzl1* and *Plp1*; Tables 2 and 3), consistent with prior reports of decreased oligodendrocyte numbers in AMY in human depression (Hamidi *et al*, 2004) and decreased oligodendrocyte-related gene expression in the adjacent temporal cortex (Aston *et al*, 2005). These findings were not present in CC and may differ from schizophrenia-related pathology, where more widespread oligodendrocyte-related findings were reported (Haroutunian *et al*, 2007). Nevertheless, these results raised the question as to the role and contribution of decreased oligodendrocyte function in neuropsychiatric disorders. Widespread decreases in elderly schizophrenic subjects may disrupt communication between multiple brain areas through altered myelination of fiber tracts (Haroutunian *et al*, 2007), but the more regionally restricted phenotype observed here suggests the presence of a local biological mechanism affecting oligodendrocyte homeostasis. Whether this mechanism occurs at the level of cellular proliferation, maturation, and/or maintenance remains to be investigated.

Functional and pathway analyses of altered gene expression in CC of UCMS-exposed mice revealed a more complex picture involving numerous cellular functions (receptors, signal-transduction pathways, transcription, metabolism, and structural components) and did not *a priori* identify specific cellular population as in AMY. In contrast, findings in DG were overall more limited in scope. UCMS-induced changes were only marginally reversed by antidepressants, and overall did not provide supporting evidence for reported changes in neurotropic function in depression and/or antidepressant effects in that brain area. Specifically, we observed no changes in *Bdnf* RNA levels after UCMS or antidepressant treatments. This absence of change was confirmed by independent qPCR measurements (not shown). Taken together, these results are consistent with a recent report showing that, in contrast to immobilization stress, UCMS in rats yielded no change in total *Bdnf* mRNA in DG, despite differentially altering specific *Bdnf* transcripts (Nair *et al*, 2007). We cannot exclude that similar changes occurred here, as exonic-dependent transcriptional regulation was not assessed in this study, thus keeping potential biological significant mechanisms undetectable (ie, post-translational, protein level, maturation, etc.). An alternate explanation for the paucity of DG findings may be that the UCMS model represents a more appropriate model for the altered mood regulation component of depression, rather than for the cognitive aspects of the disease, which may correlate more closely with changes in stress- or antidepressant-induced modulation of cellular proliferation and neurogenesis in DG (Saxe *et al*, 2006). Nevertheless, it is important to note that even if more limited in scope, molecular findings in DG may include relevant changes that could be critical to the altered mood-related phenotype. Collecting samples from brain tissue may also lead to missing signals and to underestimating the dynamic range for some changes, due to signal dilution in heterogeneous tissues, such as DG. An alternative approach would be to investigate altered gene expression based on laser-capture-selected cell populations (Ginsberg *et al*, 2004), although this approach requires *a priori* knowledge as to which cellular population to target, and has the disadvantage of being less quantitative, due to very small amount of RNA collected and partial mRNA decay during the time required for the procedure.

Summary, Limitations

Taken together, our strategy in this study was to maximize the discovery process by maintaining statistical thresholds at moderate stringency and by relying on cumulative effects over larger groups of genes, as previously demonstrated by the investigation of a robust biological phenomenon such as aging (Sibille *et al*, 2007b). This approach was helpful here at reliably identifying global features of UCMS and antidepressant treatments, which were otherwise not discernable by other approaches. Specifically, results provided answers to critical questions relating to research on the neurobiology of neuropsychiatric disorder, such as (1) antidepressant drug treatments in 'non-depressed' animals are probably not relevant to the disease and its treatment, (2) mechanisms of disease and drug treatments will differ across brain regions investigated, and (3) overall gene expression plasticity decreased from control to UCMS states. On the other hand, we also suggest that results at the level of single genes should be viewed with caution. Indeed, in the absence of formal control for false discovery and without independent confirmation in other cohorts and models, the microarray results will include false positives for individual genes. Rates of false discovery are difficult to assess due to the overall inter-dependence of genes in biological systems (Lee *et al*, 2003; Li *et al*, 2004) and since the observed effects of 'depression' and treatments are

typically large in the numbers of genes being affected, but modest in the extent of transcript changes and statistical robustness for individual genes (see current results, and Landgrebe *et al*, 2002; Rausch *et al*, 2002; Newton *et al*, 2003; Drigues *et al*, 2003; Palotas *et al*, 2004; Altar *et al*, 2004; Wong *et al*, 2004; Ploski *et al*, 2006; Takahashi *et al*, 2006). In other words, controlling for false discovery would exclude large numbers of genes of interest, while maintaining medium statistical stringency will necessarily include false-positive results. So, while the overall state-dependent and region-specific effects of depression and antidepressant treatments are robust and rely on large number of genes, results for individual genes need to be independently verified. Accordingly, we make available large-scale data (Table 2 and Supplementary Information) so that other groups can compare their results in other cohorts, models, and species. To facilitate the identification of changes that are most relevant to altered mood regulation, and as potential leads for future cell-specific targeted approaches, we are currently comparing the present results to array findings in the homologous brain areas in human depressed subjects. These results will be described elsewhere. Additional limitations of the present study that will also need to be addressed in future studies include (1) correlations of RNA findings with protein levels, (2) which genes and pathways correspond to causative changes in mood regulation, therapeutic reversal, drug side effects or epiphenomena, and (3) the extent to which these findings extrapolate to other antidepressant treatments.

CONCLUSION

Using a naturalistic animal model of depression, we demonstrate that the gene expression-related effects of two antidepressant treatments (fluoxetine and CRF_1 antagonism) are strongly influenced by the intrinsic biology of different brain areas (ie, brain region-specificity), and vary greatly depending on whether treated animals are in a control or 'depressed' state (ie, state-dependency). Correlations between behavioral states, drug exposure, and altered gene transcripts suggested candidate genes and pathways for region-specific contributions to mood regulation and therapeutic improvement, confirmed several prior depression-related findings, and highlighted the critical role of AMY in investigating the molecular pathophysiology of depression.

ACKNOWLEDGEMENTS

This study was supported by the National Institute of Mental Health or the National Institutes of Health NIMH KO1MH067721 (ES) and 5P50MH066171 (ES). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Mental Health or the National Institutes of Health. We thank Sunghee Oh for statistical support. Guy Griebel is an employee of Sanofi-Aventis.

DISCLOSURE/CONFLICT OF INTEREST

The authors of this paper have no conflict of interests.

REFERENCES

Alonso R, Griebel G, Pavone G, Stemmelin J, Le Fur G, Soubrie P (2004). Blockade of CRF(1) or V(1b) receptors reverses stress-induced suppression of neurogenesis in a mouse model of depression. *Mol Psychiatry* **9**: 278–286, 224.

Altar CA, Laeng P, Jurata LW, Brockman JA, Lemire A, Bullard J *et al* (2004). Electroconvulsive seizures regulate gene expression of distinct neurotrophic signaling pathways. *J Neurosci* **24**: 2667–2677.

Arzt E, Holsboer F (2006). CRF signaling: molecular specificity for drug targeting in the CNS. *Trends Pharmacol Sci* **27**: 531–538.

Aston C, Jiang L, Sokolov BP (2005). Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder. *Mol Psychiatry* **10**: 309–322.

Ayensu WK, Pucilowski O, Mason GA, Overstreet DH, Rezvani AH, Janowsky DS (1995). Effects of chronic mild stress on serum complement activity, saccharin preference, and corticosterone levels in Flinders lines of rats. *Physiol Behav* **57**: 165–169.

Banasr M, Valentine GW, Li XY, Gourley SL, Taylor JR, Duman RS (2007). Chronic unpredictable stress decreases cell proliferation in the cerebral cortex of the adult rat. *Biol Psychiatry* **62**: 496–504.

Belmaker RH, Agam G (2008). Major depressive disorder. *N Engl J Med* **358**: 55–68.

Berton O, McClung CA, DiLeone RJ, Krishnan V, Renthal W, Russo SJ *et al* (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science* **311**: 864–868.

Bowley MP, Drevets WC, Ongur D, Price JL (2002). Low glial numbers in the amygdala in major depressive disorder. *Biol Psychiatry* **52**: 404–412.

Castren E (2004). Neurotrophic effects of antidepressant drugs. *Curr Opin Pharmacol* **4**: 58–64.

Conti B, Maier R, Barr AM, Morale MC, Lu X, Sanna PP *et al* (2007). Region-specific transcriptional changes following the three antidepressant treatments electro convulsive therapy, sleep deprivation and fluoxetine. *Mol Psychiatry* **12**: 167–189.

Cryan JF, Holmes A (2005). The ascent of mouse: advances in modelling human depression and anxiety. *Nat Rev Drug Discov* **4**: 775–790.

Dennis Jr G, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC *et al* (2003). DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* **4**: 3.

Drevets WC, Price JL, Simpson Jr JR, Todd RD, Reich T, Vannier M *et al* (1997). Subgenual prefrontal cortex abnormalities in mood disorders. *Nature* **386**: 824–827.

Drigues N, Poltyrev T, Bejar C, Weinstock M, Youdim MB (2003). cDNA gene expression profile of rat hippocampus after chronic treatment with antidepressant drugs. *J Neural Transm* **110**: 1413–1436.

Ducottet C, Aubert A, Belzung C (2004). Susceptibility to subchronic unpredictable stress is related to individual reactivity to threat stimuli in mice. *Behav Brain Res* **155**: 291–299.

Ducottet C, Griebel G, Belzung C (2003). Effects of the selective nonpeptide corticotropin-releasing factor receptor 1 antagonist antalarmin in the chronic mild stress model of depression in mice. *Prog Neuropsychopharmacol Biol Psychiatry* **27**: 625–631.

Duman RS, Heninger GR, Nestler EJ (1997). A molecular and cellular theory of depression. *Arch Gen Psychiatry* **54**: 597–606.

Duman RS, Malberg J, Thome J (1999). Neural plasticity to stress and antidepressant treatment. *Biol Psychiatry* **46**: 1181–1191.

Erraji-BenChekroun L, Underwood MD, Arango V, Galfalvy HC, Pavlidis P, Smyrniotopoulos P *et al* (2005). Molecular aging in human prefrontal cortex is selective and continuous throughout adult life. *Biol Psychiatry* **57**: 549–558.

Galfalvy HC, Erraji-Benchekroun L, Smyrniotopoulos P, Pavlidis P, Ellis SP, Mann JJ *et al* (2003). Sex genes for genomic analysis in human brain: internal controls for comparison of probe level data extraction. *BMC Bioinformatics* **4**: 37.

Ginsberg SD, Elarova I, Ruben M, Tan F, Counts SE, Eberwine JH *et al* (2004). Single-cell gene expression analysis: implications for neurodegenerative and neuropsychiatric disorders. *Neurochem Res* **29**: 1053–1064.

Gourley SL, Wu FJ, Kiraly DD, Ploski JE, Kedves AT, Duman RS *et al* (2007). Regionally specific regulation of ERK MAP kinase in a model of antidepressant-sensitive chronic depression. *Biol Psychiatry* **4**: 353–359.

Griebel G, Simiand J, Steinberg R, Jung M, Gully D, Roger P *et al* (2002). 4-(2-Chloro-4-methoxy-5-methylphenyl)-*N*-[(1*S*)-2-cyclopropyl-1-(3-fluoro-4-methylp henyl)ethyl]5-methyl-*N*-(2-propynyl)-1,3-thiazol-2-amine hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing factor(1) receptor antagonist. II. Characterization in rodent models of stress-related disorders. *J Pharmacol Exp Ther* **301**: 333–345.

Guillot PV, Roubertoux PL, Crusio WE (1994). Hippocampal mossy fiber distributions and intermale aggression in seven inbred mouse strains. *Brain Res* **660**: 167–169.

Hamidi M, Drevets WC, Price JL (2004). Glial reduction in amygdala in major depressive disorder is due to oligodendrocytes. *Biol Psychiatry* **55**: 563–569.

Haroutunian V, Katsel P, Dracheva S, Stewart DG, Davis KL (2007). Variations in oligodendrocyte-related gene expression across multiple cortical regions: implications for the pathophysiology of schizophrenia. *Int J Neuropsychopharmacol* **10**: 565–573.

Holsboer F (2000). The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology* **23**: 477–501.

Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP (2003). Summaries of Affymetrix GeneChip probe level data. *Nucleic Acids Res* **31**: e15.

Ising M, Zimmermann US, Kunzel HE, Uhr M, Foster AC, Learned-Coughlin SM *et al* (2007). High-affinity CRF1 receptor antagonist NBI-34041: preclinical and clinical data suggest safety and efficacy in attenuating elevated stress response. *Neuropsychopharmacology* **32**: 1941–1949.

Jankowski MP, Sesack SR (2004). Prefrontal cortical projections to the rat dorsal raphe nucleus: ultrastructural features and associations with serotonin and gamma-aminobutyric acid neurons. *J Comp Neurol* **468**: 518–529.

Kirby LG, Rice KC, Valentino RJ (2000). Effects of corticotropin-releasing factor on neuronal activity in the serotonergic dorsal raphe nucleus. *Neuropsychopharmacology* **22**: 148–162.

Kulkarni SK, Dhir A (2007). Effect of various classes of antidepressants in behavioral paradigms of despair. *Prog Neuropsychopharmacol Biol Psychiatry* **31**: 1248–1254.

Kunzel HE, Zobel AW, Nickel T, Ackl N, Uhr M, Sonntag A *et al* (2003). Treatment of depression with the CRH-1-receptor antagonist R121919: endocrine changes and side effects. *J Psychiatr Res* **37**: 525–533.

Landgrebe J, Welzl G, Metz T, van Gaalen MM, Ropers H, Wurst W *et al* (2002). Molecular characterisation of antidepressant effects in the mouse brain using gene expression profiling. *J Psychiatr Res* **36**: 119–129.

Lee HK, Hsu AK, Sajdak J, Qin J, Pavlidis P (2003). Coexpression analysis of human genes across many microarray data sets. *Genome Res* **14**: 1085–1094.

Li KC, Liu CT, Sun W, Yuan S, Yu T (2004). A system for enhancing genome-wide coexpression dynamics study. *Proc Natl Acad Sci USA* **101**: 15561–15566.

Li S, Wang C, Wang M, Li W, Matsumoto K, Tang Y (2007). Antidepressant like effects of piperine in chronic mild stress treated mice and its possible mechanisms. *Life Sci* **80**: 1373–1381.

Linkowski P, Mendlewicz J, Kerkhofs M, Leclercq R, Golstein J, Brasseur M *et al* (1987). 24-Hour profiles of adrenocorticotropin, cortisol, and growth hormone in major depressive illness: effect of antidepressant treatment. *J Clin Endocrinol Metab* **65**: 141–152.

Lukkes JL, Forster GL, Renner KJ, Summers CH (2007). Corticotropin-releasing factor 1 and 2 receptors in the dorsal raphe differentially affect serotonin release in the nucleus accumbens. *Eur J Pharmacol* **578**: 185–193.

Manji HK, Drevets WC, Charney DS (2001). The cellular neurobiology of depression. *Nat Med* **7**: 541–547.

Mayberg HS (1997). Limbic-cortical dysregulation: a proposed model of depression. *J Neuropsychiatry Clin Neurosci* **9**: 471–481.

Mineur YS, Belzung C, Crusio WE (2006). Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. *Behav Brain Res* **175**: 43–50.

Mineur YS, Prasol DJ, Belzung C, Crusio WE (2003). Agonistic behavior and unpredictable chronic mild stress in mice. *Behav Genet* **33**: 513–519.

Nair A, Vadodaria KC, Banerjee SB, Benekareddy M, Dias BG, Duman RS *et al* (2007). Stressor-specific regulation of distinct brain-derived neurotrophic factor transcripts and cyclic AMP response element-binding protein expression in the postnatal and adult rat hippocampus. *Neuropsychopharmacology* **32**: 1504–1519.

Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM (2002). Neurobiology of depression. *Neuron* **34**: 13–25.

Newton SS, Collier EF, Hunsberger J, Adams D, Terwilliger R, Selvanayagam E *et al* (2003). Gene profile of electroconvulsive seizures: induction of neurotrophic and angiogenic factors. *J Neurosci* **23**: 10841–10851.

Palotas M, Palotas A, Puskas LG, Kitajka K, Pakaski M, Janka Z *et al* (2004). Gene expression profile analysis of the rat cortex following treatment with imipramine and citalopram. *Int J Neuropsychopharmacol* **7**: 401–413.

Pariante CM, Miller AH (2001). Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biol Psychiatry* **49**: 391–404.

Paxinos G, Franklin KBJ (2001). *The Mouse Brain in Stereotoxic Coordinates*. Academic Press: San Diego, CA.

Pezawas L, Meyer-Lindenberg A, Drabant EM, Verchinski BA, Munoz KE, Kolachana BS *et al* (2005). 5-HTTLPR polymorphism impacts human cingulate–amygdala interactions: a genetic susceptibility mechanism for depression. *Nat Neurosci* **8**: 828–834.

Ploski JE, Newton SS, Duman RS (2006). Electroconvulsive seizure-induced gene expression profile of the hippocampus dentate gyrus granule cell layer. *J Neurochem* **99**: 1122–1132.

Pothion S, Bizot JC, Trovero F, Belzung C (2004). Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. *Behav Brain Res* **155**: 135–146.

Price ML, Curtis AL, Kirby LG, Valentino RJ, Lucki I (1998). Effects of corticotropin-releasing factor on brain serotonergic activity. *Neuropsychopharmacology* **18**: 492–502.

Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dilley G, Pittman SD, Meltzer HY *et al* (1999). Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol Psychiatry* **45**: 1085–1098.

Rausch JL, Gillespie CF, Fei Y, Hobby HM, Stoming T, Ganapathy V *et al* (2002). Antidepressant effects on kinase gene expression patterns in rat brain. *Neurosci Lett* **334**: 91–94.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S *et al* (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**: 805–809.

Saxe MD, Battaglia F, Wang JW, Malleret G, David DJ, Monckton JE *et al* (2006). Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. *Proc Natl Acad Sci USA* **103**: 17501–17506.

Seminowicz DA, Mayberg HS, McIntosh AR, Goldapple K, Kennedy S, Segal Z *et al* (2004). Limbic-frontal circuitry in

major depression: a path modeling metaanalysis. *Neuroimage* **22**: 409–418.

Sheline YI, Gado MH, Price JL (1998). Amygdala core nuclei volumes are decreased in recurrent major depression. *NeuroReport* **9**: 2023–2028.

Shelton RC (2007). The molecular neurobiology of depression. *Psychiatr Clin North Am* **30**: 1–11.

Sibille E, Arango V, Joeyen-Waldorf J, Wang Y, Leman S, Surget A *et al* (2008). Large-scale estimates of cellular origins of nRNAs: enhancing the yield of transcriptome analyses. *Neurobiol Dis* **167**: 198–206.

Sibille E, Su J, Leman S, Le Guisquet AM, Ibarguen-Vargas Y, Joeyen-Waldorf J *et al* (2007). Lack of serotonin(1B) receptor expression leads to age-related motor dysfunction, early onset of brain molecular aging and reduced longevity. *Mol Psychiatry* **12**: 1042–1056.

Surget A, Saxe M, Leman S, Ibarguen-Vargas Y, Chalon S, Griebel G *et al* (2008). Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. *Biol Psychiatry* 2008 Apr 10 [E-pub ahead of print] doi: 10.1016/j.biopsych.2008.02.022.

Takahashi Y, Washiyama K, Kobayashi T, Hayashi S (2006). Gene expression in the brain from fluoxetine-injected mouse using DNA microarray. *Ann NY Acad Sci* **1074**: 42–51.

Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ (2006). Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nat Neurosci* **9**: 519–525.

Waselus M, Valentino RJ, Van Bockstaele EJ (2005). Ultrastructural evidence for a role of gamma-aminobutyric acid in mediating the effects of corticotropin-releasing factor on the rat dorsal raphe serotonin system. *J Comp Neurol* **482**: 155–165.

Willner P (2005). Chronic mild stress (CMS) revisited: consistency and behavioural–neurobiological concordance in the effects of CMS. *Neuropsychobiology* **52**: 90–110.

Wodarz N, Rupprecht R, Kornhuber J, Schmitz B, Wild K, Riederer P (1992). Cell-mediated immunity and its glucocorticoid-sensitivity after clinical recovery from severe major depressive disorder. *J Affect Disord* **25**: 31–38.

Wong ML, Licinio J (2001). Research and treatment approaches to depression. *Nat Rev Neurosci* **2**: 343–351.

Wong ML, O'Kirwan F, Hannestad JP, Irizarry KJ, Elashoff D, Licinio J (2004). St John's wort and imipramine-induced gene expression profiles identify cellular functions relevant to antidepressant action and novel pharmacogenetic candidates for the phenotype of antidepressant treatment response. *Mol Psychiatry* **9**: 237–251.

Yalcin I, Aksu F, Belzung C (2005). Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol. *Eur J Pharmacol* **514**: 165–174.

Zobel AW, Nickel T, Kunzel HE, Ackl N, Sonntag A, Ising M *et al* (2000). Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *J Psychiatr Res* **34**: 171–181.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)

Molecular Psychiatry (2007) 12, 1042–1056

www.nature.com/mp

ORIGINAL ARTICLE

Lack of serotonin$_{1B}$ receptor expression leads to age-related motor dysfunction, early onset of brain molecular aging and reduced longevity

E Sibille[1,2], J Su[1], S Leman[3], AM Le Guisquet[3], Y Ibarguen-Vargas[3], J Joeyen-Waldorf[1], C Glorioso[2], GC Tseng[4], M Pezzone[5], R Hen[6] and C Belzung[3]

[1]*Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA;* [2]*Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA, USA;* [3]*EA3248 Psychobiologie des émotions, Faculté des Sciences et Techniques, Université François Rabelais, Tours, France;* [4]*Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA, USA;* [5]*Department of Medicine, University of Pittsburgh, Pittsburgh, PA, USA and* [6]*Center for Neurobiology and Behavior, Columbia University, New York, NY, USA*

Normal aging of the brain differs from pathological conditions and is associated with increased risk for psychiatric and neurological disorders. In addition to its role in the etiology and treatment of mood disorders, altered serotonin (5-HT) signaling is considered a contributing factor to aging; however, no causative role has been identified in aging. We hypothesized that a deregulation of the 5-HT system would reveal its contribution to age-related processes and investigated behavioral and molecular changes throughout adult life in mice lacking the regulatory presynaptic 5-HT$_{1B}$ receptor (5-HT$_{1B}$R), a candidate gene for 5-HT-mediated age-related functions. We show that the lack of 5-HT$_{1B}$R (*Htr1b*^{KO} mice) induced an early age-related motor decline and resulted in decreased longevity. Analysis of life-long transcriptome changes revealed an early and global shift of the gene expression signature of aging in the brain of *Htr1b*^{KO} mice. Moreover, molecular changes reached an apparent maximum effect at 18-months in *Htr1b*^{KO} mice, corresponding to the onset of early death in that group. A comparative analysis with our previous characterization of aging in the human brain revealed a phylogenetic conservation of age-effect from mice to humans, and confirmed the early onset of molecular aging in *Htr1b*^{KO} mice. Potential mechanisms appear independent of known central mechanisms (Bdnf, inflammation), but may include interactions with previously identified age-related systems (IGF-1, sirtuins). In summary, our findings suggest that the onset of age-related events can be influenced by altered 5-HT function, thus identifying 5-HT as a modulator of brain aging, and suggesting age-related consequences to chronic manipulation of 5-HT.

Molecular Psychiatry (2007) **12,** 1042–1056; doi:10.1038/sj.mp.4001990; published online 10 April 2007

Keywords: serotonin; aging; longevity; cortex; Bdnf; sirtuin

Introduction

Aging leads to morphological[1–5] and functional[6–9] changes in the brain and is associated with increased risk for psychiatric and neurological disorders.[10–13] However, the mechanisms underlying normal aging of the brain likely differ from those associated with neurodegenerative and pathological conditions and are still poorly understood.[14] Several lines of evidence suggest a role for 5-HT during aging, including structural and functional age-related changes in the 5-HT system in rodents[15–17] and in humans, as documented by post-mortem receptor binding studies,[18–20] RNA level studies,[21] *in vivo* imaging studies[22] and neuroendocrine challenges.[10] Depending on the brain region investigated, the 5-HT modulation of cerebral glucose metabolism increases or decreases during normal aging, suggesting a deregulated control of 5-HT[23] (see also[24]). The mechanisms for age-related changes in 5-HT function are not known and may include gene variants, pharmacological manipulation in adult/old population or late-onset functional declines. Based on converging roles in energy metabolism, cellular signaling pathways and synaptic plasticity, interactions between 5-HT, neurotrophic function (brain-derived neurotrophic factor, Bdnf) and insulin-like growth factor (IGF) have been

Correspondence: Dr E Sibille, Department of Psychiatry, University of Pittsburgh, 3811 O'Hara Street, BST W1643, Pittsburgh, PA 15213, USA.
E-mail: sibilleel@upmc.edu or
Professor C Belzung, EA3248 Psychobiologie des émotions, Université François Rabelais de Tours, Parc de Granmont, Tours 37200, France.
E-mail: catherine.belzung@univ-tours.fr
Received 30 October 2006; revised 18 January 2007; accepted 8 February 2007; published online 10 April 2007

proposed as potential determinants of homeostasis and health during aging.[25] Therefore, due to the critical role of 5-HT in mood regulation, age-related changes in 5-HT function are considered a risk factor for developing mood disorders in older subjects.[12]

A candidate gene for deregulated 5-HT control in aging is the $5HT_{1B}$ receptor ($5HT_{1B}R$).[26] $5HT_{1B}R$ is the predominant presynaptic autoreceptor modulating 5-HT release in the brain.[27] Decrease in $5HT_{1B}R$ function, and not in its somatodendritic counterpart ($5HT_{1A}R$), has been reported in aged rodents,[26] consistently with the role of this receptor subtype in motor function[28] and with the well-characterized decline in motor function during aging. As aging can be viewed as the accumulation of a variety of events that together create a chronic challenge to the brain, and since 5-HT is a key factor for adaptation to stress,[29] we hypothesized that a central deregulation of the 5-HT system in *Htr1b*^{KO} mice would affect the brain response to this challenge and reveal the contribution of 5-HT to age-related processes. Accordingly, inactivation of $5HT_{1B}R$ in *Htr1b*^{KO} mice[30] results in mostly normal baseline, but altered 5-HT kinetics upon recruitment of the 5-HT system (i.e. increased release and higher synaptic levels), as revealed by pharmacological challenges and microdialysis studies.[27,31,32] Here we addressed the issue of causality versus correlation between 5-HT and aging, by investigating age-related behavioral and molecular changes as a result of the disruption of serotonin signaling through the $5HT_{1B}R$ in *Htr1b*^{KO} mice. We now show that the lack of $5HT_{1B}R$-mediated signaling induced both an early age-related motor decline and a global early shift of the characteristic gene expression signature of aging in the brain, ultimately resulting in decreased longevity, thus identifying 5-HT as a modulator of brain aging.

Materials and methods

Animals
All animals were raised under standard conditions: temperature $21 \pm 2^{\circ}C$, controlled humidity 20–25%, 12:12 photoperiod with lights on at 20:00 to test animals during their scotophase. Food and water were available *ad libitum*. Weaning took place at 21 ± 1 days. At this age, animals were ear-punched and genotyped. Littermate wild-type (WT) and *Htr1b*^{KO} mice were used for all behavior and microarray experiments, with the exception of animals used for the 3-month time point microarray analysis and for the serum-level measurements. These latter groups were no more than two generations away from heterozygous breeding. To avoid putative confounding effects of the previously reported increased aggressiveness of *Htr1b*^{KO} mice,[30] WT and knockout (KO) mice were housed under reduced cage density, resulting in normal or low intra-cage aggression in both experimental groups, as revealed by the absence of bite marks or wounds. All experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and with the University of Pittsburgh Animal Care and Use Committee.

Behavior
Separate groups of mice were tested at the age of 2, 6, 12 and 18 months, although all animals were generated and born approximately at the same time. This means that all animals were submitted to a battery of tests only once, according to the following schedule: open field, elevated-plus maze, rotarod and coat hanger tests. At least 1 week separated two different tests.

Open field. The apparatus consisted of a gray polyvinyl chloride circular open field, 40 cm in diameter and 30 cm high. The floor was divided into six peripheral and one circular central sectors, all of the same area (180 cm^2) and covered by a white sheet of paper, which was changed after each mouse. A black–white stripped pattern, 30×20 cm, was present on the wall and provided a local cue. The device was lit by a red bulb placed 80 cm above the floor of the open field. Each mouse was introduced in the center of the open field and recorded for a period of 5 min. Numbers of peripheral and central sector crossings (total locomotion) and of rearing were recorded.

Elevated plus maze. The apparatus consists on two open and two closed 40×10 cm arms, located 40 cm above the floor. Mice were placed in the center and the time and number of entries in the closed and open arms were recorded for 5 min.

Rotarod. The apparatus consisted of a rotating horizontal rod located 25 cm above the floor. A fixed and relatively slow rotating speed was chosen (10 revolution per minute) to increase the sensitivity of this assay at older ages. One block of 10 trials was applied with an inter-trial interval of 10 min. The latency before falling was recorded with a cut-off point of 60 s.

Coat hanger. The triangular-shaped apparatus consisted of a horizontal steel wire (diameter: 2 mm, length: 41 cm) flanked at each end by two sidebars (length: 19 cm; inclination: 35° from the horizontal axis).[33] The horizontal bar was placed at a 45 cm height from the floor. The mice were placed upside-down in the middle of the horizontal wire and released only after gripping with all four paws. Latency before falling was recorded. A trial ended when the mice fell or reached the top of the apparatus, from which it was retrieved and the maximal score of 1 min given for latencies before falling. A block of five consecutive trials was applied with a 15-min inter-trial interval and a 1-min cut-off period per trial.

All behavioral assays were analyzed by analysis of variance (ANOVA) with age and genotype as fixed factors.

Enzyme-linked immunosorbent assays
Blood samples were collected, clotted and centrifuged at room temperature to obtain serum samples,

which were aliquoted into microcentrifuge tubes and stored at −20°C. Serum samples were thawed and diluted in duplicate, and quantitative determination of mouse serum albumin and immunoglobulin (IgG) (Alpha Diagnostic International, San Antonio, TX, USA), IGF-1 (Quantikine, R&D Systems, Inc., Minneapolis, MN, USA) and insulin (Crystal Chem Inc., Downers Grove, IL, USA) were measured using their respective enzyme-linked immunosorbent assay (ELISA) kits according to specific manufacturer instructions. Within 30 min of terminating each reaction assay, optical densities were measured on an ELISA plate reader (SpectraMax Gemini XS, Molecular Devices Corp, Sunnyvale, CA, USA) at a wavelength of 450 nm. Mean absorbance for each duplicate sample was compared with standard curves to obtain concentration values.

Intestinal histology
Small and large intestine were harvested, rinsed gently in saline to remove food and fecal material, and fixed in 4% buffered formaldehyde for 4 h. After washing twice in phosphate-buffered solution (PBS) for 10 min, tissue samples were then cryoprotected in 30% sucrose in PBS overnight at 4°C. After paraffin embedding, tissues were sectioned at 10 μm using a sliding microtome, mounted on poly-lysine-coated slides, dried and stained with hematoxylin and eosin (Sigma, St Louis, MO, USA).

Immunocytochemistry
Small and large intestine were processed for 5-HT$_{1B}$R immunocytochemistry. After cryoprotection with sucrose, intestinal samples were frozen in optimum cutting temperature-embedding medium (Miles Laboratories, Elkhart, IN, USA), cut in 8 μm sections on a cryostat and thaw mounted on poly-lysine-coated slides. Tissue sections were re-hydrated in potassium–phosphate-buffered solution (KPBS) at room temperature, blocked with 10% normal goat serum and incubated overnight at 4°C with a rabbit polyclonal IgG antibody to the rat 5-HT$_{1B}$R (Acris Antibodies, Hiddenhausen, Germany), diluted 1:100 in KPBS, 0.05% goat serum and 0.1% Triton X-100. This primary antibody recognizes rat, mouse and human epitopes corresponding to amino acids 8–26 and 263–278 of the rat 5-HT$_{1B}$R. The following day, slides were rinsed with KPBS three times and then incubated with a Cy3-conjugated goat anti-rabbit IgG secondary antibody (Jackson ImmunoResearch, West Grove, PA, USA) at room temperature for 2 h at a dilution of 1:800 in KPBS, 0.05% goat serum and 0.1% Triton X-100. Slides were washed three times with KPBS, coverslipped and imaged using an Olympus Fluoview 500 scanning confocal microscope in the Center for Biological Imaging at the University of Pittsburgh. Optimal antibody concentrations were determined by serial dilutions. Controls for the specificity of the antisera consisted of incubation of the tissue with normal rabbit serum substituted for the primary antiserum. Using this substitution, no nonspecific staining was seen. Positive control consisted of substituting mouse cortex (CTX) for intestinal tissue.

For the dopamine transporter (DAT), a similar protocol was applied on 20-μm post-fixed coronal brain sections incubated with a rat monoclonal anti-DAT antibody (Chemicon International Inc., Temecula, CA, USA) in the presence of avidin and biotin blocking solutions (Vector Laboratories, Burlingame, CA, USA). Slides were developed with the ABC kit (Vector Laboratories, Burlingame, CA, USA). Optimal development time was determined on parallel sections. All experimental samples were processed simultaneously with pairs of aged-matched WT and KO sections on the same slides. Optical densities were quantified with the ImageJ software (http://rsb.info.nih.gov/ij/).

Microarray samples and processing
Mice were killed by cervical dislocation. Brains were split along the sagittal line, frozen in isopentane and stored at −80°C. To collect samples, frozen brains were cut on a cryostat to the appropriate anatomical level where series of 1 or 2 mm diameter micropunches (Sample corer, Fine Science Tools, Foster City, CA, USA) were collected from frontal CTX and striatum (STR) and immediately stored in Trizol reagent (Invitrogen, Carlsbad, CA, USA). CTX samples were collected from prelimbic and cingulate cortices corresponding mostly to non-motor areas between figures 18 and 23 (Bregma $\sim +2$ to $+1$ mm) in the Paxinos–Franklin Mouse Brain Atlas.[34] Dorsal STR samples were collected starting at figure 23 in the same atlas (Bregma $\sim +1$ to 0 mm). Total RNA was extracted using the Trizol protocol, cleaned with Rneasy microcolumns (QIAGEN, Hilden, Germany), quantified and verified by chromatography using the Agilent Bioanalyzer system (Santa Clara, CA, USA). Microarray samples ($n = 3$–4 per age-, genotype- and brain regions; total, $n = \sim 60$ arrays) were prepared according to the manufacturer's protocol. In brief, 2 μg of total RNA were reverse transcribed and converted into double-stranded complementary DNA (cDNA). A biotinylated cRNA was then transcribed *in vitro*, using an RNA polymerase T7 promoter site, which was introduced during the reverse transcription of RNA into cDNA. Twenty micrograms of fragmented-labeled cRNA sample was hybridized onto MOE 430–2.0 Affymetrix oligonucleotide microarrays (Affymetrix, Santa Clara, CA, USA). A high-resolution image of the hybridization pattern on the probe array was obtained by laser scanning, and fluorescence intensity data were automatically stored in a raw file. To reduce the influence of technical variability, samples were randomly distributed at all experimental steps to avoid any simultaneous processing of related samples. For data extraction, single arrays were analyzed with the Affymetrix Microarray GCOS software. Microarray quality control parameters were as follows: noise (RawQ) < 5 (CTX: 1.53 ± 0.03; STR: 1.65 ± 0.05), background signal < 100 (250 targeted intensity for array scaling; CTX: 46.2 ± 0.9; STR:

45.3 ± 0.8), consistent number of genes detected as present across arrays (CTX: 49.7 ± 0.4; STR: 52.6 ± 0.4), consistent scaling factors (CTX: 1.80 ± 0.05; STR: 1.52 ± 0.06), Actin and GAPDH 3′/5′ signal ratios <3 (CTX: ACT, 2.15 ± 0.19, GAPDH, 1.18 ± 0.09; STR: ACT, 1.43 ± 0.04, GAPDH, 0.90 ± 0.03) and consistent detection of BioB and BioC hybridization spiked controls.

Array statistical analysis

For statistical analysis, probeset signal intensities were extracted with the robust multi-array average algorithm[35] (http://www.bioconductor.org). The 45 101 probesets were reduced to ~20 000 probesets after preprocessing and filtering (present calls ⩾10%, coefficient of variation superior than 0.1 and averaged expression ⩾20). The 3-month WT and KO groups were bred at a different experimental time and were not combined in a single large-scale analysis.

Denote by $\{x_{gkti}\}$ the expression intensities from microarray, where $1 \leqslant g \leqslant 45\,101$ labels indexes for genes ($k = 0$, 1), for genotype ($k = 0$: WT; $k = 1$: KO), $t = 10$, 18, 24 for age and $i = 1$, 2, 3, 4 for biological replicates. Genes with age-related expression changes were selected by the following three analytical procedures.

- *First*, expression intensities of each gene g were fitted to a two-way ANOVA model in 10-, 18- and 24-month groups and WT and KO groups: $x_{gkti} = \mu_g + \alpha_{gk} + \beta_{gt} + \gamma_{gkt} + \varepsilon_{gkti}$.
  In the model, α represents the genotype effect, β the age-effect and γ the interaction term.
- *Second*, genotype differences were tested (two-group *t*-tests) at 3 month of age for all genes identified in (1) and,
- *Third*, one-way ANOVA models within genotype groups (WT and KO) were fitted to genes identified in steps 1 and 2 to characterize the age-related effects in the respective WT or KO experimental groups: $x_{gkti} = \mu_{gk} + \alpha_{gkt} + \varepsilon_{gkti}$, $k = 0.1$.

The goal of the overall analysis was to use the profiles of expression of large groups of genes as an 'experimental assay' to identify and measure age-related molecular effects, and to assess the cumulative effects of changes over groups of genes (i.e. correlation, functional analysis …). Therefore thresholds for gene selection were kept at medium stringencies ($P < 0.01$, changes greater than 20%). This approach has the advantage of allowing the investigation of such patterns, although the extent and levels of correlations may have been slightly underestimated.

Mouse–human age-effect correlation

We have previously reported changes in gene expression with age in the human prefrontal CTX using U133Plus-2.0 arrays.[36] Human orthologs of genes with age-effect in the mouse were identified between the MOE-430-2.0 and U133Plus-2.0 arrays using the NetAFFX webtools (Affymetrix, Santa Clara, CA, USA). In the case of multiple human probesets for a single-mouse probeset, the human probeset with the lowest age-related *P*-value was retained. Correlations of age-effects were calculated using $\log_2$ ratio values. In rodents, the ratios were as described in the text and figures. For humans, the effect of age was calculated as the signal ratio between subjects over 65 years of age versus subjects under 30 years.[36] Similar results were obtained using different threshold criteria for gene selection in the mouse data sets (in Figure 2: $P < 0.001$, changes greater than 20%). A similar approach was applied to assess correlations in transcriptome changes in CTX between *Bdnf*^{KO} mice[37] and age-related profiles in *Htr1b*^{KO} mice, as mouse probesets were directly comparable between the two studies.

Age–pattern correlation

To identify patterns of changes in gene expression in relationship to the occurrence of WT/KO behavioral differences (i.e. 10 and 18 months of age), correlation levels were systematically calculated between WT and *Htr1b*^{KO} mice gene expression at the 10- and 18-month time points and all possible transcript profiles. Profiles were designed based on two groups (WT and *Htr1b*^{KO}), two time points (10- and 18 months) and three ordinal expression levels (high, medium and low) for a total of $(3^2)^2 = 81$ possible patterns. The analysis was limited to the 1097 genes with identified age-effects in either experimental groups. With the exception of a very few probesets (See Supplementary Table S5), all identified genes were expressed at the same level at the 3-month time point. Ninety-nine percent of the genes had correlation levels greater or equal to 0.7 with at least one pattern. Patterns were then reduced to three major profiles: (i) initial WT/KO differences at 10 months or 'early' pattern, (ii) initial WT/KO differences at 18 months or 'late' pattern and (iii) overlapping profiles (no WT/KO differences). This approach was more comprehensive than simple group comparisons (i.e. difference or not at 10 or 18 months), although the vast majority of genes displayed correlation levels only with a very few patterns that corresponded closely to the profiles displayed for averaged values in Figure 5.

Functional class scoring analysis

See details at http://www.bioinformatics.ubc.ca/ermineJ/.[38,39] Rather than analyzing genes one at a time, gene functional class scoring gives scores to classes or groups of genes, representing the overall effect of age on these groups of genes. Gene groups were organized according to the Gene Ontology (GO) classification[40] and GO groups with greater than 200 or fewer than eight genes were screened out. GO groups were scored as described,[38] using age-related *P*-values as gene scores. Briefly, a raw score for each set of genes with a GO family or custom gene group is calculated as the mean of the negative log of the gene scores for all genes in each gene class. When a gene is represented more than once, only the best score is counted. The raw score is transformed into a *P*-value for age-effect

on that group by comparing it to an empirically determined distribution of raw scores. This distribution is obtained by randomly generating gene classes of the same size as the class being tested; this is repeated 100 000 times to generate the distribution of scores expected if high gene scores are not concentrated in the class.

Real-time quantitative polymerase chain reaction

Real-time quantitative polymerase chain reaction (qPCR) was performed as described previously.[41] In brief, small PCR products (80–120 base-pairs) were amplified in quadruplets on an Opticon real-time PCR machine (MJ Research, Waltham, MA, USA), using universal PCR conditions (65C–59C touch-down, followed by 35 cycles (15 s at 95C, 10 s at 59C and 10 s at 72C)). cDNA (150 pg) was amplified in 20 μl reactions (0.3 $\times$ Sybr-green, 3 mM $MgCl_2$, 200 μm dNTPs, 200 μm primers, 0.5 unit Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA)). Primer dimers were assessed by amplifying primers without cDNA. Primers were retained if they produced no primer dimers or nonspecific signal only after 35 cycles. Results were calculated as relative intensity compared to actin.

In situ *hybridization*

In situ hybridization was as described previously.[42,43] Primers were designed using the primer 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) to amplify half of the gene-coding region and half of the 3′ untranslated region of the Sirt5 cDNA (NM 178848; base-pairs 510–1144). Primers included SP6 and T7 RNA polymerase promoter tails for sense and antisense *in vitro* transcription from the amplified PCR products. PCR products were amplified from mouse brain cDNA and verified by sequence analysis. Labeled *in vitro* transcription was performed using the Ambion maxiscript kit (Ambion, Inc., Austin, TX, USA) in the presence of ^{35}S-CTP. Probes were purified using the Qiagen Rneasy kit (Qiagen, Inc., Valencia, CA, USA) and the amount of incorporated radioactivity was quantified on a liquid scintillation counter. *In situ* hybridization was performed on five coronal sections per mouse. Five mice were used per genotype and per age group (3, 6, 18 and 24 months) according to a standard protocol.[42,43] Briefly, slides were incubated for 10 min at room temperature in 4% buffered paraformaldehyde, washed in 0.1 M PBS, serially dehydrated in increasing concentrations of ethanol, and then incubated in hybridization buffer overnight at 56°C in the presence of antisense or sense probe (2 000 000 counts per slide). The following day, the slides were washed, RNAse treated, dried and exposed to film. Kodak BioMAx MR film intensities were determined to be optimal after a 4-day exposure. Images were standardized to C^{14} standards (ARC-146A and ARC-146D; American Radiolabeled Chemicals, Inc, St Louis, MO, USA) and areas corresponding to CTX and STR were quantified using the Microcomputer Imaging Device analysis software (Imaging Research, London, ON, Canada).

Results

Early onset of age-related motor deficits and reduced longevity in Htr1b^{KO} *mice*

Htr1b^{KO} mice presented no obvious developmental changes,[28,30] exhibited a normal behavior in young adulthood (Figure 1a–d, 2-month time points), but displayed an early onset of characteristic age-related motor decline, which became significant at 6 months of age in the rotarod test and at 12 months in the coat hanger test (Figure 1a and b). Total activity in the open field and the elevated plus maze (EPM) tests were not different from WT controls and declined similarly with age (Figure 1c and d). This early decline in motor behavior in *Htr1b*^{KO} mice was not due to learning deficits,[44] as procedural learning curves were essentially parallel between genotypes (Figure 1e). Consistent with previous reports,[28] *Htr1b*^{KO} mice displayed normal anxiety-like behavior in young adulthood (Supplementary Figure S1). Assessing the progression of anxiety-related behaviors over time revealed no age-related genotype difference, although the interpretation of these measurements was limited by the very low activity of older animals in both experimental groups in the challenging compartments of the behavioral apparatus (center of open field and open arms of the elevated-plus maze; Supplementary Figure S1).

The first death events occurred between 16 and 20 months of age in *Htr1b*^{KO} mice and after 21 months of age in WT mice. *Htr1b*^{KO} mice displayed significantly decreased longevity ($P<0.0001$; Figure 1f), with reductions in maximum (−14%) and average (−19%) life spans. The largest difference was observed at 30 months of age, where ~60% of WT mice but only ~5% of *Htr1b*^{KO} mice were still alive. Inspection of complementary log-mortality plots (Figure 1g) and of a Cox proportional hazard model revealed that on average *Htr1b*^{KO} mice had a 3.75-fold increase of hazard ratio ($P<0.0005$). Our analysis also revealed that changes in hazard were time dependent, which means that the increase of hazard could be larger at some points but smaller at other times. However, the averaged slopes of the mortality curves were identical (WT: 0.159 (0.159–0.177), KO: 0.160 (0.160–0.177)) and the time-related differences in estimates were very small, as the *Htr1b*^{KO} mortality curve was virtually superimposable on the WT curve (Figure 1g, hatched curve). Thus, together these results demonstrated a shift of longevity and mortality curves toward earlier ages in *Htr1b*^{KO} mice, and suggested a causative and modulatory role for 5-HT in age-related motor behavior and longevity.

The 'age-related' phenotype of Htr1b^{KO} *mice appears mediated by brain mechanisms*

The 5-HT$_{1B}$R is the main 5-HT presynaptic autoreceptor in the brain[27] and has limited functions in

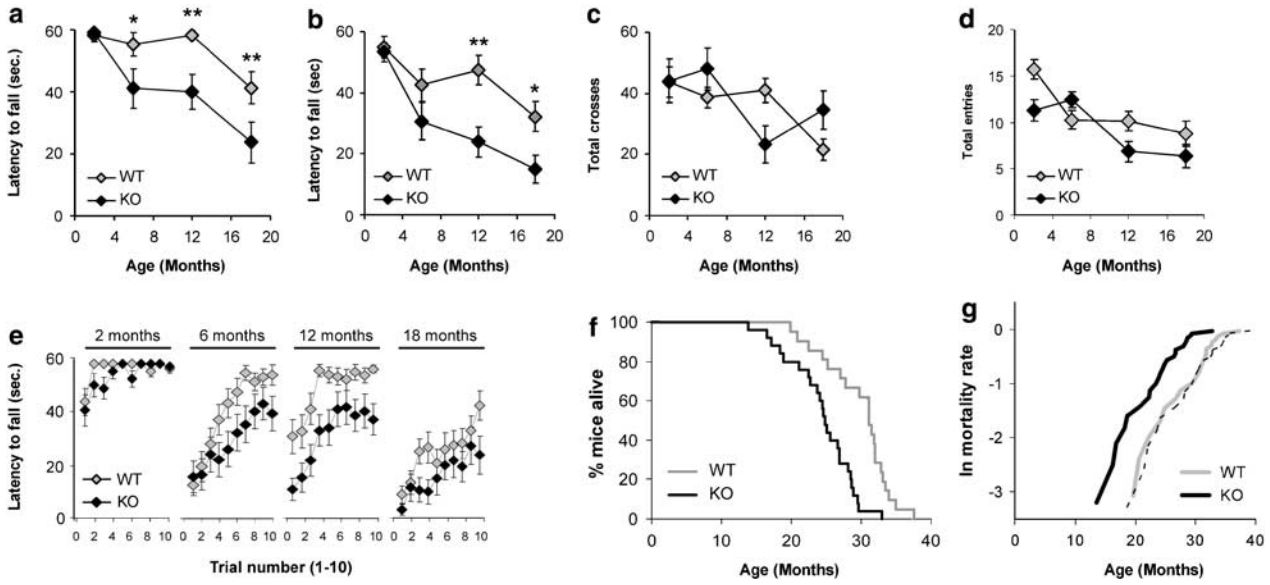

Figure 1 Early onset of age-related motor decline and reduced longevity in *Htr1b*^{KO} mice. (**a** and **b**) *Htr1b*^{KO} mice displayed early age-related motor impairment in the rotarod test ((**a**) latency to fall: $F_{3,117}=11.37$, $P<e^{-6}$; genotype effect: $F=15.04$, $P<0.0005$; genotype*age: $F=2.05$, $P=0.11$) and the coat hanger test ((**b**) latency to fall: $F_{3,117}=8.67$, $P<0.0001$; genotype effect: $F_{1,120}=16.29$, $P<0.0001$; genotype*age: $F_{3,117}=1.98$, $P=0.12$). *Post hoc* tests: $*P<0.05$, $**P<0.005$. (**c** and **d**) Total activity decreased with age in WT and *Htr1b*^{KO} mice in the open field (OF) (**c**) and EPM (**d**) tests (age-effect. OF: $F=4.12$, $P=0.008$; EPM: $F=20.78$, $P<e^{-5}$), although variability in the 12- and 18-month age groups suggested a potential age*genotype interaction (genotype*age-effect. OF: $F=3.09$, $P=0.03$; EPM: $F=3.4$, $P=0.02$; all other effects, $P>0.05$). Different cohort of mice was used for each time point to avoid memory savings between experiments (**a–d**: $n=13$–18 per group and per age). (**e**) Procedural learning curves in the rotarod test were essentially similar, but reached lower maximal values in *Htr1b*^{KO} mice (see (**a**)). (**f**) Kaplan–Meyer survival curves revealed a significant decreased in longevity in *Htr1b*^{KO} mice ($P<0.0001$). (**g**) Mortality curves: *Htr1b*^{KO} mice displayed a 3.75-fold increased hazard ratio ($P<0.0005$). Hatched curve represents *Htr1b*^{KO} mortality curve superimposed on the WT curve (**f** and **g**, WT, $n=21$; KO, $n=24$).

the periphery. Thus we hypothesized that the age-related phenotype might be mediated by central mechanisms, but first, we investigated selected peripheral systems as potential contributors to the phenotype. WT and *Htr1b*^{KO} mice had indistinguishable morphologic features at all ages, including body weights (Figure 2a). Mild alopecia and kyphosis appeared in both genotypes after 2 years of age (not shown). Necropsy procedures revealed no genotype changes in organ's appearance or weight (not shown), including in kidney and lung, two organs with reported roles for 5-HT$_{1B}$R signaling.[45,46] 5-HT modulates gastrointestinal and immune functions, but the 5-HT$_{1B}$R plays little-to-no role in these systems.[47,48] Correspondingly, 5-HT$_{1B}$R immunoreactivity was undetected in the intestinal tract (not shown) and no changes in colonic morphology were identified (Figure 2b). ELISA immunoassays on serum obtained from young and old mice revealed normal albumin content (Figure 2c), suggesting normal absorptive capacity in *Htr1b*^{KO} mice as compared to controls.

Due to the endocrine regulation of aging[49] and potential interaction with the 5-HT system,[25] we measured circulating levels of insulin and IGF-1. Both hormones displayed lower levels in young and old KO mice when compared to age-matched control mice (Figure 2c and d), although these differences reached statistical significance only for insulin. Serum levels for insulin increased with age in *Htr1b*^{KO} mice, suggesting that the lower levels in young KO mice were not due to primary deficits in hormone production. The direction of changes also suggests that hormonal levels are not mediating the age-related phenotype of *Htr1b*^{KO} mice for two reasons. First, increased levels above normal, rather than lower insulin levels, are associated with deleterious effects of aging.[50] Second, earlier studies in model organisms,[49] including mice,[51] predict a protective effect of decreased IGF-1 levels against aging.

Finally, an immunoassay for circulating IgG revealed a smaller age-related increase in IgG levels in serum of old KO mice (2.3-fold increase) versus age-matched WT controls (9.1-fold increase; Figure 2f), thus suggesting a reduced inflammatory load, or reduced recruitment of the immune system in aging *Htr1b*^{KO} mice. Thus, combined with the limited functions of 5-HT$_{1B}$R in the periphery, these results suggested that the age-related behavioral phenotype and reduced longevity of *Htr1b*^{KO} mice may have originated from a central deficiency.

Dopaminergic terminal density and area are not affected in old Htr1b^{KO} *mice*

In the brain, 5-HT$_{1B}$R modulates the synaptic release of 5-HT in serotonergic projection fields, but also acts as a heterologous autoreceptor indirectly regulating

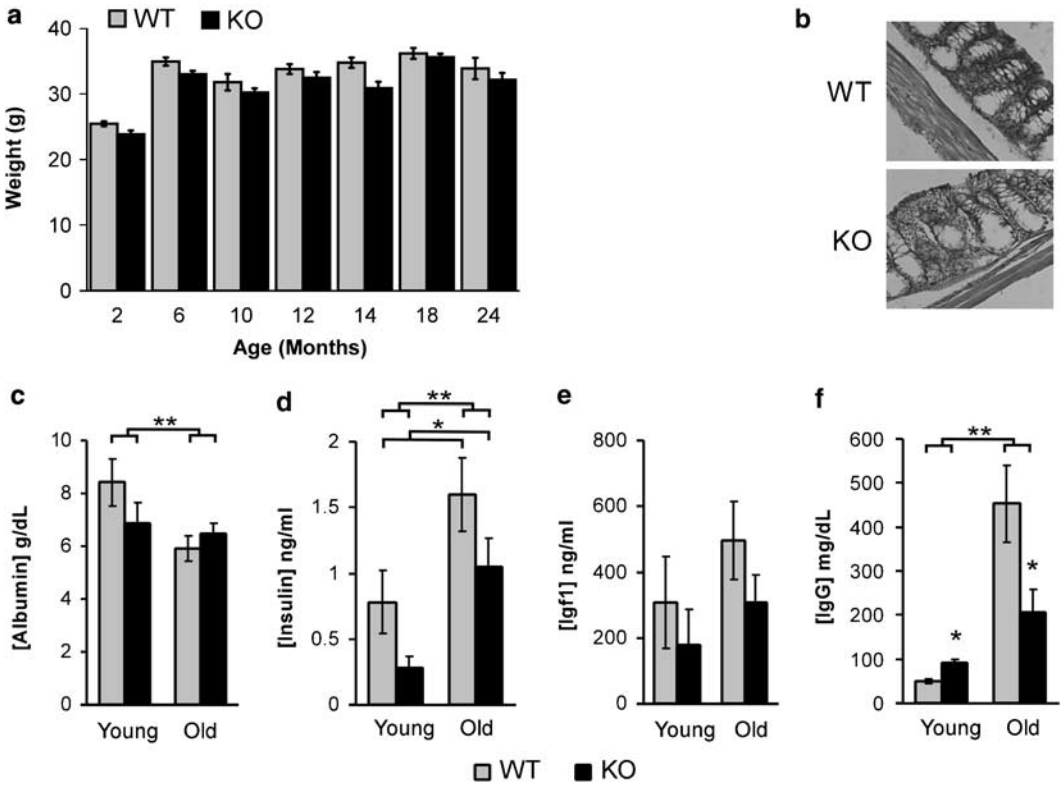

Figure 2 Peripheral markers in *Htr1b*^{KO} and WT mice. (**a**) Body weight ($n = 14$–16 per group, $P > 0.05$). (**b**) Colon mucosal and smooth muscle layers and lumen were equivalent in WT and KO mice (hematoxylin/eosin staining; representative sections from old WT and KO groups). (**c**–**e**) Serum levels of albumin (**c**), insulin (**d**), insulin-like growth factor 1 (IGF-1; **e**) and IgG (**f**). (**c**–**e**): Young (3 months; WT, $n = 5$; KO, $n = 5$) and old (18 months; WT, $n = 6$; KO, $n = 6$). Statistical significance: main-age effects for albumin ($F_{1,22} = 10.2$, $P = 0.005$), insulin ($F_{1,22} = 11.8$, $P < 0.005$) and IgG ($F_{1,22} = 21.27$, $P < 0.001$). Main genotype effect for albumin ($F_{1,22} = 4.4$, $P = 0.08$), genotype*age interaction ($F_{1,22} = 9.2$, $P = 0.02$). Main genotype effect for insulin ($F_{1,22} = 5.02$, $P < 0.05$) and age–genotype interaction for IgG ($F_{3,22} = 6.5$, $P < 0.05$). All other effects, $P > 0.05$. *Post hoc* tests; $^{**}P < 0.01$, $^{*}P < 0.05$. Error bars represent s.e.m.

dopamine (DA) functions. Indeed, *Htr1b*^{KO} mice displayed elevated basal DA levels and increased striatal overflow after cocaine challenge,[31] suggesting potential interactions between DA and the age-related motor phenotype. Using immunohistochemistry against the DA transporter, we found no differences in DA terminals density in the ventral or dorsal STR of young and old WT or KO mice ($P > 0.5$) (Figure 3a and b). DA terminal striatal areas were also unchanged between genotype groups (Figure 3c). Representative figures and measurements are provided at the 18-month time point, long after the onset of behavioral differences (6–10 months of age) and corresponding to the time of early onset of death events in the KO group. Thus, together these results suggested that the early adulthood onset of the motor phenotype in *Htr1b*^{KO} mice was not due to a structural downregulation of the DA system.

Altered gene expression in the brain of aging Htr1b^{KO} *mice corresponds to 'normal' aging*

Altered 5-HT signaling in *Htr1b*^{KO} mice could induce brain deficits that are detrimental to long-term brain homeostasis and survival and that are yet unrelated to age-related processes. Thus, as aging is accompanied by characteristic changes in gene expression in the brain,[36,52,53] we predicted that the 'molecular signature of aging' might occur earlier in *Htr1b*^{KO} mice. To this goal we first investigated the nature of life-long gene expression changes in CTX and STR, two brain areas with well-characterized roles for the 5-HT$_{1B}$R, and then assessed putative differences in the trajectories of age-related changes in *Htr1b*^{KO} mice.

Roughly twice as many genes were affected in correlation with age in CTX of *Htr1b*^{KO} mice, compared with WT mice, with fewer genes affected in STR in both experimental groups (Figure 4a; Supplementary Tables S1 and S2). Despite a considerable overlap (especially in CTX), some genes were identified only in the WT or in the KO group, reflecting either the presence of different age-related effects, or the limitation of the analytical procedures at detecting milder effects in one or the other group. To address this question, we hypothesized that if selected genes were age related, then the overall changes in transcript levels should correlate across groups, regardless of whether genes passed statistical thresholds or not. Indeed, genes identified only in

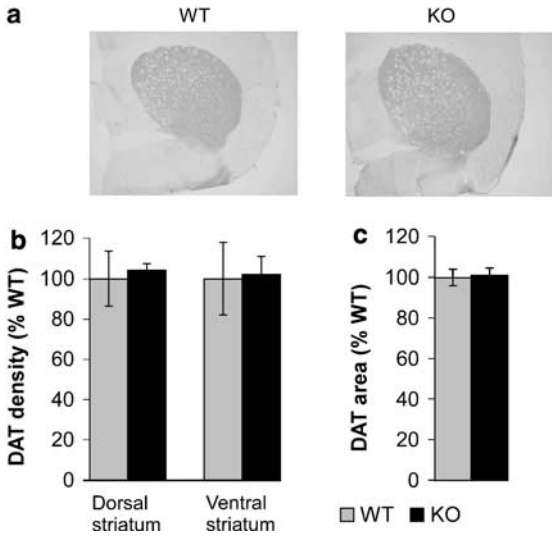

Figure 3 Intact dopaminergic terminal density in old *Htr1b*^{KO} mice. (**a**) Representative photographs of DAT immunohistochemistry in 18-month-old WT and *Htr1b*^{KO} mice ($n=4$ per group, $P>0.05$). (**b**) DAT immunoreactivity density. (**c**) DAT striatal area (similar results were obtained at 3, 6 and 24 months of age).

Htr1b^{KO} mice demonstrated high correlation levels with the effect of aging on the same genes in WT mice (Figure 4b, middle panel), and conversely age-related changes in transcript levels identified only in WT mice correlated highly with changes in KO mice (Figure 4b, left panel), thus revealing that a similar pool of genes was affected across genotypes. The higher slope values in the correlation graphs of KO-only selected genes (CTX, KO = 1.5, WT = 1.2; STR (not shown), KO = 2.3, WT = 1.4) further suggested a larger and more extensive age-effect in *Htr1b*^{KO} mice. Interestingly, correlations were the highest when comparing 18-month-old KO mice to 24-month-old WT mice (Figure 4c), suggesting that *Htr1b*^{KO} mice reached a pronounced age-effect earlier than WT mice. This 'maximum' age-effect corresponded to the period where death events started to occur in *Htr1b*^{KO} mice (Figure 1f).

Mouse–human phylogenetic conservation of age-effect in brain CTX

Comparing results to our previous study of the molecular correlates of aging in the human brain,[36] we identified highly significant correlations between age-related effects in WT or *Htr1b*^{KO} mice and the two investigated areas of the human prefrontal CTX (Figure 4d–f). Mouse–human correlations of age-effects increased from 18 to 24 months in WT mice, but again were highest for 18-month-old *Htr1b*^{KO} mice (Figure 4e). Selected genes with conserved age-effects are displayed in Figure 4f and in the Supplementary Information (Supplementary Table S3). Together, these results demonstrated a phylogenetic conservation of age-effect in mammalian CTX and confirmed the age-related nature and early peak of the brain molecular phenotype of *Htr1b*^{KO} mice.

Early occurrence of the age-related gene expression signature in Htr1b^{KO} *mice*

With very few exceptions (see last paragraph), all identified genes were expressed at similar levels in WT and KO mice at 3 months of age (Figure 5, 3-month time point), indicating that the molecular correlates of the differential age-related phenotype in *Htr1b*^{KO} mice were initiated later in adulthood, and consistently with the lack of behavioral differences in young adulthood. As predicted, investigating the appearance and progression of age-related changes revealed that over 98% of individual genes affected in CTX displayed early changes in *Htr1b*^{KO} mice when compared to WT controls. Changes (25.6%) occurred initially at 10 months (Figure 5a and b, 'early' pattern) and 72.6% at 18 months of age (Figure 5c and d, 'late' pattern). Likewise, over 97% of age-related genes identified in STR displayed similar anticipated age-related profiles in *Htr1b*^{KO} mice (not shown). This early onset of age-related pattern is illustrated for glial fibrillary acidic protein (Gfap; Figure 5e and f), a marker of age- and brain-related events[54] (i.e. inflammation), which displayed a 'late' pattern of changes, with initial WT–KO differences at 18 months of age.

The presence of different age-related patterns of changes in transcript levels raised the question as to whether specific biological functions were associated with these patterns, and could thus have mediated the early onset of behavioral changes in *Htr1b*^{KO} mice. To address this question, we (a) systematically identified groups of related genes displaying high representation of age-affected genes,[36,39] (b) compared results between WT and KO mice and (c) further investigated whether any of the identified gene groups displayed over- or under-representation of genes with changes occurring in parallel to the onset of behavioral differences (i.e. 'early' or 'late' patterns). The cumulative effect of aging on gene groups was assessed according to the GO classification[40] and is presented in a color-coded fashion for the 25 most affected gene groups in WT or *Htr1b*^{KO} mice (Figure 5g and Supplementary Table S4). The nature of the identified gene groups revealed that very similar biological functions were affected during aging in WT and *Htr1b*^{KO} mice, albeit with minor differences. Translation-related gene groups (blue bars in Figure 5g) were prominent in both groups, but displayed increased 'ranked' representation in *Htr1b*^{KO} mice, whereas the representation of inflammation-related gene groups (red bars in Figure 5g) was decreased in *Htr1b*^{KO} mice, in reminiscence of previous evidence suggesting reduced inflammation in the periphery (Figure 1e). Results are presented for CTX and were highly similar in STR (not shown). Importantly, the proportion of genes with 'early' or 'late' onset of WT/KO changes differed only marginally from their expected proportions within

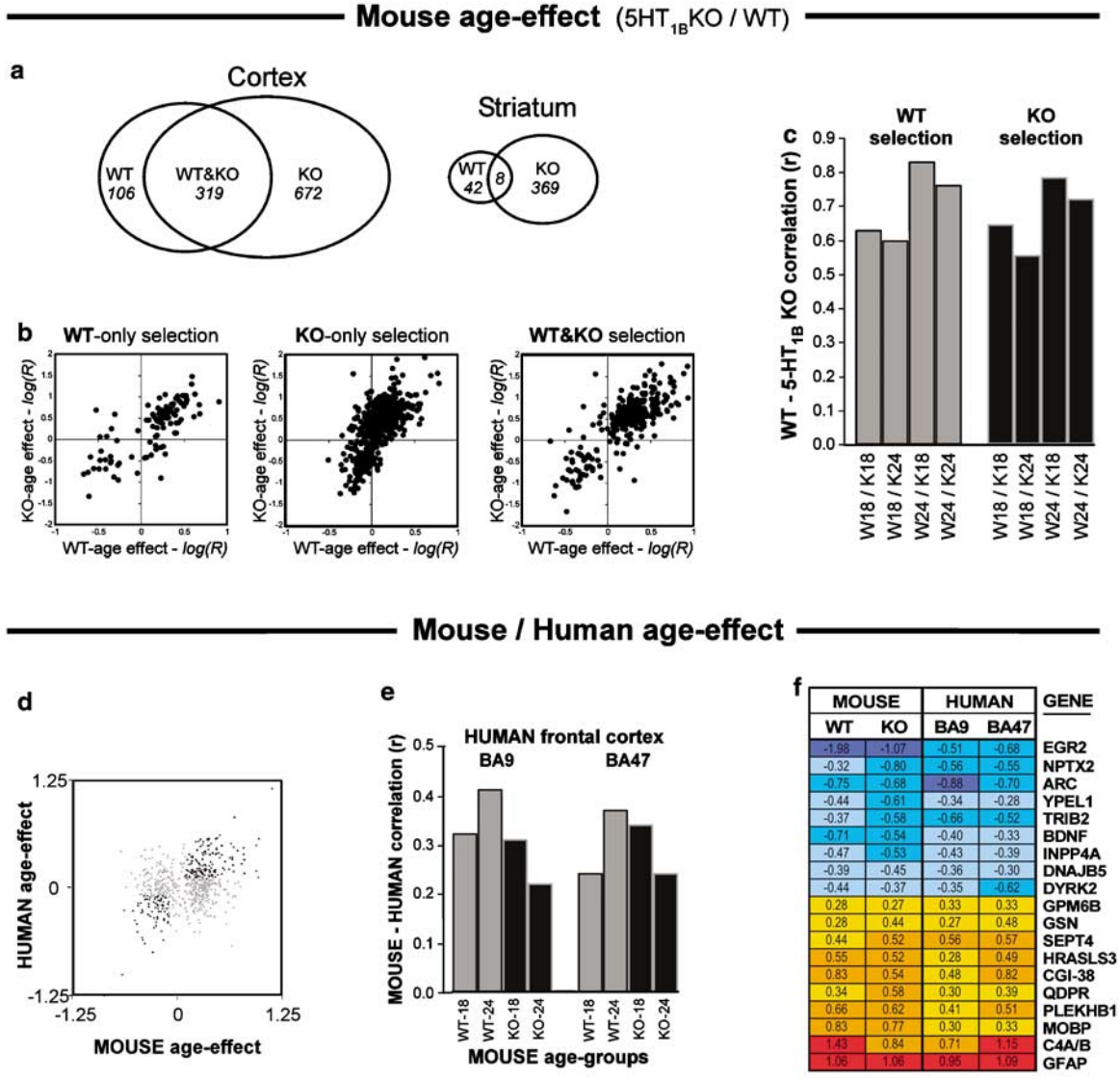

Figure 4 Correlation of age-related gene expression 'signatures' in the brains of WT and *Htr1b*^{KO} mice, and phylogenetic conservation of age-effect between mice and humans. (**a–c**) WT/*Htr1b*^{KO} age-effect comparison. (**a**) Venn diagrams of age-related transcript changes in CTX and STR ($n = 3$–4 arrays per age-, genotype- and brain regions; total, $n = \sim 60$ arrays). (**b**) Correlation graphs between age-effects (LogR = $\log_2$Old/Young) in CTX between genes identified only in WT ($n = 106$, left), only in KO ($n = 672$, middle) and in both groups ($n = 319$, right). (**c**) Correlation levels (r) of age-effects between indicated WT and *Htr1b*^{KO} age groups. All $P < 0.0001$ (**d–f**) Mouse/human age-effect comparison. (**d**) Age-effect correlation graph between genes identified in the mouse and for which age-related expression levels of orthologous genes were available in the human CTX.[36] Black dots indicate genes with most conserved age-effects that are likely to support a large proportion of the overall correlation (see (**f**) and Supplementary Table S3). (**e**) Correlation levels between age-related transcript changes in WT or *Htr1b*^{KO} mouse CTX and two areas of the human prefrontal CTX. All $P < 0.005$, except WT18 versus BA47, $P = 0.01$. Correlations were based on age-effect in rodent ($P < 0.001$) and identifiable human orthologs (WT, $n = 88$ genes; KO, $n = 271$ genes). (**f**) Selected genes with conserved age-effects in CTX between mouse and human. Values are in average $\log_2$ (old/young ratio) (red: increased; blue: decreased). See Supplementary Table S3 for additional genes and details. BA9/47, Brodmann areas 9/47.

the five main identified age-related functions (Figure 5h), with the exception of translation-related gene groups that displayed significantly more genes with late-onset differences. Genes with early WT/KO age-related differences were slightly, but non-significantly, over-represented in inflammation-related functions.

Taken together, results from our temporal and functional analyses of age-related changes in gene expression did not identify any specific biological function as the potential source or mediator of the early onset of the age-related phenotype, but rather suggested a global and early shift of the molecular signature of aging in the brain of *Htr1b*^{KO} mice.

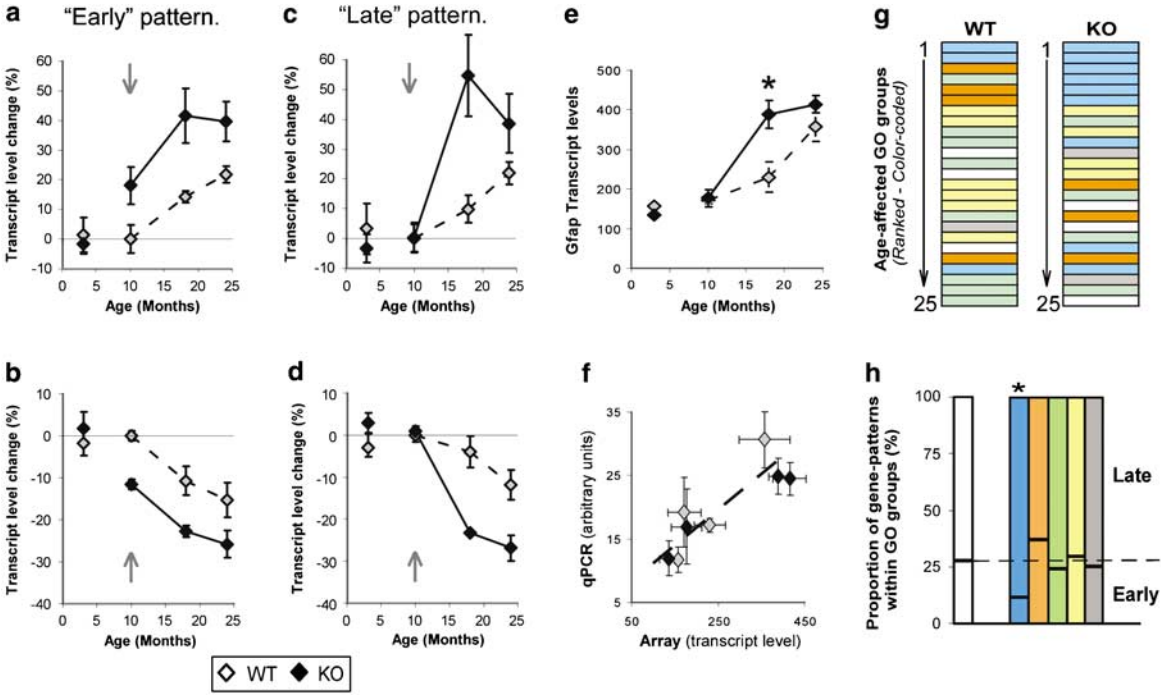

Figure 5 Over 98% of age-related genes displayed early onset of age-related trajectories in *Htr1b*^{KO} mice. (**a–d**) Averaged transcripts profiles for genes with onset of age-related effects in KO mice occurring initially at 10 months ('Early': 198 increased (**a**) and 81 decreased (**b**) genes; 25.6% of age-related genes) or 18 months ('late': 670 increased (**c**) and 126 decreased (**d**) genes; 72.6% of age-related genes), compared to the onset of behavioral differences (vertical gray arrow). Less than 2% of age-affected genes had similar profiles in WT and KO mice (not shown). Values are in percentage of WT levels. The 3-month-old groups used for array analysis were bred at a different time and analyzed separately. (**e**) Gfap age-related transcript profiles as an example of 'late' pattern (age-effect, $P<0.0005$ in WT and KO; *WT/KO at 18 month, $P<0.05$). (**f**) Confirmation of altered Gfap transcript levels by real-time qPCR: Array/qPCR correlation ($r=0.87$, $P=0.005$). Values are mean $\pm$ s.e.m. (**g** and **h**) Functional analysis of molecular aging. (**g**) The 25 most affected color-coded gene groups in WT and *Htr1b*^{KO} mice are regrouped in five main functions: translation (blue), inflammation (red), metabolism (green), cell growth (yellow), cellular respiration (gray), miscellaneous (white). Details in Supplementary Table S4. (**h**) Proportional representation of genes with 'early' (10 months) or 'late' (18 months) patterns of initial WT–KO differences in age-related trajectories within the main age-related functions. *$P<0.001$, difference from expected proportions (white column and hatched bar).

A role for Bdnf in age-related transcriptome changes and in the Htr1b^{KO} *molecular phenotype?*

Altered neurotrophic function, including 5-HT/Bdnf interactions, has been suggested to mediate some of the age-related changes occurring in the brain.[25] Here, we confirmed the previously reported down-regulation of Bdnf transcripts with age in CTX[36,43,55] (WT: −1.63-fold change, $P=0.006$; KO: −2.24-fold change, $P=0.002$; Supplementary Tables S1 and S3) and showed that changes followed an 'early' pattern in *Htr1b*^{KO} mice (Supplementary Table S1), thus suggesting a potential causative role for altered Bdnf function in the *Htr1b*^{KO} age-related phenotype. Therefore, to investigate the potential contribution of Bdnf to the molecular phenotype of *Htr1b*^{KO} mice, we took advantage of a recent report describing CTX gene expression changes occurring downstream from altered Bdnf function in Bdnf KO mice (embryonic or adult *Bdnf*^{KO}, see[37]) and investigated similarities between Bdnf- and age-induced transcriptome changes. In particular, if changes in Bdnf function participated in the age-related *Htr1b*^{KO} molecular phenotype, then the effect of *Bdnf*^{KO} on altered gene transcripts would correlate with the effect of aging, and should mostly follow an early pattern of changes in *Htr1b*^{KO} mice. Here, we identified a moderate, but significant, correlation between changes in *Bdnf*^{KO} mice and age-related changes in *Htr1b*^{KO} or WT mice ($r=\sim 0.20$, $P<0.05$). Correlations with age-related profiles were slightly higher for adult ($r=0.22$, $P<0.05$, $n=98$ genes) versus embryonic *Bdnf*^{KO}-induced changes ($r=0.17$, $P<0.05$, $n=173$ genes). Correlations with *Bdnf*^{KO}-induced changes were also slightly higher when compared to age-related changes in *Htr1b*^{KO} ($r=0.23$, $P<0.05$, $n=271$ genes) versus WT mice ($r=0.20$, $P<0.05$, $n=271$ genes). Importantly, genes affected by both Bdnf and aging displayed age-related trajectories that were evenly distributed between 'early' and 'late' patterns on WT/*Htr1b*^{KO} differences (i.e. 50% 'early' patterns genes), suggesting that Bdnf downregulation did not play a major role in the early onset of age-related events in *Htr1b*^{KO} mice.

Together, these comparative studies revealed a potential active role for Bdnf in age-related changes, as altered gene expression induced by decreased Bdnf correlated with aspects of the molecular correlates of aging in the brain ($r = \sim 0.20$, $P < 0.05$). However, our studies also suggested that the early age-related phenotype in *Htr1b*^{KO} mice was independent of the role of Bdnf in aging.

Increased age-related sirtuin 5 gene expression

What possible mechanism could induce the early onset of age-related events in *Htr1b*^{KO} mice? Out of ~45 000 transcripts tested, only eight gene transcripts displayed consistent genotype differences in CTX and STR (Supplementary Table S5), including at the 3-month time point that preceded the behavioral differences. Two of these probes corresponded to the sirtuin 5 gene (*Sirt5*), which belongs to a family of protein deacetylases that regulate life span in yeast, *Caenorhabditis elegans* and drosophila.[56] Increased *Sirt5* transcripts were confirmed by qPCR and *in situ* hybridization and displayed a pattern of increased levels in *Htr1b*^{KO} mice, converging toward WT levels at 24 months (Figure 6). The role of sirtuin genes in replicative and chronological aging in lower eukaryotes and mammalian cells is complex[57,58] and whether the reported increased *Sirt5* transcripts, and potentially sirtuin 5 function, may mediate the early onset of aging in *Htr1b*^{KO} mice or represent an early adaptive mechanism is currently under investigation.

Discussion

Together our studies have demonstrated that altering 5-HT signaling through the disruption of the 5-HT$_{1B}$ presynaptic autoreceptor can modulate the onset of selected age-related events in the central nervous system. We have shown that the lack of 5HT$_{1B}$R-mediated signaling induced both an early age-related motor decline (Figure 1) and an early shift of the characteristic gene expression signature of aging in the brain (Figure 5), ultimately resulting in decreased longevity (Figure 1). Our results also suggested that the age-related phenotype in *Htr1b*^{KO} mice may have originated in the brain, as peripheral markers revealed no effect (intestinal tract), or changes suggesting protection or adaptive mechanisms (insulin and IGF-1 systems, Figure 2) against deleterious aspects of the early onset of age-related events. As aging can be viewed as the accumulation of a variety of events that together create in essence a chronic challenge to the brain, and since the 5-HT system is a key system for homeostatic control and adaptation to stress,[59] we have provided here evidence supporting the notion that the interaction of 5-HT with this challenge influences age-dependent behavior and molecular events.

Investigating the nature and progression of age-related changes in brain gene expression throughout the adult life, we have uncovered profound changes in *Htr1b*^{KO} mice, which were characteristic of an early

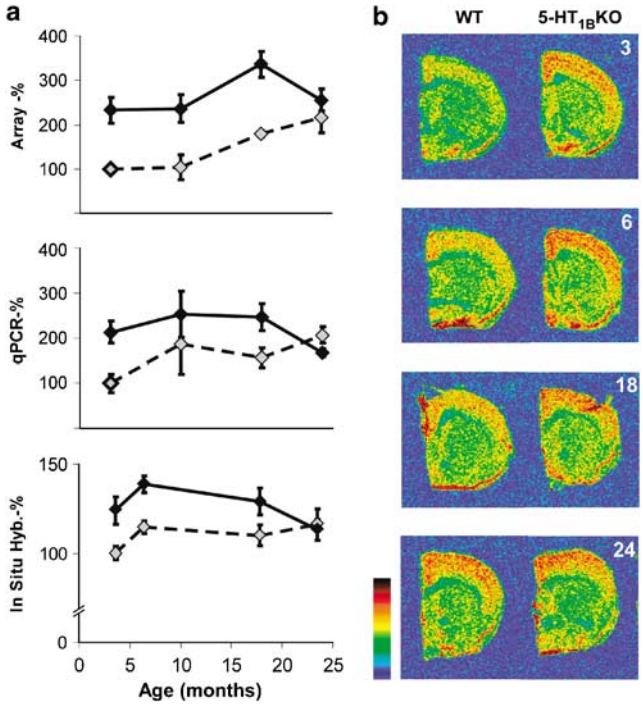

Figure 6 Upregulated *Sirt5* gene expression in CTX of *Htr1b*^{KO} mice. (**a**) Microarray (top), qPCR (middle) and *in situ* hybridization (ISH, bottom) analyses revealed significant increased Sirt5 transcript levels in *Htr1b*^{KO} mice, with normalized differences at 24 months of age. Smaller differences by ISH were mostly due to Sirt5 expression throughout the brain, which precluded background subtraction and likely underestimated specific signal differences. All values are in percentage of young WT controls. Genotype effect: array, $P < 0.01$; qPCR, $P < 0.05$; ISH, $P < 0.01$. Pair-wise correlations (array–qPCR–ISH), all $r > 0.65$, $P < 0.05$. (**b**) Representative color-coded photomicrographs of Sirt5 ^{35}S-ISH at 3, 6, 18 and 24 months of age. Color barcode indicates increased signal intensity.

onset of brain molecular aging (Figure 4). The age-related nature and early onset of the brain molecular phenotype in *Htr1b*^{KO} mice was further confirmed by cross-species comparisons with the correlates of aging in the human brain.[36] Indeed age-related changes occurring in the CTX of WT or *Htr1b*^{KO} mice significantly predicted age-related changes in two areas of the human CTX, and identified numerous individual genes with similar age-effects across species (Figure 4 and Supplementary Table S3), thus demonstrating a phylogenetic conservation of the molecular correlates of aging from mouse to human. These analyses confirmed numerous prior findings at the level of individual genes (e.g. Gfap, Bdnf, MOBP, complement activation, etc.; Supplementary Table S3), but to our knowledge, this is the first large-scale demonstration of a rodent–human phylogenetic conservation of age-effect in the mammalian CTX, thus validating the use of rodent models to recapitulate aspects of aging of the human brain. Importantly, these cross-species comparisons confirmed the age-related nature and the early onset of the brain

molecular phenotype in *Htr1b*^{KO} mice, as *Htr1b*^{KO}–human correlations of age-effect reached similar levels than in WT–human comparisons, albeit at an earlier age (Figure 4e).

Imaging and molecular studies suggest that age-related changes are continuous and progressive throughout the human adult life span.[1,2,36,53] Here we provided evidence supporting the notion of a 'maximum' age-related molecular effect. Indeed, *Htr1b*^{KO} mice reached an apparent peak in age-related changes in gene transcript levels at 18 month of age (Figure 5), which had not yet been attained at the latest time point investigated in WT mice (24 month of age). Interestingly, the timing of this 'maximum' age-effect corresponded to the age where death events started to occur in the KO group, suggesting a failure of the system at maintaining proper homeostasis past this point. Indeed, the decreased correlations of age-related transcriptome profiles that were observed between very old *Htr1b*^{KO} mice and either control mice or human subjects (Figure 2) may have reflected the presence of secondary and less-specific events occurring beyond a certain maximum age-related effect. Nevertheless, despite the potential occurrence of a late breakdown in homeostasis, our results demonstrated mostly a parallel and early age-related phenotype in *Htr1b*^{KO} mice. This trajectory was different from the exponential acceleration in age-related phenotypes and mortality that is commonly observed in mouse models of peripheral/somatic age-related mechanisms.[60–63] In particular, the increased hazard ratio in *Htr1b*^{KO} mice (Figure 1h) appears to be mostly due to a shift of death events toward earlier ages, rather than a progressive increase in frailty leading to an exponential homeostatic failure.[60–63] This parallel, rather than exponential, trajectory is consistent with a progressive and cumulative effect over time, rather than an acute and early mechanistic switch leading to a catastrophic failure of the system. Here we suggest that this parallel age-related trajectory in *Htr1b*^{KO} mice is consistent with an altered capacity of the 5-HT system to initially respond to the chronic challenge that is formed over time by various events occurring during brain aging. Indeed, as typical longevity and molecular curves display early 'buffer' periods where the cumulative effects of aging are not yet observed (i.e. 3- to 12-month behavioral and gene expression time points in WT), *Htr1b*^{KO} mice display a reduced 'buffer' period (3-months time point), resulting in an early onset, rather than altered progression, of age-related phenotypes.

To identify potential mechanisms mediating the early onset of the *Htr1b*^{KO} age-related phenotype, we proceeded with both unbiased surveys of cellular and biological functions and with directed evaluation of markers for candidate biological systems. Systematic analyses of functional relationships and of temporal patterns between age-affected genes (Figure 5) did not identify any novel biological functions being recruited during aging in *Htr1b*^{KO} mice, but rather suggested a global and early onset of the molecular signature of 'normal' aging in the brain of *Htr1b*^{KO} mice. Regarding candidate systems, a leading hypothesis for age-related mechanisms centers on the role of oxidative stress and inflammation, as mediators of neuronal damage and subsequent increase in reactive astrocytes.[14,64] Here, our combined observations of decreased ranked representation of inflammation-related functions in the brain, of late appearance of WT/KO differences of a cellular marker of inflammation (Gfap; Figure 5e and f) and of evidence of reduced peripheral inflammation (IgG, Figure 2f) did not support the notion that inflammation-related events mediated the early onset of aging in *Htr1b*^{KO} mice. Nevertheless, we cannot rule out the possibility that additional peripheral mechanisms may have contributed to the phenotype, especially in the absence of more direct measurements of reactive oxygen species-mediated damage such as lipid peroxidation and protein carbonylation, which may have contributed to the shortened life expectancy.

Bdnf is a neurotrophic factor supporting neuronal functions during development and in the mature brain. Correlative evidence of decreased Bdnf transcript levels with age in the human frontal CTX[36,43,55] has suggested the possibility of a causative role in brain aging. Here, we confirmed the downregulation of Bdnf with aging in the mouse CTX. We also identified a modest but significant correlation between the effects of Bdnf hypofunction in *Bdnf*^{KO} mice[37] and the gene expression correlates of aging in mouse CTX ($r = \sim 0.20$, $P < 0.05$), suggesting that aspects of aging in the brain may occur as a consequence of downregulated Bdnf function. However, although interactions between 5-HT and Bdnf have been documented[65] and hypothesized to both concur with IGF-1 to influence age-related events,[25] our results suggest an age-related phenotype in *Htr1b*^{KO} mice that is independent of Bdnf function.

On the other hand, our studies provided supporting evidence for potential interacting mechanisms between 5-HT and the previously identified age-related IGF-1 and sirtuin systems. A nonsignificant trend toward downregulated levels of circulating IGF-1 levels in *Htr1b*^{KO} supports the notion of 5-HT/IGF-1 interaction during aging. In this case, lower IGF-1 levels would be protective against aging[51] and thus it is not known whether lower IGF-1 levels represented a direct consequence of altered 5-HT function or rather a reactive/protective process against the detection of an early onset of age-related symptoms, as recently hypothesized for age-related processes in peripheral organs.[66] Interestingly, a clear upregulation of sirtuin 5 gene expression was identified in the brain of *Htr1b*^{KO} mice. Sirtuin genes are major cellular components of age-related pathways; however, their role in replicative and chronological aging in lower eukaryotes and mammalian cells is complex.[57,58] The role of altered Sirt5 transcripts in the age-related phenotype in *Htr1b*^{KO} mice is currently under investigation, including as a potential mediator of 5-HT control over homeostasis, or as an early

mechanism for altering the onset of age-related phenotypes, since Sirt5 transcript changes preceded the appearance of age-related behavioral and molecular changes. Nevertheless, it suggests that the differentiated central nervous system may share common molecular and/or cellular components with age-related mechanisms in peripheral somatic tissues.

Although the observed phenotype originated from a deficiency in 5-HT-mediated presynaptic inhibition, a limitation in the analysis of potential mechanisms supporting the observed phenotype resides in the fact that the deletion of this receptor altered the kinetics of the 5-HT system,[27,31,32] and thus could have influenced the behavior, molecular and longevity phenotypes through altered signaling at other 5-HT receptors. Therefore, the observed phenotypes should be considered in the context of altered 5-HT-mediated functions in *Htr1b*^{KO} mice. This observation relates to our initial choice of the *Htr1b*^{KO} mice for investigating the role of 5-HT into aging, as we hypothesized that a disrupted 5-HT homeostasis may initially more closely model the evidence of a deregulated control of 5-HT during aging (see Introduction). Moreover, other neurotransmitters systems interacting with 5HT$_{1B}$R and not investigated here may have played a role in the mutant phenotype. In particular, changes in cholinergic functions may have occurred, as suggested by Buhot *et al.*,[44] in view of complex changes in memory functions in aging *Htr1b*^{KO} mice.[44]

The presence of an early deficit in motor behavior was consistent with the role of 5-HT$_{1B}$R in motor function.[28] The distribution and functional contribution of 5-HT$_{1B}$R in the spinal cord and cerebellum are low,[67,68] whereas the nature of the phenotype indicated a deficit in complex motor behavior (rotarod and coat hanger tests), rather than a decrease in locomotor and/or muscle strength (total activity and rearing in open field and EPM; early occurrence rather than accelerated frailty). This suggested a deficit in higher coordination, rather than a potential degeneration of spinal motor neurons, although a direct examination of the integrity of these neurons will be necessary to rule out this possibility. In the central nervous system, 5-HT$_{1B}$R indirectly regulates DA release in STR.[31] No evidence of altered DA terminal density and area was identified in STR of old (and young) WT or KO mice (Figure 3), thus also excluding a structural downregulation of the DA system as the cause of the motor deficits in young *Htr1b*^{KO} mice, although a role for altered DA kinetics in aging KO animals can not be ruled out. Moreover, the behavior decline could also be mediated through altered signaling at several other 5-HT receptors, due to altered 5-HT kinetics.[27,31] Thus, currently the exact mechanism leading to the motor phenotype is unknown, and may involve additional brain areas and systems not investigated here. Due to the complex biological nature of motor coordination and for the purpose of this study, we have considered the *Htr1b*^{KO} motor phenotype as an indicator of changes in overall age-related functions, while we have concentrated our molecular studies on FC and STR, as two brain areas with well-described roles for 5-HT$_{1B}$R. Furthermore, investigating the FC allowed for the direct comparison of age-related molecular changes in WT and *Htr1b*^{KO} mice with our prior characterization of aging in the human brain.[36] Eventually, studies using regional-, cell type- and time-specific targeted manipulations of 5-HT$_{1B}$R in conditional mutant mice will be necessary to investigate the more specific roles of critical brain regions, time periods and interacting neurotransmitter systems that may participate in the different aspects of the behavioral and molecular phenotypes, and that underscore the increased vulnerability to age-related events in *Htr1b*^{KO} mice.

In conclusion, our studies suggest a brain-driven age-related phenotype in mice lacking the presynaptic 5-HT$_{1B}$R. The notion of altered longevity and early onset of age-dependent events due to changes in the brain represents an important novel finding, which is however not unprecedented. For instance, in lower eukaryotes, the well-characterized changes in longevity due to mutations in the IGF-1 pathway is rescued only when signal transduction is restored in neurons and not in other cell types of nematodes[69] and flies,[70] therefore providing supporting evidence for a control of age-related processes by the brain. Here, as the 'onset' rather than the 'nature' of aging was affected in *Htr1b*^{KO} mice, 5-HT can be considered a modulator of 'normal' aging of the brain, consistently with its role in adapting to environmental challenges and maintaining homeostasis. As the 5-HT system is the target of chronic pharmacological interventions for psychiatric and other diseases, these results raise the possibility of long-term and/or age-related consequences to 5-HT manipulation that will need to be addressed in future studies. Pursuant is the potential for altered molecular and/or behavioral age-related trajectories in correlation with genetic polymorphisms in the Htr1b gene or other key regulators[71] of the 5-HT system.

Acknowledgments

We thank Irwin Lucki and Anita Bechtholt for help with additional rodent support, Ruomei Liang for technical help, and David Lewis and Karoly Mirnics for helpful comments on the manuscript. This work was supported by NIMH (ES).

References

1 Resnick SM, Pham DL, Kraut MA, Zonderman AB, Davatzikos C. Longitudinal magnetic resonance imaging studies of older adults: a shrinking brain. *J Neurosci* 2003; **23**: 3295–3301.
2 Sowell ER, Peterson BS, Thompson PM, Welcome SE, Henkenius AL, Toga AW. Mapping cortical change across the human life span. *Nat Neurosci* 2003; **6**: 309–315.
3 Terry RD, DeTeresa R, Hansen LA. Neocortical cell counts in normal human adult aging. *Ann Neurol* 1987; **21**: 530–539.

4 Bertoni-Freddari C, Fattoretti P, Paoloni R, Caselli U, Galeazzi L, Meier-Ruge W. Synaptic structural dynamics and aging. *Gerontology* 1996; **42**: 170–180.
5 Pakkenberg B, Gundersen HJ. Neocortical neuron number in humans: effect of sex and age. *J Comp Neurol* 1997; **384**: 312–320.
6 Park DC, Lautenschlager G, Hedden T, Davidson NS, Smith AD, Smith PK. Models of visuospatial and verbal memory across the adult life span. *Psychol Aging* 2002; **17**: 299–320.
7 Thibault O, Hadley R, Landfield PW. Elevated postsynaptic [Ca^{2+}]i and L-type calcium channel activity in aged hippocampal neurons: relationship to impaired synaptic plasticity. *J Neurosci* 2001; **21**: 9744–9756.
8 Davare MA, Hell JW. Increased phosphorylation of the neuronal L-type Ca2+ channel Cav1.2 during aging. *Proc Natl Acad Sci USA* 2003; **100**: 16018–16023.
9 Verkhratsky A, Toescu EC. Calcium and neuronal ageing. *Trends Neurosci* 1998; **21**: 2–7.
10 Lerer B, Gillon D, Lichtenberg P, Gorfine M, Gelfin Y, Shapira B. Interrelationship of age, depression, and central serotonergic function: evidence from fenfluramine challenge studies. *Int Psychogeriatr* 1996; **8**: 83–102.
11 Mattson MP, Magnus T. Ageing and neuronal vulnerability. *Nat Rev Neurosci* 2006; **7**: 278–294.
12 Meltzer CC, Smith G, DeKosky ST, Pollock BG, Mathis CA, Moore RY *et al.* Serotonin in aging, late-life depression, and Alzheimer's disease: the emerging role of functional imaging. *Neuropsychopharmacology* 1998; **18**: 407–430.
13 Reynolds III CF, Kupfer DJ. Depression and aging: a look to the future. *Psychiatr Serv* 1999; **50**: 1167–1172.
14 Morrison JH, Hof PR. Life and death of neurons in the aging brain. *Science* 1997; **278**: 412–419.
15 Carlsson A. Neurotransmitter changes in the aging brain. *Dan Med Bull* 1985; **32**(Suppl 1): 40–43.
16 Davidoff MS, Lolova IS. Age-related changes in serotonin-immunoreactivity in the telencephalon and diencephalon of rats. *J Hirnforsch* 1991; **32**: 745–753.
17 Nishimura A, Ueda S, Takeuchi Y, Matsushita H, Sawada T, Kawata M. Vulnerability to aging in the rat serotonergic system. *Acta Neuropathol (Berlin)* 1998; **96**: 581–595.
18 Arango V, Ernsberger P, Marzuk PM, Chen JS, Tierney H, Stanley M *et al.* Autoradiographic demonstration of increased serotonin 5-HT2 and beta-adrenergic receptor binding sites in the brain of suicide victims. *Arch Gen Psychiatry* 1990; **47**: 1038–1047.
19 Marcusson J, Oreland L, Winblad B. Effect of age on human brain serotonin (S-1) binding sites. *J Neurochem* 1984; **43**: 1699–1705.
20 Marcusson JO, Morgan DG, Winblad B, Finch CE. Serotonin-2 binding sites in human frontal cortex and hippocampus. Selective loss of S-2A sites with age. *Brain Res* 1984; **311**: 51–56.
21 Bach-Mizrachi H, Underwood MD, Kassir SA, Bakalian MJ, Sibille E, Tamir H *et al.* Neuronal tryptophan hydroxylase mRNA expression in the human dorsal and median Raphe nuclei: major depression and suicide. *Neuropsychopharmacology* 2005; **31**: 814–824.
22 Meltzer CC, Smith G, Price JC, Reynolds III CF, Mathis CA, Greer P *et al.* Reduced binding of [^{18}F]altanserin to serotonin type 2A receptors in aging: persistence of effect after partial volume correction. *Brain Res* 1998; **813**: 167–171.
23 Goldberg S, Smith GS, Barnes A, Ma Y, Kramer E, Robeson K *et al.* Serotonin modulation of cerebral glucose metabolism in normal aging. *Neurobiol Aging* 2004; **25**: 167–174.
24 van Luijtelaar MG, Tonnaer JA, Steinbusch HW. Aging of the serotonergic system in the rat forebrain: an immunocytochemical and neurochemical study. *Neurobiol Aging* 1992; **13**: 201–215.
25 Mattson MP, Maudsley S, Martin B. A neural signaling triumvirate that influences ageing and age-related disease: insulin/IGF-1, BDNF and serotonin. *Ageing Res Rev* 2004; **3**: 445–464.
26 David DJ, Bourin M, Hascoet M, Colombel MC, Baker GB, Jolliet P. Comparison of antidepressant activity in 4- and 40-week-old male mice in the forced swimming test: involvement of 5-HT1A and 5-HT1B receptors in old mice. *Psychopharmacology (Berlin)* 2001; **153**: 443–449.
27 Trillat AC, Malagie I, Scearce K, Pons D, Anmella MC, Jacquot C *et al.* Regulation of serotonin release in the frontal cortex and ventral hippocampus of homozygous mice lacking 5-HT1B receptors: *in vivo* microdialysis studies. *J Neurochem* 1997; **69**: 2019–2025.
28 Ramboz S, Saudou F, Amara DA, Belzung C, Dierich A, LeMeur M *et al.* Behavioral characterization of mice lacking the 5-HT1B receptor. *NIDA Res Monogr* 1996; **161**: 39–57.
29 Caspi A, Moffitt TE. Gene-environment interactions in psychiatry: joining forces with neuroscience. *Nat Rev Neurosci* 2006; **7**: 583–590.
30 Saudou F, Amara DA, Dierich A, LeMeur M, Ramboz S, Segu L *et al.* Enhanced aggressive behavior in mice lacking 5-HT1B receptor. *Science* 1994; **265**: 1875–1878.
31 Shippenberg TS, Hen R, He M. Region-specific enhancement of basal extracellular and cocaine-evoked dopamine levels following constitutive deletion of the serotonin(1B) receptor. *J Neurochem* 2000; **75**: 258–265.
32 Ase AR, Reader TA, Hen R, Riad M, Descarries L. Altered serotonin and dopamine metabolism in the CNS of serotonin 5-HT(1A) or 5-HT(1B) receptor knockout mice. *J Neurochem* 2000; **75**: 2415–2426.
33 Joyal CC, Beaudin S, Lalonde R. Longitudinal age-related changes in motor activities and spatial orientation in CD-1 mice. *Arch Physiol Biochem* 2000; **108**: 248–256.
34 Paxinos G, Franklin KBJ. *The Mouse Brain in Stereotoxic Coordinates*. Academic Press: San Diego, CA, 2001.
35 Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP. Summaries of Affymetrix GeneChip probe level data. *Nucleic Acids Res* 2003; **31**: e15.
36 Erraji-BenChekroun L, Underwood MD, Arango V, Galfalvy HC, Pavlidis P, Smyrniotopoulos P *et al.* Molecular aging in human prefrontal cortex is selective and continuous throughout adult life. *Biol Psychiatry* 2005; **57**: 549–558.
37 Glorioso C, Sabatini M, Unger T, Hashimoto T, Monteggia LM, Lewis DA *et al.* Specificity and timing of neocortical transcriptome changes in response to BDNF gene ablation during embryogenesis or adulthood. *Mol Psychiatry* 2006; **11**: 633–648.
38 Pavlidis P, Lewis DP, Noble WS. Exploring gene expression data with class scores. *Pac Symp Biocomput* 2002; **1**: 474–485.
39 Pavlidis P, Qin J, Arango V, Mann JJ, Sibille E. Using the Gene Ontology for microarray data mining: a comparison of methods and application to age effects in the human prefrontal cortex. *Neurochem Res* 2004; **29**: 1213–1222.
40 Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM *et al.* Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000; **25**: 25–29.
41 Galfalvy HC, Erraji-Benchekroun L, Smyrniotopoulos P, Pavlidis P, Ellis SP, Mann JJ *et al.* Sex genes for genomic analysis in human brain: internal controls for comparison of probe level data extraction. *BMC Bioinformatics* 2003; **4**: 37.
42 Sibille E, Hen R. Serotonin(1A) receptors in mood disorders: a combined genetic and genomic approach. *Behav Pharmacol* 2001; **12**: 429–438.
43 Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z *et al.* Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. *J Neurosci* 2003; **23**: 6315–6326.
44 Buhot MC, Wolff M, Benhassine N, Costet P, Hen R, Segu L. Spatial learning in the 5-HT1B receptor knockout mouse: selective facilitation/impairment depending on the cognitive demand. *Learn Mem* 2003; **10**: 466–477.
45 Keegan A, Morecroft I, Smillie D, Hicks MN, MacLean MR. Contribution of the 5-HT(1B) receptor to hypoxia-induced pulmonary hypertension: converging evidence using 5-HT(1B)-receptor knockout mice and the 5-HT(1B/1D)-receptor antagonist GR127935. *Circ Res* 2001; **89**: 1231–1239.
46 Ciaranello RD, Tan GL, Dean R. G-protein-linked serotonin receptors in mouse kidney exhibit identical properties to 5-HT1b receptors in brain. *J Pharmacol Exp Ther* 1990; **252**: 1347–1354.
47 Gershon MD. Review article: serotonin receptors and transporters – roles in normal and abnormal gastrointestinal motility. *Aliment Pharmacol Ther* 2004; **20**(Suppl 7): 3–14.
48 Mossner R, Lesch KP. Role of serotonin in the immune system and in neuroimmune interactions. *Brain Behav Immun* 1998; **12**: 249–271.
49 Tatar M, Bartke A, Antebi A. The endocrine regulation of aging by insulin-like signals. *Science* 2003; **299**: 1346–1351.
50 Suji G, Sivakami S. Glucose, glycation and aging. *Biogerontology* 2004; **5**: 365–373.

51 Holzenberger M, Dupont J, Ducos B, Leneuve P, Geloen A, Even PC *et al.* IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 2003; **421**: 182–187.
52 Lee CK, Weindruch R, Prolla TA. Gene-expression profile of the ageing brain in mice. *Nat Genet* 2000; **25**: 294–297.
53 Lu T, Pan Y, Kao SY, Li C, Kohane I, Chan J *et al.* Gene regulation and DNA damage in the ageing human brain. *Nature* 2004; **429**: 883–891.
54 DeVellis JS. *Neuroglia in the Aging Brain*. Humana Press: Totowa, NJ, 2002.
55 Webster MJ, Weickert CS, Herman MM, Kleinman JE. BDNF mRNA expression during postnatal development, maturation and aging of the human prefrontal cortex. *Brain Res Dev Brain Res* 2002; **139**: 139–150.
56 Hekimi S, Guarente L. Genetics and the specificity of the aging process. *Science* 2003; **299**: 1351–1354.
57 Fabrizio P, Gattazzo C, Battistella L, Wei M, Cheng C, McGrew K *et al.* Sir2 blocks extreme life-span extension. *Cell* 2005; **123**: 655–667.
58 Parker JA, Arango M, Abderrahmane S, Lambert E, Tourette C, Catoire H *et al.* Resveratrol rescues mutant polyglutamine cytotoxicity in nematode and mammalian neurons. *Nat Genet* 2005; **37**: 349–350.
59 Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H *et al.* Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 2003; **301**: 386–389.
60 Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE *et al.* Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science* 2005; **309**: 481–484.
61 Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T *et al.* Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* 1997; **390**: 45–51.
62 Mounkes LC, Kozlov S, Hernandez L, Sullivan T, Stewart CL. A progeroid syndrome in mice is caused by defects in A-type lamins. *Nature* 2003; **423**: 298–301.
63 Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE *et al.* Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* 2004; **429**: 417–423.
64 Finch CE. Neurons, glia, and plasticity in normal brain aging. *Adv Gerontol* 2002; **10**: 35–39.
65 Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci* 2000; **20**: 9104–9110.
66 Niedernhofer LJ, Garinis GA, Raams A, Lalai AS, Robinson AR, Appeldoorn E *et al.* A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. *Nature* 2006; **444**: 1038–1043.
67 Alvarez FJ, Pearson JC, Harrington D, Dewey D, Torbeck L, Fyffe RE. Distribution of 5-hydroxytryptamine-immunoreactive boutons on alpha-motoneurons in the lumbar spinal cord of adult cats. *J Comp Neurol* 1998; **393**: 69–83.
68 Wallis DI. 5-HT receptors involved in initiation or modulation of motor patterns: opportunities for drug development. *Trends Pharmacol Sci* 1994; **15**: 288–292.
69 Wolkow CA, Kimura KD, Lee MS, Ruvkun G. Regulation of *C. elegans* life-span by insulin like signaling in the nervous system. *Science* 2000; **290**: 147–150.
70 Hwangbo DS, Gershman B, Tu MP, Palmer M, Tatar M. Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. *Nature* 2004; **429**: 562–566.
71 Payton A, Gibbons L, Davidson Y, Ollier W, Rabbitt P, Worthington J *et al.* Influence of serotonin transporter gene polymorphisms on cognitive decline and cognitive abilities in a nondemented elderly population. *Mol Psychiatry* 2005; **10**: 1133–1139.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)

RESUME

Bien que les troubles anxio-dépressifs représentent une des principales causes d'invalidité et un des plus sérieux problèmes de santé dans le monde, les mécanismes neurobiologiques à l'origine de ces affections demeurent méconnus. Les processus mis en jeu semblent multiples et complexes : passant par des déséquilibres au niveau des neurotransmetteurs jusqu'à des modifications de la plasticité neurale et du remodelage cellulaire. Les neurotrophines étant considérées comme les principaux régulateurs de la plasticité, l'hypothèse d'un lien causal entre le niveau de neurotrophine, principalement de l'une d'entre elles, le Brain-Derived Neurotrophic Factor (BDNF) et l'apparition de troubles anxio-dépressifs a ainsi été proposée. Notre travail a donc eu pour objectif l'étude de l'implication du BDNF dans l'étiopathogenèse et le traitement des troubles anxio-dépressifs à travers l'utilisation de modèles murins.

Etant donné que plusieurs études cliniques et précliniques ont montré une implication du BDNF dans plusieurs traits psychologiques et comportementaux, nous avons cherché à déterminer si des différences dans le gène BDNF pouvaient être à l'origine de la grande hétérogénéité comportementale des différentes souches de souris. Nous rapportons dans cette étude l'existence d'un polymorphisme sur un seul nucléotide (SNP) à l'origine d'un changement d'acide aminé (une leucine est remplacé par une méthionine) en position 32 dans la séquence du prodomaine du BDNF. Nous démontrons ensuite que, bien que ce SNP ne modifie par l'expression basale de BDNF dans le cerveau, ce polymorphisme est associé au phénotype « anxieux » des souris. Par contre, il n'est pas impliqué dans le comportement alimentaire, le toilettage, l'activité, l'apprentissage et la mémoire. Une analyse précise des données montre que les souris portant l'allèle Met présentent une propension plus importante à développer des réactions néophobiques et des comportements d'anxiété de trait. Ainsi, ce polymorphisme pourrait contribuer aux différences de profils comportementaux des différentes lignées de souris dans les tests d'anxiété.

Ensuite, nous avons réalisé une comparaison de sept lignées de souris dans un modèle chronique de la dépression et de l'action thérapeutique des antidépresseurs. Ce paradigme, le stress chronique léger imprédictible (UCMS), modélise l'influence des stress socio-environnementaux dans l'étiopathogenèse de la dépression et est sensible à l'action chronique des antidépresseurs. Nous montrons dans cette étude que l'application d'un même facteur étiologique (les stresseurs) est capable d'induire presque autant de profils différents d'altérations physiques, comportementales et physiologiques, et de réponses au traitement que de lignées de souris testées. De plus, les différents profils obtenus se rapprochent des différents sous-types de la pathologie humaine suggérant que le patrimoine génétique des patients pourrait être déterminant dans l'apparition clinique d'un sous-type particulier. Par contre, aucune association entre le polymorphisme Leu32Met du BDNF et les altérations « dépression-like » ou la réponse au traitement antidépresseur n'émerge de notre étude.

Nous avons enfin étudié l'effet d'un déficit en BDNF sur la vulnérabilité au stress chronique et la réponse aux antidépresseurs par l'utilisation de souris hétérozygotes BDNF $^{+/-}$. Nous montrons ici que l'expression monoallélique du BDNF n'induit pas de différences dans la sensibilité à l'UCMS sur toutes les mesures physiques (état du pelage, poids), comportementales (alimentations, activité, anxiété, agressivité, résignation) et neuroendocrines (corticostéronémie). Par contre, certains effets du traitement sont perturbés chez les souris BDNF $^{+/-}$. Finalement, notre étude suggère que le BDNF n'est pas impliqué dans l'apparition des altérations « dépression-like » mais qu'il est nécessaire à l'efficacité des antidépresseurs.

Ce travail a donc permis de mettre en évidence une relation entre le BDNF et les comportements anxieux ainsi que l'action des antidépresseurs. De plus, en utilisant deux modèles différents (le polymorphisme d'un gène ou son ablation partielle), nous avons montré l'absence de lien entre le BDNF et l'étiopathogenèse de la dépression.

ABSTRACT

Although anxio-depressive disorders are a major cause of disability with important health consequences, the underlying neurobiological mechanisms remain largely unknown. Neurotransmitter imbalances can change numerous intracellular signaling pathways that ultimately result in lasting modifications in neural plasticity and cellular remodeling. Since neurotrophins, and particularly brain-derived neurotrophic factor (BDNF), are considered main regulators of neural plasticity, changes in the expression of these genes are an attractive mechanism by which normally differentiated brain cells may transform into a pathological anxio-depressive phenotype.

A first study seven strains of mice with large behavioral differences were used to investigate whether differences in the expression and sequence BNDF may be associated with anxiety and depressive traits. A change of a single nucleotide in position 32 of prodomain sequence of BNDF, which resulted in a leucine being replaced by methionine, was associated with mice exhibiting the anxious phenotype. Mice carrying the Met allele had greater propensity to neophobic reactions and behaviors related to anxiety. However, this polymorphism did not alter the basal expression of the gene and other behavioral activities including appetite, thirst, grooming, learning and memory. Furthemore, when chronic stress slightly unpredictable (UCMS) was used in seven mouse lines, no association was observed between Leu 32 Met and depression-like effectors due to antidepressant treatments. When heterozygous (+/-) BNDF mice were used in the UCMS model, no changes were noted in physical observations (i.e, hair coat, weight), behavioral responses (to mimic anxiety, aggressiveness and resignation) and in the stress hormones; by contrast, some effects to the anti-depressants were different from the ones seen in homozygous (+/+) mice.

In conclusion, BDNF polymorphisms can contribute to the various behavioral profiles exhibited by different strains of mice in test of anxiety. However, it does not appear to be involved in the etiopathogenesis of depression.