Synaptic identity of neocortical circuits involving Martinotti cells in healthy conditions and in Down syndrome

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THESE DE DOCTORAT EN NEUROSCIENCE

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Science knows no country, because knowledge belongs to humanity, and is the torch which illuminates the world.

Louis Pasteur

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LIST OF ABBREVIATIONS

AIS: axon initial segment

AP: Action Potential

bAP: back propagating action potential

BDZ: benzodiazepine

CCK: colecistokinin

CGE: caudal ganglionic eminence

DS: Down Syndrome

EPSC: excitatory postsynaptic current

FDDI: frequency-dependent disynaptic inhibition

GABA: gamma-aminobutyric acid

$GABA_AR$: $GABA_A$ receptor

IN: interneuron

IPSC: inhibitory postsynaptic current

IT PN: intraenchephalic pyramidal neuron

L1-6: Layer 1-6

MC: Martinotti cell

MGE: medial ganglionic eminence

NMDAR: N-methyl-D-aspartate receptor

NOR: novel object recognition

NOS: nitric oxide synthase

NT: neurotransmitter

PFC: Prefrontal cortex

PN: principal neuron

PPR: Paire pulse ratio

PT cell: pyramidal tract cell

CT cell: corticothalamic cell

PV cell: parvalbumin cell

sEPSC/sIPSC: spontaneous EPSC/IPSC

SST: somatostatin

STDP: spike timing-dependent synaptic plasticity

TC: thalamocortical

uEPSC/uIPSC: unitary EPSC/IPSC

VIP: Vaso Intestinal Peptide

CHAPTER 1:
INTRODUCTION

1.1 The Neocortex

The cerebral cortex is a phylogenetically recent brain structure, which is unique to mammals, and due to its expansion and circuit specificity, makes humans different from other mammals (Fig. 1.1). It is the most superficial brain structure and covers both brain hemispheres. Here high cognitive processes occur: consciousness, awareness, attention, memory, language, calculations, judgment, emotions, abstraction, generation of motor commands, and processing of sensory information (Adolphs et al., 2003; Rakic et al., 2007; Abdel-Mannan et al., 2008). These diverse functions are accomplished in highly differentiated cortical areas. We can distinguish two types of cortex: the allocortex and the neocortex (NCx) (Fig 1.2). The allocortex is phylogenetically older and is a three-layered structure, consisting in the paleocortex (piriform cortex) and the archicortex (hippocampus, dentate gyrus and cingulate cortex). However, the

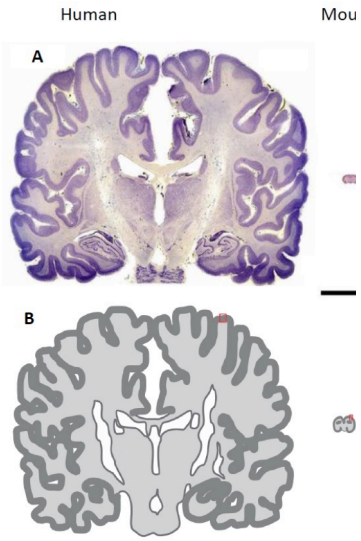


Fig. 1.1. The human and mouse neocortex. (A) Coronal sections of the human and mouse brain (Thionin staining, scale bar: 10 cm) (adapted from DeFelipe, 2011) (B) The dark superficial region shows the neocortex, although it does not reflect actual cortical thickness. Note the difference in size and convolutions between these two mammalian species, resulting in a ~1,000-fold increase in the human (adapted from Lourenço & Bacci, 2017)

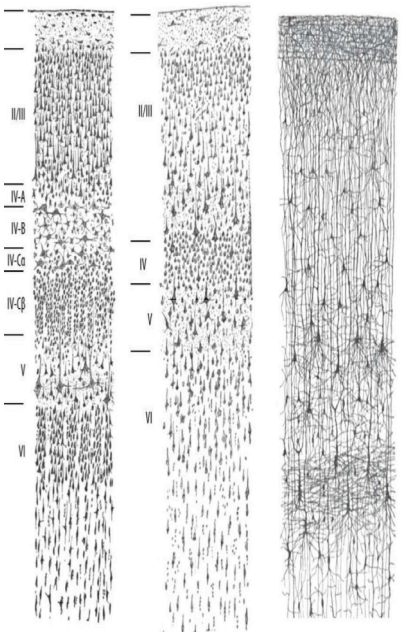


Fig. 1.2 Drawing of neocortical layers from Ramon y Cajal 1911. Left: Nissl staining of the adult visual cortex of human. Middle: Nissl staining of the adult motor cortex of human. Right: Golgi staining of the infant (1 month and ½) human neocortex.

largest portion of the cerebral cortex is represented by the phylogenetically younger neocortex (NCx) that contains about 50.000 neurons/mm^3. This structure is unique to the mammalian brain and constitutes more than 80% of the total brain volume (Kaas JH., 2012). It is 1-4 mm thick (depending on the species), and is stratified into six distinct layers, from *pia* to the white matter (Fig 1.2). Importantly, the expansion of the neocortex and the expression of species-specific circuits correlate with the cognitive and behavioral complexity of different mammalian species (Willemet et al., 2013; Rakic, 2009; Dicke & Roth, 2016; Herculano-Houzel, 2009). Cortical expansion culminates with *Homo Sapiens*, and this is believed to underlie its ability to perform highly complex cognitive tasks and behaviors, such as language, abstract thinking and cultural diversity (Carrol, 2013; Lourenço & Bacci, 2017).

Cortical function relies on the interplay of heterogeneous but stereotyped organization of networks, composed of multiple cell types constituting neuronal circuits (Silberberg et al., 2002; Potjans & Diesmann, 2014; Bartolini et al., 2013; Tremblay et al., 2016). Two principal types of neuron form cortical circuits (Fig.1.3): excitatory (≃ 80% of all cortical neurons) that use glutamate as

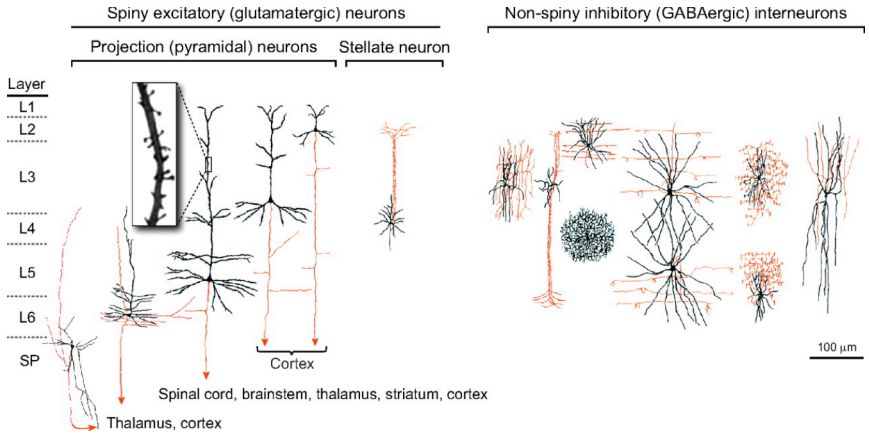


Fig. 1.3 Major neuronal cell types of the adult cerebral cortex. Cortical neurons (shown here for primates) are categorized into two major classes: spiny excitatory (glutamatergic) neurons (left panel) and non-spiny inhibitory (GABAergic) interneurons (right panel). Projection neurons display marked layer- and subtype-specific differences in the morphology of their dendrites (black) and in the targets of their axonal projections (red). The non-spiny interneurons, which are highly diverse in morphology, neurochemistry and electrophysiology, project axons within a local circuit (adapted from Jones, 1986).

neurotransmitter and inhibitory interneurons (≃ 20%), using gamma-aminobutyric acid (GABA) (Meinecke & Peters, 1987, DeFelipe & Fariñas, 1992).
Excitatory glutamatergic neurons make up the large majority of all cortical neurons: for this reason, because they can relay to long distances in the central nervous systems, and because they effectively transfer information through their excitatory synapses, they are often referred to as principal neurons (PNs). They are generally characterized by pyramidal morphologies (except in L4) and generally form both short and long-range projections with their axons. Conversely, inhibitory GABAergic interneurons display multipolar, non-pyramidal morphologies and project locally with often-dense axonal plexuses; hence their "interneurons" denomination. In addition to being both activated by feed-forward long-range connections, pyramidal neurons and interneurons are reciprocally connected: interneurons inhibit principal cells and are excited by them (Ascoli et al., 2008; Tremblay et al., 2016; Freund & Buzsáki, 1996; Gupta et al., 2000; Crandal & Connors, 2016; Isaacson & Scanziani, 2011), generating prominent feedforward and feedback inhibitory loops. The density, the morphological (soma and dendritic arborization) and electrophysiological properties of both cell types are layer-dependent. Importantly, the connectivity between these two neuronal classes is quite high: individual interneurons can inhibit more than of 50% PNs. This produces a highly orchestrated and well-regulated activity of neural circuits, thus shaping all cortical functions (Isaacson & Scanziani, 2011; Swadlow, 2003; Wilent & Contreras, 2004). Notably, the anatomical and functional organization of the cortex remains similar across regions accomplishing distinct functions.
In the following paragraphs, I will provide a detailed description of the cortical organization by the different neocortical cell types.

1.1.1. How does the neocortex work? Mechanisms of cortical processing

As we mentioned above, the cerebral cortex is the brain region where high order functions take place. One of the most studied and well-described basic mechanisms of this region consists in the ability to associate aspects of perceived experience with an internal representation of the world, and make an educated

guess about the imminent future (Larkum et al., 2013). This is possible because the cortex works via a combination of feedforward drive (bottom-up), encoding sensory input from the external world, and feedback drive (top-down), carrying information about context and prior knowledge, conferring expectation (Fig. 1.4). Precisely, the feedforward stream is driven by external information, acting on the sensory machinery, whereas the feedback pathway is conveyed by an internal context, built from previous experiences (Larkum, 2013).

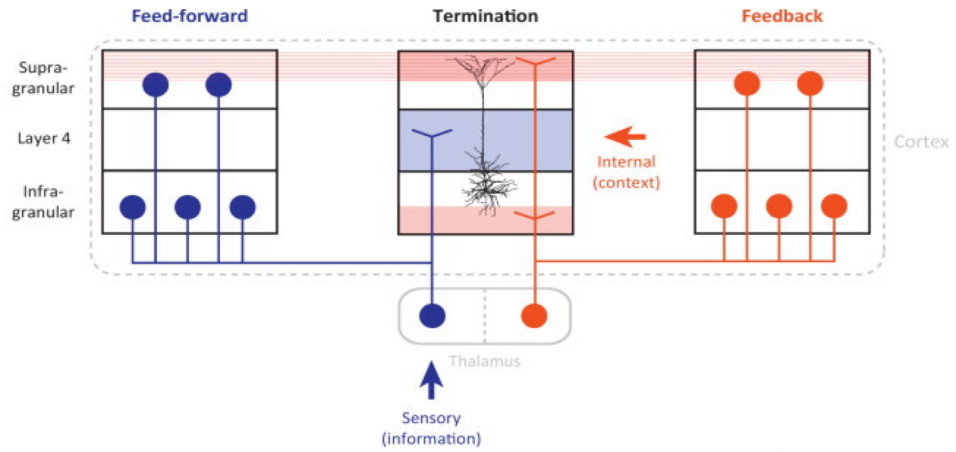


Fig.1.4. General scheme for feed-forward and feedback connectivity between cortical areas. In the middle panel, a L5 pyramidal neuron (black) has been superimposed to coloured rectangles to highlight the location of the dendrites relative to the large-scale wiring of the cortex. From (Larkum, 2013).

1.1.2. Neocortical architecture: the cortical column

In the 1950s, Vernon Mountcastle noticed that neurons located along the vertical path of a recording electrode in cat somatosensory cortex, shared similar response properties (Mountcastle 1957). These functionally related neurons were grouped in columns and represented functional modules that were repeated across the neocortex. Today, it is known that each of these cortical columns is formed by a group of basic ontogenic units, linked together by short horizontal connections, representing both an anatomical and functional module of the cortex (Fig 1.5.). These columns are characteristic of sensory cortices of all mammals. Interestingly, the size of individual columns is relatively constant from one species to another but the number of columns has expanded during

evolution, providing higher order species with larger cortices (Hoffman et al, 2011). Indeed, brain evolution results from the cooperative association by the self-similar compartmentalization and hierarchical organization of neural circuits and cortical folding, fundamental for the reduction of interconnective axonal distances. The design of the primate brain allows performing a great number of complex functions using a relatively low amount of energy (Hoffman et al, 2014).

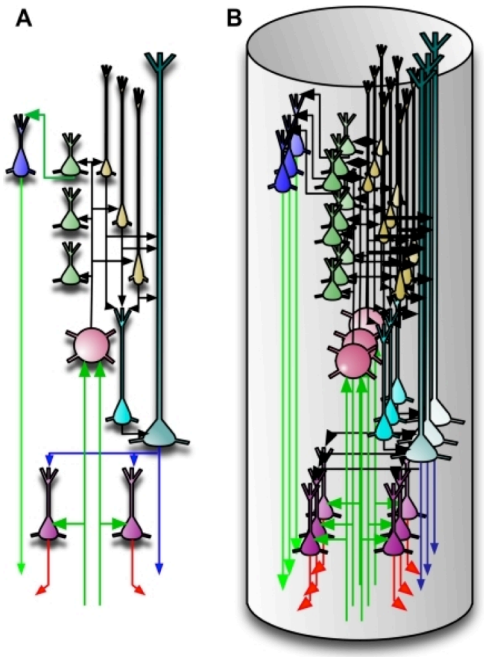


Fig. 1.5 Columnar organization cortical microcircuits. (A) A single cortical column. (B) A more complex arrangement of cells comprising several copies of the column (A) (adapted from George and Hawkins, 2009)

1.1.3. Connectivity within the cortical columns

Sensory cortical circuits are characterized by a precise scheme of connectivity throughout layers (Fig.1.6). Precisely, in primary sensory cortical circuits, the granular L4 serves as the fundamental input station for thalamocortical connections. Conversely, superficial L1 and L5 are mostly targeted by associative thalamus (Larkum, 2013). Consequently, sensory information is conveyed within cortical columns. In L4, excitatory thalamic fibers contact both excitatory and inhibitory neurons. This layer projects to supra-granular L2/3, which is considered an integrative layer. Indeed it receives feedforward information from L4 and feedback input from other cortical areas. Furthermore, L2/3 projections reach L5, where pyramidal neurons project back to subcortical regions. Finally, L6 provides both a direct strong feedback excitatory modulation to thalamic nucleus and an indirect feedback via a monosynaptic intracortical connection from L4 (Feldmeyer, 2012; Qi and Feldmeyer, 2016; Thomson, 2010). Moreover, it was demonstrated that in the mouse visual cortex, L6 exerts a strong suppressive action on primary visual cortex (V1). Precisely, it was shown that L6 PNs, called L6 cortico-thalamic

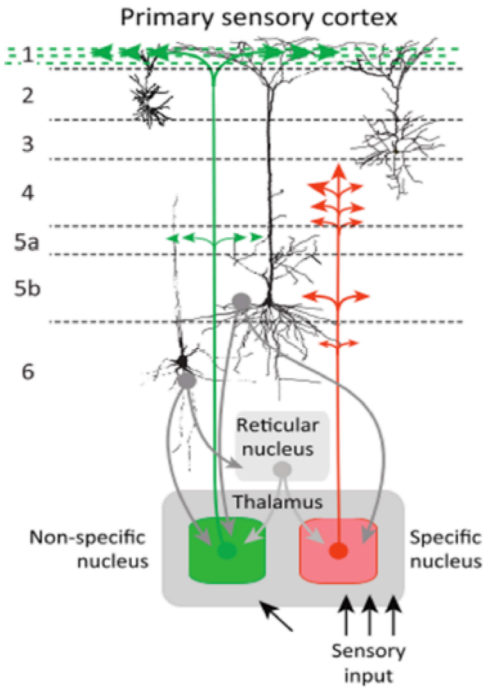


Fig.1.6 Canonical excitatory microcircuit in the neocortex Example of a circuit in somatosensory cortex but all sensory circuits share the same architecture (from Larkum et al., 2013)

(L6-CT) neurons, mediate intracortical suppression of visually evoked activity in the superficial layers of V1 (Bolz and Gilbert, 1986; Olsen et al., 2012) by recruiting fast spiking (FS) cells with translaminar axons (Bortone et al., 2014). For review on the cortical organization described above see (Bence and Levelt, 2004; Allene et al., 2015). Even though the thalamus is the main source of input to the neocortex, more than 20 different subcortical structures projecting to the neocortex have been identified (Tigges, 1985). Moreover, cortical neurons receive excitation from different cortical areas. Thus, the thalamus and neocortex work together to shape sensory responses (Reinhold et al., 2015).

I will now describe separately the two cortical areas were the topic of my thesis work: the barrel cortex and the prefrontal cortex (PFC).

1.2 The barrel cortex

As described in the previous section, the mammalian cortex can be considered as multiple repeats of canonical circuits, each composed of modules organized into vertical columns and piled alongside each other. Due to its experimental accessibility, the somatosensory cortex became a classical model system of cortical microcircuitry. This region represents the set of modules of the neocortex responsible for processing sensations of touch (Markram et al., 2015). Rodents are nocturnal animals that live underground in tunnels. Therefore, their somatosensory system is adapted for tactile exploration. Indeed, they use their vibrissae to navigate and collect information from the environment and

immediate surroundings (Kleinfeld et al., 2006; Petersen et al., 2007). Unsurprisingly, a large part of the mouse brain is dedicated to the processing of sensorimotor information coming from whiskers (Fig 1.7 B to D). Importantly, in rodent somatosensory cortex, a well-defined region named the barrel fields (or barrel cortex), is observed. Similar to other sensory cortices, the barrel cortex shares also a columnar organization and is composed of six layers presenting different cellular types and connectivity patterns. Its curious name is due to the presence of characteristic neuronal clusters in the L4 whose 3D shapes resemble a barrel of wine. This highly organized cytoarchitectonic pattern is fundamental for

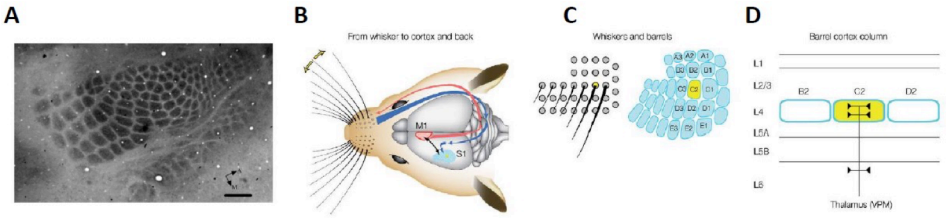


Fig. 1.7 The rodent barrel cortex. (A) Barrel fields in mouse somatosensory cortex. Example of a CO stained barrel field. Scale 500 μm. (From Jan et al., 2008) CO is a mitochondrial enzyme. Since mitochondria are particularly dense at synapses a stronger staining in the center of the barrel where the thalamic inputs arrive can be appreciated. (B) Whiskers present on the snout of the rodent sends sensory information to the primary somatosensory barrel cortex (S1) via the brainstem and the thalamus. The barrel cortex signals to motor cortex (M1), which regulates whisker movements. (C) The layout of the whiskers (left) is precisely matched by the layout of the barrels (right) in primary somatosensory cortex. The C2 whisker and barrel are highlighted in yellow. (D) A barrel column is arranged in different layers. Single whisker sensory information from ventral posterior medial (VPM) thalamus arrives in a single L IV barrel. The supragranular layer II/III and the infragranular layers V/VI perform integrative functions. (Modified from Petersen,2007)

the coding of sensory information coming from whiskers. Barrels are easily identifiable as cytochrome oxidase (CO^{-}) rich regions in L 4 (Fig. 1.7 A) and correspond one-to-one with sensory inputs from a single whisker on the contralateral face (Woolsey and Van der Loos, 1970; Wong-Riley and Welt, 1980). These 'barrels' define the lateral extent of an individual cortical column in rodent primary somatosensory cortex.

1.2.1 Citoarchitectonics of the barrel cortex

As underlined before, the barrel cortex contains six layers (L1 to L6). The most superficial layer, L1, shows the lowest cell density of all. The L2 and L3 are difficult

to dissociate in rodents, and usually referred as L2/3 or supragranular layers as they are in top of L4. L5 and L6 are subcategorized in L5a, L5b, L6a, L6b, and they are termed infragranular layers. As mentioned before, at the depth of L4 a group of ordered cytoarchitectonic structures can be identified as the barrels. Thanks to their visibility, they make L4 the most visible of the cortical layers of S1. This visibility is conferred by the many thalamic afferents that terminate and cluster in the center of the barrels (Woolsey and Van der Loos, 1970; Welker and Woolsey, 1974). In the walls of the barrels, several layers of cells (2 or 3) tend to project their dendrites towards the center (Simons and Woolsey, 1984) where they are synaptically contacted by thalamic afferents (Figure 1.7). L4 is characterized by a high density of spiny stellate cells, also named granule cells, and hence this layer is also termed "granular layer". In the barrel cortex about 80% of excitatory glutamatergic cells are localized across all layers from L2 to L6, but preferentially in L2/3 and L5/6 (Feldmeyer et al., 2002; Lefort et al., 2009). Furthermore, the barrel cortex contains also inhibitory interneurons present within all layers. These neurons are GABAergic, non-pyramidal cells with low spine density, and have locally projecting axons making synaptic contacts within the cortical column and to direct neighboring columns. Barrel cortex interneurons encompass all types of inhibitory interneurons present also in other areas of the neocortex (Markram et al., 2004; Sakmann et al., 2008).

I will give a more detailed description of glutamatergic pyramidal cells and inhibitory interneurons in the following sections.

1.2.2 Intracortical connectivity of mouse somatosensory cortex

While the vertical processing and projections typical of a cortical column are responsible for the integration of distinct features of sensory environment, the horizontal connections linking neighbouring cortical domains, are fundamental to allow these features to be processed in a contex-dependent manner (Tucker & Katz, 2003; Adesnik and Scanziani, 2010). The intracortical circuitry is represented by local intralaminar connections (intracolumnar and transcolumnar), and translaminar input. Excitation arriving from the ventral posterior medial thalamus (VPM) activates mainly L4 spiny stellate cells. These cells have very confined connections within the barrel column. They mainly project vertically to L2/3 and

to a lesser extent to L5a (Lübke et al., 2000). Both projections do not spread much to other columns. Furthermore, as part of the corticothalamic feedback, both spiny stellate neurons and star pyramidal neurons of L4 project to L6 pyramidal cells (Qi and Feldmeyer, 2015). The axons of excitatory pyramidal cells from L2/3 can extend horizontally for several millimeters within this layer covering numerous columns (Gottlieb and Keller, 1997). Electrophysiological studies of these cells demonstrated that they form synaptic contacts with other pyramidal cells mainly in the L2/3 and in L5 (Reyes and Sakmann, 1999). This suggests that after the initial excitation of L4, the cells of the supragranular layers are responsible for spreading the activity all along the column. The circuits of L2/3 can then be interpreted as linking ongoing tactile information processing in primary somatosensory cortex (S1) with the related activity of a multitude of afferent and efferent columns, and different functional cortical areas outside S1.

In L5, pyramidal cells send one apical dendrite up to L1, whereas the basal dendrites extend over an area corresponding to several barrels within L5/6 (Ito, 1992; Lübke et al., 2000). Importantly, these cells are in charge of collecting information from all layers of the cortical column and from several barrel columns. Moreover, it has been shown that all the cortical layers send connections to L5 (Schubert et al., 2001), confirming the integrative role of these neurons. Both L2/3 and L5 pyramidal cells show a strong and prominent horizontal projection domain, sending their axons across the entire barrel field (Bruno et al., 2009; Oberlaender et al., 2011; Narayanan et al., 2015). In L2/3, the axons of PNs project both vertically and horizontally to connect the specific domains that represent each whisker (Gottlieb et al., 1997; Petersen et al., 2003). Recently the group of Massimo Scanziani provided important information on the role of horizontal interactions. They demonstrated that horizontal projections from L2/3 PNs suppress L2/3 and, at the same time, facilitate L5. This layer-specific modulation is not the result of a spatial separation of excitation and inhibition, but from a layer-specific ratio between these two opposing conductances (Adesnik & Scanziani, 2010). Extensive research identified a descending connection from PN in L2/3 as a prominent source of intracortical excitation to L5 (Adesnik and Scanziani, 2010; Feldmeyer, 2012; Hooks et al., 2011; Lefort et al., 2009; Otskuka and Kawaguchi, 2009; Petreanu et al., 2009). A

common assumption from these data is that L2/3 provides a critical component of sensory facilitation to L5. However, a recent study demonstrated that, surprisingly both superficial layers primarily suppress L5 rather than providing facilitation (Pluta et al., 2019). Pluta e al., showed that this translaminar suppression sharpens stimulus representations in L5 through different translaminar inhibitory mechanisms: L4 relies on L5 parvalbumin (PV) interneurons while L2/3 depends, in part, on SST. For more detailed descriptions of these connections see the Scholarpedia review article by Feldmeyer: S1 microcircuits (Feldmeyer, 2015).

1.3 The prefrontal cortex

The barrel cortex represents a clear example of primary sensory area decoding a specific sensory modality (touch from the animal's whiskers). In contrast, the prefrontal cortex (PFC) is the quintessential associative, higher-order cortical area. During evolution, the prefrontal cortex (PFC) grew in size compared to the rest of the cortex. In the human brain it constitutes 30% of the total cortical area (Fuster, 2001). It is known that the PFC is involved in higher order cognitive functions. It integrates incoming sensory input with memorized information to form an internal representation of the external world. Moreover, the PFC generates internal goals (desires, expectations) and coordinates behavior. In addition, it is the place where the sensory-motor cycle links the organism with its environment by integrating representations of perception (Fuster, 2001). In humans, the PFC is implicated in expression of personality and social behavior.

1.3.1 Historical facts: the discovery of the PFC

The term "prefrontal" was used for the first time in 1884 by Ferrier and Yeo (Ferrier and Yeo, 1884). However, today we consider "prefrontal" what was initially described by Brodmann (Brodmann, 1909). Based on cytoarchitectural criteria, he used the terms "frontal" and "precentral" to describe two main regions of the primate frontal lobe. The precentral region was characterized by a distinct granular L4. Surprisingly, he found that the frontal region (i.e. granular) was poorly developed or even absent in non-primates. This led him to conclude

that the granular frontal region is unique to primates (Brodman, 1909; Preuss, 1995).

1.3.2 Anatomy

The anatomy of the prefrontal cortex in different species has been extensively studied (Uylings et al., 2003). Depending on the species, different terminologies can be used to subdivide the structure (Preuss, 1995; Uylings et al., 2003; Kolb, 2015; Wilson et al., 2010). Brodmann's cortical scheme and cytoarchitectonic

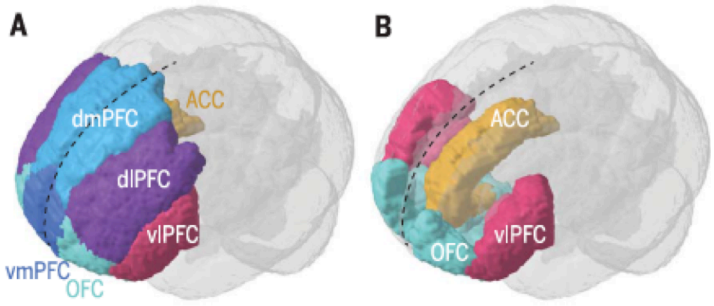


Fig. 1.8. Functional division of the human prefrontal cortex. (A and B) Tilted frontal-side view (left) of the human brain with illustration of common functional divisions of the prefrontal cortex, including ACC. The delineation of functional areas differs between studies (from Carlén, 2017)

numbers are still widely used for primates. Even though the subdivisions and their extent are highly variable, dorsolateral, dorsomedial, ventromedial, and orbital prefrontal cortex are common functional divisions (Kolb, 2015) (Fig. 1.8, A and B). Interestingly, the dorsolateral part in primates receives projections from the medio-dorsal thalamus (MD), whereas the MD in rodents does not target dorsolateral frontal areas. The granular dorsolateral prefrontal cortex is thus considered a primate specialization (Wise, 2008). Additionally, the MD targets medial and orbital cortices in rats, as in primates. Mice and rats possess fewer areas in the frontal lobe than primates, and all areas in the prefrontal cortex of mice and rats are agranular. Thus, sometimes the agranular cytoarchitecture is used as a definition of the rodent prefrontal cortex (Van de Werd et al., 2010 and 2014). Several studies have been conducted to provide a precise parcellation of

the mouse prefrotal cortex (Rose, 1929; Caviness, 1975; Van de Werd et al., 2010) (Fig. 1.9, A to C).

For instance, a recent study showed that different terminologies have been used for the same structure, and areas with similar terminology display different extent and location in different atlases (Van de Werd, 2014). Because there is not yet a consensus surrounding the anatomy (or nomenclature) of the prefrontal cortex in *Mus musculus*, stereotaxic coordinates are still indispensable for communication and comparison of experimental findings. The lack of a conclusive definition (Kolb, 2015) prevents direct comparisons of the prefrontal cortex between species. Even with a definition, the vast variation in anatomy, connectivity, and possibly also function across species constitutes a major challenge to the establishment of what can be considered equivalent prefrontal regions between different species (Dalley et al., 2004). However, despite the limited expansion of the PFC in rodents, they can still accomplish complex executive functions and they show cognitive flexibility (Schmitt et al., 2017, Muir et al., 1996; Shaw and Aggleton, 1993; Bussey et al., 1997; for review, see Laubach et al., 2018). For this reason, they can be used as models to study complex behaviors.

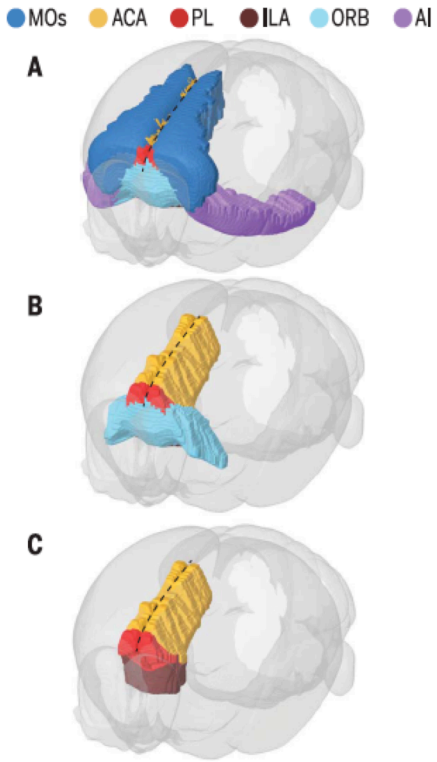


Fig. 1.9. The mouse prefrontal cortex. (A to C) Tilted frontal-side view (left) of the mouse brain with schematic illustration of the subdivisions of the agranular mouse prefrontal cortex. (A) All areas, (B) with MOs removed, (C) with MOs and ORB removed. Nomenclature: MOs, secondary motor area; ACA, anterior cingulate area; PL, prelimbic area; ILA, infralimbic area, ORB, orbital area; AI, agranular insular area (from Carlén, 2017).

1.4 Neuronal diversity in the neocortex

I have just described two neocortical areas both important for different aspect of behavior. As already mentioned above, cortical function relies on the interplay of the heterogeneous but stereotyped organization of functional networks, composed by excitatory principal neurons and GABAergic interneurons (Douglas & Martin, 1991; Mountcastle et al., 1997; Kozlowski et al., 2001; Silberberg & Gupta, 2002). These cell types are precisely distributed across layers. Importantly, both glutamatergic and GABAergic cortical neurons are diverse, conferring each layer with distinct functional features and specific subnetworks. I will now describe both excitatory and inhibitory cell types in details.

1.4.1 Diversity of excitatory principal neurons

Excitatory neurons: pyramidal neurons classification

Excitatory cortical neurons, using glutamate as a neurotransmitter are considered the principal neurons (PNs) of the neocortex as they receive and transmit information within cortical networks and to and from subcortical brain areas. PNs are characterized by distinct apical and basal dendritic trees and a soma with a pyramidal shape. These cells share similarities in their morphological and electrophysiological properties (Fig. 1.10) and form a more homogenous group if

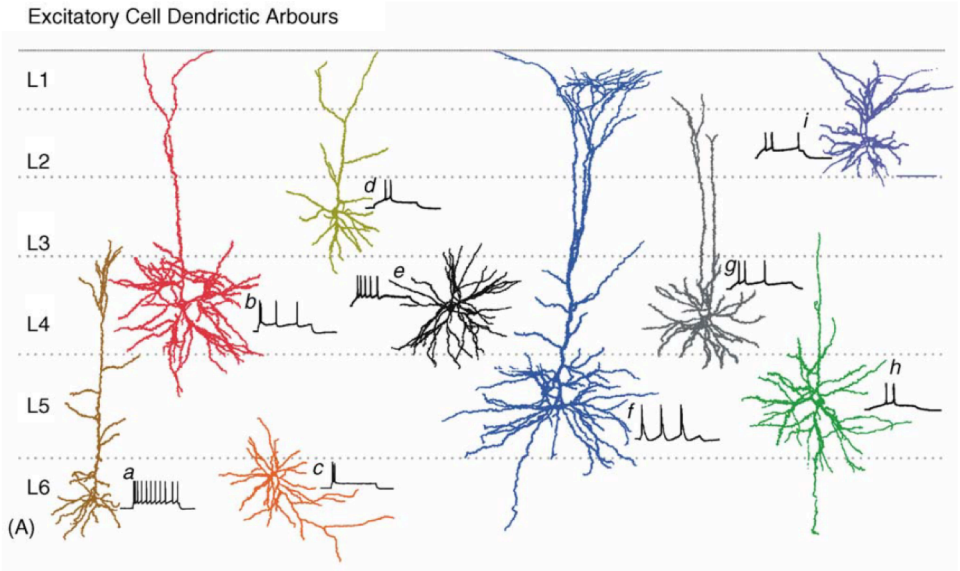


Fig. 1.10 Diversity of excitatory neurons across cortical layers. Several examples of reconstruction of dendritic tree of excitatory neurons of L1-6 shown together with the corresponding firing pattern (from Thomson & Bannister, 2003).

compared to interneurons (Peters and Jones, 1981; Connors and Gutnick, 1990). Electrophysiologically (Fig 1.11), most neocortical excitatory neurons typically fire in a regular spiking (RS) manner, meaning that they show adapting action potentials (AP), followed by a steady-state regular firing in response to depolarizing current pulses (McCormick et al., 1985).

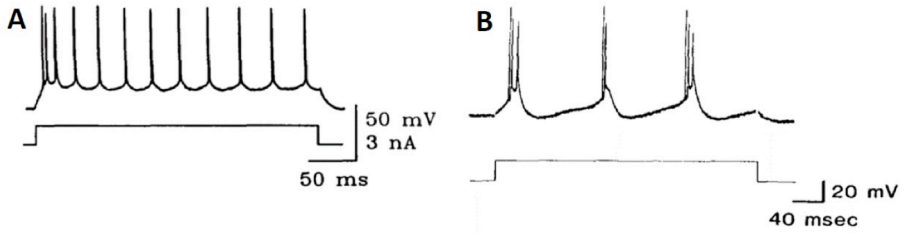


Fig. 1.11 Example firing pattern of two types of excitatory neurons. (A) Representative regular spiking discharge of excitatory neocortical neurons in response to depolarizing current injection (from Connors and Gutnick, 1990) (B) Representative intrinsically bursting neuron of L5, in response to current injection (from Agmon & Connors, 1989).

However, some deep L5 pyramidal neurons fire repetitive bursts, and they are therefore classified as intrinsically bursting neurons (Agmon and Connors, 1989). Despite their homogeneity, two main groups of principal neurons can be identified, based on morphological features: the pyramidal cells, and the spiny stellates (SS) cells (Feldman, 1984), which essentially differ from each other across and within layers by the size and shape of their cell body, extent of their dendritic arborization and spine density (DeFelipe and Fariñas, 1992; Spruston, 2008). Pyramidal somata are situated in L2–6 whereas spiny stellate (SS) cells are within L4 of primary sensory areas.

A pyramidal neuron differs from an SS cell because it presents an apical dendrite that extends through several layers above the soma. Another important factor distinguishing different pyramidal neuron types is their input-output connectivity (Fig 1.12). Following this functional classification, it is possible to distinguish: the intratelencephalic (IT) PNs located in L2-6, projecting within the telencephalon and to other cortical areas or the striatum, the pyramidal tract (PT) cells, located in L5b, that connect through the PT to the entire neuraxis and finally, the corticothalamic (CT) cells, restricted to L6, that connect to the thalamus (Oberlaender et al., 2012; Harris and Shepherd, 2015).

Accordingly, PNs with similar morpho-functional features, and embedded within a cortical networks, can be differentially recruited by long-range connections such as cortico-cortical, corticofugal and thalamocortical (TC) (Smith et al., 2012, Glickfield et al., 2013; Kita & Kita, 2012; Malmierca et al., 2014; Smith et al., 2015). This input-output specificity confers a differential treatment of information to specific subgroups of PNs.

In addition, cortical excitatory principal neurons can be classified according to their projection patterns (e.g. cortico-cortical vs. cortico-striatal vs. cortico-tectal) (Brown and Hestrin, 2009) (Fig 1.12) or their functional connectivity in response to sensory stimulation (Ko et al., 2013). The specific connectivity blueprint of different pyramidal neurons is reviewed in Allene et al., 2015 (Allene et al., 2015).

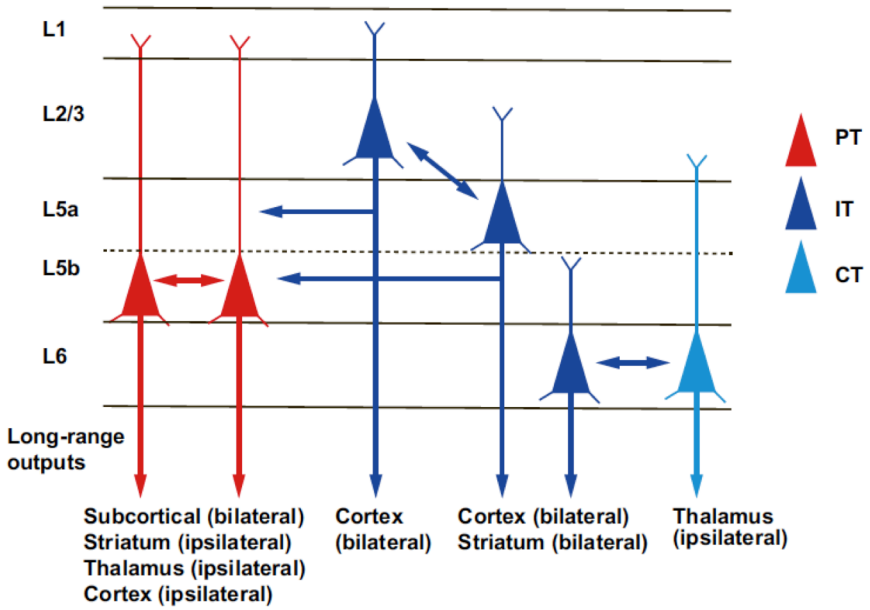


Fig. 1.12 PN classification based on long-range projections. Local connectivity patterns of PN subtypes and their long-range projection targets (From Kamigaki, 2018)

1.4.2 Neocortical inhibitory interneurons

Glutamatergic, excitatory PNs are considered the principal cellular elements of the cerebral cortex, because: i) they make up the vast majority of cortical neurons; ii) they are glutamatergic and thus their spike trains is responsible for the flow of information across different cortical and/or brain areas. However, the activity of PNs is constantly shaped and controlled by the activity of inhibitory GABAergic interneurons (INs). Despite their heterogeneity, PNs are not as

spectacularly diverse as GABAergic interneurons (Figure 1.13) (Ascoli & Alonso-Nanclares, 2008; Cauli & Audinat, 1997; Kawaguchi & Shindou, 1998; Markram et al., 2004; Somogyi & Kausberger, 2005; Yuste et al, 2005). The specific properties of cortical interneurons are determined during development and mainly depend on their embryonic origins, either from the medial (MGE) or the caudal (CGE) ganglionic eminences (Marin and Mueller, 2014).

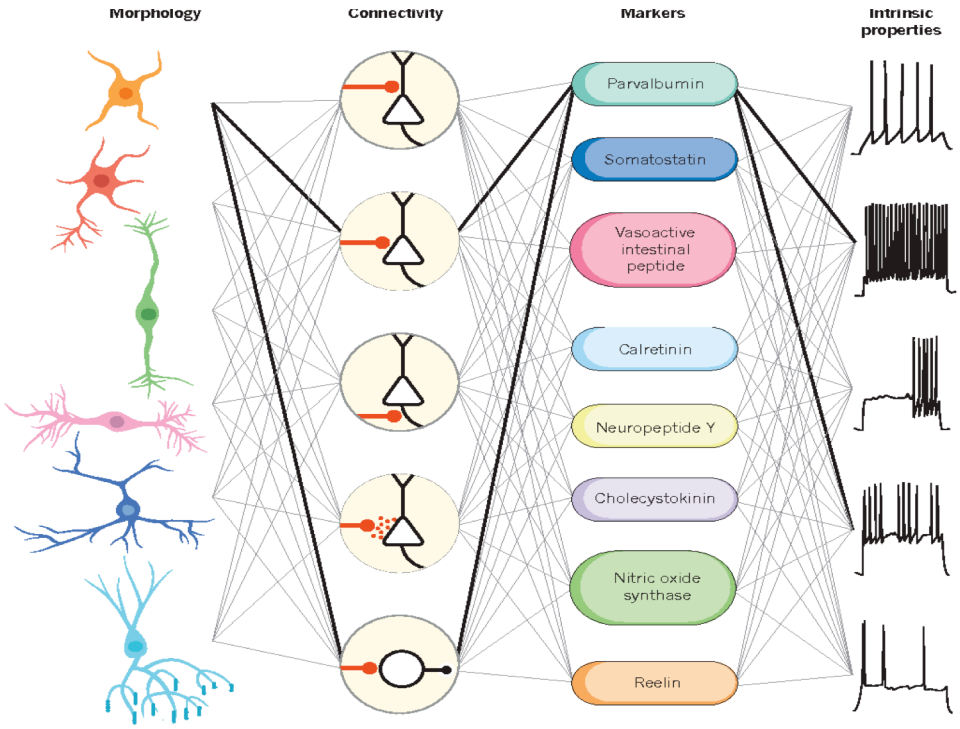


Fig. 1.13 Multiple dimensions of interneuron diversity. Interneuron cell types are usually defined using a combination of criteria based on morphology, connectivity pattern, synaptic properties, marker expression and intrinsic firing properties. The highlighted connections define fast-spiking cortical basket cells (From Kepecs and Fischell, 2014).

GABAergic interneurons' remarkable diversity is based on their morphological, electrophysiological and connectivity properties, as well as the expression of molecular markers such as parvalbumin (PV), calbindin (CB) and calretinin (CR), that are Ca^{2+}-binding proteins, and neuropeptides, mainly represented by somatostatin (SST), cholecystokinin (CCK) and vasoactive intestinal peptide (VIP) (Gupta, 2000; Ascoli and Alonso-Nanclares, 2008; DeFelipe et al., 2013) (Fig. 1.13). However, due the overlap of the different morphological and functional features, attempting to define different IN subclasses, to date a clear classification of the many cortical IN subtypes is far from being established (De Felipe et al., 2013). Even though the classification of cortical GABAergic interneurons is problematic, perhaps one relevant functional classification relies on their specialized

connectivity with different domains of PNs (Fig. 1.14) that generates an efficient division of labor of different forms of inhibition during cortical activity. Consequently, they can provide different levels of control: precisely, dendrite-targeting interneurons contribute in controlling the input to PNs, by modulating dendritic spike generation and synaptic integration (Murayama et al., 2009; Silver, 2010; Tran-Van-Min et al., 2015; Lovett-Barron et al., 2011), whereas perisomatic-targeting interneurons control the rhytmic outputs of PNs (Haider & McCormick, 2009; Markram et al., 2004; McBain & Fisahn, 2001; Pouille & Scanziani, 2001; Peter Somogyi & Klausberger, 2005; Wehr & Zador, 2003). Indeed, we can typically distinguish the perisomatic-targeting basket cells (BCs) and the axo-axonic chandeliers cells. The precise targeting of BCs and chandelier cells on the output region of PNs allows a precise control of PN output spiking activity (Freund & Katona, 2007; Freund, 2003).

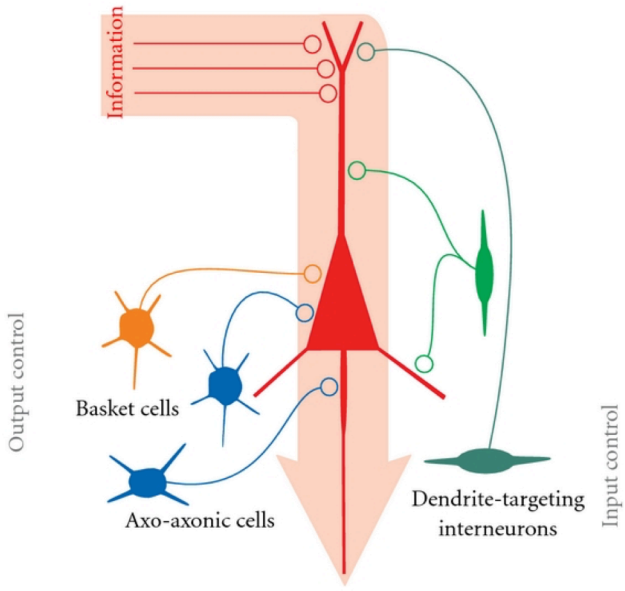


Fig. 1.14. Oversimplified scheme of cortical GABAergic circuits controlling principal pyramidal neurons directly and indirectly. The information coming onto principal cells can be specifically and directly filtered by different interneuron types, which can be specialized in output (left) or input (right) control. From (Méndez and Bacci, 2011).

Basket cells, which represent the largest population of INs (about 50%) can be divided into two large subclasses: the PV-expressing and the CCK- expressing basket cells that express cannabinoid receptor type 1 (CB1R) (Freund and Katona, 2007). PV+ basket cells sustain high-frequency firing, receive strong excitation, release GABA very reliably, and are considered the clockwork of cortical networks, as they synchronize a large population of principal cells (Buzsáki and Draguhn,

2004; Freund and Katona, 2007; Klausberger and Somogyi, 2008). Conversely, basket cells expressing CB1Rs (and CCK) receive less excitation, cannot sustain high-frequency firing, release GABA more asynchronously and unreliably (Hefft and Jonas, 2005), and are negatively modulated by endocannabinoids (Kano et al., 2009). Notably, CCK+ cells are the specific target of subcortical neuromodulators, such as acetylcholine and serotonin, and this, together with their less reliable GABAergic transmission led to the hypothesis that CCK+ cells exert a fine-tuning of cortical activities and might play a key role in the control of mood (Freund and Katona, 2007; Varga et al., 2009). This functional classification of PV and CCK BCs derive mostly from studies in the hippocampus (Freund, 2003; Szabadics Jet al., 2006; Freund and Katona, 2007). Indeed, a deep knowledge of the different distribution of CCK and PV cells in different neocortical layers and areas is missing. Yet, we know that CCK/CB1 BCs are mostly located in superficial cortical layers (L1 and L2/3), where they share the perisomatic control of PN excitability with PV BCs. In contrast, L5 PNs are almost exclusively modulated by PV BCs (Allene et al., 2015). Importantly, both PV and CCK cells include several subtypes that can be classified by their specific connectivity patterns.

Another important class of GABAergic interneurons is represented by SST-expressing dendrite-targeting cells. SST cells broadly encompass neurons that have been identified — using various anatomical and electrophysiological criteria — as so-called Martinotti cells (MCs), bitufted cells, regular-spiking non-pyramidal cells or low-threshold spiking cells (Kawaguchi & Kubota, 1997; Wang Y, et al., 2004; Fanselow et al., 2008; Reyes et al., 1998). Since my thesis work was mainly focused on this particular interneuron type, I will present SST-cell in a separate section.

Distal dendritic inhibition is also provided by neurogliaform cells (NGFCs) and other interneurons whose cell bodies are located in L1 (Overstreet-Wadiche & McBain, 2015; Tremblay et al., 2016; Kawaguchi and Kubota, 1997; Kubota, 2014; Olah et al., 2007, 2009; Tamas et al., 2003; Price et al., 2008; Schuman et al., 2019). In addition to the connectivity logic of interneurons onto PNs, cortical inhibitory neurons can be recruited by distinct excitatory circuits (Isaacson and Scanziani, 2011; Roux and Buzsáki, 2015). Excitatory inputs originating from

cortical and subcortical regions can diverge onto both principal cells and interneurons, providing strong feed-forward inhibition (Fig. 1.15 A).

This form of inhibition, triggered by long-range connections, is ubiquitous and plays an important role in shaping and controlling the precise time window of PN spiking activity. This type of inhibition is particularly strong in L4 in which PV interneurons are potently recruited by thalamic fibers (Sun QQ et al., 2006;

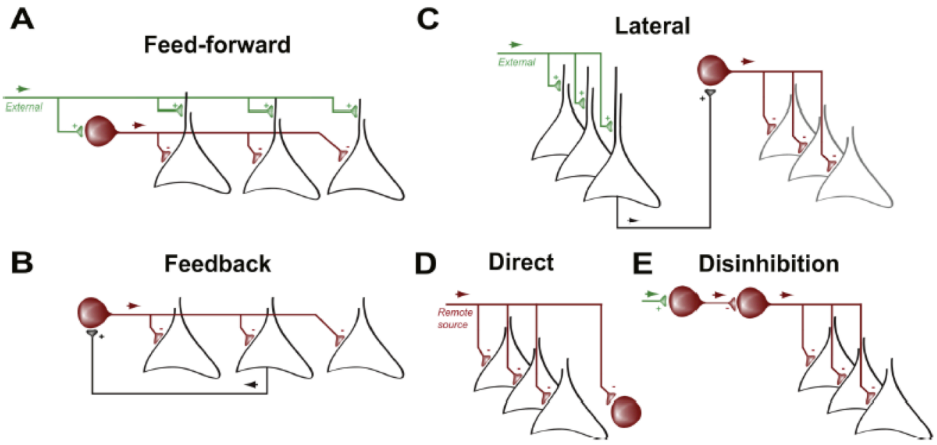


Fig. 1.15 Principal types of inhibitory microcircuits. (A) Feed-forward inhibition (B) Feedback inhibition (C) Lateral inhibition (D) Direct inhibition (E) Dishinbition. Interneurons are represented in red, afferent external excitatory inputs are in green whereas local PNs are in black. Modified from (Roux and Buszàki, 2015)

Gabernet et al., 2005; Cruikshank et al., 2007). Feedback inhibition is divided in recurrent inhibition (Fig. 1.15 B) or lateral inhibition (Fig. 1.15 C). In both cases a PN fires first and recruits a postsynaptic inhibitory neuron, which in turn suppresses the activity of the same (recurrent) or a neighboring PN (lateral) (Silberberg and Markram, 2007; Adesnik & Scanziani, 2010). Lateral inhibition is important for example in the visual cortex where it drives surround inhibition (Adesnik et al., 2012), which is a basic mechanism for setting and modulating the receptive fields. Furthermore, a form of direct inhibition arises when long-range GABAergic inputs from distant regions drive local inhibition in the circuit (Fig. 1.15 D). This form of lateral inhibition involves majorly SST-INs (Silberberg and Markram, 2007; Adesnik & Scanziani, 2010).

Another important circuit, in which inhibitory neurons are involved, is disinhibition, which takes place when GABAergic neurons target other GABAergic neurons (Fig. 1.15 E). This can mediate network synchrony or disinhibition of principal neurons (Sohn et al., 2016). Inhibition of inhibition is a common feature

in cortical circuits (Tremblay et al., 2016; Pfeffer et al., 2013; Letzkus et al., 2015). For example, we know that PV INs strongly inhibit themselves via autaptic transmission and mutual inhibition between PV cells (Deleuze et al., 2014; Jiang et al., 2013; Manseau et al., 2010; Bacci & Huguenard, 2006; Bacci et al., 2003; Tamas et al., 1997; Connelly & Lees, 2010; Bekkers, 2003; Van der Loos & Glaser, 1972). Particularly, an important disinhibitory cortical circuit involves interneurons expressing the vasoactive intestinal polypeptide (VIP). These interneurons are specialized in contacting other GABAergic neurons selectively, and they have a particular preference for SST cells, although they also inhibit PV cells with a lower extent (Pfeffer et al., 2013; Kepecs and Fishell, 2014). VIP IN-dependent disinhibition has been recently described to underlie several cognitive functions, including auditory discrimination (Pi et al., 2013), sensory-motor integration (Lee et al., 2013) and working memory (Kamigaki and Dan, 2017; Turi et al., 2019).

The rich diversity of cortical INs, their highly specialized division of labor and the various inhibitory circuits that they form, contribute to the formation of a delicate ecosystem responsible for all cortical functions underlying behavior. Indeed, alterations of specific interneuron types lead to an imbalance between excitation and inhibition. This disequilibrium has been associated with a range of diseases and conditions such as epilepsy, Down syndrome, anxiety, schizophrenia and autism (Cobos et al., 2005; Cossart et al., 2001; Gonzalez-Burgos & Lewis, 2008; Han et al., 2014; Levitt, Eagleson & Powell, 2004; Noebels et al., 2003).

Since my thesis work revolves around the study of this particular dendrite-targeting neocortical microcircuit, in the following section, I will give a more detailed description of SST-dendrite-targeting interneurons, with a special attention for Martinotti cells.

1.4.3 Somatostatin positive interneurons

It has been clear for some time that the cortical SST-inhibitory interneuron population is not homogeneous. Although, the somatostatin (SST) interneuron group of the neocortex has typically been associated with Martinotti cells (MCs), several distinct SST-population have already been described in the mouse neocortex (Ma et al., 2006; McGarry et al., 2010; Halabisky et al., 2006; Xu et al.,

2006). This distinction has been made by looking at different electrophysiological, anatomical (Kawaguchi et al., 1997) and molecular properties (Gonchar & Burkhalter, 1997) of SST-cells. Martinotti cells were described for the first time in 1889 by the Italian anatomist Carlo Martinotti (Martinotti, 1889). These cells represent the largest and best-known population of SST interneurons. In fact, approximately 15% of neocortical interneurons and 50% of SST cells are MCs (Druga, 2009; Wang et al., 2004). They are mostly located in L5 and then in L2/3 (Kawaguchi & Kubota, 1997; Uematsu et al., 2008; Wang et al., 2004) and display bitufted dendritic morphology and an extensive axonal arborization towards L1 where they inhibit the tuft dendrites of pyramidal cells. Moreover, MCs can reach different domains: their axons can extend horizontally within L1 in to neighboring columns for millimeters, providing also cross-columnar inhibition (Beaulieu, 1993). Moreover, MCs of L5 can also project selectively to L4 (Wang et al., 2004). They make synaptic contacts mainly on dendritic shafts and on spines of PNs. MCs express SST and never express PV or VIP. Importantly, excitatory inputs onto MCs are generally strongly facilitating, which is a key feature that distinguishes these interneurons from FS neurons, whose excitatory inputs are depressing (Fig. 1.16)(Beierlein et al., 2003; Fanselow, Richardson et al., 2008; Kapfer et al., 2007;

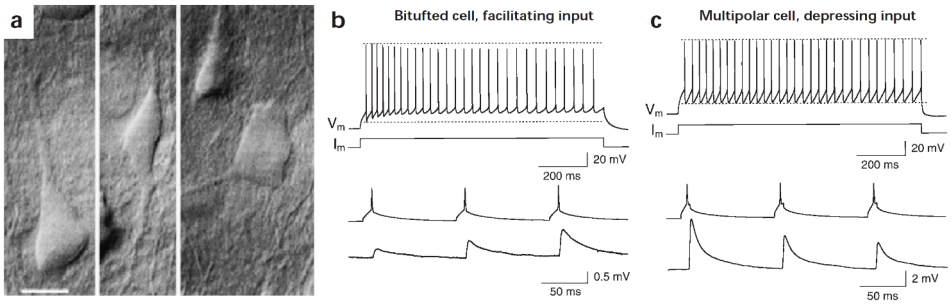


Fig. 1.16. Selection of three classes of neurons in layer 2/3. (a) Morphological selection. Representative infrared differential interference contrast enhanced video images of a pyramidal (left), bitufted (middle) and multipolar cell (right) in a slice of the somatosensory cortex taken from a two-week-old (P14) rat. Calibration bar is 10 µm and applies to all three images. (b c) Functional selection. Upper pair of traces show action potential patterns of bitufted (b) and multipolar (c) neurons following injection of depolarizing current steps. The resting potentials were -68 mV and -70 mV. Lower pair of traces show the presynaptic action potentials and associated EPSPs evoked in bitufted and multipolar neurons during repetitive stimulation of the presynaptic pyramidal cell. The EPSPs evoked in the bitufted cell facilitated, whereas those evoked in the multipolar cell depressed. Amplitude calibrations refer to EPSPs. The EPSPs in this and subsequent figures are averages compiled from 50–200 sweeps and were evoked by delivering a 10 Hz train of brief current pulses to the presynaptic cells (from Reyes et al., 1998).

Reyes et al., 1998; Silberberg & Markram, 2007). Interestingly, since facilitation and depression involve presynaptic mechanisms, and because a single neuron can express both behaviors simultaneously, Reyes and collaborators speculated that local differences in the molecular structure of presynaptic nerve terminals were induced by retrograde signals from different classes of target neurons (Reyes et al., 1998; Sylwestrak et al., 2012). Since MCs and PV-cells both form reciprocal inhibitory connections with pyramidal cells, different rates of action potentials in PN activate different local inhibitory pathways (Kawaguchi and Kubota, 1997; Somogyi et al., 1998) PNs discharging at a low rate would thus preferentially excite PV cells, which will inhibit pyramidal cells via a perisomatic feedback circuit. At higher rates, the facilitation of MCs inputs would increasingly ensure recruitment of this population of neurons, which will then inhibit apical dendrites of pyramidal cells. One explanation of why these cells have pronounced frequency facilitation of their inputs may lie in their position in the cortical network. Their excitatory input could be dominated primarily by the level of local pyramidal cell activity, which is fed back to the distal dendrites of the pyramidal cells as GABAergic inhibition (Han et al., 1993; Blasco-Ibanez et al., 1995; Maccaferri et al., 1996; Kawaguchi and Kubota, 1997; Somogyi et al., 1998).

Furthermore, SST interneurons, together with PVs and VIP-expressing interneurons were proposed to form a so-called “blanket of inhibition” by connecting to local PNs in a promiscuous and extensive fashion (Fino and Yuste, 2011; Karnani et al., 2014). It was then proposed that VIP-INs are responsible for making “holes” in this blanket of inhibition by contacting in an extensive manner SST-INs, thus allowing the propagation of excitatory activity (Karnani et al., 2016). Whereas interneurons broadly contact PNs, the rules of connectivity between interneurons are less well understood. Some studies reported a high degree of connectivity between PV-INs, from PV-Ins to SST-INs and SST-INs to PV-INs (Avermann et al., 2012; Gibson JR et al., 1999; Hu et al., 2011). Therefore, this inhibitory blanket might extend to interneurons too, with the exception that MCs never inhibit each other. Finally, MCs were shown to be the most important actors in lateral frequency dependent dysinaptic inhibition (FDDI) in the rodent neocortex (Kapfer et al., 2007). This mechanism is unique to MCs and allows PNs to inhibit each other via intermediate MC activation.

However, in contrast to the notion of a non-specific 'blanket of inhibition' a detailed blueprint of connectivity between different IN subtypes and PNs was shown. For example, PV cells make stronger contacts with thick-tufted than thin-tufted PNs in the PFC (Lee et al., 2014). Likewise it has been reported that MCs preferentially contact thick-tufted but not thin-tufted PNs and participate in defined cortical circuits (Hilscher et al.,2017). In addition, there is growing evidence that the strength of inhibitory synapses can be modulated in an activity-dependent manner, altering single PN sensitivity to both PV cells and SST cells (Lourenço et al 2014; Xue et al., 2014). Finally, also IN-IN connectivity exhibits marked specificity. VIP interneurons are believed to selectively contact other INs, with a preference for SST interneurons, avoiding PNs (Ramaswamy et al., 2017). Furthermore, PV cells tend to connect with other PV cells more strongly and curiously, as mentioned in the previous section, they form massive self-innervation with autaptic contacts, conferring a unique, fast and powerful form of disinhibition to this cell type (Bacci et al., 2003; Bacci and Huguenard, 2006; Manseau et al., 2010; Deleuze et al., 2014). As mentioned before, the SST-IN population is highly heterogeneous. In 2006, Ma et al., generated five new mouse lines in which different SST-INs were labeled by GFP (Fig 1.17) (Ma et al., 2006).

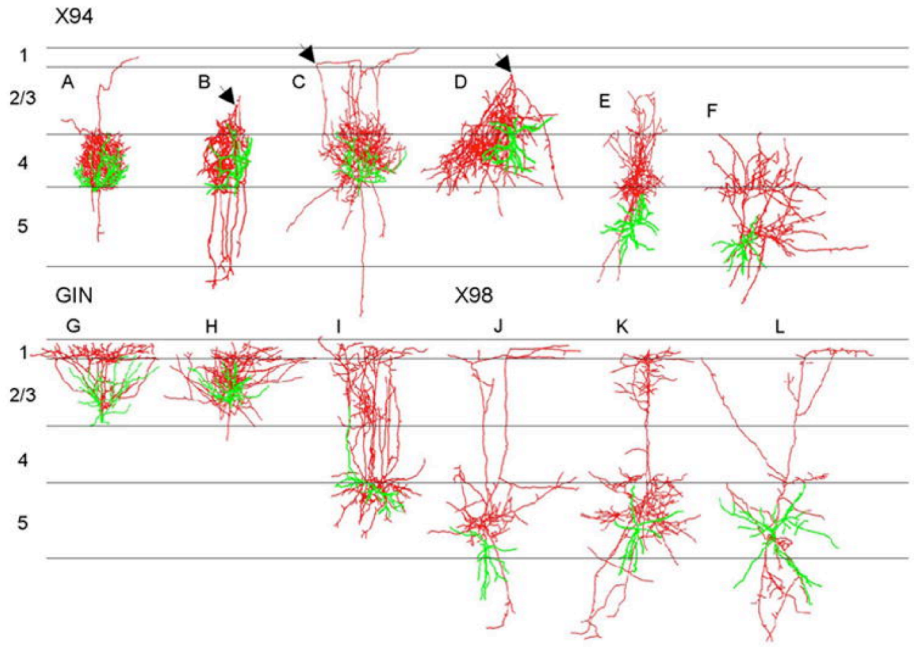


Fig 1.17 Morphological reconstruction of SST-interneurons subtypes. Neurons were reconstructed Neurolucida; cell bodies and dendrites are shown in *green*, axons in *red*. Arrowheads in B–D point to a turning point of the axon, from the upper layers back to layer 4 (from Ma et al., 2006)

Precisely, they generated these lines by expressing GFP under control of the GAD67 promoter. Even though the same techniques and the same DNA construct were used to obtain these lines, GFP positive INs were distinct in each line. Interestingly, a new subpopulation of SST-expressing cells was first described in the somatosensory cortex of the transgenic mouse line X94 (Ma et al., 2006). For instance, GFP^+ cells in X94 mice, were mainly located in L 4 and 5, and their innervations were abundant in L4. They showed lower input resistance compared to FS cells and spikes of shorter duration. They could fire at higher frequency than Martinotti cells but they were characterized by spike frequency adaptation (Ma et al., 2006). In addition, based on the differences observed in the expression of molecular markers, intrinsic firing properties, and connectivity, it has been suggested that there might be other additional subpopulations of SST neurons in the neocortex (Fig 1.18) (McGarry et al., 2010; Miyoshi et al., 2007).

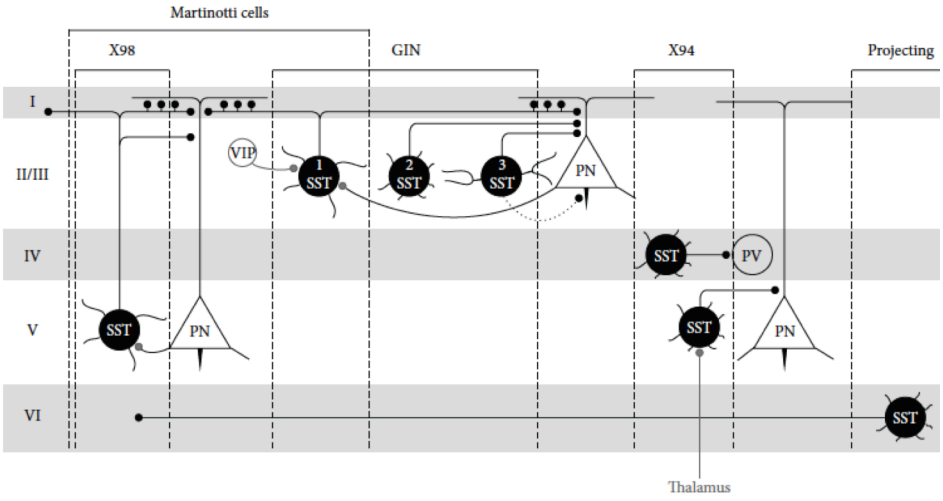


Fig. 1.18 SST-interneurons labeled in X98-, GIN-, and X94-transgenic mice have distinct laminar distributions and wiring patterns. X98-SST-interneurons mainly reside in L5 whereas the GIN-SST-interneuron subpopulation resides in L2/3. Both subtypes are considered Martinotti cells since they project to L1 where they target dendrites of layer 2/3 and 5 pyramidal neurons. The second and third population of GIN-SST-interneurons avoid L 1 but target pyramidal neurons within L 2/3. GIN-type 2 interneurons are characterized by small, multipolar dendritic arbors, whereas GIN-type 3 interneurons have larger, bitufted dendritic arbors. Some layer 2/3 GIN-SST-neurons target the axon initial segment of pyramidal neurons. L 2/3 VIP-interneurons somatically target SST-interneurons within this layer. X94-SST-interneurons reside either in L 4, where they mainly target fast-spiking PV-interneurons, or in L 5, where they dendritically target L 5 pyramidal neurons. Specifically L 5 X94-neurons can receive thalamic input, whereas L 4 X94-neurons are intracortically driven. Finally, SST-projecting-neurons are mainly described in layer 6. (From Scheyltjens and Arckens, 2016)

In fact, approximately a third of SST interneurons in frontal, somatosensory (S1), and visual cortex (V1) contain calretinin (CR)(Xu et al., 2006). Although they exhibited similar Martinotti cell anatomical features and had similar adapting spike-firing patterns, these cells differed in the horizontal extension of dendrites, number of primary processes (Xu et al., 2006), connectivity and faster AHPs (Xu & Callaway, 2009).

Other two subgroups were found and specifically labeled by GFP expression in X98 and GIN mouse models. These two subclasses of SST interneurons mainly target L1 dendrites and like MCs they colocalize with calbindin and NPY (Kawaguchi and Kubota, 1996; Gabbott et al., 1997; Naka et al., 2018). They show low-threshold spiking behavior and they could be seen as MCs residing mostly in L5 and 2/3 respectively. The delineation between SST^{+}/CR^{-} and SST^{+}/CR^{+} MCs is still difficult and unclear, but there is evidence suggesting that the two populations originate from different regions during development (Fogarty et al., 2007; Sousa et al., 2009).

1.4.4 Dendritic integration and its modulation by dendritic inhibition

As described above, MCs are dendrite-targeting inhibitory interneurons. They control the integration of the inputs onto PNs. But what does this mean? Why is dendritic inhibition important? First, dendrites are the main receiving elements of neurons. They act like antennas picking up information from thousands of presynaptic glutamatergic inputs mostly onto dendritic spines (Stuart and Spruston, 2015). Action potentials (APs) are generated close to the soma, in the axon initial segment (Coombs et al., 1957; Fuortes et al., 1957). Therefore, the capacity of synaptic input to influence AP output depends on how excitatory synapses modulate membrane potential at their location, and how synaptic perturbations of membrane potential are transferred to the soma. Importantly, dendrites attenuate and filter synaptic potentials as they propagate to the soma (Rall, 1964), influencing their effect on AP output via the axon. Moreover, the passive membrane properties of dendrites (resistance and capacitance as well as their geometry) influence the way neurons integrate synaptic inputs in complex

ways, enabling a wide range of nonlinear operations (Rall, 1962; Koch et al., 1983). In addition, the location of synaptic inputs on the dendritic tree can have important functional consequences (Magee, 2000; Spruston, 2008; Williams and Stuart, 2003). Importantly, when synaptic inputs are distributed over the dendritic tree, they tend to sum approximately linearly owing to the passive properties of the dendrites (Tamas et al., 2002; Gasparini and Magee, 2006; Nevian et al., 2007, Tran-Van-Minh et al., 2016). However, when inputs are clustered, sublinear EPSP summation owing to a greater reduction in driving force and membrane shunting would be expected (Abrahamsson et al., 2012). Surprisingly, nonlinear dendritic conductances - such as NMDA (N-methyl-D-asparate) receptors, Na^{+}, Ca^{2+} channels – can boost synaptic potentials when depolarized, whereas A-type K+ can dampen them (Fig 1.19)(Schiller et al., 1997; Eilers and Konnerth, 1997; Harnett et al., 2015; Cash & Yuste, 1999; Hoffman et al., 1997; Golding & Sprouston, 1998). The presence of these channels allows

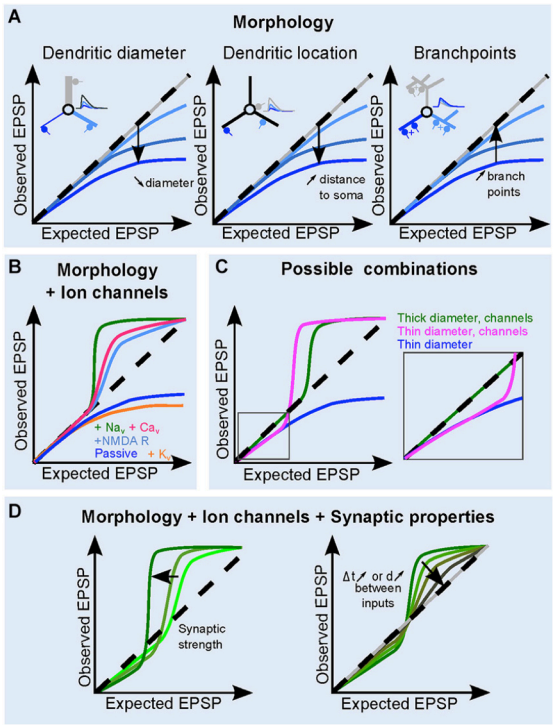


Fig 1.19. Contribution of dendritic and synaptic properties to EPSP summation. (A) Influence of morphological parameters dendritic: diameter (left), increasing distance to soma (middle) and increasing dendritic branching (right) on the dendritic subthreshold input/output (sI/O). Dashed line shows a linear I/O for reference. (B) The role of ion channels on the shape of the sI/O, for a given morphology (C) Example of sI/O in three realistic combinations: thick (>2 µm) dendrites with active conductances (blue curve, as in Branco and Häusser, 2011), thinner dendrites with active conductances (brown curve, <1 µm, Losonczy and Magee, 2006), or thin dendrites with only passive properties (blue curve, Abrahamsson et al., 2012). (D) Influence of synaptic properties on the sI/O for a given morphology and ion channel combination. An increase in synaptic strength makes the sI/O diverge from linearity both in the sublinear and the supralinear regime, whereas increasing the interval or the distance between synaptic inputs tends to linearize the curve (right) (from Tran-Van-Minh et al., 2016)

dendrites to be excitable and thus modulate EPSP propagation, via back-propagating APs (bAPs). In some cases, the synergistic function of voltage-gated and ligand-gated ion channels can generate complex dendritic spikes, mostly produced by Ca^{2+}-dependent conductances (NMDARs and L-type Ca^{2+} channels) (Larkum et al., 2009; Antic et al., 2010; Major et al., 2013). One important function of this active dendritic excitability is promoting synaptic plasticity. Indeed, bAPs invading the distal dendritic tree trigger a form of synaptic plasticity called spike timing–dependent synaptic plasticity (STDP) (Magee and Johnston, 1997; Bi and Poo, 1998; Markram et al., 1997). Other work demonstrated that, under some conditions, synaptic plasticity requires the generation of dendritic spikes (Golding et al., 2002; Letkzus et al., 2006; Sjostrom and Hausser, 2006; Remy and Spruston, 2007; Takahashi and Magee, 2009). Recent work *in vivo* has confirmed both of these findings, showing that STDP has similar properties *in vivo* and *in vitro* (Pawlak et al., 2013) and that, during sensory input, NMDA spikes are important for the induction of synaptic plasticity *in vivo* (Gambino et al., 2014). Another recent *in vivo* study found that, in the motor cortex, dendritic calcium spikes play a key role in specific dendritic branches during specific motor learning tasks (Cichon and Gan, 2015). Finally, the intrinsic properties of dendrites are also subject to plasticity (Frick et al., 2004; Losonczy et al., 2008) providing an additional mechanism by which synaptic plasticity can influence the effect of synaptic input on neuronal output.

Particularly, depending on the type of dendritic operations different important function can be mediated. For instance, numerical simulations suggest that supralinear dendritic operations are essential for translation-invariant orientation tuning (Mel et al., 1998) and binocular disparity tuning (Archie and Mel, 2000), while sublinear dendritic operations contribute to coincidence detection of auditory stimuli (Agmon-Snir et al., 1998). Recently, state-of-the-art *in vivo* recordings have shown that dendritic supralinearities are associated with various other neuronal computations: formation of hippocampal place fields (Lee et al., 2012), detection of multi-modal sensory stimuli (Xu et al., 2012), angular tuning of barrel cortex pyramidal neurons (Lavzin et al., 2012), and enhancement of orientation tuning (Smith et al., 2013). Sublinear operations have also been

shown to underlie orientation selectivity of binocular neurons in visual cortex *in vivo* (Longordo et al., 2013).

1.4.5 5HT3AR interneurons

In 2010, Lee et al., described a group of interneurons defined by the expression of the 5HT3a serotonin receptor (Lee et al., 2010). This GABAergic subpopulation accounts for 30% of GABAergic cells in the somatosensory cortex. They represent the largest IN population in supragranular layers. Then, the 5HT3aR group can be divided in two subgroups based on the expression of the neuropeptide VIP. All neurons in L1 are GABAergic INs: most of them belong to the 5HT3aR group and are largely non-VIP-expressing. This layer contains the distal dendritic tufts of pyramidal cells, as well as intracortical axons from local PNs, long-range inputs from other areas and corticopetal axons from high order thalamic nuclei and neuromodulatory centers. There is a great interest in this layer because of its presumed associative role and in top-down regulation of cortical processing because of the presence of projections from high order structures (Larkum, 2013). Based on their supragranular location, it has been suggested that 5HT3aR INs might be important mediators of such operations. Two main groups of 5HTaR expressing INs can be identified: VIP and non-VIP. VIP interneurons represent about 40% of 5HT3aR INs in the barrel cortex. They are present mainly in L2/3. They are mostly vertically oriented, have bipolar-like dendritic morphology, the remaining being multipolar (Bayraktar et al., 2000; Pronneke et al., 2015). The most important intrinsic electrophysiological feature of these cells is their input resistance, which is higher than most cortical neurons, making this IN population particularly sensitive to excitatory inputs (Tremblay et al., 2016). Importantly, these cells are involved in the mechanisms of disinhibition. It was shown that they have a striking preference to target dendritic targeting SST interneurons in L2/3 of S1, V1, A1 and prefrontal cortices (Lee et al., 2013; Pfeffer et al., 2013; Pi et al., 2013).

Non-VIP 5HT3aR INs represent about 60% of 5HTaR INs and about 90% of all L1 INs. They include the neurogliaform cells (NGFC), CCK-expressing INs (presumably non-VIP CCK basket cells) and other less clearly defined types. NGFCs, called spiderweb cells by Cajal, have a characteristic multipolar morphology consisting of

a small, round soma from which multiple, very short dendrites spread radially in all directions and have a wider, spherical, very dense axonal plexus composed of fine branches (Kawaguchi and Kubota, 1997; Kubota, 2014; Olah et al., 2017). These INs have been described in all layers, but might be more prevalent in supragranular layers and are a major component of L1. Interestingly, $GABA_A$ responses from NGFCs exhibit unusually slow kinetics as compared to other INs, with a decay time constant in the order of tens of milliseconds (Price et al., 2008; Szabadics et al., 2007; Tamas et al., 2003). In addition, NGFCs are the only interneurons that have been shown so far to elicit unitary $GABA_B$ currents in response to a single action potential (Price et al., 2005; Price et al., 2008; Tamas et al., 2003).

Overall, interneuron diversity is crucial for providing sufficient sensitivity, complexity and dynamic range for the inhibitory system to match excitation regardless of the intensity and complexity of the stimulus (Silberberg et al., 2004). Each interneuron subtype innervates its target cell by distributing several synapses in a characteristic manner and onto specific selected domains: e.g., axon initial segments (AIS), soma, proximal and distal dendritic shafts and spines, and dendritic tufts (White, 1989; Somogyi et al., 1998; DeFelipe, 1997). Interneurons that target the AIS provide editing of the output of the postsynaptic neuron by affecting the generation and the timing of action potential. Whereas, innervation of the perisomatic membrane allow the control of the action potential discharge of target cells (Wang et al., 2002; Miles et al., 1996; Buhl et al., 1995). Moreover, perisomatic-targeting interneurons are involved in phasing and synchronizing neuronal activity (Pouille et al., 2001; Cobb et al., 1995; Tarczy-Hornoch et al., 1998; Freund, 2007). Importantly, dendrite-targeting interneurons are crucial in many different post-synaptic processes. For example, they participate in dendritic processing and integration of synaptic inputs (Segev et al., 1998; Segev & London, 1999), they influence synaptic plasticity, locally or by interacting with back-propagating action potentials (Magee & Johnston, 1997). Then they affect the generation and propagation of dendritic calcium spikes (Larkum, 1999; Traub, 1995). Finally, the preferential innervation of distal dendritic and tuft regions affect dendritic integration.

1.4.6 Synaptic diversity in neocortical circuits

Synapses are highly specialized structures fundamental for the propagation of information between neurons and importantly, they are critical for circuit formation (Chen and Cheng, 2009). It is possible to identify fundamental types of synaptic circuits at successive levels of organization. These types are called basic or canonical circuits (Tau and Peterson, 2010). Most synapses involve the apposition of the plasma membranes of two neurons to form a junction named the *active zone*. The orientation of the junction defines the presynaptic and the postsynaptic compartments. The flow of information between neurons relies on a tight balance between excitatory and inhibitory neurotransmission. Synaptic transmission is accomplished through a sequence of specific steps that can be resumed as follows: (1) depolarization of the presynaptic membrane; (2) influx of Ca^{2+} ions in the presynaptic terminal; (3) fusion of synaptic vesicles with the plasma membrane; (4) release of SVs (*quanta*) of neurotransmitter molecules; (5) diffusion of the neurotransmitter across the synaptic cleft separating pre- and post-synaptic compartments; (6) activation of postsynaptic receptors; (7) effect on the postsynaptic neuron. As a result of the interaction between NT and its postsynaptic receptors, some changes of the membrane potential and, consequently, of the excitability can be observed. Particularly, when the change is depolarizing, an excitatory postsynaptic potential (EPSP) is generated. On the other hand, if the change is hyperpolarizing, the excitability is decreased, thus generating an inhibitory postsynaptic potential (IPSP). Ionotropic receptors mediate rapid transmission of information (1-20 msec) whereas the activation of metabotropic receptors involves a second-messenger pathway modulating a membrane conductance or inducing other metabolic effects. Despite the functional diversity, synapses display a high degree of morphological uniformity throughout the nervous system. Two groups can be identified: synapses with asymmetrical thickening of their presynaptic and postsynaptic membranes (type 1) and synapses with symmetrical densification (type 2) (Gray et al., 1959). Type 1 synapses are frequently associated with small, round, clear synaptic vesicles and in some cases they have been identified as excitatory glutamatergic synapses. Conversely, type 2 are usually identified by small, clear, flattened or pleomorphic vesicles and are implicated in inhibitory events. The synapse can be described as

an integrative micro-unit. In fact, thanks to its small size (0.5-2 μm) large numbers of synapses can be packed into limited spaces. I will now focus on inhibitory synapses by describing GABAergic transmission that occurs thanks to $GABA_A$ receptors.

1.5 Inhibitory GABAergic synaptic transmission

In the mammalian brain, GABA is the most prominent inhibitory neurotransmitter. GABAergic synaptic transmission is mediated by three receptor classes: $GABA_A$, $GABA_B$ and $GABA_C$ receptors.

$GABA_A$ receptors ($GABA_ARs$) represent the most expressed receptor subtype (Laurie et al., 1992; Wisden et al., 1992) and are the target of several neuromodulators, which have profound effects on brain function, such as benzodiazepines (BDZs), barbiturates, anesthetics, neurosteroids and ethanol (Collingridge et al., 2009; Macdonald & Botzolakis, 2010; Rabow et al., 1995). In adult neurons, activation of $GABA_ARs$ promotes membrane hyperpolarization through the inflow of negative charges (Cl^- ions), or shunting inhibition through increase of membrane resistance. This mechanisms, occurs because in adult neurons, the chloride equilibrium potential is typically more hyperpolarized than or very similar to the resting membrane potential. In fact, it reflects a relatively low concentration of intracellular Cl^- due to expression of the potassium-chloride exporter, KCC2 (Rivera et al., 1999). Importantly, at early developmental stages (P0-P7), neurons express low levels of KCC2 (Watanabe and Fukuda, 2015; Achilles et al., 2007). In this particular condition, $GABA_ARs$ activation causes membrane depolarization (excitation) through outflow of Cl^- ions upon $GABA_AR$ channel opening (Ben-Ari, 2002; Stein & Nicoll, 2003). Generally, $GABA_ARs$ are expressed in the postsynaptic compartments but they can also be presynaptic and inhibit neurotransmitter release (Kullmann et al., 2005). On the contrary, $GABA_B$ receptors are metabotropic G-protein-coupled receptors that provide slow inhibition either by activating inwardly rectifying potassium channels (Wagner & Dekin, 1993) or by inhibiting voltage-gated calcium channels (Mintz & Bean, 1993). They can be found at both pre and postsynaptic neuronal membrane where they inhibit neurotransmitter release and neuronal firing, respectively. However, these receptors are not widely distributed. They could be mainly found

in cerebral cortex, thalamus, cerebellum and spinal cord (Wilkin et al., 1981; Bowery et al., 1987; Chu et al., 1990). $GABA_C$ receptors have been identified in the retina and brainstem (Enz & Cutting, 1998; Bormann, 2000; Milligan et al., 2004). Although $GABA_A$ and $GABA_C$ receptors ($GABA_AR$ and $GABA_CR$) how different functional and pharmacological properties, they are both ligand-gated ion chloride channels assembled from the same family of homologous subunits (Collingridge et al., 2008).

1.5.1 Heterogeneity of $GABA_A$ receptors

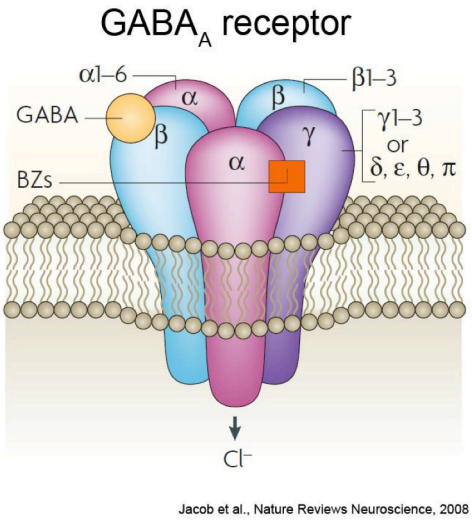


Fig. 1.20 $GABA_AR$ structure. Five subunits from 7 subunit subfamilies (α,β,γ,δ,ε,θ,π) assemble to form a heteropentameric chloride-permeable channel. Binding of the neurotransmitter GABA occurs at the interface between the α and β subunits and triggers the opening of the channel, allowing the rapid influx of chloride ions. (from Jacob et al., 2008)

$GABA_ARs$ mediate the majority of GABAergic signaling and they are responsible for maintaining the inhibitory tone in the mammalian brain. These particular receptors are pentamers resulting from the assembly of homologous subunits that form a central ion channel permeable to chloride (Figure 1.20) (Schofield et al., 1987; Nayeem et al., 1994; Knight et al., 1998; Barrera et al., 2008). A large body of experimental work revealed the existence of eight subunit families (α, β, γ, δ, ε, θ, π and ρ), thus providing a potentially enormous molecular heterogeneity of these receptors. Moreover, each subunit occurs in multiple isoforms (α1-6, β1-3, γ1-3, ρ1-3), splicing variants (e.g., β2S and β2L; β3-v1 and β3-v2; γ2S and γ2L) and alternatively edited transcripts (e.g., α3I and α3M). This further increases the heterogeneity of these receptors (Schofield et al., 1987; Levitan et al., 1988; Pritchett et al., 1989; Shivers et al., 1989; Ymer et al., 1990; Ymer et al., 1989; Whiting et al., 1990; Kirkness & Fraser, 1993; Davies

et al., 1997; Hedblom & Kirkness, 1997; Bonnert et al., 1999; Simon et al., 2004; Ohlson et al., 2007).

Many subunit combinations are theoretically possible but only a few dozen were shown to exist, reflecting the differential distribution of subunit types in different brain regions and neuronal populations (Wisden et al., 1992; Fritschy & Mohler, 1995; Pirker et al., 2000), but also implying some basic rules of assembly (Luscher et al., 2004; Kittler et al., 2000). Despite the huge amount of subunit subtypes, specific requirements to assemble $GABA_A$Rs could limit their heterogeneity (Angelotti et al., 1993). It was shown that, for example, only a small subset of subunits could form pentamers, a fundamental characteristic to obtaining a functional receptor (Gorrie et al, 1997; Connolly et al., 1996; Connolly et al., 1999; Taylor et al., 2000; Bollan et al., 2003; Lo et al., 2008; Sarto-Jackson & Sieghart, 2008). Moreover, it was observed that ternary receptors assemble with higher efficiency than binary receptors and in most of the cases, the presence of both α and β subunits is mandatory to form pentameric receptors (Angelotti et al., 1993). Importantly, the most broadly expressed subunits combination are $2\alpha2\beta2\gamma$ but in some cases, the γ subunit can be substituted by α δ, ε, or π. Morevover π and θ might be capable to be co-assembled with α, β and γ subunits to form receptors containing subunits from four different families (Bonnert et al., 2002). The $\alpha1\beta2\gamma2$ isofrom is the most abundant, representing about 40% of all $GABA_A$Rs (McKernan and Whiting, 1996; Olsen and Sieghart, 2008).

1.5.2 Pharmacological properties of $GABA_A$Rs

Early drugs targeting $GABA_A$Rs had generalized non-specific effects in the CNS. Given the huge heterogeneity and distribution of $GABA_A$Rs, the pharmacology linked to these receptors is dense and rich (Macdonald and Olsen, 1994; Johnston, 1996; Moehler, 2006). $GABA_A$Rs can be activated by GABA and several GABA analogues or agonists and they can be blocked or antagonized by diverse compounds. For instance, GABAergic currents mediated by $GABA_A$Rs can be antagonized, in a competitive manner, by bicuculline and by gabazine (SR95531). Molecules such as picrotoxin are classified as non-competitive antagonists (Johnston, 2013). Then, the anticonvulsant barbiturates and benzodiazepines

(BDZs) provide allosteric modulation of $GABA_A$Rs and enhance inhibitory currents but by binding at different and sites through different mechanisms (Twyman et al., 1989). In fact, barbiturates increase the fraction of long openings by increasing channel mean open time (Gage & McKinnon, 1985; Dilger et al., 1997). On the contrary, BDZs increase the microscopic affinity of GABA for the receptor, without altering channel mean open time, thus increasing the opening frequency of the channel (Bianchi et al., 2009). Interestingly, the pharmacological properties of $GABA_A$Rs depend on the specific subunit composition (Hevers & Lueddens, 1998). For instance, BDZ modulation requires γ subunit but only α(1-2-3 or 5)$\beta\gamma$ receptor isoforms are sensitive to the compound (Poncer et al., 1996; Rudolph et al., 1999; Mohler et al., 2002; Tan et al., 2011). On the contrary, these receptor isoforms are less sensitive to furosemide, while those containing α4 or α6 subunits are highly sensitive (Hevers et al., 2008). Furthermore, zolpidem, an imidazolpyridine, has highest affinity for α1 subunit, low for α2 and α3 and almost no affinity for α5-containing receptors (Hanson et al., 2008). If we consider the β subunit, it was shown that both β2 and β3 isoforms provide high sensitivity to loreclezole whereas β1 subtypes are almost insensitive (Wingrove et al., 2006). Interestingly, incorporation of the γ subunit increases receptor sensitivity to neurosteroids and zinc, whereas inclusion of δ subunits intensifies the sensitivity to neurosteroids and ethanol (Han et al., 2009; Matthew and Samba, 2013).

1.5.3 Localization of $GABA_A$Rs

$GABA_A$R-mediate inhibitory postsynaptic currents (IPSCs) observed in many neurons of the central nervous system are characterized by rapid onset and rise time. These fast kinetics are due to the presence of a high densitity of receptors clustered in close apposition to presynaptic terminals, responsible for GABA release. To better understand and reveal the localization of $GABA_A$Rs, several studies performed in the 80s used monoclonal antibodies against $GABA_A$Rs subunits together with electron microscopic (EM) immunoperoxidase reactions. Interestingly, it was shown that $\alpha 1$ and $\beta 2/3$ occupied non-synaptic sites on membranes (extrasynaptic localization) (Richards et al., 1987; Somogyi et al.,

1983; Waldvogel et al., 1990; Soltesz et al., 1990). Yet, technical limitations could not allow showing and better demonstrating the synaptic localization of these subunits. Later, thanks to the development of new techniques, such as light-microscopic immunofluorescence and EM immunogold methods, more accurate data were obtained. For example, enrichment of $\alpha1$, $\alpha2$, $\alpha3$, $\alpha6$, $\beta2/3$ and $\gamma2$ subunits within the postsynaptic specialization of inhibitory synapses was underlined in many brain regions such as cerebellum, globus pallidus, hippocampus and neocortex (Nusser et al., 1995, 1996, 1998; Craig et al., 1994; Fritschy et al., 1998). Nevertheless it should be taken into account that each of the subunit mentioned above was also found in extrasynaptic plasma membranes, thus underlining the fact that no $GABA_A$ receptor subunit has an exclusively synaptic location. However, for example, the δ subunit is exclusively present in the extrasynaptic somatic and dendritic membranes of cerebellar granule cells (Nusser et al., 1998) and at extrasynaptic and perisynaptic locations in granule cells of hippocampal dentate gyrus (Wei et al., 2003).

Interestingly, δ subunit-containing receptors are likely to be purely extrasynaptic but it was shown that other subunit might also be predominantly or exclusively located outside synapses. For example, hippocampal pyramidal neurons, the $\alpha5$ subunit is not detected at synaptic clusters and does not colocalize with gephyrin (Brunig et al., 2002; Crestani et al., 2002), a protein responsible for the anchoring of $GABA_A$Rs at postsynaptic sites (Tyagarajan & Fritschy, 2014; Pennacchietti et al., 2017). In this particular case, $\alpha5$ overrides the ability of $\gamma2$ subunit to promote synaptic localization. Overall, these data show that receptors containing a $\gamma2$ subunit in association with $\alpha1$, $\alpha2$ or $\alpha3$ subunits are the predominant receptor subtypes providing phasic synaptic inhibition (reviewed in Rudolph & Möhler, 2013). Importantly, receptors containing $\alpha4$, $\alpha5$ or $\alpha6$ subunits are predominantly or exclusively extrasynaptic (reviewed in Rudolph & Möhler, 2013). However, even if $\alpha5$ is mainly described as an extrasynaptic subunit, there is evidence for its presence at synaptic sites. Importantly, by using immunofluorescence and EM immunogold, it was shown that in hippocampal and neocortical pyramidal neurons, $\alpha5$-$GABA_A$Rs are also located at GABAergic synapses (Servanski et al., 2006). Interestingly, it was also demonstrated that neocortical synapses made by dendrite preferring, bitufted cells (SST-positive MCs) onto pyramidal neurons, use

$\alpha 5$ containing $GABA_A$Rs (Ali & Thomson, 2008; Schulz et al., 2018). More in details, IPSCs evoked by MC onto PNs were sensitive to a specific $\alpha 5$ selective inverse agonist (IAalpha5) and Zolpidem had no significant effect at $\alpha 5$-containing GABAergic synapses. On the contrary, IPSCSs evoked by perisomatic targeting basket cells, were highly sensitive to Zolpidem and insensitive to the $\alpha 5$ selective inverse agonist, thus underlying exclusive presence of $\alpha 1$ selective subunit a this GABAergic synapse (Ali & Thomson, 2008).

The presence of specific subunits at the synapse and outside the synaptic junction implies the possibility of distinct $GABA_A$Rs modulating neuronal excitability outside the specialized sites of communication between neurons. This can happen if GABA spills over the synaptic cleft during sustained synaptic activity, or if the excitability of extrasynaptic compartments of a neuron is constantly controlled by ambient levels of GABA. In the next section I will introduce the functional difference between phasic (synaptic) and tonic (that might be extrasynaptic) inhibition.

1.5.4 How do interneurons perform inhibitory control in the circuit?

The good functioning of the adult mammalian brain depends on the coordinated regulation of neural activity provided by a diversified population of GABA-releasing neurons. The main action of this neurotransmitter in mature neurons is to increase membrane permeability to chloride and bicarbonate ions, thus evoking an inward flow of anions and a hyperpolarizing post-synaptic response, the inhibitory post synaptic potential (IPSP). Depending on the modes of activation of $GABA_A$ receptors and on their location and composition, two major types of inhibition can be defined: phasic inhibition and tonic inhibition. Phasic inhibition results from the activation of $GABA_A$ receptors, briefly exposed to high concentration of GABA that is released by presynaptic terminals. This $GABA_A$ receptor-mediated communication is fundamental to realize a rapid and precise transmission of presynaptic activity into a postsynaptic signal. However, it is known that neurotransmitters that are traditionally described to participate in rapid point-to-point communication through the activation of ionotropic

receptors might also participate in slower forms of signaling (Mody et al., 2001), including tonic activation of receptors. This form of activity is guaranteed by the presence of receptors in somatic, dendritic and axonal compartments of neurons, located far away from the sites in which the neurotransmitter is released (Kullmann et al., 2005).

1.5.5 $GABA_A$Rs activation

$GABA_A$Rs can be activated by two main modalities: phasic and tonic activation. These different mechanisms are determined by the subcellular location and biophysical properties of receptor subtypes (Farrant and Nusser, 2005). In the following sections, I will describe how these mechanisms occur and what functional roles they play in the brain.

Phasic and Tonic receptor activation

When an action potential reaches a nerve terminal, a local calcium influx triggers the fusion of synaptic vesicles with the presynaptic membrane at the release site. Each vesicles contains several thousand GABA molecules that are release in the synaptic cleft, thus generating a peak GABA concentration in the millimolar range (Mody et al., 1994). Opposite to the release site, a small number of receptor can be observed (Mody et al., 1994; Nusser et al., 1997; Brickley et al., 1999). In some of these receptors, the binding of GABA triggers the near-synchronous opening of their ion channels and a defining feature of this phasic mode of receptor activation is the short duration of GABA signaling, inducing a transient response (Fig. 1.21 A and B).

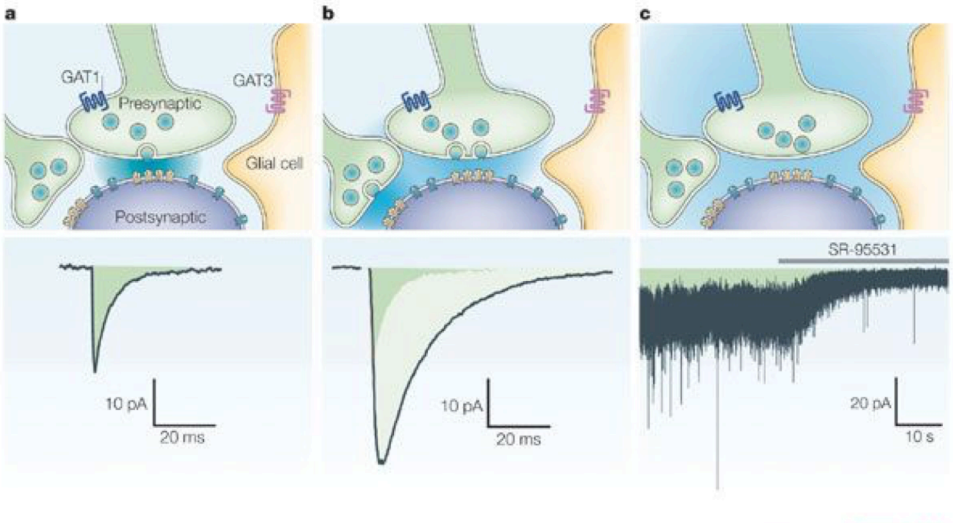


Fig. 1.21 Different mode of $GABA_A$Rs activation. (A) A sigle vesicle from a presynaptic terminal activates only those post-synaptic $GABA_A$Rs that cluster in the membrane immediately beneath the release site (yellow). Below, an averaged waveform of miniature inhibitory postsynaptic currents (mIPSCs). (B) Synaptic and perisynaptic receptors (blue) are activated by action-potential dependent release of multiple vesicles or evoked release from several terminals. (C) Low concentration of ambient GABA mediate tonic activation of high-affinity $GABA_A$Rs. High concentration of gabazine (SR-95531) blocks phasic IPSCs and tonic channel activity, leading to a change in the holding current (from Farrant & Nusser, 2005).

The rapid diffusion of GABA away from the release site may be due to the short dwell time of the neurotransmitter within the cleft (Overstreet et al., 2002). The binding rate of GABA is slow compared to diffusion (Jones et al., 1998). Moreover, the short exposure time to GABA means that not all the postsynaptic receptors will be completely occupied. Although postsynaptic receptor full occupancy occurs at certain synapses, the degree of receptor occupancy may vary between synapses on different neurons and even between synapses on a single neuron (Nusser et al., 1997; Mozrzymas et al., 2003; Frerking et al., 1996; Perrais et al. 1999; Hajos et al.; 2000). Moreover, the vesicles size and content, the nature of vesicle fusion, the geometry of the synaptic cleft and the number and position of GABA transporters and postsynaptic receptors can influence the time course of the GABA transient in the synaptic cleft. This description addresses only the situation in which a single vesicle is released from an active zone and the liberated transmitter activates only those receptors that are clustered on the postsynaptic membrane (Fig. 1.21 A). In reality, there are more levels of complexity. For example, if an action potential causes a multivesicular release at a single active

zone, the postsynaptic receptors would be exposed to a different GABA concentration. The time course of the synaptic GABA transient will be considerably modified if the vesicle release is temporally dispersed (asynchronous release). After diffusing from its release site(s), GABA could activate peri-synaptic receptors, receptors at other postsynaptic densities made by the same bouton, more distal extrasynaptic receptors or receptors at nearby synapses. In this case, the GABA waveform will be determined by its location relative to the release site, the geometry and spatial arrangement of the neighboring cellular elements, diffusional barriers and the proximity of GABA transporters in neurons and astroglia (Overstreet et al., 2002; Barbour and Hausser, 1997; Telgkamp et al., 2004). Currents derived from GABA spillover can be considered phasic because time-locked to the release event.

AP-dependent GABA release underlies phasic inhibition, which is time-locked to presynaptic spiking. The functional role of phasic synaptic inhibition depends on the location of GABAergic synapses (e.g. dendritic vs. somatic), the biophysical properties of the presynaptic neuron and its GABAergic terminals. These were discussed in detail above (section 1.4.2). Overall, fast synaptic inhibition modulates input and output information onto a receiving, postsynaptic neuron at synaptic junctions.

In addition to fast synaptic inhibition, GABAergic signaling can be also tonic and extrasynaptic.

This particular form of inhibition is present in different embryonic neurons before the start of synapse formation (Valeyev et al., 1993; LoTurco et al., 1995; Owens et al., 1999; Demarque et al., 2002). Tonic activation of $GABA_A$Rs in mature neurons was identified in voltage-clamp recordings from rat cerebellar granule cells (Kaneda et al., 1995). SR-95531 (gabazine) and bicuculline, two $GABA_A$R antagonists, blocked spontaneous IPSCs and, importantly, they also decreased the "holding" current necessary to clamp the cells at a given membrane potential (Fig. 1.21 C). The reduction of the input conductance was linked with a reduction of current variance that was consistent with a decrease in the number of open $GABA_A$Rs channels (Kaneda et al., 1995; Tia et al., 1996; Wall & Usowicz, 1997). Further studies identified GABA-mediated tonic conductances in several cell types such as granule cells of the dentate gyrus (Nusser & Mody, 2002), thalamocortical

relay neurons of the ventral basal complex (Porcello et al., 2003), L5 pyramidal neurons in the somatosensory cortex (Yamada et al., 2007), CA1 pyramidal cells (Bai et al., 2001) and certain inhibitory interneurons in the CA1 region of the hippocampus (Semyanov e al., 2003). Despite certain recombinant (MacDonald et al., 2010; Sigel et al., 1989; Maksay et al., 2003; Lindquist et al., 2004) and native $GABA_A$Rs (Birnir et al., 2000) open spontaneously with low probability in the absence of agonists, most $GABA_A$Rs require the binding of agonist molecules to promote entry into open states. Therefore, GABA (or some $GABA_A$R agonist) should be present in the extracellular space at a sufficiently high concentration to cause persistent receptor activation. Moreover, the concentration of GABA in the extracellular space reflects the number and activity of GABA-releasing elements and, importantly, also the action of GABA transporters. This is the case for the Na^+ and Cl^- symporters that normally remove GABA from the extracellular space but that could also operate in the reverse direction, thus providing a source of GABA (Attwell et al., 1993). However, the pharmacological blockade of transport (Wall et al., 1997; Nusser et al., 2002; Semyanov et al, 2003; Rossi et al., 2003) and in transporter deficient mice (Jensen et al., 2003), the size of tonic current increases, indicating that that the activity of reversed transporter is not fundamental in contributing to ambient GABA.

Functional roles of tonic inhibition

Tonic activation of $GABA_A$Rs has one unequivocal outcome: a persistent increase in cell input conductance. This condition causes the increase of the magnitude and duration of the voltage response to an injected current and the decrease of voltage with distance. Therefore, for a given excitatory input, such as an excitatory postsynaptic current (EPSC), the size and duration of the EPSP will be decreased and the temporal and spatial window over which the signal is integrated will be narrowed, reducing the probability to generate an action potential. Several groups investigated how tonic inhibition affects neuronal excitability. For example, in cerebellar granule cells, blockade of tonic inhibition result in a leftward shift of the input-out curve. In other words, in the presence of tonic inhibition, neurons are leakier and they require stronger stimuli to induce AP firing (Brickley et al., 1996; Hamann et al., 2002; Chadderton et al., 2004).

In the neocortex, different levels of heterogeneity co-exist. Indeed, the correct functioning of cortical microcircuits strongly relies on the well-orchestrated activity of PNs and inhibitory interneurons. Both these two cell populations are heterogeneous, but inhibitory GABAergic interneurons seem to be significantly more diverse as compared to PNs. In addition to the rich neocortical diversity of cell types, another level of heterogeneity occurs at the synaptic level due to the sensationally assortment of $GABA_AR$ subunit isoforms and splice variants. The differential expression of $GABA_AR$ subunits ultimately shapes fast GABAergic transmission at specific synapses, and can confer high affinity to extrasynaptic receptors, thus mediating tonic inhibition. Overall, these specific mechanisms for phasic and tonic inhibition result in a tight balance between excitation and inhibition in each neuron. In the next section, I will point out that modifications of this equilibrium in specific microcircuits can lead to the emergence of pathological conditions of the brain.

1.6 Involvement of $GABA_A$Rs in pathological brain states

As we already mentioned, normal cognitive functions rely on a balanced and coordinated activity of neuronal networks composed by a rich diversity of excitatory and inhibitory neurons connecting with each other, following a detailed blueprint. In particular, in the neocortex, fast synaptic inhibition shapes all forms of spontaneous and sensory-evoked activity (Isaacson & Scanziani, 2011). It was demonstrated that, perturbations of this inhibition/excitation equilibrium and alterations of specific inhibitory circuits lead to network desynchronization, and thus to brain diseases characterized by cognitive dysfunctions, such as for example schizophrenia, autism spectrum disorders (ASD) and Down Syndrome (DS) (reviewed in Brat & Kooy, 2005; Del Pino et al., 2013; Zorrilla de San Martin et al., 2018; Selten et al 2018).

Particularly, in the past years research successfully identified some genes underlying syndromic forms of neurodevelopmental disorders, such as in patients with a combination of ASD, epilepsy and intellectual disability (ID) (Krumm et al., 2015). Emerging evidence suggests that many of the these genes cluster in a relatively limited numbers of modules, operating in the same molecular processes (Epi4K Consortium and Epilepsy Phenome/Genome Project, 2013; Gilman et al., 2011; O'Roak et al., 2012; Voineagu et al., 2011). Strikingly, the encoded mutated proteins are mainly involved in chromatin remodeling and importantly, in synaptic functioning (De Rubeis et al., 2014; Krumm et al., 2014). In this perspective, the GABAergic system is a key pathway that is commonly altered in many neurodevelopmental disorders (for a more detailed description see: Braat & Kooy, 2015, Neuron). Importantly, several lines of evidence suggest that inhibitory neurotransmission in the central nervous system (CNS) plays important roles in modulating circuits in the brain that are involved in the manifestation of symptoms of schizophrenia. A downregulation of the biosynthesis of cortical GABA leads to a defective GABAergic cortical function in schizophrenia and a compensatory (but insufficient) upregulation of $GABA_A$ receptors (Guidotti e al., 2005). Furthermore, a deficit in the glutamatergic activation of GABAergic interneurons in the PFC, which synapse on pyramidal neurons at the axon initial

segment, results in upregulation of the α_2 subunit of the $GABA_A$ receptor in the axon initial segment (Lewis et al., 2005).

In addition, α1 is the most expressed subunit of the $GABA_A$Rs and mediates fast synaptic transmission ubiquitously in the neocortex. Interestingly, it was shown that mutations in the α1 subunit were found in patients affected by early infantile epileptic encephalopathy, juvenile myoclonic epilepsy and other seizure disorders (Carvill et al., 2014; Cossette et al., 2002; Maljevic et al., 2008; Epi4K Consortium and Epilepsy Phenome/Genome Project, 2013).

Importantly, it was shown that manipulations of other subunits can have profound effects on brain function. Notably, it was shown that partial pharmacological and genetic manipulation of α5-containing $GABA_A$Rs lead to improved hippocampus-dependent performance, as shown by trace fear conditioning, appetitive conditioning, and novel object recognition (Clément et al., 2012; Crestani et al., 2002). Similarly, in 2002 Collinson and colleagues demonstrate that mice with a full deficit of α_5 receptors ($\alpha_5^{-/-}$ mice), learning and memory were enhanced (Collinson et al., 2002). Particularly, also synaptic transmission was altered: in the CA1 region of hippocampal brain slices from α5 −/− mice, the amplitude of the IPSCs was decreased, and paired-pulse facilitation of field EPSP (fEPSP) amplitudes was enhanced. Altogether, these results indicate that, despite α5- $GABA_A$Rs only represent less than 5% of all receptors (Rudolph & Möhler, 2013), play a fundamental role in cognitive processes.

Importantly, it was also demonstrated that changes in the normal functioning of α5-$GABA_A$Rs can lead to pathological conditions. For instance, although dysfunctional parvalbumin (PV) cells were suggested to contribute to the emergence of schizophrenia (Kalus, 2002), it was shown that schizophrenic subjects display low levels of α5-$GABA_A$Rs, suggesting impaired dendritic inhibition, involved in network synchronization (Duncan et al., 2010). Similarly, it has been shown that abnormal micro-duplications the chromosomic human locus 15q11-13 yields altered α5 expression, resulting in a significant reduction of α5-$GABA_A$Rs availability in individuals with ASD (Voineagu, 2011). This prompts the question whether dysfunctions of inhibitory activity using the α5 subunit of the $GABA_A$R are involved in conditions characterized by intellectual disabilities.

1.6.1 The case of α5-$GABA_A$Rs and DS

Down Syndrome (DS or trisomy 21) is the most common genetic cause of intellectual disability and occurs when an individual has a full or partial extra copy of chromosome 21 (Lejeune et al., 1959; Antonarakis et al., 1997). DS patients face various health issues including learning and memory, congenital heart diseases (CHD), Alzheimer's diseases (AD), leukemia, cancers and Hirschprung disease (HD). Furthermore, they are often diagnosed with ASD-like traits. In particular, it was shown that long-lasting changes of synaptic strength are the cellular substrates for learning and memory (Pastalkova et al., 2006; Whitlock et al., 2006). Interestingly, recent evidence suggests that an excessive GABAergic inhibition could interfere with these changes of synaptic strength, thus leading to cognitive and learning deficits in DS (Wigstrom and Gustafsson, 1986; Zorrilla de San Martin et al., 2018). Moreover, altered GABAergic function was shown in DS mouse models, namely a significant alteration of KCC2 leading to excitatory actions of GABA in DS (Deidda et al., 2015). To investigate these mechanisms, several groups started testing several hypotheses in animal models of DS, such as the Ts65Dn mouse. This murine model recapitulates several fundamental features of DS, especially cognitive deficits and alterations in brain morphology and function (Bartesaghi et al., 2011; Haydar and Reeves, 2012; Rueda et al., 2012). Precisely, these animals are characterized by a segmental trisomy of murine chromosome 16, containing 92 human orthologues between Mrp139 and Zfn295 (Sturgeon and Gardiner, 2011). Interestingly, early studies in hippocampal tissue from Ts65Dn animals, showed a relevant deficit in long-term potentiation (LTP) of synaptic strength in CA1 neurons (Fig. 1.22 B) (Siarey et al, 1997, 1999). Moreover, an enhanced long-term depression (LTD) was also detected while stimulating Schaffer collateral at low frequencies (Fig. 1.22 C) (Siarey et al., 1999). Importantly, these alterations were confirmed in other mouse models of DS (O'Doherty et al., 2005; Siarey et al., 2006; Belichenko et al., 2007; Belichenko et al., 2009).

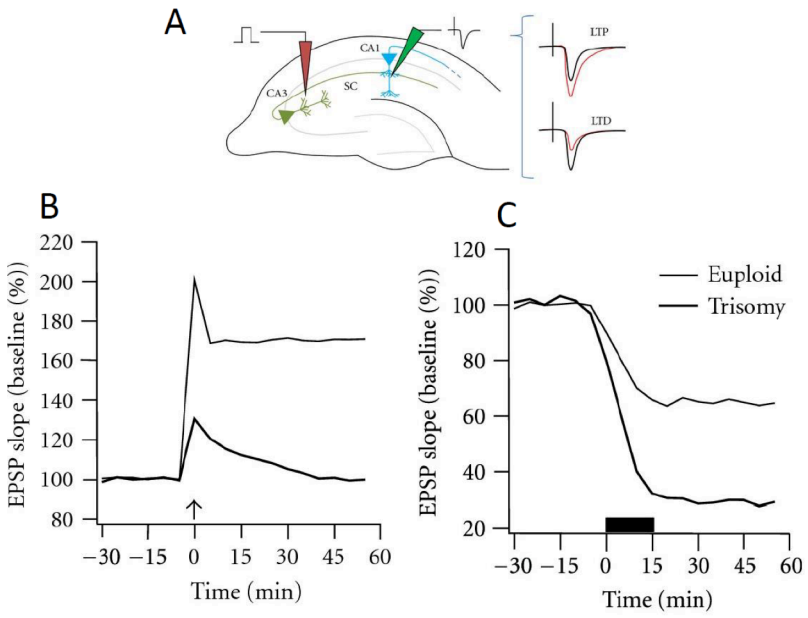


Fig. 1.22 Altered CA1 hippocampal plasticity in Ts65Dn mice. (A) Diagram indicating electrode placement for stimulating Schaffer collaterals arising from CA3 and recording the evoked field excitatory postsynaptic potential (EPSP) in CA1. Traces to the right indicate the typical change in evoked responses (red) following LTP and LTD. (B) Simulated data depicting suppressed LTP in Ts65Dn mice. After high-frequency stimulation of SC (at arrow head), the field EPSP increases and remains enhanced in euploid mice but fails to remain elevated in Ts65Dn mice. (c) Simulated LTD data depicting exaggerated depression of evoked EPSPs following low-frequency stimulation of SC in Ts65Dn mice. (Traces in B and C based on data from [Siarey et al, 1997, 1999].)

Recently, Marie-Claude Potier's group demonstrated that highly specific α5-inverse agonists (α5IA) restored cognitive deficits in Ts65Dn mice (Fig.1.23) (Braudeau et al., 2011). Despite many advances have been made indicating malfunction in DS at the circuit level, it is not yet clear how general these alterations are. Particularly, further investigations are required to understand which specific circuits are affected.

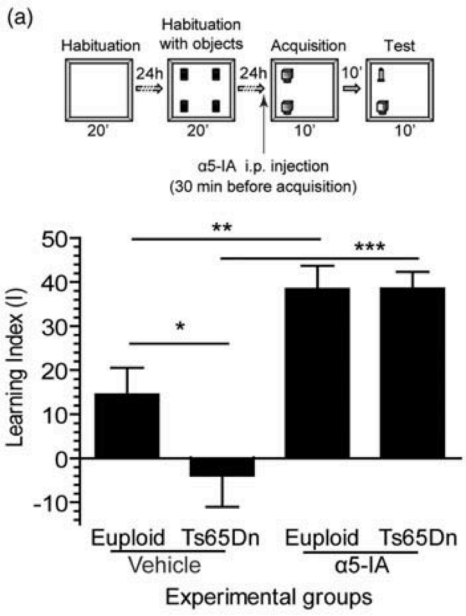


Fig. 1.23 α5IA rescues recognition memory deficits in Ts65Dn mice. Upper part: general protocol of the novel object recognition (NOR) Lower part: Learning index). Under vehicle, Ts65Dn mice were found to be impaired. Following i.p. injection of α5IA, both euploid and Ts65Dn mice improved their NOR performance and the deficit of Ts65Dn mice was rescued.

AIM OF THE THESIS

Why studying α5-GABA$_A$Rs?

Despite the numerous studies revolving around GABA$_A$Rs and inhibitory circuits, the role of α5-GABA$_A$Rs is still controversial. As mentioned before, these receptors were mostly believed to participate to tonic inhibition. For instance, it was clearly demonstrated that α5-GABA$_A$Rs mediate tonic currents in the central nucleus in the amygdala (Botta et al., 2015). Moreover, α5-GABA$_A$Rs generate tonic conductance that regulates the excitability of pyramidal neurons in CA1 and CA3 regions of the hippocampus (Caraiscos et al., 2004; Glykys and Mody, 2006, 2007; Pavlov et al., 2009; Prenosil et al., 2006; Semyanov et al., 2004) and layer 5 cortical neurons (Yamada et al, 2007). Conversely, the study conducted by Ali and Thomson in 2008, clearly states the involvement of these receptor subtype in dendric synaptic inhibition from Martinotti cells onto neocortical PNs (Ali and Thomson, 2008). Furthermore, a more recent study describes the pivotal role of synaptic α5-GABA$_A$Rs in controlling dendritic postsynaptic integration and action potential firing of hippocampal CA1 pyramidal cells (Schulz et al., 2018). Considering this controversy and given the strong interest of α5-GABA$_A$Rs as a therapeutic target of several brain diseases, it is fundamental to pinpoint the properties of this receptor subunit within cortical circuits in healthy and pathological conditions. For this reason, my thesis work is focused on two specific aims:

1) ***Studying the role of α5-GABA$_A$Rs in L 2/3 of somatosensory cortex in physiological conditions.***

The diversity of GABAergic interneurons is paralleled by a diversity of GABA$_A$ receptors that display structural, functional, and positional specifications geared toward the requirement of the respective synapse operation. Therefore, the GABA$_A$ receptor subtypes are pharmacological targets that provide diverse opportunities for modulating the spatiotemporal pattern of network activity (Rudolph & Möhler, 2014). Importantly, it was widely demonstrated that α5-GABA$_A$Rs play an in important role in cognitive processes. In this context, and given the involvement of α5-GABA$_A$Rs is pathologies characterized by cognitive impairment, it is fundamental to reveal if these receptors are specific to distinct cortical inhibitory circuits. Moreover, their contribution in shaping the activity of

the mouse somatosensory cortex remains elusive and their participation in tonic or phasic inhibition is still controversial (Ali and Thomson, 2008; Botta et al., 2015; Schulz et al., 2018; Serwanski et al., 2006; Mody and Pearce, 2004). Here, we wanted to investigate the involvement of α5-$GABA_A$Rs in fast synaptic and/or tonic inhibition in this particular cortical area. Importantly, this region is characterized by sparse action potential firing in excitatory neurons, thus providing a simple and reliable neural code useful for associative learning. Sparse coding is enforced by strong GABAergic inhibition, recruited by firing of a few excitatory L2/3 PNs and represents a common rule for representation of sensory information in L2/3 of primary sensory cortices (Sakata and Harris, 2009; O'Connor et al., 2010; Crochet et al., 2011; Haider et al., 2013). Studying the role of α5-$GABA_A$Rs in specific microcortical circuits would help to better understand how sensory representation is processed and especially integrated, since these receptors are thought to be present on the dendrites of PNs (Ali and Thomson, 2008).

2) ***Study of specific inhibitory microcircuits in L 2/3 of the PFC in a mouse model of DS***

In recent years, GABAergic over-inhibition appeared as an emerging hypothesis supporting the underlying mechanisms leading to cognitive deficits in DS. A reduction of this excessive inhibition could therefore represent a possible solution to alleviate the cognitive symptoms in these subjects. However, non-specific interventions on GABAergic signaling often result in massive and unwanted effects, such as seizure activity, anxiety and convulsions (Dorow et al., 1983; Horowski & Dorow, 2002; Velísková & Velísek, 1992; Khalilov et al., 2003; Bradford, 1995). This led to the development of milder approaches to diminish the inhibitory tone in DS subjects. The α5 subunit of the $GABA_A$R has been a target for this intervention, due to the relatively lower expression of this subunit, as compared to more prominent ones (e.g. α1), and because α5 was considered to be expressed extrasynaptically, mainly mediating tonic, non-specific inhibition. Accordingly, highly specific and partial pharmacological modulation of α5$GABA_A$Rs, cognitive deficits in Ts65Dn mice were rescued (Braudeau et al., 2011, Martínez-Cué et al., 2014). This specific partial blockade obtained by a

single administration of the α5IA, not only resulted in a complete recovery of cognitive impairment, but also did not result in epileptiform activity.

Results outlined in the first part of my thesis work demonstrated that α5-$GABA_A$Rs are selectively expressed at the specific GABAergic circuit formed by MC-PN synapses. This, and the notion that cortical GABAergic inhibition results from the activity of a plethora of interneuron subtypes, led us to hypothesize that over-inhibition in DS might result from circuit-specific alterations. In particular, given the preferential expression of α5-$GABA_A$Rs at MC-PN synapses, we hypothesized that inhibitory responses from these interneurons is potentiated in DS. Here, together with my colleague Javier Zorrilla de San Martin, we dissected and studied the morpho-functional properties of different inhibitory circuits in the PFC of DS mice. Understanding the mechanisms underlying circuit-specific alterations of GABAergic signalling will be fundamental to develop a therapeutic strategy to ameliorate the cognitive and learning impairment in DS.

CHAPTER 2:
MATERIALS AND METHODS

2.1 Animals

Experimental procedures followed National and European guidelines, and have been approved by the authors' institutional review boards. In order to identify GABAergic transmission from different interneurons we used several mouse models: to record from PV interneurons we used PV-Cre mice (Jackson Laboratory Stock Number 008069). To selectively express tdTomato in PV-positive cells, we bred PV:Cre with Tdtomato mice (Jackson Laboratory Stock Number 007909).To record from MC we used X98 mice that express EGFP principally in MCs (Jackson Laboratory Stock Number 006340). To perform simultaneous recordings from MCs and PV cells we crossed X98 mice with PvAlb-tdtomato. Furthermore, in order to record from synaptically connected VIP interneurons and MCs we crossed VIP Cre mice (*Vip*$^{tm1(cre)Zjh}$/J, Jackson Laboratory Stock Number 010908) with X98. Later, we expressed ChR2 virus in VIP Cre positive interneurons (see section 2.2). To study GABAergic transmission from MCs in DS, we crossed X98 mice with Ts65Dn (B6EiC3Sn a/A-Ts(17^{16})65Dn/J, Jackson Laboratory Stock Number 5252). Moreover, to investigate inhibition mediated by PV interneurons in DS, we crossed Pvalb-Tdtomato mice with Ts65Dn. In all the experiments, both female and male from 18- to 25-d-old mice were used.

2.2 Virus-Mediated Gene Delivery and Optogenetics

To selectively express the light-sensitive ion channel channelrhodopsin 2 (ChR2) in VIP- expressing cortical interneurons, VIPCre::X98 pups (P0–3) were anesthetized on ice, and a beveled injection pipette, attached to a micromanipulator, was gently inserted 300 µm deep in the somatosensory cortex through intact skin and skull. We then delivered 300 nL of viral particles (in PBS) using an injector (Nanoliter 2000 Injector, WPI Inc., USA). The pipette was left in place for an additional 30 s, before it was retracted. The adeno-associated viral (AAV) particles expressed floxed ChR2 (AAV9.EF1.dflox.hChR2(H134R)-mCherry.WPRE.hGH; Addgene 20297) and were obtained from the Penn Vector Core (University of Pennsylvania). At the end of the procedure, pups were returned to their mother until P18–25, when they were sacrificed to obtain slices for electrophysiological experiments, as described in section 2.4. ChR2 activation was obtained by brief

(ranging between 0.5 and 2 ms) light flashes on cortical slices, using a 5W LED (λ = 470 nm, Cairn) collimated and coupled to the epifluorescence path of a Zeiss AxioExaminer microscope. Experiments were performed using a 60× water immersion lens. Light-evoked responses were recorded in L 2/3 MCs and were completely abolished by gabazine (not shown).

2.3 Immunofluorescence

Slices used for electrophysiology experiments were fixed overnight in 4% paraformaldehyde in phosphate buffered saline (PBS, pH 7.4) at 4°C. Slices were then rinsed three times at room temperature (10 min each time) in PBS and incubated overnight at 4°C in PB with 0.3% Triton X-1000, 0.1% normal goat serum (NGS), primary rabbit anti-GFP antibody (1:400, AB3080,Millipore) and primary mouse anti-SST antibody (1:250, G10 sc-55565, Santa Cruz). Slices were then rinsed three times in PBS (10 min each) at room temperature and incubated with Alexa rabbit 488 (1:500, A11034, Life technologies) and Alexa mouse 633 (1:500, A21052, Life technologies) for 2 h at room temperature. Slices were then rinsed three times in PBS (10 min each) at room temperature and coverslipped in mounting medium. Immunofluorescence was then observed with a confocal microscope (Leica) and images were acquired.

2.4 *In Vitro* Slice Preparation and Electrophysiology

Coronal slices (350 µm thick) from somatosensory and prefrontal cortices were obtained from 18- to 25-d-old mice. Animals were deeply anesthetized with isofluorane and decapitated. Brains were quickly removed and immersed in "cutting" solution (4°C) containing the following (in mM): 126 choline, 11 glucose, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 10 $MgSO_4$ and 0.5 $CaCl_2$ (equilibrated with 95% O_2/5% CO_2). Slices were cut with a vibratome (Leica) in cutting solution and then incubated in oxygenated artificial cerebrospinal fluid (aSCF) containing the following (in mM): 126 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 16 mM glucose (pH 7.4), initially at 34°C for 30 min, and subsequently at room temperature, before being transferred to the recording chamber. Recordings were obtained at 30°C. Synaptic currents were recorded in whole-cell voltage- or current-clamp mode from layer 1 interneurons, layer 2/3

pyramidal neurons, MCs, PV cells and VIP interneurons of mouse primary barrel somatosensory cortex and L 2/3 PN, and from PV cells and MCs of mouse prefrontal cortex. Pyamidal neurons were visually identified using infrared video microscopy by their large somata and pia-oriented apical dendrites. Also layer 1 interneurons were visually identified using infrared video microscopy, being the only cell type with the soma present in L1. MCs (labeled with GFP), and PV-expressing interneurons (labeled with TdTomato), were identified using LED illumination (OptoLED, blue, λ=470nm, green λ=530nm, Cairn Research, Faversham, UK). We used different intracellular solutions depending on the type of experiment and the nature of the responses we wanted to assess. For voltage-clamp experiments, in which tonic currents were analyzed, electrodes (with a tip resistance of 2–4 MΩ) were filled with an intracellular solution containing (in mM): 145 CsCl, 4.6 $MgCl_2$,, 10 HEPES, 1 EGTA, , 0.1 $CaCl_2$, 4 Na-ATP, 0.4 Na-GTP, pH adjusted to 7.2 with CsOH, 280–300 mOsm. The estimated E_{Cl} was approximately + 3 mV based on the Nernst equation. In these experiments, GABA (5 μM) was added in the aCSF. In order to isolate $GABA_A$-receptor-mediated currents, DNQX (10 μM) was present in the superfusate of all experiments, unless otherwise indicated. To study the role of α5-$GABA_A$Rs in tonic inhibition and at specific GABAergic synapses, α5IA (L-822179, Triazolophthalazine) (50-100 nM) was added to the aCFS-DNQX solution. Conversely, to exclude the presence of α1 subunit at GABAergic synapses formed by MC onto PN and to confirm its action at PV-PN connections, we added Zolpidem (100 nM) to the bath.

For voltage-clamp whole cell paired recordings electrodes were filled with an intracellular solution containing (in mM): 70 K-gluconate, 70 KCl, 10 HEPES, 1 EGTA, 2 $MgCl_2$, 4 Mg-ATP, 0.3 Na-GTP, pH adjusted to 7.2 with KOH, 280–300 mOsm. The estimated E_{Cl} was approximately -15 mV based on the Nernst equation. For paired recordings between pyramidal neurons and MC, post-synaptic MC, these latter were recorded using a Cs based solution (see Cs based solution described above). To completely block inhibitory currents, gabazine (10 μM) was added to the aCFS at the end of the experiment. To study both GABAergic currents and glutamatergic inputs in TsDn65 mice, DNQX was not added to the bath. To record inhibitory currents from pyramidal neurons, electrodes were filled with a high Cl^- intracellular solution (see K-gluconate

solution described above). To record glutamatergic currents from interneurons, a low chloride solution was used. It contained (in mM): 150 K-gluconate, 4.6 $MgCl_2$, 10 HEPES, 1 EGTA, 0.1 $CaCl_2$, 4 Na-ATP, 0.4 Na-GTP, pH adjusted to 7.2 with KOH, 280–300 mOsm.

Signals were amplified, using a Multiclamp 700B patch-clamp amplifier (Axon Instruments, Foster City, CA), sampled at 20 kHz and filtered at 4 KHz (for voltage-clamp experiments) and 10 KHz (for current clamp experiments). All drugs were obtained from Tocris Cookson (Bristol, UK) or Sigma (Bristol, UK). α5IA was provided by Marie-Claude Potier.

In voltage-clamp experiments, access resistance was on average <15 MΩ and monitored throughout the experiment. Recordings were discarded from analysis if the resistance changed by >20% over the course of the experiment. Unitary synaptic responses were elicited in voltage-clamp mode by brief somatic depolarizing. A train of 5 presynaptic spikes at 50 Hz was applied to infer short-term plasticity of synaptic responses. The paired pulse ratio (PPR) was obtained as the peak amplitude of the second uEPSC divided by that of the first.

2.5 Morphological reconstruction

To reconstruct and quantify anatomical features of different cortical neurons, biocytin (Sigma) was included in the intracellular solution at a high concentration (10mg/mL), which required extensive sonication. To avoid excessive degradation of fragile molecules such as ATP, sonication was performed in an ice bath. The intracellular solution was then filtered twice to prevent the presence undissolved lumps of biocytin in the patch pipette. Extra care was applied in verifying that the micro manipulators and slice were stable for recordings of at least 30 min. During that time, access resistance was continuously monitored throughout the experiment. At the end of recordings, the patch pipette was removed carefully with the aim of resealing the cell properly, equivalent to obtaining an inside out patch. The slice was then left in the recording chamber for a further 5-10 min to allow further diffusion. Slices were then fixed with 4% paraformaldehyde in phosphate buffer saline (PBS, Sigma) for at least 48 h. Following fixation, slices were incubated with the avidin-biotin complex (Vector Labs) and a high

concentration of detergent (Triton-X100, 5%) for at least two days before staining with 3,3'-Diaminobenzidine (DAB, AbCam). Cells were then reconstructed and cortical layers delimited using Neurolucida (MBF Bioscience). Neuronal reconstructions were aligned to a mouse atlas from the Allen Institute. Two different analyses were performed by Neurolucida Explorer: we determined the length of axons and dendrites of MCs in L 2/3 and L1 of somatosensory cortex, and we performed a Sholl analysis of dendrites and axons of MCs in L 2/3 of PFC both in euploid and trisomic animals. In both cases, data were exported and processed in Origin.

2.6 Data analysis

Experiments on firing dynamics, tonic currents and unitary paired recordings were analyzed with Clampfit (Molecular Devices), Origin (Microcal) and custom-made scripts in Matlab (the Mathworks). Spontaneous synaptic events were detected using custom written software (Wdetecta, courtesy J. R. Huguenard, Stanford University https://hlab.stanford.edu/wdetecta.php) based on an algorithm that calculates the derivative of the current trace to find events that cross a certain defined threshold. Three type of events were detected: type I events were isolated sIPSCs, the rising and decay phases of which did not overlap other events; type II events were followed by sIPSCs on their decay phase; and type III events were those rising on the decay phase of a previous IPSC. Here we only considered type I events. Amplitude and rise times of the events were then binned and sorted, using other custom written routines (courtesy J. R. Huguenard, Stanford University).

The peak-to-baseline decay phase of uIPSCs was fitted by the following double exponential function:

$$F(t) = (A_{fast}e^{-t/\tau_{fast}}) + (A_{slow}e^{-t/\tau_{slow}}), (1)$$

where A_{fast} and A_{slow} are the fast and slow amplitude components, and τ_{fast} and τ_{slow} are the fast and slow decay time constants, respectively. The weighted decay time constant ($\tau_{d,w}$) was calculated using the following equation:

$\tau_{d,w} = [(A_{fast}\tau_{fast}) + (A_{slow}\tau_{slow})] / ((A_{fast} + A_{slow})$ (2).

AP waveforms were investigated using a phase plot analysis (Bean, 2007). Several parameters can be extrapolated from the loop (dV/dt plotted in function of Vm) obtained: i) AP threshold, measured as the potential at which the slope of the AP exceeds a certain threshold ii) depolarization slope (ascending phase) iii) AP peak, which is the maximum potential reached iv) repolarization slope (descending phase) v) after-hyperpolarization vi) AP width, measured at the midpoint of the rising phase. Passive properties as well as optical stimulation experiments were analyzed with Clampfit and custom-made scripts in Matlab. Both unitary and light-induced IPSCs were averaged across at least 20 trials for control and 20 for the treatment with α5IA.

Results are presented as means ± SEM.

2.7 Statistical tests

All statistical analysis were performed in Origin (Microcal). Normality of the data was systematically assessed (Shapiro-Wilk normality test). Normal distributions were statistically compared using Paired t-Test or Two-sample t-Test. When data distributions were not normal or n was small, non-parametric tests were performed (Mann-Whitney, Wilcoxon Signed Ranks Test, 2 way ANOVA). Two-way ANOVA tests were followed by Bonferroni's multiple comparison *post hoc*. Differences were considered significant if $p < 0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

CHAPTER 3:
RESULTS
Part 1

3.1 GAD-67 GFP X98 mice: a mouse model to study L2/3 Martinotti cells

Even though inhibitory interneurons account for only 20% of the total number of cortical neurons, their diversity is much richer as compared to excitatory principal cells (Gonchar et al., 2008; Rudy et al., 2011; Tremblay et al., 2016; Butt e al., 2017). Despite being broadly classified as dendrite-targeting interneurons, SST-expressing cells exhibit significant heterogeneity (Tremblay et al., 2016; Ma et al., 2006; Scheyltjens & Lutgarde, 2016). Indeed, several interneurons expressing this neuropeptide have been described in the mouse cortex, based on distinct electrophysiological, anatomical (Kawaguchi and Kubota., 1996) and molecular properties (Gonchar and Burkhalter, 1997). Importantly, previous studies identified α5-GABA$_A$Rs as unique to synapses made by Martinotti cells onto the dendrites of PNs (Ali and Thomson, 2008).

To study these specific receptor subtypes at these specific synapses, we used a mouse model in which only MCs were labelled: the GAD-67 GFP X98 mice (here termed X98 mice). In this mouse line, GFP is predominantly expressed in layers 5B and 6, and, to a lesser extent, in layer 2/3. A detailed characterization of X98 mice was provided by Agmon's group but it was mainly related to L5 Martinotti cells (Ma et al., 2006). Here we set out to confirm that GFP-expressing cells in L2/3 belong to the specific SST-positive interneuron subtype defined as the MCs.

We performed immunofluorescence staining on microtome-cut sections from mouse brains of 18-25-d-old mice and showed that virtually all GFP-expressing cells also expressed SST (example in Fig. 3.1 A). Then, several GFP-expressing neurons were filled with biocytin (n=11) during whole-cell patch-clamp recordings, and reconstructed to assess their somato-dendritic structures and axonal projections. Layers were defined by referring to the Allen Brain References Atlas. We considered only those cells whose cell body was located in L2/3 (n=11) and measured the length of both axon and dendrites. We found that axons of GFP-expressing neurons were oriented towards superficial layers and consistently reached L1 (Fig. 3.1 C and D). Conversely, dendrites were mostly located in L2/3 without reaching L1 (Fig 3.1. C and E).

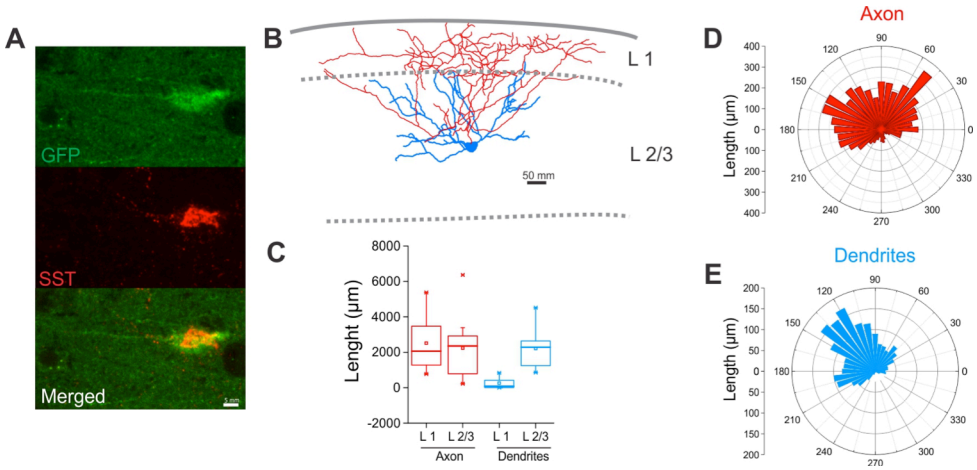


Fig. 3.1 Morphological characterization of MC. (A) SST staining (red) overlaps with GFP (green) in X98 mice (scale bar: 5 µm) (B) Morphological reconstruction of MC. Dendrites are represented in blue whereas axon in red. (C) Comparison of axon (red) and dendrites (blue) lengths in L1 and L 2/3 (lengths were obtained following reconstruction in Neurolucida).(D) and (E): Polar plots representing the characteristic orientation of MC axon (red) and dendrites (blue) respectively. Axon mostly projects to L1.

We performed patch-clamp recordings of GFP positive cells in X98 mice (n=22) and compared their firing pattern with PV-INs analyzed in PVcre::tdTomato mice (n=14). As previously described, the majority of the GFP-positive cells in X98 mice displayed accommodating and adapting responses when depolarizing currents were injected (Kawaguchi, 1995; Cauli et al. 1997) (Fig 3.2 A), and they displayed a typical sag in response to hyperpolarizing currents (2.81941 mV ± 0.39809) (Fig. 3.2 A, asterisk). Conversely, PV cells were characterized by much higher firing frequencies in response to depolarizing currents (Fig. 3.2 B), more prominent spike frequency adaptation (Fig. 3.2 E, p=1.09486E-5, Mann-Whitney test, Table 1), and lower input resistance (Fig. 3.2 D, p=8.1138E-6, Mann-Whitney test, Table 1).

One hallmark of MCs is that they receive highly facilitating glutamatergic synaptic responses from nearby PNs, as opposed to PV cells (Reyex et al., 1998; Markram et al., 1998; Ascoli et al., 2008). We therefore performed paired recordings from PNs and connected GFP-cells in X98 mice, and compared them with PN-PV glutamatergic responses in PVcre::tdTomato mice. We found that excitatory inputs to GFP-expressing cells in X98 mice were invariably strongly facilitating (Fig

3.3 A-C-D, paired pulse ratio= 1.84695 ± 0.19426, Table 2). In contrast, unitary excitatory postsynaptic currents onto PV cells were depressing (Fig 3.3 B-C-D, paired pulse ratio= 0.44649 ± 0.04717, Table 2).

Furthermore, we analyzed the kinetics of the inhibitory responses elicited by MCs and PV-cells in L2/3 PNs (Fig 3.3 A to D) and found that uIPSCs evoked from MCs had significantly slower rise times on average as compared to PV cells (Fig. 3.3. D, 1.89 **±** 0.25 ms versus 0.57 **±** 0.02 ms, Table 7, p = 2.22307E-5, Mann-Whitney test). uIPSCs elicited by MCs show slower rise times because of dendritic filtering. Conversly, uIPSCs elicited by PV-cells display faster rise times because they are evoked on the perisomatic compartment of PNs.

Altogether, these results indicate that GFP expressing neurons in X98 mice encompass a homogeneous SST-positive interneuron subtype, exhibiting the typical anatomical, intrinsic excitability and synaptic features of MCs.

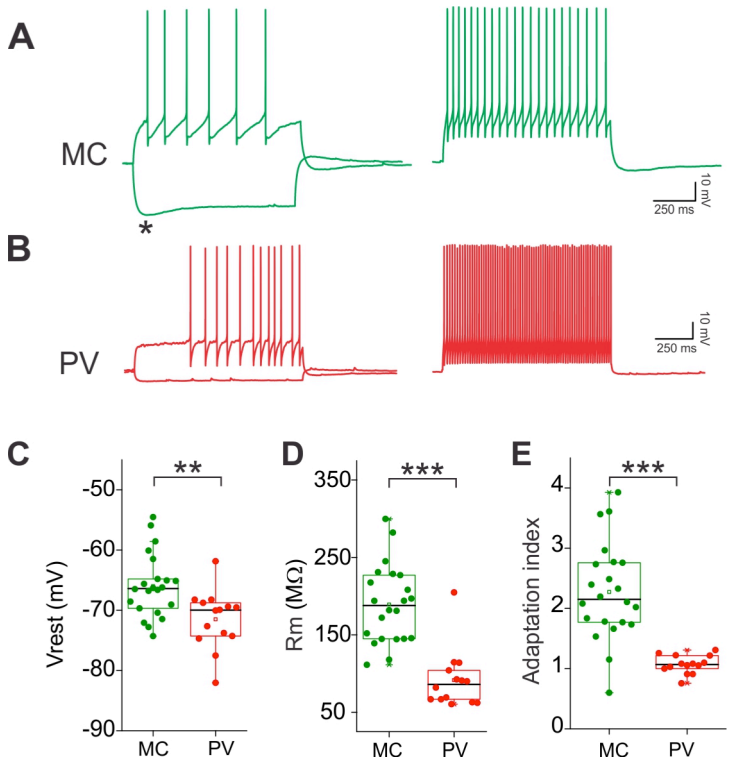


Fig. 3.2. Firing dynamics of X98 GFP and PV cells. Representative current-clamp recordings from a GFP-expressing interneuron in X98 mice (A) and a PV cell (B). X98 GFP cells display a characteristic sag (A, asterisk) in response to hyperpolarizing current injection whereas PV-cells show fast-spiking patterns in response to depolarizing current (C-E) Passive properties of X98 GFP (green) and PV (red) interneurons. (G) Adaptation index of X98 GFP and PV cells. MC: Martinotti cell; PV: parvalbumin cell.

Table 1. Comparison of intrinsic membrane properties of MCs and PV-cells.

	MC		PV		Statistics	
	Mean	**± SEM**	**Mean**	**± SEM**	**P value**	**Test**
Vrest (mV)	-65.99	1.12	-71.49	1.30	**0.00168	Unpaired *t*-test
Rm (MΩ)	189.07	10.74	91.54	10.08	***8.1138E-6	Mann-Whitney
Adapt. index	2.27	0.17	1.07	0.04	***1.09486E-5	Mann-Whitney

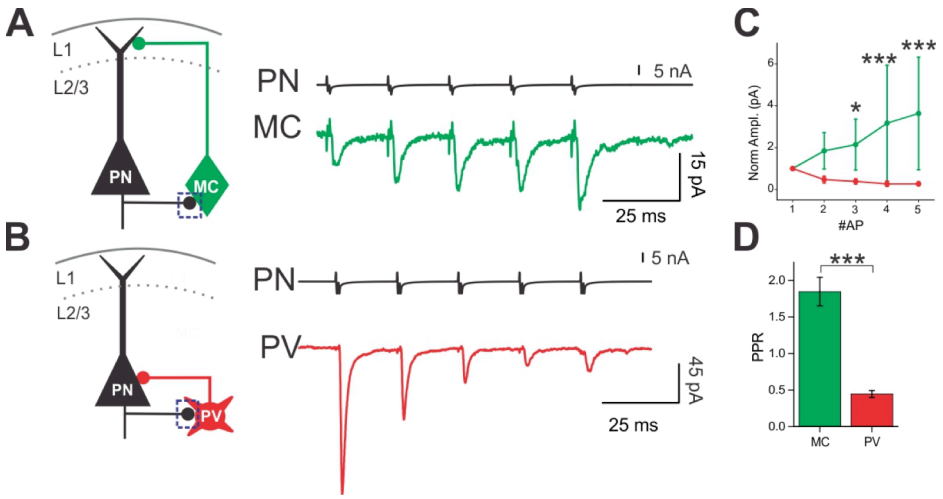


Fig. 3.3 EPSPs evoked in L2/3 by stimulation of L2/3 PNs. (A) Glutamatergic synapse onto a MC in L2/3 (left panel). Representative averaged voltage clamp trace of unitary EPSCs stimulated by 5APs at 40Hz in a PN and evoked in a GFP-positive cell from a X98 mouse (green)(right panel). (B) Same as in A, but in a PV cell from a PVcre::tdTomato mouse (C) Plot of short term plasticity of uEPSCs evoked in X98 GFP (n=20, green) and PV cells (n=11, red). (D) Bar graph showing paired-pulse ratio (PPR) of X98 GFP (n=20, green) and PV cells (n=11, red). The PPR is significantly different between the two cell populations: X98 GFP cells (n=20) displayed paired-pulse facilitation whereas PV-cells (n=11) were characterized by paired-pulse depression (***p=1.82703E-6, Mann-Whitney test). MC: Martinotti cell; PV: parvalbumin cell.

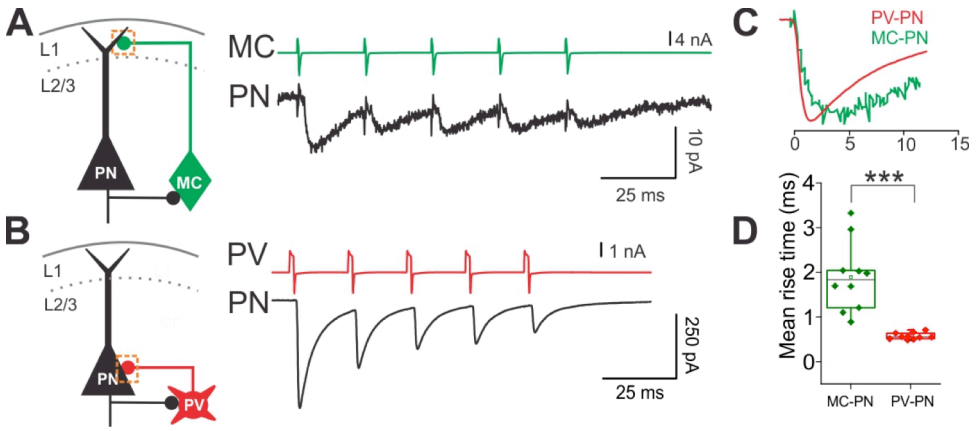


Fig. 3.4 IPSPs evoked in L2/3 by stimulation of L2/3 MCs and PV-cells. (A) GABAergic synapse made by a MC onto the dendrites of a L2/3 PN (left panel). Representative averaged voltage clamp trace of unitary IPSCs stimulated by 5APs at 40Hz in a MC and evoked in a PN in L2/3(black). (B) Same as in A, but the GABAergic synapse is made by a PV-cell on the somatic compartment of a L2/3 PN. (C) Voltage clamp traces representing the different rise time kinetics of uIPSCs elicited by MCs (green) and PV-cells (red) recorded from PN PV-IN (D) Box plot of the mean Rt (for ***p= 1.82672E-4, two sample *t*-test).

Table 2. Short term plasticity of glutamatergic synapses onto MCs and PV cells.

	MC		PV		Statistics	
	Mean	**± SEM**	**Mean**	**± SEM**	**P value**	**Test**
Pulse 2 (pA)	1.85	0.19	0.47	0.045	0.1228	2 way ANOVA
Pulse 3 (pA)	2.14247	0.27159	0.38581	0.04	*0.0246	2 way ANOVA
Pulse 4 (pA)	3.17	0.62	0.27	0.07	***<0.0001	2 way ANOVA
Pulse 5(pA)	3.63	0.60	0.27	0.03	***<0.0001	2 way ANOVA
PPR	1.85	0.19	0.46	0.05	***1.82703E-6	Mann-Whitney

3.2 α5-GABA$_A$Rs are expressed at MC-PN synapses in L2/3 of mouse somatosensory cortex

3.2.1 Synapses between MCs and PNs use GABA$_A$Rs expressing α5 subunit

α5-GABA$_A$Rs have been hypothesized as being extrasynaptic, mainly mediating tonic inhibition whereas α1-GABA$_A$Rs are known to be present at perisomatic synapses (Lee and Maguire, 2014). However, there is growing evidence that α5-GABA$_A$R are also involved in dendritic inhibition at specific synapses made by MCs in the cortex and by oriens-lacunosum molecolare (OLM) interneurons in the hippocampus (Ali and Thomson, 2008; Schulz et al., 2018).

To test whether the α5 subunit was specifically present at MC-PN synapses in the mouse somatosensory cortex, we performed paired whole-cell voltage-clamp recordings between presynaptic MCs and postsynaptic PNs. Inhibitory currents were isolated by adding DNQX (10 μM) to the perfused solution and, at the end of the experiment, inhibitory responses where suppressed by gabazine (SR-95531, 10 μM – not shown). To exclude the presence of α5-GABA$_A$Rs at perisomatic synapses, we recorded from postsynaptic PNs synaptically connected with presynaptic PV-cells. Importantly, these synapses express α1-GABA$_A$Rs (Ali and Thomson, 2008). Two specific drugs were used: α5IA, a highly specific inverse agonist for α5 subunit (α5IA, 50-100 nM) and Zolpidem (100 nM), an allosteric modulator of GABA$_A$Rs containing the α1 subunit (Depoortere et al., 1986; Rudolph & Mohler, 2013; Arbilla et al., 1985). Importantly, α5IA is a partial inverse agonist and displays 40% efficacy, thus not providing a complete blockade of α5-dependent inhibition (Chambers et al., 2004). In addition, α5-GABA$_A$Rs have extremely low affinity to Zolpidem (Puia et al., 1991; Burgard et al., 1996).
We used PVCre::RCE and X98 mouse lines to study PV-PN and MC-PN connections respectively. We found that unitary inhibitory post-synaptic currents (uIPSCs) mediated by PV interneurons onto PNs were sensitive to Zolpidem. Indeed, the decay time constant ($\tau_{d,w}$) of uIPSCs was significantly increased by Zolpidem (Fig 3.5 A and B n=6, p=0.014, Paired t-test Table 3). In contrast, PV-PN uIPSCs, were unaffected by α5IA (Fig. 3.5 A and C, Table 3). Conversely, the amplitudes of

uIPSCs elicited from MCs were highly sensitive to α5IA (Fig 3.5 D and F, n=11, p=0.003, Wilcoxon Signed-Ranks test, Table 3), and Zolpidem did not affect the decay time constant of the MC-PN uIPSCs (Fig 3.5 D and E, n=6, Table 3). Importantly, the overall reduction of MC-PN unitary transmission by α5IA was of ~60% (65,7% ± 5,36%; Fig. 3.5 D and F), which is in line with the efficacy of this highly selective inverse agonist (Chambers et al., 2004).

Altogether, these results suggest that synapses formed by dendrite-targeting MCs onto PNs, specifically express α5-$GABA_A$Rs. Importantly, these receptor subtypes do not contribute to PV-mediated fast perisomatic inhibition onto PNs: PV-PN synapses are therefore characterized by α1-$GABA_A$Rs.

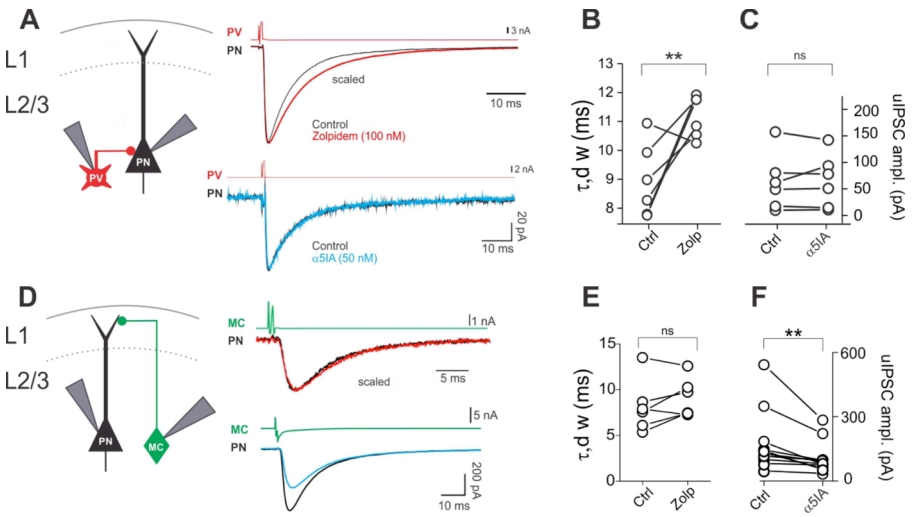


Fig. 3.5. Pharmacology of MC and PV-cell uIPSCs. (A) Representative average uIPSCs elicited by PV cells onto PN are shown in each condition, uIPSPs are sensitive to Zolpidem (red scaled trace) but insensitive to α5IA (blue trace). (B) Population data showing a significant effect of Zolpidem on the weighted decay time constant (τ,dw – Methods) of uIPSCs mediated by PV interneurons (Paired t-test,*p=0.014). (C) Plot of uIPSC amplitudes in control and after the addition of α5IA. (D) Average uIPSCs elicited by MCs onto PNs. Amplitudes of uIPSCs elicited by MC are reduced after incubation with α5IA (blue trace).uIPSCs are insensitive to Zolpidem (red scaled trace). (E) Plot of uIPSCs elicited by MC in control and after incubation with Zolpidem. No significant effects on decay time are reported. (F) Plot of uIPSCs amplitudes in control and after the addition of α5IA, which induced a significant reduction of uIPSCs amplitudes (Wilcoxon Signed-Ranks Test, **p=0.003).

Table 3. Pharmacological properties of uIPSCs generated by presynaptic MCs and PV-cells onto L2/3 PNs

	Control ampl. (pA)		α5IA ampl. (pA)		Statistics		n
	Mean	± SEM	Mean	± SEM	P value	Test	
MC-PN	176.77	43.51	104.41	23.24	**0.003	Wilcoxon Signed-Ranks	11
PV-PN	62.74	21.86	65.07	20.49	0.729	Paired t-Test	6
	Control $\tau_{(d,w)}$ (ms)		Zolpidem $\tau_{(d,w)}$(ms)		Statistics		
	Mean	± SEM	Mean	± SEM	P value	Test	n
MC-PN	8.18	1.17	9.12	0.87	0.173	Paired t-Test	6
PV-PN	8.95	1.28	11.17	0.72	*0.014	Paired t-Test	6

3.2.2 Slow spontaneous inhibitory postsynaptic currents recorded from L 2/3 PNs are selectively affected by α5IA

The results illustrated in the previous figure suggest that α5-$GABA_A$Rs are expressed at synaptic contacts between MCs and PNs. However, it is possible that sensitivity of uIPSCs to α5IA could be partially of fully due to activation of extrasynaptic $GABA_A$Rs activated by GABA spillover, induced by AP-evoked synaptic transmission. To further study the role of synaptic α5-$GABA_A$Rs, we measured spontaneous inhibitory postsynaptic currents (sIPSCs) recorded from PNs. Quantal, AP-independent synaptic events make up a large fraction (although not all) of sIPSCs. In other words, sIPSCs are less likely shaped by activation of extrasynaptic receptors. Therefore, if α5-$GABA_A$Rs are expressed at synapses between MCs and PNs, α5IA will affect the amplitudes of slow sIPSCs originating from distal dendritic GABAergic synapses. Events were detected using a custom written software (Wdetecta, courtesy J. R. Huguenard, see Methods) and classed into two separated groups based on their mean rise times (Rt) (Fig 3.6 B). We considered as "slow" the events with rise times (Rt) ≥ than 1.8 ms (1.89 ms ±

0.25), whereas the ones with Rt <1.8 ms were defined as “fast”. We divided slow and fast events, based on rise times of uIPSCs recorded in MC-PN and PV-PN connected pairs (Fig. 3.4). Events with Rt > 8 ms were considered as artifacts and removed by the analysis. Recordings were performed from the soma of PNs, both in control conditions and in the presence of α5IA. Slower events, affected by dendritic filtering, were considered as putative “dendritic” and likely generated at distal synapses. Conversely, fast events were likely generated at perisomatic compartments. Interestingly, only amplitudes of slow sIPSCs were significantly affected by α5IA (Fig 3.6 C, n=11, *p= 0.030, Wilcoxon Signed-Ranks test, Table 4). Conversely, α5IA did not produce any significant effect on fast sIPSCs (Fig 3.6 C, n=11, Table 4). This indicates that fast, perisomatic events are generated by other interneurons types, not using α5-$GABA_A$Rs.

Overall, these results, together with the ones described above in section 3.2.1, suggest that α5-$GABA_A$Rs specifically mediate slow dendritic GABAergic synaptic transmission without being involved in fast perisomatic inhibition.

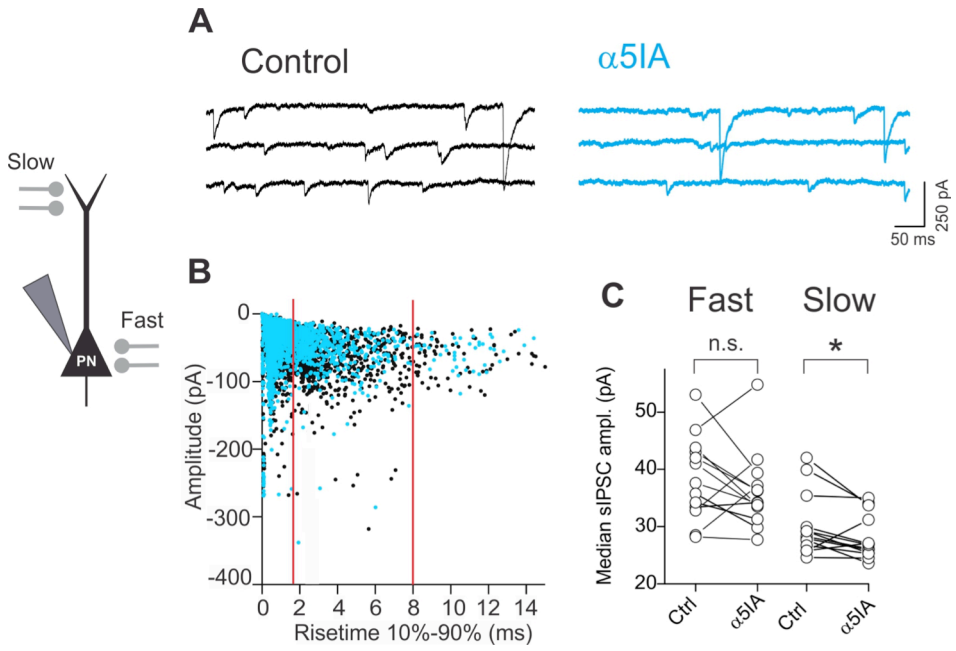


Fig. 3.6. Effect of α5IA on spontaneous synaptic events recorded from PNs. (A) Representative voltage-clamp traces of sIPSCs onto PN in control (aCSF, black) and α5IA-treated (blue). (B) Representative plot of sIPSCs in control (black) and α5IA (blue). The two red lines represents the cut-off made at 1.8 ms and at 8 ms. (C-D) plot of the median amplitudes of fast and slow sIPSCs measured in control and after incubation with α5IA. Fast events are not affected by the drug, whereas the slow ones display a significant reduction of their amplitudes (*p< 0.030)

Table 4. Pharmacology of fast and slow sIPSCs on PNs

	Control ampl. (pA)		α5IA ampl. (pA)		Statistics	
	Mean	**± SEM**	**Mean**	**± SEM**	**P value**	**Test**
Fast events	38.47	2.02	36.15	1.87	0.3636	Wilcoxon Signed-Ranks
Slow events	30.17	1.52	28.14	1.09	*0.030	Wilcoxon Signed-Ranks

3.3 α5-GABA$_A$Rs do not contribute to tonic inhibition in L 2/3 of mouse somatosensory cortex

A low concentration of ambient GABA, which persists despite the activity of the neuronal and glial GABA transporters (GAT1 and GAT3), tonically activates high-affinity extrasynaptic receptors (Farrant and Nusser, 2005). It has been proposed that specific subunits are exclusively present in extrasynaptic receptors. For instance, if we consider the α subunit of the GABA$_A$R, α4, α6, and α5 are mainly known for their role in mediating tonic inhibition (Brickley and Mody 2012; Lee and Maguire 2014; Botta et al., 2015). We found that α5-GABA$_A$Rs are present at synapses between MCs and PNs. We therefore tested whether this subunit is responsible for tonic inhibition of L 2/3 PNs of the barrel cortex.

We recorded tonic GABAergic currents in voltage-clamp from L2/3 PNs (clamped at -70 mV). For these recordings, we added GABA (5 μM) to the artificial cerebrospinal fluid (aCSF). Importantly, it was shown that by adding this specific concentration of GABA, active GABA uptake within the slice may significantly reduce the ambient concentration to levels close to those measured *in vivo* (0.2–2.5 μM), thus providing a way of standardizing tonic GABA measurements (Glykys and Mody, 2007).

We set up two different experimental conditions: a control group, in which brain slice were perfused with aCSF (Fig.3.7 A) and a treated group (Fig.3.7 B), in which brain slices were pre-incubated for 10-15 min with α5IA (100 nM). In this group, α5IA was kept in the bath solution during the recording. Pre-incubation was necessary because the effect of the drug can be detected only after about 10 min incubation. Moreover, highly stable baselines were required to measure tonic currents and this was possible only by reducing the duration of the recordings to 20 min maximum. Thus, we recorded tonic currents for 10-15 min, and then we blocked inhibition by using a solution containing gabazine (10 μM). Tonic inhibition was measured as the shift in holding current ($I_{hold,}$) induced by gabazine both in controls and α5IA-treated slices. These shifts in the holding currents were defined as ΔI_{hold}. We compared the two groups and surprisingly found that α5IA did not significantly affect the shift of the I_{hold} (Fig 3.7 A-C, Table 5). This result

suggests that α5-GABA$_A$Rs do not significantly contribute to tonic inhibition of L 2/3 PNs in this particular cortical region.

Importantly, blockade of tonic inhibition can increase membrane resistance (Rm). We therefore tested whether Rm changed upon α5IA and gabazine treatment and surprisingly, no significant differences were observed (Fig 3.7 D, Table 5). Considerably, changes in the current noise reflect changes in tonic GABA$_A$R-mediated conductance. We therefore analyzed the noise in our recordings and did not find any significant differences between control and α5IA-treated groups (Fig. 3.7 E, Table 5). Importantly, in both experimental conditions gabazine induced a significant reduction of the noise (Fig. 3.3 E, *** p< 9.03828E-10, ***p< 5.68428E-7, paired t-test, Table 5).

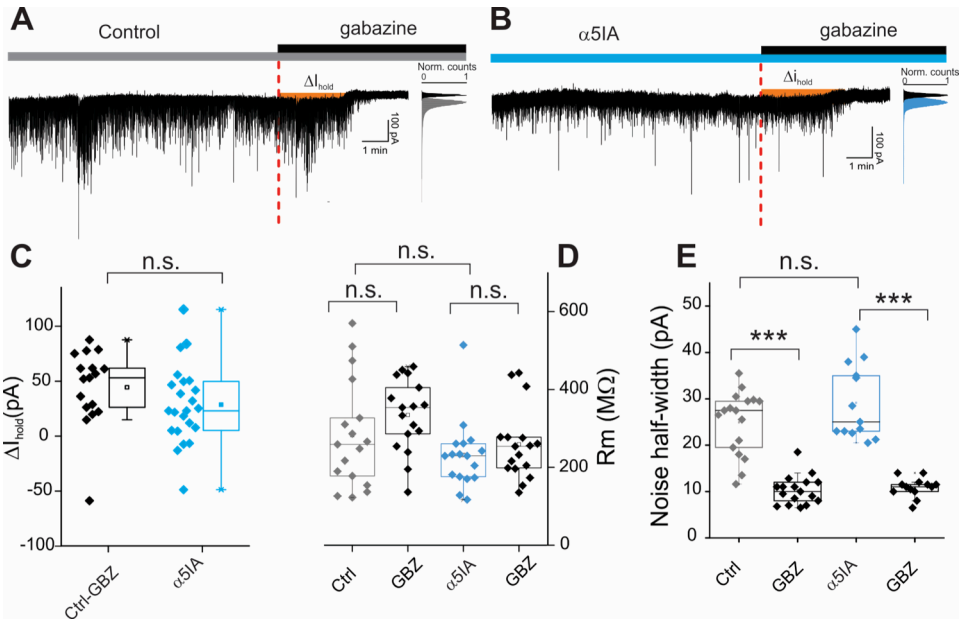


Fig. 3.7. α5GABA$_A$Rs do not contribute to tonic inhibition in L 2/3 of mouse somatosensory cortex. (A) and (B) whole-cell voltage-clamp recordings from two distinct L2/3 PN of somatosensory cortex (Vm =−70 mV) in the presence of 10 μM DNQX and 5 μM GABA. In B, cells were pre-incubated for 10-15 min with 100 nM α5IA. Horizontal bars over the recording denote the time of aCSF or drug perfusions. Right panel: Gaussian fits to all-points histograms derived from 360 ms recording in control (grey in A) or α5IA (blue in B) condition and a 15 s recording period during the perfusion of gabazine (black) used to determine the tonic current. (C) No significant differences between the delta values obtained from control and treated group. The same result was obtained after comparison of membrane resistance (Rm) values (D). (E) Analysis of the halfwidth of gaussian histograms. No significant differences between control and α5IA currents. Gabazine was used as a control (*** p< 9.03828E-10, ***p< 5.68428E-7)

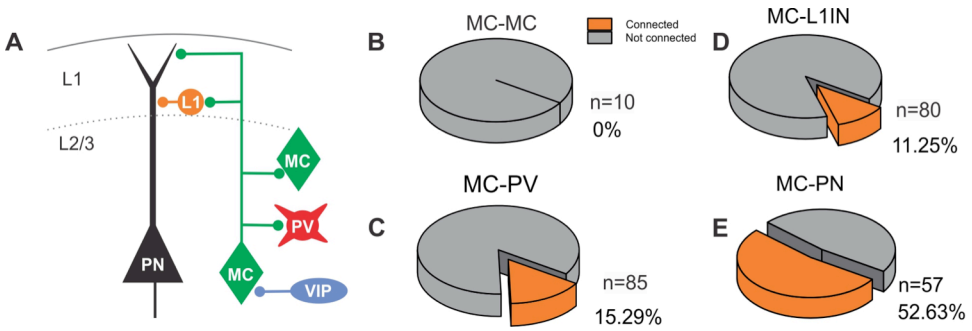
Table 5. Pharmachology of tonic inhibition: ΔI_{hold}, Rm and noise half-width

	Control		α5IA		Statistics	
	Mean	**± SEM**	**Mean**	**± SEM**	**P value**	**Test**
Δ (pA)	44.45	8.47	28.72	7.42	0.173	Unpaired *t*-test
	aCSF in ctrl – α5IA in treated		**Gabazine**		**Statistics**	
	Mean	**± SEM**	**Mean**	**± SEM**	**P value**	**Test**
Rm ctrl group (MΩ)	282.46	33.68	334.67	22.57	0.15557	Wilcoxon signed ranks
Rm treated group (MΩ)	231.32	21.55	258.75	22.36	0.10751	Wilcoxon signed ranks
Noise hw ctrl group (pA)	24.87	1.67	10.27	0.75	***9.03828E-10	Paired *t*-test
Noise hw treated group (pA)	29.14	2.26	10.88	0.57	***5.68428E-7	Paired *t*-test
	Control (aCSF)		**α5IA**		**Statistics**	
	Mean	**± SEM**	**Mean**	**± SEM**	**P value**	**Test**
Rm ctrl – α5IA (MΩ)	282.46	33.68	231.32	21.55	0.31786	Mann-Whitney
Noise hw ctrl - α5IA (pA)	24.87	1.67	29.14	2.26	0.93434	Unpaired *t* - test

3.4 α5-GABA$_A$Rs are exclusively expressed at synapses made by MCs onto PN dendrites

We found that α5-GABA$_A$Rs do not significantly contribute to tonic inhibition in L 2/3 PNs of somatosensory cortex but, instead, they play an important role at synapses made by MCs onto dendrites of PNs. However, MCs contact other elements of the cortical microcircuits, and are seemingly preferentially targeted by VIP-expressing interneurons (Pfeffer et al., 2013; Tremblay et al., 2016; Walker et al., 2016). Do MCs preferentially connect with α5-GABA$_A$Rs also at synapses other than with PNs? To address this question, we crossed a mouse line, in which PV cells are labeled by the tdTomato (PVAlbTdTomato) with X98 mice to label PV-cells and MCs in the same preparation. This mouse line allowed testing MC-PV and MC-MC connections. Moreover, given the extensive axonal arborization of MCs onto L1 (Fig. 3.1 B), we tested GABAergic synapses formed by MCs onto L1 interneurons (Fig. 3.8 A). L1 interneurons were identified using infrared videomicroscopy, being the only cellular elements of this superficial cortical layer.

We recorded uIPSCs from MCs synaptically connected with PV-INs and L1 interneurons (L1INs) (Fig 3.8 A). We could not find functional synaptic transmission between MCs (n = 0/10; Fig 3.8 B), in line with previous reports, indicating that SST interneurons do not contact other SST-cells, (Cottam et al., 2013; Pfeffer et al., 2013; Pi et al. 2013; Kepecs and Fischell, 2014). However, we found significant connectivity between MCs and PV cells (n = 13/85; Fig. 3.8 C) and between MCs and L1INs (n = 5/85, Fig. 3.8 D). Yet, the connectivity rate between MCs and these interneuron types was lower than functional connections with dendrites of PNs (n = 30/57; Fig. 3.8 F).



F

	PN	PV	L1-INs	MC
MC	53,63%	15,29%	11,25%	0%

Fig. 3.8. Connectivity of MCs in L2/3 of somatosensory cortex. (A) Schematic representation of the connections that were tested. (B to E) Pie charts showing the connectivity rate of between MCs (B), MCs to PV cells (C), MC to L1INs (D) and MCs to PNs (E) (grey: not connected pair, orange: connected pairs). (F) Table summarizing the connectivity rates of MCs onto other cell types.

We then tested whether GABAergic synaptic transmission between MCs and other interneurons relied on α5-$GABA_A$Rs, as in MC-PN connections. We found that uIPSCs elicited by MCs onto PV interneurons were not significantly affected by α5IA (Fig. 3.9 A-B, n=7, Table 5). Similarly, uIPSCs recorded from L1-INs were not sensitive to the drug (Fig. 3.9. C-E, n=5, Table 6) were not affected by α5IA.

These results suggest that MCs do not connect extensively with other members of cortical circuits, as they do with PN dendrites. Moreover, these results indicate that α5-GABA$_A$Rs are a unique signature of MC-PN synapses.

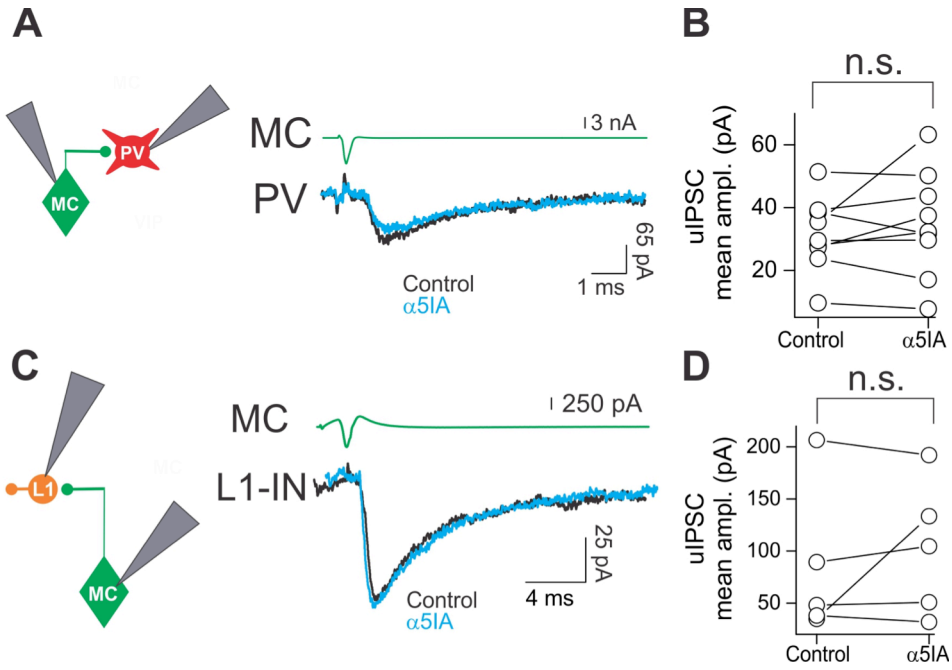


Fig. 3.9. Pharmachology of MC connections. (A) and (D): schematic representation of the experimental procedure. (B) and (E): average uIPSPs elicited by MC onto PV and L1-IN respecitively. In both synapses, uIPSCs are insensitive to α5IA. (blue trace). (C) and (F): Plot of uIPSCs amplitude recorded from PV and L1-IN respectively. No significant effect of α5IA is reported.

3.5 Synaptic inhibition on MCs does not involve α5-GABA$_A$Rs.

MCs are preferentially innervated by interneuron-preferring VIP cells (Walker et al., 2016; Lee et al., 2013; Pfeffer et al., 2013; Pi et al., 2013; Tremblay et al., 2016) and to a lesser extent also PV-INs (Lee SH et al., 2013). Since VIP-MC synapses represent an important disinhibitory cortical circuit, we asked whether α5-GABA$_A$Rs mediate inhibitory inputs from VIP-INs. To assess this question, we crossed VIPCre mice with X98 mice and we expressed a the light-sensitive opsin ChR2 via injection of flexed-ChR2 AAV particles in the barrel cortex of VIPCre:X98 pups(Fig 3.10 A). This approach allowed us to specifically activate VIP interneurons while recording from GFP-expressing MCs. We recorded light evoked IPSCs (leIPSCs) in MCs, and we found that these inhibitory responses from VIP cells were not sensitive to α5IA (Fig 3.10 B-C, n=7 Table 6).

To provide further evidence that MCs were not receiving any inhibitory input mediated by α5-$GABA_A$Rs, we recorded spontaneous inhibitory events from MCs (Fig 3.10 D). We sorted the sIPSCs and analyzed their amplitudes in control and after incubation with α5IA. Importantly, also in this case, the drug did not produce any significant effect (Fig. 3.10 E, Table 6). These results suggest that α5-$GABA_A$Rs do not participate fast inhibitory synaptic transmission onto MCs.

Table 6. Action of α5IA in specific inhibitory circuits involving MCs: effects on uIPSCs amplitudes and on sIPSCs recorded from MCs

	Control (aCSF)		α5IA		Statistics	
	Mean	**± SEM**	**Mean**	**± SEM**	**P value**	**Test**
MC-PV ampl. (pA)	31.40	3.90	34.74	5.56	0.3757	Paired *t*-test
MC-L1INs ampl. (pA)	83.29	32.29	102.47	28.87	0.5896	Wilcoxon Signed ranks
VIP-MC ampl. (pA)	170.46	46.11	166.26	51.99	0.7432	Paired *t*-test
sIPSCs (recorded from MCs)	32.31	1.37	29.07	3.26	0.06595	Paired *t*-test

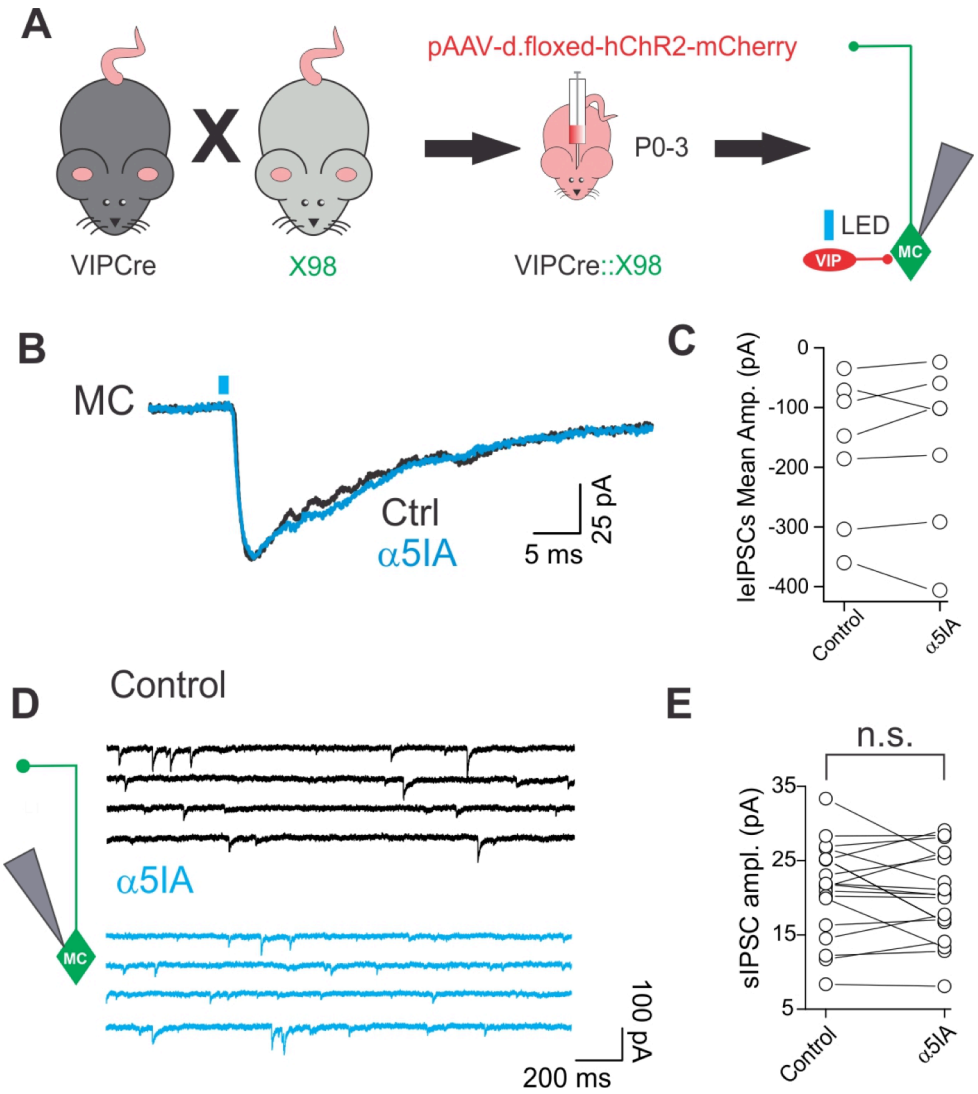


Fig. 3.10. Pharmachology of VIP-MC connections. (A) Schematic representation of the experimental procedure. VIPCre mice were crossed with X98GFP mice. Pups were injected with flexed AAVs carrying ChR2 and mCherry. (B) Average representative trace of light-evoked IPSC (leIPSC) from VIP interneurons. leIPSCs were insensitive to α5IA (blue trace). (C) Population data of leIPSCs amplitude in control and after incubation with α5IA. (D) Representative voltage clamp traces of sIPSCs recorded from MCs in control (black) and α5IA (blue). (E) Population data of sIPSCs amplitude in control and after incubation with α5IA.

3.6 MCs mediate slow inhibitory currents onto PNs and faster inhibition onto PV- and L1- interneurons

One hallmark of MCs is their exclusive targeting of distal dendrites of PNs in L1 (Markram et al., 2004; Wang et al., 2004; Goldberg et al., 2004). This allows MCs to control the electrogenesis of PNs and the supralinear integration and plasticity

of top-down glutamatergic input onto principal cortical neurons (Tran-van-Minh et al., 2015; Abrahamsson et al., 2012). However, MCs connect also to other elements of cortical circuits, namely PV cells and L1 interneurons (Fig. 3.11 B and C). Are MCs dendrite-targeting also when they contact other interneurons? To test this, we analyzed the rise times of uIPSCs obtained in our pair recordings with PV cells and L1-INs and compared them with MC-PN synaptic responses. uIPSCs recorded at the level of the soma of PNs but generated at distal dendrites, are passively conducted along the apical dendrite before reaching the soma (Maccaferri and Dingledine, 2002). This does not happen to perisomatic uIPSCs, generated close to the recording electrode. We measured the mean 10%-90% rise times (Rt) of uIPSCs elicited by MCs onto PNs, PV- and L1-INs. Events were all recorded from the soma. Interestingly, we found that uIPSCs recorded from PNs were significantly slower than the ones recorded from PV-interneurons and L1-INs (Fig 3.11 from A to H, p=0.00541, p=0.004 respectively, Mann-Whitney test, Table 7). Whereas the mean uIPSC rise time onto PNs was consistent with the known dendritic targeting of MCs (1.89 ± 0.25 ms; Fig. 3.11 A and D, Table 7), uIPSCs rise times onto both PV cells and L1-INs was ~ 1ms (0.73 ± 0.10 ms and 0.63 ± 0.13 ms, MC-PV and MC-L1INs, respectively; Fig. 3.11 B-E and C-F, Table 7). This is consistent with perisomatic targeting of both interneuron types by MCs. Overall uIPSC rise times between MCs and PV or L1-INs were not significantly different (Table 7).

Altogether, these findings indicate that the dendritic connectivity logic of MCs differ between PNs and other cortical interneurons. This can result in different functional roles of MCs while inhibiting PNs and other inhibitory neurons embedded in the cortical circuit.

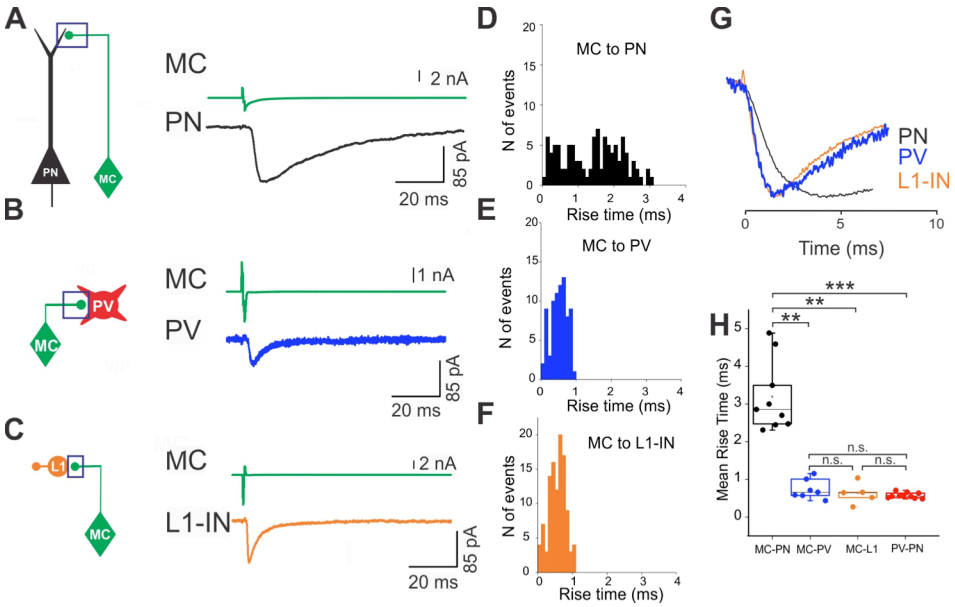


Fig. 3.11 Kinetics of sIPSCs from MCs at different synapses (A-C) Representative scheme of the inhibitory synapses made by MCs (left panel). Representative voltage-clamp traces of uIPSCs from MCs onto PNs (A, black), PV-INs (B, blue) and L1-INs (C, orange) (D-F) (right panel) Distributions of uIPSCs 10%-90% Rt from a single MC-PN (D), MC-PV (E) and MC-L1INs connection. (G) Voltage clamp traces representing the different rise time kinetics of uIPSCs recorded from PN (black), PV-IN (blue) and L1-INs (orange) (H) Box plot of the mean Rt (for MC-PN and PV-PN ***p=1.82672E-4, for MC-PN and MC-L1IN **p=0.00541, for MC-PN and MC-PV **p=0.004, Mann-Whitney test).

Table 7. Classification of MCs mediated uIPSCs based on mean risetime values

	uIPSCs Risetime (ms)	
	Mean	**± SEM**
MC-PN	1.89	0.25
PV-PN	0.57	0.02
MC-PV	0.73	0.10
MC-L1IN	0.63	0.13

CHAPTER 3:
RESULTS
PART 2

4.1. MC-PN synapses in mouse PFC contain α5-GABA$_A$Rs

In the past few years, growing evidence indicate the importance of over-inhibition as underlying cognitive deficits in Down syndrome (reviewed in Zorrilla de San Martin et al., 2018). In particular, already euploid mice lacking α5-GABA$_A$Rs displayed increased learning and memory (Collinson et al., 2002). Interestingly, Braudeau and colleagues demonstrated that by a single injection of α5IA, cognitive impairment in trisomic mice was rescued and even euploid animals were performing better in a novel object recognition task (Braudeau et al., 2011). Even though the involvement of α5-GABA$_A$Rs in cognitive processes is clear, the mechanisms leading to better memory and learning performances remain unknown at the circuit level.

Many of the intellectual deficits, which are present in DS, can be ascribed to alterations of prefrontal cortical circuits (Rowe et al., 2006; Grieco et al., 2015). Moreover, cognitive behavioral deficits involving the PFC were recovered in mouse models of DS by administration of α5IA (Braudeau et al., 2011). In the previous section, we found that α5-GABA$_A$Rs underlie fast dendritic inhibition at synapses made by MCs onto PNs in L2/3 of the mouse barrel cortex. We therefore hypothesize that this specific circuit could be altered in DS subjects.

We tested this hypothesis on Ts65Dn mice (hereinafter defined as DS mice), an established model of DS, in which several fundamental features of DS, especially cognitive deficits and alterations in brain morphology and function are recapitulated (Bartesaghi et al., 2011; Haydar and Reeves, 2012; Rueda et al., 2012). Precisely, these animals are characterized by a segmental trisomy of murine chromosome 16, containing 92 human orthologues between Mrp139 and Zfn295 (Sturgeon and Gardiner, 2011).

To test whether the improvement of cognitive behavior in the presence of α5IA was due to the specific MC-PN GABAergic circuit in the prefrontal cortex, we crossed DS with X98 mice. This allowed us targeting MCs in a mouse model of trisomy.

We first verified the presence of α5-GABA$_A$Rs at MC-PN synapses in the mouse PFC. We performed simultaneous whole-cell voltage-clamp recordings from L2/3 PNs mutually connected to MCs. Brain slices were obtained from Ts65Dn mice (Fig

4.1) and uIPSCs were recorded both in control and after incubation with α5IA. We found that uIPSCs amplitudes were significantly reduced after the treatment with α5IA (Fig 4.1 B, n=11, p=6.98016E-4, paired *t*-test, Table 1). These results indicate that also in the PFC and in trisomic animals, MCs provide dendritic synaptic inhibition onto PNs by using GABAergic receptors containing the α5 subunit, similarly to what we observed in the somatosensory cortex.

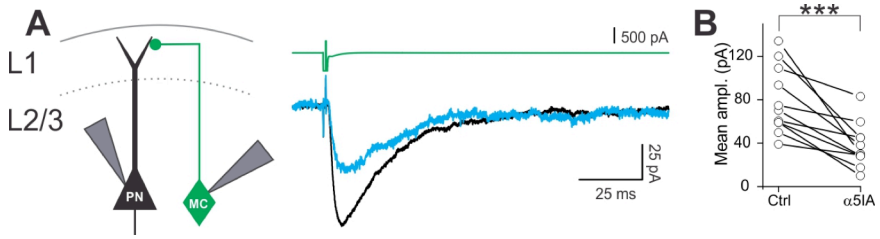


Fig. 4.1. Pharmacology of MC-PN synapses in Ts65Dn mice. (A) Representative experimental procedure left panel). Averaged representative trace of uIPSCs elicited from MCs onto PNs in control (black) and α5IA (blue) (right panel). (B) Plot of uIPSCs mean amplitudes in control and after incubation with α5IA. A significant reduction of uIPSCs amplitudes is observed (n=11, *p=0.00795, Mann-Whitney test).

Table 1. Pharmacology of MC-PN synapses in L2/3 of the mouse PFC

	Control ampl. (pA)		α5IA ampl. (pA)		Statistics	
	Mean	**± SEM**	**Mean**	**± SEM**	**P value**	**Test**
MC - PN	78.94	9.30	37.59	6.13	***6.98016E-4	Paired *t*-test

4.2. Increased dendritic inhibition from MCs is potentiated in Ts65Dn animals

After confirming the presence of α5GABA$_A$Rs at MC-PN synapses, and given the effect that was observed *in vivo* after a single administration of α5IA (Braudeau et al., 2011), we investigated if alterations of this specific microcircuit in Ts65Dn animals. We recorded uIPSCs elicited by MCs onto PNs in Ts65Dn animals and in their euploid littermates (Fig 4.2 A). uIPSCs recorded from DS mice exhibited significantly larger amplitudes compared to euploid (Fig. 4.2 B and D n=19; p=0.0265, Mann-Whitney test, Table 2). Furthermore, we found that the value of uIPSC rise times in DS were significantly larger too (Fig 4.2. E, n=19, p=0.01493,

Mann-Whitney test, Table 2). Conversely, no differences in the decay time were observed (Fig. 4.2. F, Table 2). Importantly, the failure rate of uIPSCs evoked by a single presynaptic spike was significantly smaller in DS (Fig 4.2 G n= 19; p=0.0487, unpaired t-test, Table 2). Additionally, presynaptic trains of 5 APs at 50Hz elicited uIPSCs with altered short-term plasticity, with stronger depression in DS mice as compared to euploid (Fig. 4.2. I, $p<0.01$, $p<0.001$; 2 way ANOVA, Table 2).

Overall, these results indicate that MC-PN synapses provide a much stronger dendritic inhibition in DS as compared to control mice, likely involving presynaptic mechanisms.

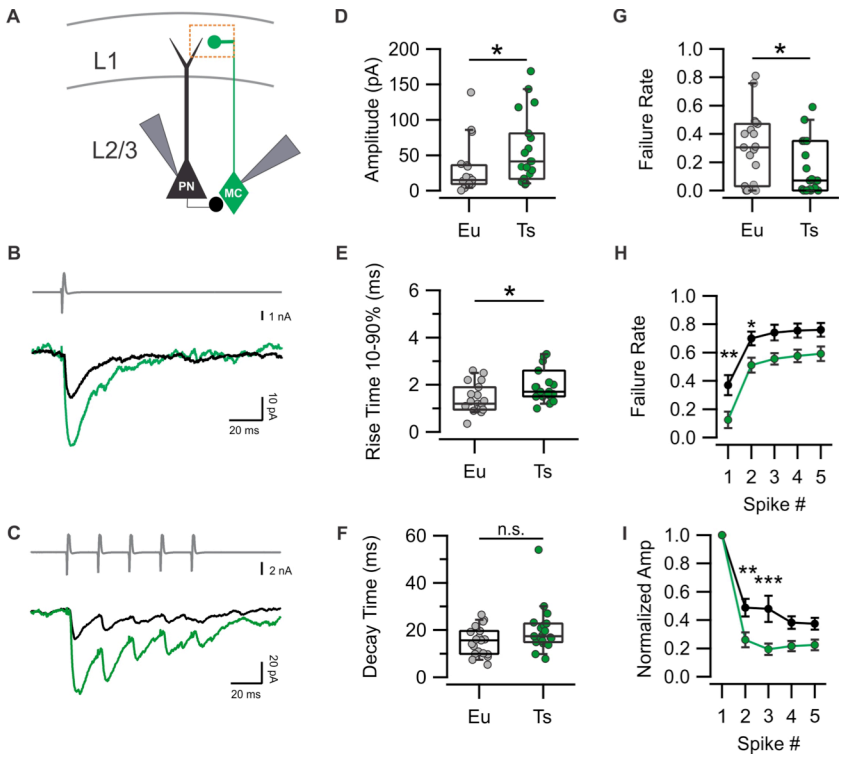


Fig. 4.2. Electrophysiological characterization of MC-PN inhibitory synapses in DS and euploid mice. (A) Schematic representation of the experimental protocol. (B) Averaged traces of inhibitory responses elicited by MC onto PN in euploid animals (black) and DS (green). (C) Average traces of inhibitory responses elicited by trains of 5 APs in euploid and DS mice. (D) Plot of uIPSCs amplitudes in euploid (grey) and DS (green). The amplitudes are significantly larger in Ts animals (*p=0.0265). (E) Population data of Rise Time values in euploid and Ts. Events recorded from MC-PN synapses in DS are significantly slower than the ones in euploid littermates (*p=0.01493). (F) Plot of decay time value in euploid and trisomic animals. No differences are observed. (G) Plot of failure rate in euploid and DS mice. (*p=0.05) (H) Failure rates of uIPSCs elicited by a 5AP train: the second and the third IPSCs show significantly different failure rate of the first and the second IPSC (**p<0.01, *p<0.05, 2 way ANOVA) (I) Normalized amplitudes of the uIPSCs elicited by 5 AP at 50 Hz. (**p<0.01, ***p<0.001).

Table 2. Properties of MC-PN synapses of L2/3 of the mouse PFC in DS and euploid mice

	Euploid		Trisomic (DS)		Statistics	
	Mean	± SEM	Mean	± SEM	P value	Test
Ampl. 1AP (pA)	29.40	8.19	57.28	11.14	*0.0265	Unpaired t-test
Failure rate 1AP	0.30	0.06	0.15	0.04	*0.0487	Unpaired t-test
Rise time 10-90% (ms)	1.40	0.14	1.92	0.16	*0.01493	Mann-Whitney
Decay time (ms)	20.04	2.26	15.20	1.40	0.09604	Mann-Whitney
PPR	0.49	0.06	0.26	0.05	**0.00501	Mann-Whitney
Pulse 2 (pA)	0.49	0.06	0.26	0.05	**<0.01	2 way ANOVA
Pulse 3 (pA)	0.48	0.09	0.19	0.04	***0.001	2 way ANOVA
Pulse 4 (pA)	0.38	0.04	0.22	0.04	>0.05	2 way ANOVA
Pulse 5 (pA)	0.37	0.04	0.22	0.04	>0.05	2 way ANOVA
Fail 1	0.37	0.06	0.12	0.05	**<0.01	2 way ANOVA
Fail 2	0.70	0.05	0.50	0.06	*<0.05	2 way ANOVA
Fail 3	0.74	0.04	0.55	0.06	>0.05	2 way ANOVA
Fail 4	0.75	0.04	0.56	0.05	>0.05	2 way ANOVA
Fail 5	0.76	0.05	0.58	0.05	>0.05	2 way ANOVA

4.3 Local glutamatergic recruitment of MCs is enhanced in DS mice

We found that GABAergic inhibition from MCs was enhanced in DS mice. MCs are recruited by local glutamatergic PNs with high probability and this PN-MC-PN is responsible for lateral inhibition (Adesnik et al., 2012; Isaacson and Scanziani, 2011) and frequency-dependent disynaptic inhibition (FDDI, Berger et al., 2009; Murayama et al., 2009) in sensory cortices. We therefore investigated whether also the glutamatergic synaptic recruitment of MCs by local PNs was altered in DS. We recorded uEPSCs from PNs and connected MCs, and found that amplitudes were significantly larger in DS as compared to euploid (Fig 4.3 B, Table 3; p =

0.0232, unpaired *t*-test, n =8 and 9, euploid and trisomic, respectively). Functionally, PN-MC synapses are loose-coupled connections characterized by low-release probability and pronounced short-term facilitation (Vyleta & Jonas, 2014). In DS mice, the failure rate PN-MC uEPSCs was significantly smaller as compared to euploid littermates (Fig 4.3 C, Table 3; n= 19, p=0.0117 Mann-Whitney test). However, short-term facilitation in response to a train of 5 APs at 50 Hz did not exhibit significant differences between DS and euploid (Fig. 4.3. E, Table 3), although the failure rate of the fourth uEPSCs was significantly smaller as compared to euploid (Fig 4.3. F, p< 0.05, 2 way ANOVA, Table 3).

These results indicate that MCs are more strongly recruited by local PNs. This finding, combined with the above-described potentiation of MC-PN inhibition indicates that the MC-PN loop is profoundly enhanced in trisomic animals as compared to euploid littermates.

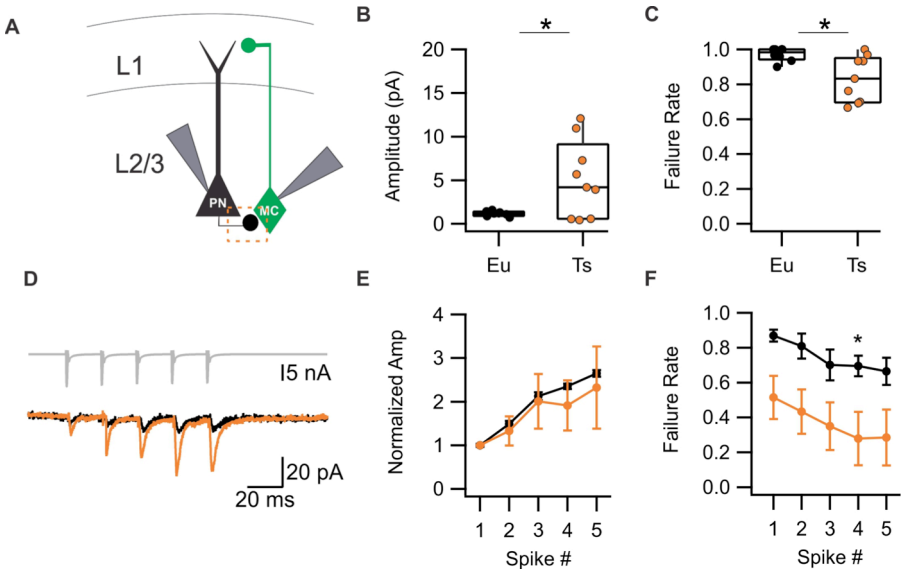


Fig. 4.3. Local recruitment of MCs by PNs is enhanced in DS mice. (A) Schematic representation of the experimental protocol. (B) Plot of uIPSCs amplitudes in euploid (grey) and DS (orange). The amplitudes are significantly bigger in DS animals (*p=0.0232). (C) Plot of failure rate index in euploid and and Ts mice. Data indicate that in DS failure rate is significantly smaller (*p=0.017). (D) Averaged traces of excitatory responses elicited by trains of 5 APs in euploid (black) and DS (orange). (E) Normalized uEPSCs amplitudes (F) Failure rate of uEPSCs evoked by 5APs at 50Hz. Failure rate on the fifth event is significantly smaller in DS as compared to euploid (*p<0.05).

Table 3. Properties of PN-MC synapses of L2/3 of the mouse PFC in DS and euploid mice

	Euploid		Trisomic (DS)		Statistics	
	Mean	**± SEM**	**Mean**	**± SEM**	**P value**	**Test**
Ampl. 1AP (pA)	1.14	0.10	5.07	1.46	*0.0232	Unpaired t-test
Failure rate 1AP	0.97	0.01	0.83	0.04	*0.0117	Unpaired t-test
PPR	1.49	0.43	1.33	0.33	0.3882	Unpaired t-test
Pulse 2 (pA)	1.49	0.43	1.33	0.33	>0.05	2 way ANOVA
Pulse 3 (pA)	2.13	0.57	2.01	0.63	>0.05	2 way ANOVA
Pulse 4 (pA)	2.35	0.54	1.91	0.58	>0.05	2 way ANOVA
Pulse 5 (pA)	2.65	0.65	2.32	0.948	>0.05	2 way ANOVA
Fail 1	0.13	0.03	0.48	0.12	>0.05	2 way ANOVA
Fail 2	0.19	0.07	0.57	0.13	>0.05	2 way ANOVA
Fail 3	0.30	0.09	0.65	0.17	>0.05	2 way ANOVA
Fail 4	0.31	0.06	0.72	0.15	*<0.05	2 way ANOVA
Fail 5	0.35	0.08	0.72	0.16	>0.05	2 way ANOVA

4.4. Firing dynamics and passive properties of MCs and PNs are not altered in Ts65Dn mice

To test whether the profound alterations of the MC-PN synaptic loop that we found in Ts65Dn mice were accompanied by alterations of intrinsic excitability of the cellular elements involved in this circuit, we analyzed the passive properties and firing dynamics of both MCs and PNs.

We patched 50 MCs (28 euploid and 22 trisomic) and 51 PNs (27 euploid and 24 trisomic).

We analyzed the membrane resting potential (V_{rest}), membrane time constant (τ_{memb}) and input resistance (R_i) and did not observe any remarkable differences in euploid MCs as compared to DS mice (Fig 4.4 C to E, table 4). Furthermore, we analyzed the properties of somatically recorded action potentials (Fig 4.4 F to J – Fig 4.5 E to I). We measured AP features, such as threshold and peak values by constructing phase plots (Fig. 4.4 G –Fig. 4.5 F– Fig. 4.9 F), in which the derivative of the spike waveform (dV/dt) was plotted against the actual membrane potential values (Vm). Conversely, AP threshold, AP amplitude and AP width at half-maximum amplitude (herein defined as AP width) were computed from actual spikes. We did not find any significant differences in MCs (Fig.4.4 C to F, Table 4) from euploid as compared to DS littermates. We then analyzed firing dynamics of MCs by injecting DC current steps of increasing amplitudes. Neurons responded with increasing firing rates (Fig. 4.4 L). Input-output curves (known as frequency-intensity or *f-i* curves) were identical in both genotypes (Fig. 4.4 L; Table 4). Both euploid and trisomic MCs displayed similar accommodating and adapting firing behavior in response to depolarizing currents in both genotypes (Fig 4.4 K, euploid: black trace, trisomic: green trace).

Similarly to MCs, passive membrane properties, single AP waveform and firing dynamics were unaltered in PNs from euploid and DS mice (Fig. 4.5, $p>0.05$ for all parameters, Table 5).

Thus, these results indicate that alterations of the MC-PN loop in DS mice were not associated to changes in passive and excitability properties of both MCs and PNs.

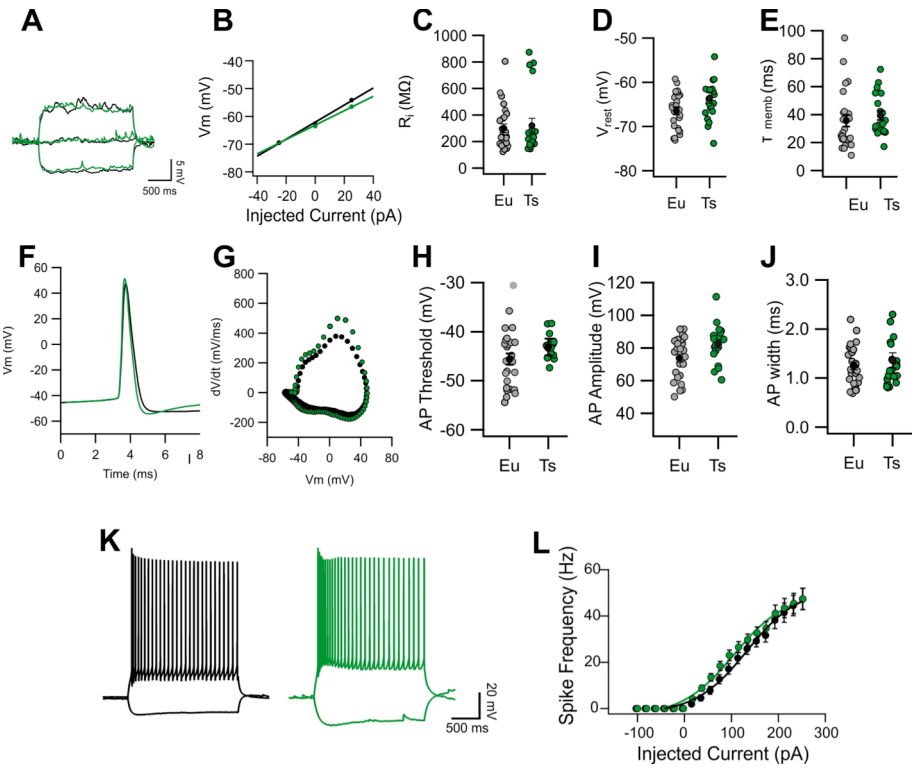


Fig. 4.4 MC firing dynamics and passive properties are not altered in DS animals. (A) Representative current clamp trace (B) Plot of the magnitude of membrane voltage deflections against the step size of corresponding current injections. No significant changes in Rm are detected. . No significant changes are observed in input resistance (R_i) (C) membrane resting potential (V_{rest}) (D), and membrane time constant (τ_{memb}) (E). (F) Example traces of single APs recorded from MC cells, in euploid (black) and DS (green) animals. AP shape is not different in DS mice. (G) Phase plot analysis. Average AP threshold (H), amplitude (I) and width (J) in euploid animals and DS (K) Characteristic firing of MC cells in response to depolarizing current injections, in control condition (black) and in DS animals (green). (L) F-I curves of MC cells in euploid (black) and Ts (green) mice. No differences in Spike Frequency between the two groups are reported.

Table 4. Passive properties and firing dynamics of MCs in Euploid and Trisomic mice

	Euploid			Trisomic (DS)			Statistics	
	Mean	**± SEM**	**n**	**Mean**	**± SEM**	**n**	**P value**	**Test**
AP thresh. (mV)	-45.59	1.15	26	-43.11	1.68	20	0.09877	Mann-Whitney
AP ampl. (mV)	38.71	0.52	26	41.29	0.87	20	0.98859	Unpaired *t*-test
AP width (ms)	1.25	0.08	26	1.28	0.10	20	0.88548	Mann-Whitney
Vrest (mV)	-66.46	0.81	26	-63.59	1.21	20	0.97633	Unpaired *t*-test
τmemb (ms)	35.70	4.19	25	39.36	3.29	20	0.14067	Mann-Whitney
Ri (MΩ)	300.72	31.86	26	320.75	55.23	20	0.57205	Mann-Whitney
I_{50} (pA)	103.07	8.80	27	92.76	9.37	21	0.36038	Mann-Whitney
Max spiking rate (Hz)	42.08	4.17	26	45.81	4.71	21	0.72243	Unpaired *t*-test

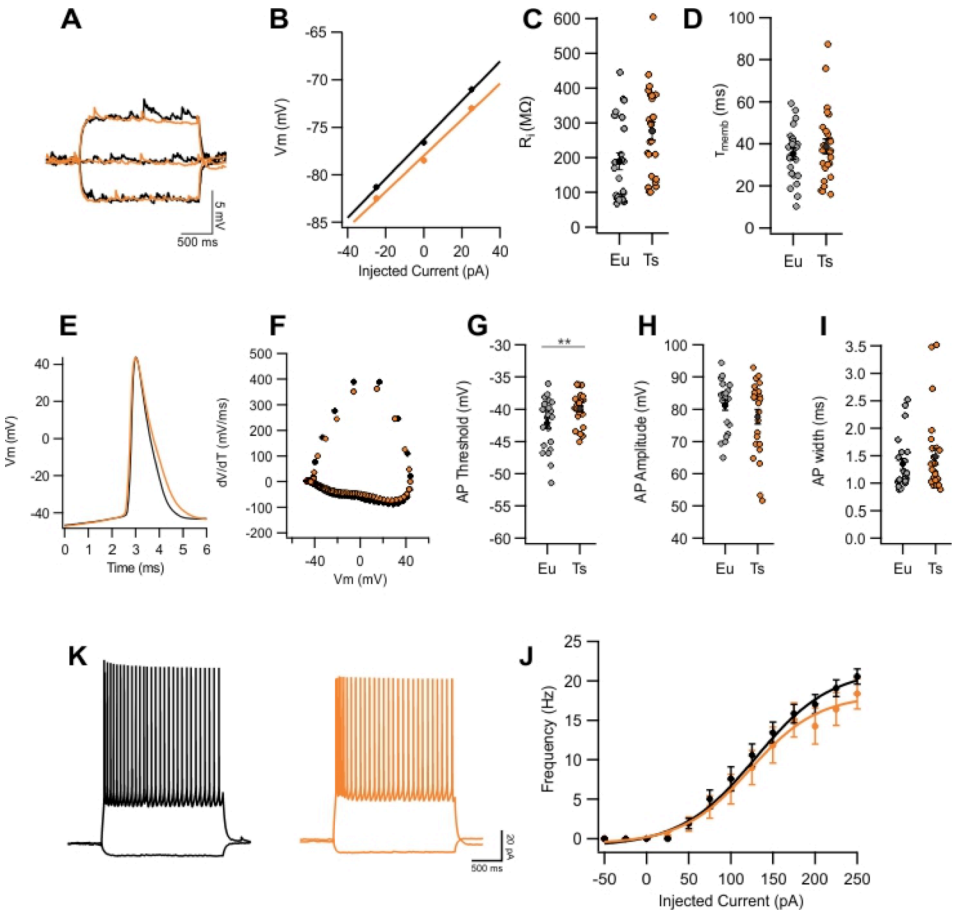


Fig. 4.5 PN firing dynamics and passive properties are not altered in DS animals. (A) Representative current clamp trace (B) Different injections of currents in Eu and Ts. No significant changes in Rm are detected No significant changes are observed in input resistance (R_i) (C) and membrane time constant (τ_{memb})(D). (E) Example traces of single APs recorded from PN cells, in euploid (black) and DS (orange) animals. AP shape is not different in DS mice. (F) Phase plot analysis. Average AP threshold (G), amplitude (H) and width (I) in euploid animals and DS. No significant changes are observed in input resistance (R_i) (K) Characteristic firing of PN cells in response to depolarizing current injections, in control condition (black) and in DS animals (orange) (J) F-I curves of MC cells in euploid (black) and Ts (orange) mice.

Table 5. Passive properties and firing dynamics of PNs in Euploid and Trisomic mice

	Euploid			Trisomic (DS)			Statistics	
	Mean	± SEM	n	Mean	± SEM	n	P value	Test
AP thresh. (mV)	-38.71	0.52	25	-41.30	0.87	22	**0.00606	Mann-Whitney
AP ampl. (mV)	76.49	2.29	25	80.08	1.68	22	0.37627	Mann-Whitney
AP width (ms)	1.49	0.15	25	1.36	0.10	22	0.725	Mann-Whitney
Vrest (mV)	-75.03	1.11	25	-73.12	1.19	22	0.87638	Unpaired *t*-test
τmemb (ms)	38.92	3.50	25	38.39	3.16	22	0.45608	Unpaired *t*-test
Ri (MΩ)	259.89	26.47	25	194.49	25.27	22	0.05918	Mann-Whitney
I_{50} (pA)	121.67	9.71	12	133.73	12.22	11	0.77773	Unpaired *t*-test
Max spiking rate (Hz)	21.08	0.93	12	20	1.68	11	0.2855	Unpaired *t*-test

4.5. PV-PN loop is not altered in Ts65Dn animals

The effects of α5IA in recovering cognitive dysfunctions in DS mice well correlate with the strong enhancement of the MC-PN-MC loop that we report in Fig 4.2 and Fig 4.3. However, fast synaptic inhibition is provided by a rich diversity of GABAergic interneurons (Ascoli & Alonso-Nanclares, 2008; Cauli & Audinat, 1997; Kawaguchi & Shindou, 1998; Markram et al., 2004; Somogyi & Kausberger, 2005; Yuste et al, 2005). Are other prominent GABAergic circuits altered in DS? In particular, is perisomatic inhibition from PV cells affected in DS mice? Addressing these questions is fundamental to reveal whether the over-inhibition of cortical circuits in DS is circuit-specific. We therefore crossed DS mice with a mouse line, in which the fluorescent protein tdTomato is expressed under the control of PV promoter. PV-tdTomato mice represent a powerful tool to identify PV cells in the mouse cortex (Kaiser et al., 2016). We performed paired whole-cell patch-clamp

recordings from PV-INs and PNs in DS-PVtdTomato mice. We found that GABAergic uIPSC amplitudes were similar in the two different genotypes (Fig 4.6 A to C, p=0.1738, Mann-Whitney test; n= 26 euploid and 8 DS, Table 6). Similarly, the failure rate calculated on these uIPSCs was not altered in both DS and euploid mice (Fig. 4.6 D, p=0.97989, Mann-Whitney test; n= 26 euploid and 8 DS, Table 6). Moreover, we elicited trains of 5 APs in presynaptic PV cells and found that uIPSCs were characterized by similar short-term depression in both genotypes (Fig 4.6 E, p>0.05, 2 way ANOVA; n= 26 euploid and 8 DS, Table 6). However, PPR shows a small albeit significant increase in DS as compared to euploid animals (Table 6). Similarly, glutamatergic synaptic transmission from PNs to PV cells was similar in both euploid and trisomic mice, in terms of uEPSC amplitudes (Fig. 4.7; p=0.64972, Mann-Whitney test; n= 16 euploid and 5 DS, Table 7) failure rate (Fig. 4.7 B-C; p=0.75492, Mann-Whitney test; n= 16 euploid and 5 DS, Table 7) and short term depression (Fig. 4.7 E; p>0.05, 2 way ANOVA test; n= 16 euploid and 5 DS, Table 7).

Importantly, however, we found that the proportion of connected pairs was significantly different in trisomic, as compared to their euploid littermates. Indeed, whereas the probability of finding connected PV→PN responses was similar in the two genotypes (Fig. 4.8 C and D; p = 0.40417, square χ-test; n =85 euploid vs 73 DS), the likelihood of finding PN→PV pairs connected by glutamatergic synapses was significantly reduced (Fig. 4.8 A and B; p = 0.012, square χ-test; n = 136 euploid vs 88 DS). Overall, our results indicate that in DS, the synaptic properties of the PV-PN loop are not altered. However, the reduced connectivity between PNs and PV cells might lead to impaired network activity involving this important GABAergic interneuron subtype.

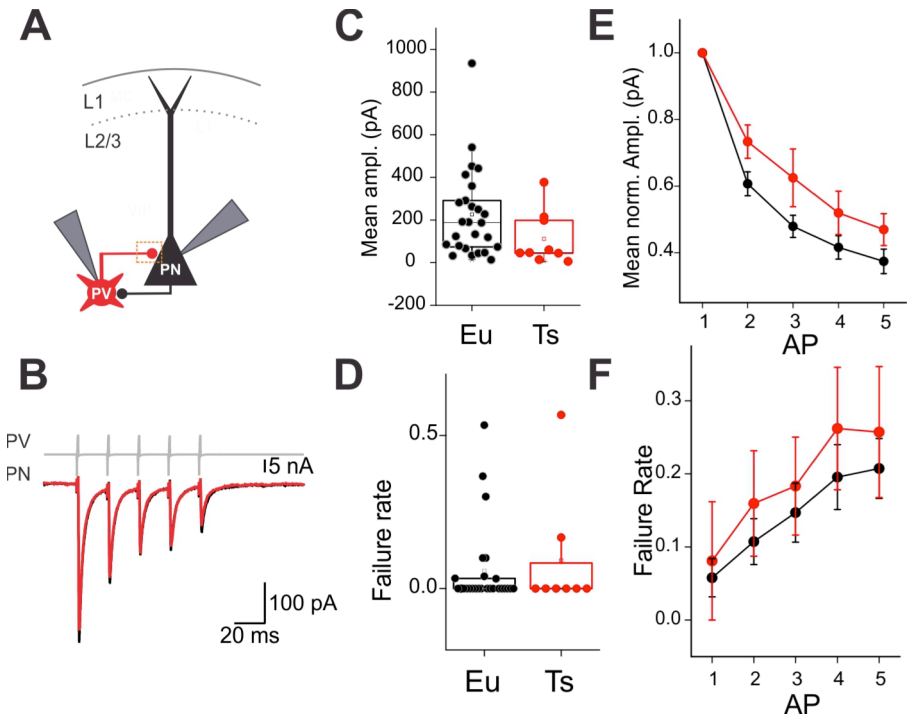


Fig 4.6 uIPSCs elicited by PV-INs are not altered in Ts65Dn mice. (A) Representation of the experimental procedure. (B) Representative averaged voltage-clamp traces of a PV-cell (grey) connected to a PN (Euploid: n= 26, Black, DS: n = 9 Red). (C) Plot representing the mean amplitudes of a single GABAergic uIPSC. Amplitudes are not significantly different between the two genotypes. (D) Plot of the failure rates of a single GABAergic uIPSC. The failure rate index is not different between genotypes. (E) Plot of the uIPCs mean normalized amplitudes. Synaptic responses were elicited by a presynaptic AP train at 50Hz. No significant differences are remarkable between euploid and DS animals. (F) Plot of the failure rate values calculated for every IPSCs elicited by a presynaptic train of 5 AP. The indexes were not significantly different between the two genotypes.

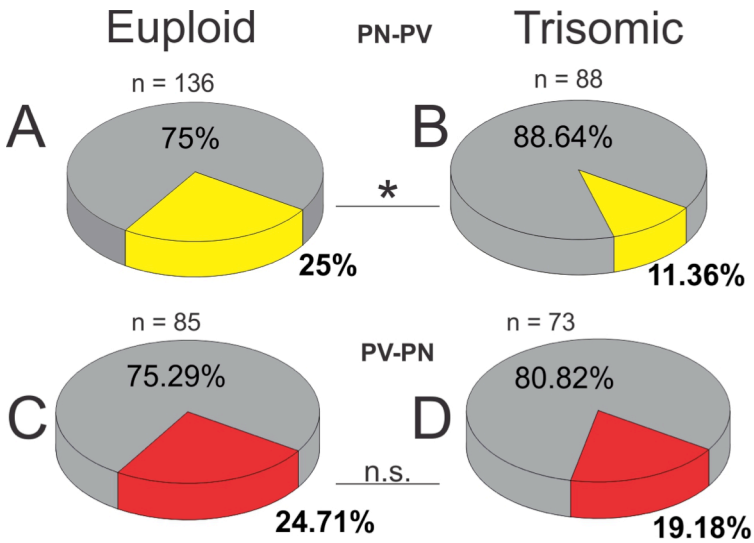


Fig 4.8 The connectivity rate of PNs to PVcells is altered in DS. Pie charts showing the connectivity percentages of PNs to PV cells in euploid (A) and trisomic (B) mice (grey: not connected, yellow: connected). Connectivity rates were significantly affected in DS mice ($\chi 2$ test, *p = 0.012). Conversely, PV cell to PNs connectivity was not affected in DS (D) as compared to euploid (C) (grey: not connected, red: connected, $\chi 2$ test, p = 0.40417)

Table 6. Properties of PV-PN synapses of L2/3 of the mouse PFC in DS and euploid mice

	Euploid		Trisomic (DS)		Statistics	
	Mean	**± SEM**	**Mean**	**± SEM**	**P value**	**Test**
Ampl. 1AP (pA)	225.79	40.58	124.918	44.78	0.1738	Mann-Whitney
Failure rate 1AP	0.06	0.03	0.09	0.07	0.97989	Mann-Whitney
PPR	0.61	0.04	0.73	0.05	*0.01957	Mann-Whitney
Pulse 2 (pA)	0.61	0.04	0.73	0.05	> 0.05	2 Way ANOVA
Pulse 3 (pA)	0.48	0.03	0.62	0.09	> 0.05	2 Way ANOVA
Pulse 4 (pA)	0.42	0.04	0.52	0.07	> 0.05	2 Way ANOVA
Pulse 5 (pA)	0.37	0.04	0.37	0.04	> 0.05	2 Way ANOVA
Fail 1	0.06	0.03	0.47	0.05	> 0.05	2 Way ANOVA
Fail 2	0.11	0.03	0.18	0.06	> 0.05	2 Way ANOVA
Fail 3	0.15	0.04	0.18	0.06	> 0.05	2 Way ANOVA
Fail 4	0.20	0.04	0.26	0.07	> 0.05	2 Way ANOVA
Fail5	0.21	0.04	0.26	0.08	> 0.05	2 Way ANOVA

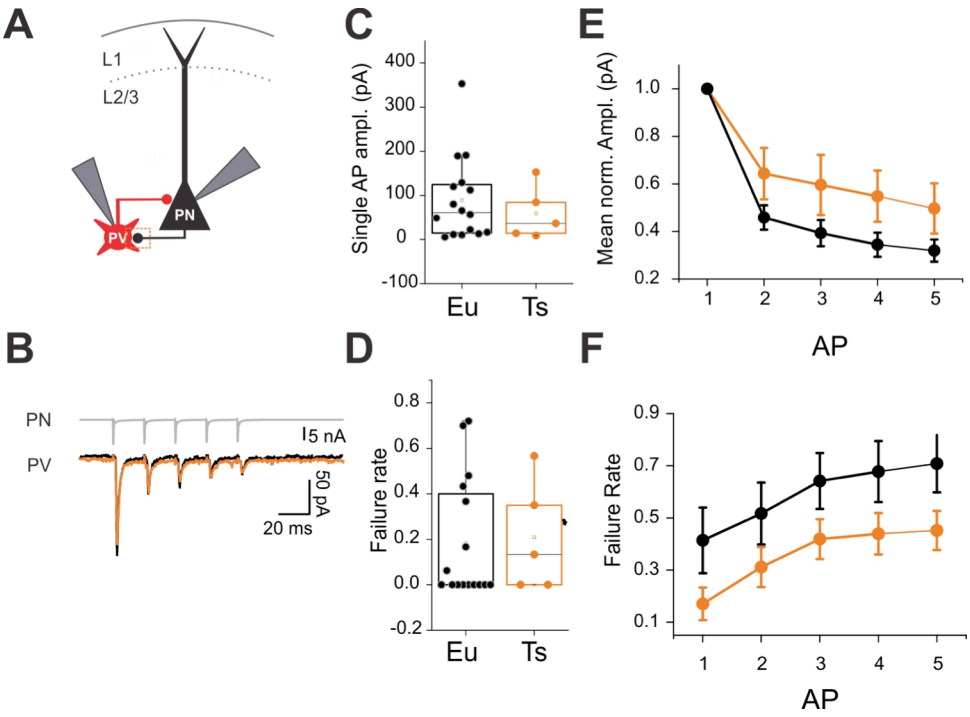


Fig 4.7 uEPSCs recorded from PV-cells are not altered in Ts65Dn mice. (A) Representation of the experimental procedure. (B) Representative voltage-clamp traces of a PN (grey) connected to a PV-cell (Euploid: n=15 Black, DS: n=6, orange). (C) Plot representing the mean amplitudes of a single GABAergic uIPSC. Amplitudes are not significantly different between the two genotypes. (D) Plot of the failure rates of a single GABAergic uIPSC. The failure rate index is not different between genotypes. (E) Plot of the uIPCs mean normalized amplitudes. Synaptic responses were elicited by a presynaptic AP train at 50Hz. No significant differences are remarkable between euploid and DS animals. (F) Plot of the failure rate values calculated for every IPSCs elicited by a presynaptic train of 5 AP. The indexes were not significantly different between the two genotypes.

Table 7. Properties of PN-PV synapses of L2/3 of the mouse PFC in DS and euploid mice

	Euploid		Trisomic (DS)		Statistics	
	Mean	**± SEM**	**Mean**	**± SEM**	**P value**	**Test**
Ampl. 1AP (pA)	88.95	23.39	59.21	26.86	0.64972	Mann-Whitney
Failure rate 1AP	0.18	0.07	0.21	0.11	0.75492	Mann-Whitney
PPR	0.46	0.05	0.64	0.11	0.14927	Mann-Whitney
Pulse 2 (pA)	0.46	0.0516	0.64	0.11	>0.05	2 way ANOVA
Pulse 3 (pA)	0.39	0.06	0.60	0.13	>0.05	2 way ANOVA
Pulse 4 (pA)	0.34	0.05	0.55	0.11	>0.05	2 way ANOVA
Pulse 5 (pA)	0.32	0.05	0.50	0.11	>0.05	2 way ANOVA
Fail 1	0.17	0.06	0.41	0.13	>0.05	2 Way ANOVA
Fail 2	0.31	0.08	0.52	0.12	>0.05	2 Way ANOVA
Fail 3	0.42	0.08	0.64	0.11	>0.05	2 Way ANOVA
Fail 4	0.44	0.08	0.68	0.12	>0.05	2 Way ANOVA
Fail5	0.45	0.07	0.71	0.11	>0.05	2 Way ANOVA

4.6 Firing dynamics and passive properties PV-interneurons are altered in Ts65Dn mice

Both GABAergic and glutamatergic synapses involved in the PV-PN-PV loop share similar properties in both euploid and DS mice (Fig. 4.6 and 4.7, Table 6 and 7). But, is the excitability of PV cells different in the two genotypes? We analyzed the firing dynamics and passive properties of PV-cells in both Ts6Dn and euploid mice from a total of 54 PV-INs (28 euploid and 26 trisomic). We found that PV cells exhibited striking alterations of their excitability in DS mice. Indeed, membrane resistance was on average increased of ~2 fold in PV cells of DS mice, as compared to euploid littermates (Fig. 4.9 A to C, $p = 1.17211E-4$, Mann-Whitney test, Table 8). In addition, when we analyzed single action potential waveform, we found that AP half-width was dramatically increased (Fig. 4.9 I, $p = 0.00267$, Mann-Whitney test; Table 8), whereas AP threshold and peak were not different in the two genotypes (Fig 4.9 G and $p > 0.05$, Mann-Whitney test; Table 8). Finally, we found profound alterations of spiking dynamics in response to DC current injections in PV cells from trisomic, as compared to euploid mice. Indeed, PV cells of DS mice fired much earlier than in euploid littermates (Fig. 4.9 J, control vs. trisomic mice; $p = 6.584E-5$; Mann-Whitney test; Table 8). However, in trisomic mice, PV cells could not sustain high-frequency firing (>80 Hz), a typical fast-spiking behavior of PV cells (Buzsáki and Draguhn, 2004; Freund and Katona, 2007; Klausberger and Somogyi, 2008) present in control conditions. Finally, PV cells in trisomic mice exhibited a stronger accommodation and spike-frequency adaptation, when compared with euploid mice.

Altogether, these results indicate that in DS mice PV-cell excitability is profoundly altered. PV cells fire earlier, their spike is broader, but they cannot sustain high-frequency firing typical of this interneuron type. These effects are associated with major increases of their input resistance. These alterations of PV-cell excitability together with their reduced recruitment by local PNs might result in significant alterations in their ability of orchestrating PFC circuits during cognitive-relevant network activity.

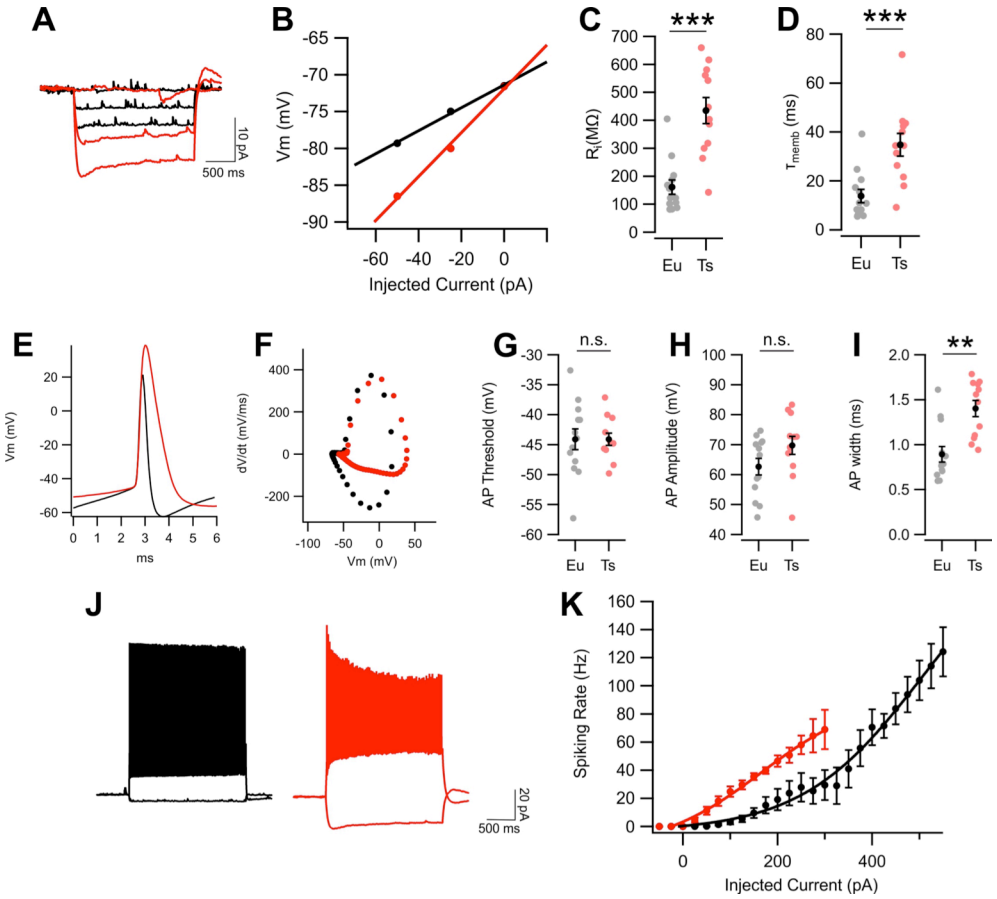


Fig. 4.9 PV firing dynamics and passive properties are altered in DS animals. (A) Characteristic firing of PV cells in response to depolarizing current injections, in control condition (black) and in DS animals (red). (B) Example traces of single APs recorded from PN cells, in euploid (black) and DS (red) animals. AP shape is different in DS mice. (C) Phase plot analysis. Passive properties of PV cells: average AP threshold (D), amplitude (E) and width (F), τ_{memb} (G) and input resistance (H) in euploid animals and DS. Significant changes are observed in AP width, membrane time constant (τ_{memb}) and R_i. (I)Membrane resistance (J) Plot of the magnitude of membrane voltage deflections versus the step size of corresponding current injections. Significant changes in Rm are displayed. (K) F-I curves of MC cells in euploid (black) and Ts (red) mice.

Table 8. Passive properties and firing dynamics of PV-INs in Euploid and Trisomic mice

	Euploid			Trisomic (DS)			Statistics	
	Mean	± SEM	n	Mean	± SEM	n	P value	Test
AP thresh. (mV)	-40.93	1.88	28	-43.38	0.80	26	0.55033	Mann-Whitney
AP ampl. (mV)	62.71	2.41	27	68.74	1.95	25	0.97035	Unpaired *t*-test
AP width (ms)	1.11	0.16	28	1.30	0.07	26	**0.00267	Mann-Whitney
Vrest (mV)	-71.06	1.12	28	-68.98	1.06	26	0.90885	Unpaired *t*-test
τmemb (ms)	15.73	2.03	28	28.96	2.86	25	***2.88019E-4	Mann-Whitney
Ri (MΩ)	216.58	37.15	28	411	35.94	26	***1.17211E-4	Mann-Whitney
I_{50} (pA)	257.64	25.89	28	105.81	10.84	26	***6.584E-5	Mann-Whitney
Max spiking rate (Hz)	89.11	10.12	28	57.69	4.90	26	0.0964	Mann-Whitney

DISCUSSION

Part 1

Role of α5-GABA$_A$Rs in L2/3 of the mouse somatosensory cortex

In this study, we examined the role of α5-GABA$_A$Rs in L2/3 of the mouse somatosensory cortex. This particular GABA$_A$ receptor subunit is not the most prominently expressed α subunit in the mouse neocortex (Fig. 5.1). Indeed, α5 has been traditionally held responsible for mediating tonic extrasynaptic inhibition in several brain areas, thereby maintaining a specific inhibitory tone and regulating membrane conductance non-specifically (Botta et al., 2015; Etherington et al., 2017; Caraiscos et al., 2004; Glykys and Mody, 2006, 2007; Pavlov et al., 2009; Prenosil et al., 2006; Semyanov et al., 2004; Yamada et al., 2007). Importantly, however, evidence has emerged implying that α5-GABA$_A$Rs mediate fast synaptic inhibition in both the neocortex and hippocampus (Ali and Thomson, 2008; Serwanski et al., 2006; Schulz et al., 2018; Salesse et al., 2011). Yet, even if synaptic localization for α5-GABA$_A$Rs is compelling, the contribution of this specific subunit to tonic inhibition is unclear. Overall, the functional role of this particular receptor subunit remains elusive and controversial (Botta et al., 2015;

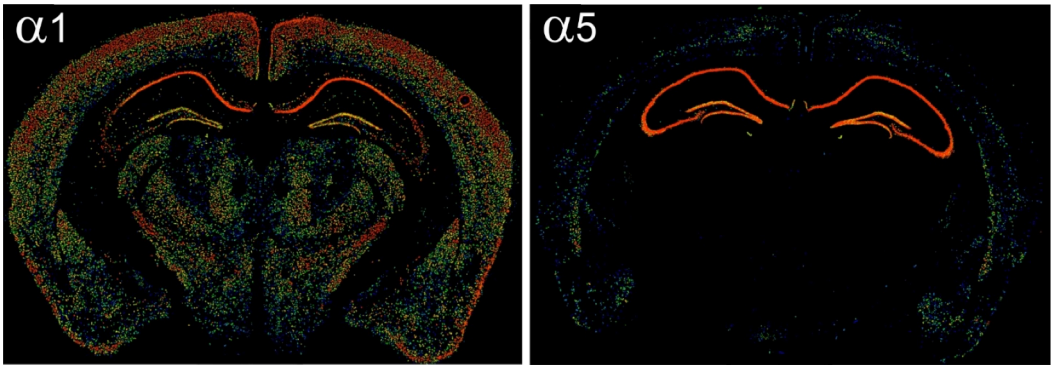


Figure 5.1 Heat map images, illustrating the differential expression of α1 (left) and α5 (right) subunits of the GABA$_A$R (*Gabra1* and *Gabra5*, respectively) in the mouse somatosensory cortex. Shown is the mRNA level. Note the relatively low expression of α5, as compared to the more ubiquitous α1 subunit. Image obtained from the Allen Brain Atlas

Etherington et al., 2017; Caraiscos et al., 2004; Glykys and Mody, 2006, 2007; Pavlov et al., 2009; Prenosil et al., 2006; Semyanov et al., 2004; Yamada et al., 2007; Serwanski et al., 2006: Ali and Thomson, 2008; Schulz et al., 2018). Here we found that in L2/3 of the mouse somatosensory cortex, α5-GABA$_A$Rs mediate fast-synaptic dendritic inhibition selectively from MCs onto PNs, without contributing to tonic inhibition. Moreover, we

found that MCs contact other cell types within L2/3, namely PV cells and L1 INs, but much less extensively than with PNs. Finally, GABAergic synapses from MCs to other interneurons are perisomatic and do not use α5-$GABA_A$Rs.

SST-cre mouse lines are widely used to study the functional role of SST-expressing INs in inhibiting PN dendrites in both neocortex and hippocampus. In particular, SST-cre mice were used *in vivo* to dissect the function of this broad class of cortical INs during sensory processing and/or cognitive function (Taniguchi et al., 2011; Lovett-Barron et al., 2012; Cottam et al., 2013; Polack et al., 2013; Neske et al., 2016; Sturgill and Isaacson, 2015). Particularly, in this mouse line, the contribution of SST INs to these brain functions can be studied by manipulating and recording their activity, using cre-driven expression of light-sensitive opsins or genetically encoded Ca^{2+} sensors. Despite its extensive use, the SST-cre mouse line target all interneuron subtypes expressing SST, which encompass several subtypes (Ma et al., 2006; McGarry et al., 2010; Halabisky et al., 2006; Xu et al., 2006) some of which do not exclusively target PN dendrites but also their perisomatic compartment (Nassar et al., 2015; Lim et al., 2018). Importantly, MCs represent a specific subpopulation of GABAergic interneurons, accounting for only 20% of all SST-expressing cells (Yavorska and Wehr, 2016). They are recruited by a stereotyped facilitating glutamatergic pattern (Reyes et al., 1998), which favor late integration (as opposed to PV cells, which might be more coincidence detectors) and target the distal portion of PN dendrites, thereby controlling dendritic integration and possibly gating plasticity of top-down glutamatergic input. Previous results suggested that synapses formed by MCs onto PN dendrites use α5-$GABA_A$Rs (Ali and Thomson, 2008; Schulz et al., 2018). To investigate the actual role of this receptor as mediating phasic and tonic inhibition, we used the X98 mouse line to study MCs specifically. Indeed, in this line it was demonstrated that GFP is specifically expressed by L5 MCs (Ma et al., 2006). We found a significant amount of GFP-expressing neurons also in L2/3, which however hosts a rich diversity of SST-expressing interneurons. Here we provide evidence that GFP-expressing neurons in the somatosensory cortex from the X98 mouse line exhibit the typical anatomical and electrophysiological properties of MCs (Tremblay et al., 2016). In addition, glutamatergic recruitment of GFP-positive cells is strongly facilitating, as opposed to PV cells, another hallmark of MCs (Reyes et al., 1998). Therefore, we conclude that this mouse line represents a specific tool to study inhibitory synapses made by MCs onto the dendrites of L2/3 PNs.

Notably, we found that MCs provide specific dendritic synaptic inhibition onto PNs using α5-$GABA_A$Rs, whereas fast perisomatic inhibition provided by PV-cells mostly uses α1-

containing receptors. These data are in line with previous results (Ali and Thomson, 2008). We used zolpidem and α5IA as specific tools to dissect the expression of α1 and α5 at these two synaptic sites. Indeed, zolpidem is a non-benzodiazepine allosteric modulator binding to the benzodiazepine site of the $GABA_AR$. It has a high affinity for α1-containing $GABA_ARs$, and 10-fold lower affinity for the α2- and α3- subunits than for α1, and no appreciable affinity for α5 subunit-containing receptors (Puia et al, 1991; Burgard et al., 1996). Conversely, α5IA (L-822179 – Atack, 2010) is a highly specific partial inverse agonist of $GABA_ARs$ expressing the α5 subunit, with an efficacy of ~40% (Chambers et al., 2004; Atack, 2010). In fact, this drug binds to for $\alpha1, \alpha2, \alpha3$ and $\alpha5$ subunits with similar high affinity; however, it reduced $GABA_AR$-mediated currents only in α5-expressing receptors (Atack, 2010; Chambers et al., 2004). Therefore, these pharmacological properties make this drug a perfect tool to study functional expression of α5-containing $GABA_ARs$. In addition, α5IA lacks proconvulsant and anxiogenic effects and was therefore used in preclinical trials in humans (Atack et al, 2010). Other studies focused on $\alpha5$-$GABA_AR$ used different compounds targeting $\alpha5$-mediated inhibition, such as L-655,708 and PWZ-029 (Botta et al., 2015; Schulz et al., 2018). Even though these molecules display higher binding affinity to the $\alpha5$ subunit as compared to $\alpha5$IA, their efficacy as inverse agonists is significantly lower (about 20%), thus inducing smaller blockade of $\alpha5$-mediated inhibition (Atack et al, 2006; Savić et al., 2008).

Unitary responses from MCs to PNs were invariably reduced by α5IA. The blockade was not total (~60%) but in good agreement with the actual efficacy of the drug (Atack, 2010; Chambers et al., 2004). However, it is possible that GABA, released by single APs, might have spilled over to peri- or extrasynaptic $GABA_ARs$ containing α5. If this were the case, we would not detect any effect on quantal events, which reflects mostly synaptic activation of $GABA_ARs$. Our results on sIPSCs corroborate the synaptic localization of α5-$GABA_ARs$. Indeed, at our extracellular K^+ concentrations, sIPSCs are dominated by AP-independent miniature events (Rusakov & Fine, 2003). Importantly, we recorded sIPSCs from the soma of L2/3 PNs, and found that only slow sIPSCs were sensitive to $\alpha5$IA, whereas fast perisomatic inhibitory events were unaffected. Slow sIPSC rise times are consistent with dendritic synaptic localizations, whose responses are more sensitive to cable filtering (Hausser, 2001). Therefore, our results suggest that $\alpha5$-$GABA_ARs$ are prominently expressed at synaptic sites of dendritic MC-PN connections.

$\alpha5$-$GABA_ARs$ have been described as mediating mostly tonic, extrasynaptic inhibition (Botta et al., 2015; Etherington et al., 2017; Caraiscos et al., 2004; Glykys and Mody,

2006, 2007; Pavlov et al., 2009; Prenosil et al., 2006; Semyanov et al., 2004; Yamada et al., 2007).

Indeed, some studies concluded that α5-$GABA_A$Rs are uniquely present at extrasynaptic sites (Botta et al., 2015; Caraiscos et al., 2004) and others indicating that this subunit is expressed at both synaptic and extrasynaptic sites (Schulz et al., Serwanski et al., 2006). Most (but not all) of these studies use tonic currents as areadout of extrasynaptic $GABA_A$R activity. However, it is important to note that this notion carries the assumption that synaptic $GABA_A$Rs have a lower affinity to GABA. Indeed, shifts in I_{hold} triggered by fluctuating concentrations of extracellular GABA can in principle result from activation of both extra- and purely synaptic receptors. Here we measured the contribution of these receptors to tonic inhibition of L2/3 PNs and found that α5IA did not affect tonic currents. To avoid spurious measurements of fluctuating I_{hold}, and because α5IA displays its effect only after about 10 min incubation, we performed chronic pre-incubation of the drug on brain slices to reduce the time of the recordings. The discrepancy with previous studies could be due to one or a combination of the following reasons: the highly specific tool used here, the specific neuron and brain areas analyzed and the age of the animals. Moreover, reported effects mediated by α5-$GABA_A$Rs on I_{hold} are often very small. For these reasons, in a separate set of experiments (not shown) we tested higher concentration of α5IA (500 nM) to assess whether tonic activation of α5-$GABA_A$Rs required higher drug concentrations. We did not notice any significant effects on tonic current, meaning that the lack of the effect was not dependent on the drug concentration. Moreover, the concentration used in our experiments (50 nM and 100 nM) remarkably affected a5-$GABA_A$Rs-mediated synaptic currents, providing a highly specific effect.

In addition, the apparent lack of effect of the drug on tonic currents may be due to other several possible reasons. Importantly, as mentioned above, α5IA is a partial inverse agonist with a 40% efficacy (Chambers et al., 2004; Atack et al., 2010). The expected effect of the drug could therefore be smaller than the natural fluctuation of I_{hold}, thus resulting non-detectable. Furthermore, dendritic filtering might have occluded partial effects, as our measurements of I_{tonic} were performed at the soma. Future experiments involving dendritic recordings will help understanding whether α5-$GABA_A$Rs provide tonic inhibition on the dendrites of L2/3 PNs.

In addition to specific pharmacology and electrophysiological recordings that here were instrumental to unmask the dendritic synaptic inhibition mediated by this receptor subtype, Serwanski et al. performed electron microscopy (EM) and found that the a5

subunit is localized both at synapses and on extrasynaptic membranes both in the hippocampus and cortex (Serwanski et al., 2006). Here we sought to reproduce the same experiment. In particular, we aimed to quantify the extrasynaptic and synaptic α5. Unfortunately, however, the antibodies that we used (both a gift from J.M. Fritschy, Univ. Zurich and commercially available from Synaptic Systems) failed to give a seemingly specific staining of $GABA_A$Rs. Future experiments using specific protocols to better use available antibodies will be required. In particular, EM or ultrastructural analysis of L2/3 PN somas and dendrites will allow measuring the actual synaptic vs. extrasynaptic localization of α5-$GABA_A$Rs. However, here we found that the use of a partial and specific inverse agonist (α5IA) had a prominent effect at synaptic (both u- and slow sIPSCs) but not tonic inhibitory responses.

It was recently shown that NOS-expressing, neurogliaform dendrite-targeting interneurons provide slow inhibition onto dendrites of CA1 PNs using α5-$GABA_A$Rs (Schulz et al., 2018). Because these interneurons provide a very-slow inhibition due to their anatomical pre-post synaptic appositions, $GABA_A$R-mediated responses are very slow (induced by volume-like transmission) and likely involving extrasynaptic $GABA_A$Rs (Price et al., 2008; Szabadics et al., 2007; Tamas et al., 2003). The α5 subunit is much more strongly expressed in the hippocampus than in the neocortex (Lingford-Hughes et al., 2002)(see Fig. 5.1). Therefore, it will be interesting to reveal whether α5 has different circuit-specificity and/or plays a different role in these two cortical areas.

MCs extensively inhibit PNs via α5-$GABA_A$Rs. Yet, these SST-expressing interneurons contact other elements of the cortical microcircuits, and, in addition, they are preferentially targeted by VIP-expressing interneurons (Tremblay et al., 2016; Walker et al., 2016; Pfeffer et al., 2013). Indeed, MCs widely project their axons into L1, but they also innervate locally connecting to several other interneuron subtypes (Ma et al., 2006; Adesnik & Scanziani, 2010; Tremblay et al., 2016). We used several mouse lines to investigate two particular microcircuits: that involving MC-PV connections and that concerning VIP-MCs. L1-INs were patched blindly since we did not use any specific mouse line. Importantly, we reported that MCs contact PV- and L1-INs and do not use α5-$GABA_A$Rs. Furthermore, VIP-INs inhibit MCs through non-α5-mediated synaptic inhibition.

Importantly, MCs dendritic inhibition seems to be a specific signature of their connections with PNs, as uIPSC rise times measured on other MC targets (interneurons) had fast (<1 ms) kinetics similar to the known PV-PN perisomatic responses. In addition to dendritic filtering, MC-PN synaptic responses might be slow due to the specific

properties of the α5-subunit itself, which is exclusively expressed at this synapse. Dendritic patch would be again necessary to test this hypothesis, although high series-resistance of dendritic patch recordings might prevent an accurate analysis of fast currents.

Our results on L1-INs suggest that MCs do not use α5-$GABA_A$Rs at these synapses. However, L1 is populated by a highly heterogeneous IN population (Schuman et al., 2019) and, since we did not use specific mouse lines to target distinct cell types, our data may have been collected from a relatively heterogeneous interneuron group.

Our results indicate a specific anatomical and molecular signature for GABAergic synapses from MCs, which are dendrite targeting and using the α5 subunit of the $GABA_A$R only when they connect with their preferred targets, the PNs. The selective use of the α5 subunit of the $GABA_A$R at dendritic synapses from MCs onto PNs reveals the molecular determinant of a specific circuit involved in controlling the flow of information to L2/3 PNs, with crucial implication in processes like learning and memory. Importantly, these experiments reveal a specific target of drugs designed to affect this subunit in several brain diseases, such as schizophrenia, ASD and Down syndrome) (reviewed in Brat & Kooy, 2005; Del Pino et al., 2018; Zorrilla de San Martin et al., 2018).

The specific expression of α5-$GABA_A$Rs at MC-PN synapses and the reported effects of α5IA in recovering behavioral deficits prompted our investigation on whether specific GABAergic circuits are altered in DS. The main responsible for this second project was a postdoctoral fellow in the lab, Dr. Javier Zorrilla de San Martin. I have extensively collaborated with him in obtaining electrophysiological recordings from different neuron types in the two genotypes, and I have filled and reconstructed several neurons in both euploid and trisomic mice. These results will be discussed in the next section.

Part 2

Circuit-specific alterations of GABAergic interneurons in DS

A large body of evidence suggests that altered GABAergic signaling might be among the pathophysiological mechanisms underlying several cognitive deficits in DS (Grover and Yan, 1999; Wigstrom and Gustafsson., 1986; Zorrilla de San Martin et al., 2018). These alterations arise during brain development, they extend into adulthood and include dysfunctions in the genesis of GABAergic neurons and inhibitory drive, leading to malfunctions in cognitive-relevant network activity (rewiewed in Contestabile et al., 2017).

Here we set out to understand whether the rescue of the cognitive deficits by α5IA in Ts65Dn mice (Braudeau et al., 2011) was due to the modulation of this drug on the highly specific GABAergic cortical circuit formed by MCs. We then crossed DS mice with X98 animals and found that in the PFC of DS animals MCs mediate dendritic inhibition, which is highly sensitive to α5IA. We found that MC-PN synapses provide much stronger dendritic inhibition in DS. Moreover, glutamatergic recruitment of MCs by local input from PNs was also enhanced. Interestingly, perisomatic inhibition from PV cells as well as their glutamatergic recruitment were overall similar in the two genotypes. Whereas firing properties were largely unaltered in PNs and MCs, PV cells exhibited profound alterations of intrinsic excitability, AP waveform and firing dynamics.

The enhanced MC-PN loop could derive from a combination of pre- and postsynaptic mechanisms, including: *i)* sprouting of GABAergic and glutamatergic axons, increasing the number of release sites; *ii)* alterations of release probability due to molecular of morphological modifications; *iii)* changes in receptor sensitivity to glutamate and GABA at postsynaptic sites. Future experiments will be necessary to pinpoint the actual site of this synaptic potentiation. We are currently performing a detailed anatomical analysis (coupled to estimate of synaptic density) of these two cell types in both euploid and trisomic mice. Classical electrophysiological experiments at different Ca^{2+} concentrations are usually done to determine quantal synaptic parameters (such as, multi-probability fluctuation analysis (Silver et al.; 2003). Whereas this is possible for somatic targeting synapses, estimate of pre- and postsynaptic parameters can be hampered when

quantifying biophysical properties of distally located (and therefore filtered) synapses. Regardless of the actual synaptic mechanism, the potentiation of this crucial feedback inhibitory loop might have profound consequences in the computational ability of cortical circuits. Indeed dendritic inhibition operated by MCs were found crucial for frequency-dependent disynaptic inhibition, lateral inhibition, surround suppression and network synchronization during slow γ-activty in primary sensory cortices (Naka et al., 2018; Pfeffer et al., 2013; Silberberg & Markram, 2007; Kapfer et al., 2007; Adesnik et al., 2012). The simultaneous enhancement of glutamatergic synapses onto MCs, and their feedback GABAergic inhibition onto PNs in DS, might decrease the threshold of activation of MCs and concurrently increase the extent of their inhibitory influence within cortical circuits in DS subjects.

Dendritic inhibition is important to modulate dendrite electrogenesis and nonlinear synaptic integration (Tran-van-Minh et al., 2015; Stuart et al., 1997; Stuart & Spruston, 2015). Enhanced dendritic inhibition might therefore dampen NMDA-dependent spikes that occur in distal dendrites elicited by top-down glutamatergic input. These inputs carry context and prior knowledge during associative tasks and are therefore cognitive relevant (Cohen, 2014). Blunting dendritic excitability will also affect the ability of inducing forms of synaptic plasticity that rely on dendritic APs (both bAPs and NMDA-dependent spikes), such as spike-timing dependent plasticity (STDP), an important mechanism of learning and memory (Feldman, 2012; Markram et al., 1997; Sjöström et al., 2001; Letzkus et al., 2006; Sjöström and Häusser, 2006). It is therefore tempting to speculate that impaired STDP could explain, at least in part, the cognitive deficits reported in DS. This is supported by indirect evidence from the α5IA-mediated effects *in vivo*, and the specific localization of α5-$GABA_A$Rs that we reported in the first part this thesis.

Remarkably, synaptic alterations in DS seem to be specific for dendritic inhibitory loop, as synapses to and from PV cells were overall unaffected in DS mice. However, these experiments are still ongoing to increase the sample size of PV-PN and PN-PV synaptic responses. Indeed, whereas uIPSCs were not significantly different, their PPR showed a small albeit significant increase (Table 6), suggesting a potential reduction of release probability of perisomatic-targeting GABAergic synapses onto PNs.

Despite these small effects of synaptic transmission (or lack thereof) to and from PV cells, the excitability of these neurons was strongly altered in DS mice: Rm was higher, APs were broader and firing dynamics did not exhibit the classical fast-spiking, non-adapting behavior of PV cells. Increased Rm explains the reduced rheobase current

necessary to make these cells fire. This can increase PV-cell reaction time –their recruitment being equal— thus affecting their gain.

The mechanisms responsible for alterations of PV cells excitability remain unknown. However, slowed repolarization, increase Rm and decreased ability of sustaining high-frequency firing can be accounted for decreased expression of voltage-dependent K^+ channels. The Kv3 channel family is believed to be important in setting and controlling firing frequency in fast-spiking neurons (Espinosa et al., 2008; Lien and Jonas, 2003, Porcello et al., 2002, Rosato-Siri et al., 2015). Particularly, Kv3.1 and Kv3.2 channels are the principal modulators of high firing rates, typical of fast-spiking interneurons (Boddum et al., 2017). It will be fundamental to reveal whether PV cells of DS mice (and possibly subjects) present altered expression of these channels via high-throughput transcriptomic analysis and/or single-cell real-time PCR. Likewise, it will be crucial to isolate whole-cell currents mediated by these channels to assess whether the functional expression of these channels is altered in DS mice.

PV cells are the metronomes of cortical circuits (Buzsáki and Draguhn, 2004; Freund and Katona, 2007; Klausberger and Somogyi, 2008). They are efficiently recruited by local and long-range glutamatergic synapses, and provide a reliable and efficient inhibition onto PNs and other interneurons –especially other PV cells (Deleuze et al., 2014; Jiang et al., 2013; Manseau et al., 2010; Bacci & Huguenard, 2006; Bacci et al., 2003; Tamas et al., 1997; Connelly & Lees, 2010; Bekkers, 2003; Van der Loos & Glaser, 1972). These cells orchestrate network activity and drive several oscillations, in the β-γ-frequency range (20 – 100 Hz) (Sohal et al., 2009; Freund, 2003; Whittington et al., 1995; Ylinen et al., 1995; Tamas et al., 2000). Alterations of their excitability might therefore strongly affect their ability to synchronize with each other and with a large population of PNs. On this line, it was recently shown that overexpression of Dyrk1A, a serine/threonine kinase involved in neuronal differentiation and synaptic plasticity, impairs the generation of decreases excitability and impairs γ-oscillations in the PFC (Ruiz-Mejias e al., 2016). Surprisingly, pharmacologically inhibition of the activity of this kinase rescues the cognitive deficits in Ts65Dn mice (Neumann et al., 2018). Indeed, affecting γ-activity might underlie several cognitive dysfunctions, including attention and sensory perception. Accordingly, malfunctions of PV-cell circuits was suggested to be among the physio-pathological mechanism underlying several brain disorders, including epilepsy, schizophrenia, major depressive disorders and ASD (Cattaud et al., 2018; Woloszynowska-Fraser etal., 2017; Lewis & Hashimoto, 2005; Lewis & González-Burgos, 2008; Lewis et al., 2012).

The specific synaptic and excitability alterations found in DS mice affecting MCs and PV cells, respectively, might underlie different aspects of cognitive deficits of DS subjects. We speculate that the increased dendritic inhibitory loop impairs learning and memory, whereas altered PV-cell excitability is responsible for autistic traits that affect DS subjects. Future experiments will be required to test this provocative hypothesis.

Ongoing work

Over-inhibition and alterations of specific circuits could be resulting from changes of synaptic mechanisms leading to altered plasticity. However, anatomical differences could also explain the alterations we observed at MC-PN synapses and in PV-cells. We are therefore quantifying the morphological properties of dendrites and axons of PNs, MCs and PV-cells from both DS and euploid mice (Fig 5.2). The results that we will obtain by further analyzing these anatomical reconstructions would be instrumental to better interpret our physiology data. For instance, in the particular case of the altered MC-PN loop, we aim to analyze the density of the axon in L1: an increased density of this structure in this layer, could mean an increased number of synaptic contact onto the dendrites of L2/3 PNs, thus explaining the increased inhibitory activity of these particular interneurons.

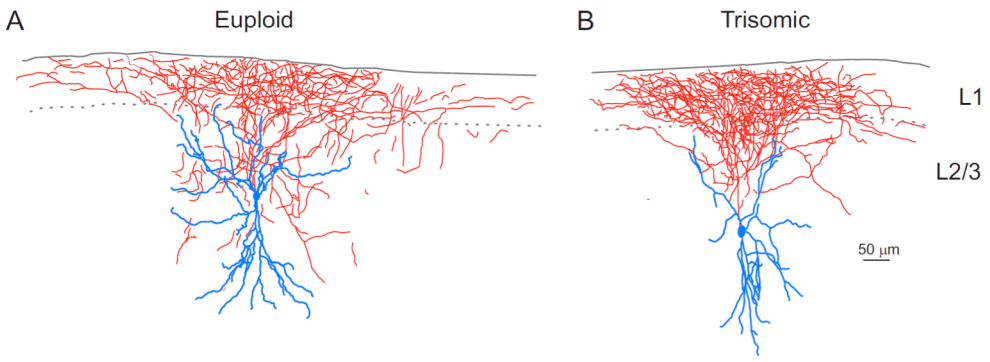


Fig 5.2 Example of reconstructed MCs. Reconstructions of two distinct MC from a euploid (A) and a trisomic (B) mouse (dendrites: blue, axon: red; scale bar: 50 µm). Solid line: pia, dotted line delimits layer 1 and layer 2/3.

Furthermore, since "over inhibition" appears as an emerging hypothesis supporting cognitive deficits in DS (Zorrilla de San Martin et al., 2018), we are performing in vivo experiments to study the general network excitability in DS. Precisely, we are collecting both local field potential (LFP) and juxta-cellular recordings from pyramidal neurons of L2/3 of the PFC. If we consider our experimental approach based on the over inhibition

hypothesis, we expect to see a decreased spiking of PNs and more generally in the neuronal network of L2/3 of PFC in Ts65Dn mice. Moreover, we expect differences in the phase coupling of PN spikes with γ-oscillations in DS mice. We are currently collecting and analyzing data in this direction.

Altogether, these experiments could help understanding the mechanisms underlying the cognitive and learning impairment in DS, thus leading to the opening of new therapeutic avenues to alleviate these symptoms in DS patients.

Future Perspectives

Does the specific segregation of α5-$GABA_A$Rs determine (or modulate) the dendrite-targeting phenotype of MCs?

The segregation of different a subunits at particular synapses formed by well-defined interneuron types (i.e. α1-$GABA_A$Rs at PV-PN synapses, α2/3-$GABA_A$RS at BC-PN and α5-$GABA_A$Rs at MC-PN – Ali and Thomson, 2008) suggests that the insertion of the different a subunit at specific synapses may depend on pre- or postsynaptic mechanisms. To test this hypothesis, experiments aimed at knocking out α5-$GABA_A$Rs in L2/3 PNs of mouse somatosensory cortex are required. In order to address this question, these experiments should yield a mosaic KO of this subunit. This can be achieved by expressing constructs that efficiently edit the GABRA5 gene, responsible for α5- subunit of $GABA_A$Rs expression. To this aim, shRNA or CRISPR/Cas9 systems can be used. A mosaic knockout would allow the simultaneous analysis of two postsynaptic neurons: one where the α5-subunit is still expressed, and one, in which α5 is efficiently knocked out. These experiments can reveal whether removal of α5 will result in the formation of ectopic, non-dendritic synapses or complete loss of synaptic contact on the dendritic tree (Fig 5.3)

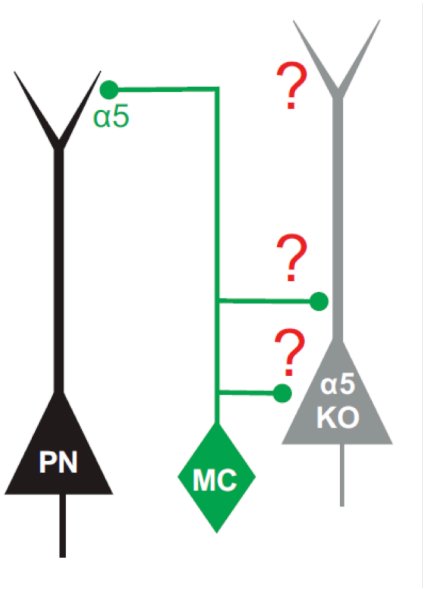


Fig 5.3 The α5 knock-out hypothesis. A wild type PN (black) is contacted by a MCs onto its dendritic compartment. On the right side of the panel, the same MC makes ectopic synapses onto PNs that do not express α5-$GABA_A$Rs (grey).

Which $GABA_A$Rs majorly contributes to tonic inhibition in L2/3 PNs of the mouse somatosensory cortex?

Here we confirmed that α5-$GABA_A$Rs do not contribute to tonic inhibition in this particular cortical region. However, we do not know which subunit is responsible for tonic inhibition in L2/3 PNs. As mentioned above, Glykys and colleagues showed that, in the hippocampus, tonic inhibition relied on α5- and δ- $GABA_A$Rs (Glykys et al., 2008). Therefore, δ subunit could be one of the first candidates to be tested. Further electrophysiological recordings from L2/3 PNs should then be performed, and specific pharmacology used to reveal whether δ-$GABA_A$Rs are involved in tonic inhibition in L2/3 PNs (d-$GABA_A$Rs pharmachology reviewed in Zheleznova et al., 2009). Particularly, neurosteroids are the most powerful modulators of αβδ-containing and act on d-containing receptors as agonist $GABA_A$Rs (Adkins et al., 2001; Brown et al., 2002; Wohlfart et al., 2002). Importantly, THDOC, one of the most used δ–agonists, increases the efficacy of the α1β3δ receptor by increasing the duration of channel opening and introducing a new open state (Wohlfart et al., 2002). Since in our previous work we used GABA-inverse agonists, we would ideally perform these experiments on δ-$GABA_A$Rs KO mouse in the presence of THDOC; tonic currents would be measured in both WT animals and KO, thus allowing a quantification of the level of tonic inhibition mediated by these particular receptor subtypes.

Does the partial blockade of α5-GABA$_A$Rs increase dendritic excitability and function in PNs?

The α5 subunit of the GABA$_A$Rs is selectively expressed at MC-PN synapses. The α5IA is a specific tool to reduce dendritic inhibition originating from this specific cell type. Therefore, it would be interesting to test whether modulation of unitary inhibitory transmission from this cell type would increase dendritic excitability and promote dendritic supralinearity. This can be tested combining patch-clamp recordings with 2-photon imaging and 2-photon glutamate uncaging. Moreover, modulation of dendritic inhibition might differentially gate long-term plasticity of glutamatergic synapses impinging L1 PNs and carrying top-down, context information. This could be tested initially in slice experiments, using STDP protocols, and eventually in head-fixed awake mice subject to sensory whisker stimulations (using dendritic 2-P imaging).

These experiments are also relevant, as they will define important functional roles in the computational ability of L2/3 PNs in the presence of plastic dendritic inhibition. In fact, understanding the actual role of these synapses will also better define the impact in pathological states, such as in DS.

What are the subunits forming the GABA$_A$Rs at synapses formed by MCs onto other IN-type?

α5-GABA$_A$Rs are exclusively present at MC-PN synaptic contacts. However, not much is known about the subunit assembly characteristic of the synapses formed by MCs onto other INs. We can speculate that disinhibitory circuits could be involved in some particular pathological brain states caused by network over-inhibition. Importantly, studying the composition of inhibitory receptors at synapses formed between INs will reveal circuit-specific subunits that can be targeted with well-defined drugs. Electrophysiological recordings combined with pharmacology, immunostainings or electron microscopy performed with reliable antibodies could be used to try to investigate the composition and the exact localization of these receptors.

Altogether, these experiments will likely advance our knowledge on the molecular underpinnings of cortical microcircuits. The molecular specificity adds another level of diversity to the so complex machine that is the brain. In addition, understanding how activity of characteristic inhibitory circuits and of particular receptors shape brain

activity in physiological conditions, could provide new ideas to investigate whether some of these mechanisms could be involved or altered in particular pathological conditions.

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RÉSUMÉ

Dans le néocortex, l'inhibition synaptique rapide sculpte toutes les formes d'activité cognitive. Les interneurones GABAergiques, responsables de l'inhibition néocorticale, englobent un grand nombre de types cellulaires. Certains interneurones innervent la région périsomatique des neurones pyramidaux corticaux (NP), tandis que d'autres ciblent les dendrites des NPs. Ici, nous avons étudié la sous-unité α5 du récepteur $GABA_A$ ($GABA_AR$), qui contribuerait de manière significative à l'inhibition tonique. Nous avons constaté que, dans les NP de la couche 2/3 du cortex somatosensoriel chez la souris, α5 a une contribution négligeable à l'inhibition tonique. Inversement, nous avons constaté que la sous-unité α5 est spécifiquement exprimée au niveau des synapses entre les interneurones ciblant les dendrites - les cellules de Martinotti (MC) - et les NP, indiquant ainsi que la transmission GABAergique médiée par les $GABA_AR$ contenant α5 est importante pour l'inhibition dendritique synaptique. Au moyen d'enregistrements en configuration patch-clamp entre MCs et différents types de neurones corticaux, nous avons montré que l'expression de α5 n'est toujours présente que dans les synapses formées par les MCs sur les PNs. Ces résultats suggèrent que les α5-$GABA_A$Rs sont une signature moléculaire spécifique des synapses dendritiques, issues de circuits inhibiteurs impliquant les MC. Il est maintenant établi que de nombreuses maladies du cerveau sont le résultat du dysfonctionnement de circuits inhibiteurs distincts et, en particulier, les souris α5-KO montrent un apprentissage amélioré. En conséquence, il a été montré que le traitement avec un agoniste inverse spécifique du récepteur α5-$GABA_A$ (α5IA) a permis la récupération des déficits cognitifs chez des souris Ts65Dn, un modèle animal de la trisomie 21 (DS). Cependant, les mécanismes sous-jacents à cette récupération cognitive sont inconnus aux niveaux synaptique et des circuits. Nos résultats préliminaires indiquent que les synapses GABAergiques des interneurones ciblant les dendrites sont spécifiquement modifiées chez les souris DS. En combinant différentes approches, notamment la génétique de la souris et plusieurs enregistrements en configuration patch-clamp, nous définirons si cette modification est spécifique à un circuit particulier. Nos résultats permettront de mieux comprendre les altérations spécifiques des circuits de la DS et d'ouvrir de nouvelles pistes thérapeutiques pour atténuer les troubles cognitifs de cette maladie. Plus largement, ces expériences contribueront à mieux définir le rôle des synapses dendritiques contenant des α5-$GABA_AR$ dans le cortex dans des conditions physiologiques et pathologiques

ABSTRACT

In the neocortex, fast synaptic inhibition sculpts all forms of cognitive-relevant activity. Neocortical inhibition is provided by GABAergic interneurons, which encompass a vast number of cell types. Some interneurons innervate the perisomatic region of cortical pyramidal neurons (PNs), whereas others target PN dendrites. Here we studied the α5 subunit of the $GABA_AR$, which is believed to contribute significantly to tonic inhibition. We found that, in L 2/3 PNs of mouse somatosensory cortex, α5 provides a negligible contribution to tonic inhibition. Conversely, we found that α5 is specifically expressed at synapses between the dendrite-targeting interneurons Martinotti cells (MCs) thus indicating that GABAergic transmission through α5-containing GABAAR subtypes is important for synaptic dendritic inhibition. Using multiple patch-clamp recordings between MCs and different cortical neuron types, we show that the expression of α5 is always present only at synapses made by MCs onto PNs. These results suggest α5-GABAARs as a prominent molecular signature of specific dendritic synapses from inhibitory circuits involving MCs. Importantly, it is well known that many brain diseases originate from dysfunctions of distinct inhibitory circuits and, in particular, α5-KO mice show improved learning. Accordingly, it was shown that the treatment with a highly specific α5 inverse agonist rescued learning and memory deficits in Ts65Dn mice, an animal model for Down syndrome (DS). Yet, the actual mechanisms underlying this cognitive rescue at the synaptic and circuit levels are unknown. Our preliminary results indicate that GABAergic synapses from dendrite-targeting interneurons are specifically altered in DS. Using a combination of approaches, including mouse genetics and multiple patch-clamp recordings we are defining whether this alteration is circuit-specific. Our results will provide a better understanding of specific circuit alterations in DS, and will likely open new therapeutic avenues to alleviate cognitive impairment of this disease.

Altogether, these experiments will contribute to better define the role of dendritic synapses containing α5-$GABA_AR$ in somatosensory cortex in physiological and pathological conditions.