Social Conditioned Place Preference in the Captive Ground Squirrel (*Ictidomys tridecemlineatus*): Social Reward as a Natural Phenotype

Garet P. Lahvis, Jules B. Panksepp,
and Bruce C. Kennedy
Oregon Health and Science University

Clarinda R. Wilson
University of Wisconsin–Madison

Dana K. Merriman
University of Wisconsin–Oshkosh

Social behaviors of wild animals are often considered within an ultimate framework of adaptive benefits versus survival risks. By contrast, studies of laboratory animals more typically focus on affective aspects of behavioral decisions, whether a rodent derives a rewarding experience from social encounter, and how this experience might be initiated and maintained by neural circuits. Artificial selection and inbreeding have rendered laboratory animals more affiliative and less aggressive than their wild conspecifics, leaving open the possibility that social reward is an artifact of domestication. We compared social behaviors of wild and captive population of juvenile 13-lined ground squirrels (*Ictidomys tridecemlineatus*), the latter being 2nd- and 3rd-generation descendants of wild individuals. At an age corresponding to emergence from the burrow, postnatal day (PD) 38, captive squirrels engaged in vigorous social approach and play and these juvenile behaviors declined significantly by PD 56. Similarly, young wild squirrels expressed social proximity and play; affiliative interactions declined with summer's progression and were replaced by agonistic chasing behaviors. Social conditioned place preference testing (conditioned PDs 40–50) indicated that adolescent squirrels derived a rewarding experience from social reunion. Our results support the contention that undomesticated rodents have the capacity for social reward and more generally suggest the possibility that positive affective experiences may support group cohesion, social cooperation, and altruism in the wild.

Keywords: social reward, classical conditioning, play-fighting, altruism, camaraderie effect

Mammals express an ontogeny of social behaviors, beginning with mother–infant interactions, continuing through playful interactions with peers, and maturing into the more stereotypical, sex-dependent patterns characteristic of adults (Wolff & Sherman, 2008). Though sharing these commonalities, the sociality of ground squirrel species varies in both duration and degree (Armitage, 1981; Hare & Murie, 2008; Rayor & Armitage, 1991). Juvenile ground squirrels engage in social play soon after they emerge from their natal burrows (Steiner, 1971; Yeaton, 1972). As sum-

mer progresses, play behaviors diminish (Betts, 1976; Nunes, Muecke, Anthony, & Batterbee, 1999), with adolescents often wandering beyond their natal range, an action that can lead to emigration (Barash, 1974; Holekamp, 1984a, 1984b; McCarley, 1966; Michener & Michener, 1977; Rayor & Armitage, 1991). Among the more social Columbian ground squirrels, *S. columbianus,* adult social interactions often appear affiliative, typically consisting of dyads and triads networked via social interactions with extra-group individuals (Manno, 2008). Among less social species, including *I. tridecemlineatus,* arctic ground squirrels, *S. parryii plesius,* and Richardson's ground squirrels, *S. richardsonii,* social interactions are expressed rather as conflicts for mating opportunities and territory (Lacey, Wieczorek, & Tucker, 1997; Schwagmeyer & Woontner, 1986; Schwagmeyer & Parker, 1987; Luna & Baird, 2004).

Mammalian social behaviors are often considered within the ultimate framework of adaptive benefits versus survival risks (Silk, 2007). For instance, juvenile play is thought to improve physical and emotional responsiveness to unexpected events (Nunes et al., 1999; Spinka, Newberry, & Bekoff, 2001), familiarize juveniles with self-handicap and fair behavior (Bekoff, 2004), improve abilities to cope with social challenges (van den Berg et al., 1999), establish dominance relationships (Blumstein, Chung, & Smith, 2013), and help refine abilities to respond to subtle and ambiguous social signals (Pellis, Pellis, & Reinhart, 2010). Such adaptive

benefits are difficult to test (Burghardt, 2005; Pellis & Pellis, 2006) but are suggested by evidence that social play promotes normal brain development (Gordon, Burke, Akil, Watson, & Panksepp, 2003; Pellis & Pellis, 2007).

Like juvenile play, dispersal also has adaptive benefits that likely vary among squirrel species (Bowler & Benton, 2005; Lawson Handley & Perrin, 2007; Wolff, 1994). Benefits include competitive mating advantages (Dobson, 1982), access to higher quality resources (Dobson, 1979), avoidance of inbreeding (Wauters & Dhondt, 1993), and evasion from parasitism (Hoogland, 1979). These benefits can offset dispersal's enormous survival risks (Byrom & Krebs, 1999). Proximate explanations for variations in adolescent dispersal include differences in environmental harshness (Barash, 1974; Ritchie & Belovsky, 1990), adult aggression toward maturing adolescents (Steiner, 1972; Wolff, 1993), and ontogenetic changes promoting excursion behavior (Holekamp, 1984a; 1984b). Proximate explanations might also include psychological changes. Exploratory activity of laboratory rodents can be greater among adolescents than either younger juveniles or adults (Macri, Adriani, Chiarotti, & Laviola, 2002). Affiliative social behaviors also change with maturation (Panksepp et al., 2007; Terranova, Laviola, & Alleva, 1993). Exploratory and social behaviors utilize neurocircuits (Laviola, Macri, Morley-Fletcher, & Adriani, 2003; Liu & Wang, 2003; Resendez, Kuhnmuench, Krzywosinski, & Aragona, 2012) activated by natural and drug rewards (Spear, 2000; Kelley & Berridge, 2002; Dölen et al., 2013).

We can infer an animal subject finds a stimulus rewarding *if* the subject returns to an environment associated with that stimulus (Glickman & Schiff, 1967; Schneirla, 1959). Measures of conditioned place preference (CPP) allow us to formally assess this possibility. In the conditioning phase, the subject is alternated between one environment paired with the presence of a stimulus and a second environment associated with its absence. During the test phase, both environments are available to the subject, though no stimulus is present, and the subject is allowed to amble back and forth between the two conditioned environments (Bardo & Bevins, 2000; Tzschentke, 2007). CPP has demonstrated that laboratory rodents typically prefer to occupy environments associated with social access, a behavior driven by anticipation of a social reward (Calcagnetti & Schechter, 1992; Douglas, Varlinskaya, & Spear, 2004) and by avoidance of environments paired with social isolation (Panksepp & Lahvis, 2007).

Though laboratory rodents can express social reward-related phenotypes during a CPP test, similar processes may not, of necessity, regulate the social behaviors of wild rodents. Confinement of laboratory rodents for generations to small cages fosters a selection bias for individuals with relatively low levels of territoriality and activity concomitant with tolerance for high housing densities (Boice, 1981; Chalfin et al., 2014; Künzl, Kaiser, Meier, & Sachser, 2003; Künzl & Sachser, 1999). Thus, conceivably, multigenerational confinement might cultivate phenotypes expressing strong responses to social CPP tests. Many laboratory rodents have been selected for "desirable" traits (Beck et al., 2000; Wade & Daly, 2005) and then inbred for over 200 generations for medical research (see http://jaxmice.jax.org/findmice/index.html). Though social CPP has been designed primarily with face and construct validity relative to psychological concepts, this test has not been utilized for rodents lacking an extensive history with domestication and genetic inbreeding. This experimental gap leads to an obvious question regarding the applicability and relevance of such testing scenarios to wild or captive rodents.

In this paper, we present findings from a novel experimental opportunity that employs laboratory tests of rodent social interaction (Panksepp et al., 2007) and social reward (Panksepp & Lahvis, 2007) to study captive 13-lined ground squirrels, *Ictidomys tridecemlineatus*, second- and third-generation descendants of wild individuals (Merriman et al., 2012). Our choice of generation for using captive-born ground squirrels is supported by studies showing that wild Norway rats robustly express wild behaviors within the first two to five generations of captivity in the absence of any direct selection for tameness (Stryjek & Pisula, 2008). Litters of 13-lined ground squirrel are born in late May to early June (McCarley, 1966; Merriman, Lahvis, Jooss, Gesicki, & Schill, 2012), emerging from their burrows at about 5 weeks of age, forage, engage in play, then disperse within a month of their emergence (McCarley, 1966; Rayor & Armitage, 1991; Wistrand, 1974). Young females more commonly remain within the colony (Schwagmeyer, 1980), whereas males typically emigrate (Armitage, 1981; McCarley, 1966).

We compared social interaction in captive, same- and mixed-sex dyads, with contemporaneous observations of wild squirrels at times approximating emergence from the burrow and dispersal. We found that captive and wild ground squirrels expressed analogous declines in affiliative behaviors with the progression of summer. Employing an adapted social CPP paradigm (Panksepp & Lahvis, 2007), we found that adolescent squirrels expressed a robust place preference for environments associated with social interactions, indicating social reward from reunion with their peers. Overall, squirrel social behaviors resemble patterns expressed by highly domesticated and inbred rodents, strengthening the value of using psychological approaches for understanding the natural developmental transitions in rodent social affiliation.

Method

Capture

Detailed descriptions of the capture, housing and husbandry of 13-lined ground squirrels have been previously published (Vaughan, Gruber, Michalski, Seidling, & Schlink, 2006). A Scientific Collector's Permit (NER SCP 144) was obtained from the Wisconsin Department of Natural Resources prior to capture. The colony of 13-lined ground squirrels used here was derived from animals trapped at cemeteries located in Oshkosh (44.02°N 88.53°W) and Neenah (44.19°N 88.46°W), Wisconsin.

Husbandry

Thirteen-lined ground squirrels were bred for two generations in the University of Wisconsin–Oshkosh colony located at the Halsey Science Center (Oshkosh, WI). Subjects used for social behavior and CPP testing were either second- or third-generation descendants of individuals captured during the initial trapping excursions. Except for purposes of breeding, adult squirrels were housed individually in standard polypropylene cages (412 × 210 × 200 mm) that contained shaved aspen bedding (Teklad Aspen Sani-Chips, Harlan, Indianapolis, IN) and a plastic rain gutter tube

(200 × 65 × 65 mm) available for use as a burrow. Squirrels received ad libitum access to water and commercial dog chow (IAMS Chunks, Dayton, OH) supplemented with sunflower seeds. Ambient temperature was maintained at 68° ± 2° F, and the light–dark cycle was periodically adjusted to correspond with sunrise and sunset in the location captured until squirrels entered hibernation in the fall. During hibernation, squirrels were housed at 38° ± 3° F in constant darkness. After arousal and recovery from hibernation, sexually mature male and female squirrels were housed together for up to 4 weeks before pregnant females were moved to single housing for birth and pup upbringing. Pups born in the enclosures received a combination of dog chow, cat chow (Purina Indoor Formula, St. Louis, MO) and sunflower seeds until weaning.

Juveniles used in this study were not handled from birth or otherwise tamed, so their handling was thus no different from that of animals recently caught from the wild (Vaughan et al., 2006). Individuals were thus highly capable of escape and expressed some skittish behaviors. All cage changes were performed with the home cage placed in a deep plastic tote bin. Soft cloth nets identical to those used for field captures were used for the occasional escapee. Whenever it was necessary to transfer or restrain them, leather welder's gloves were used to protect from inevitable bites.

On postnatal day (PD) 35/36, squirrels were weaned into mixed-sex social groups (two males and two females) from either a single litter (six groups) or a combination of multiple litters (eight groups), and these groupings served as the subsequent housing arrangement between all of the behavioral procedures. Statistical differences between squirrels from full-sibling versus partial-sibling groups were not detected and are not considered further. All cages of weaned juveniles were moved to a separate room outside the main colony that received natural lighting through room windows.

Social Interaction Test

Social interaction tests (SI tests) were conducted on PD 38/39 and again on PD 56/57 (see Figure 1). To engender social motivation, each individual within a social group was isolated into a clean cage 24 hr prior to testing (see Panksepp, Wong, Kennedy, & Lahvis, 2008 for rationale). One male and one female individual from each social group were randomly designated as test squirrels and the remaining two as stimulus squirrels. All test/stimulus combinations were evaluated (i.e., F–F, F–M, M–F and M–M), and these designations were maintained for the tests on PD 38/39 and PD 56/57. To identify individuals, the back of each squirrel was marked with hair dye. On test day, squirrels were habituated to a windowless experimental room (approximately 4 × 4 m) illuminated with dim fluorescent lighting (adjacent to the colony room) for at least 90 min before testing. Five min prior to testing, the cage top was replaced with clear acrylic glass. The SI test
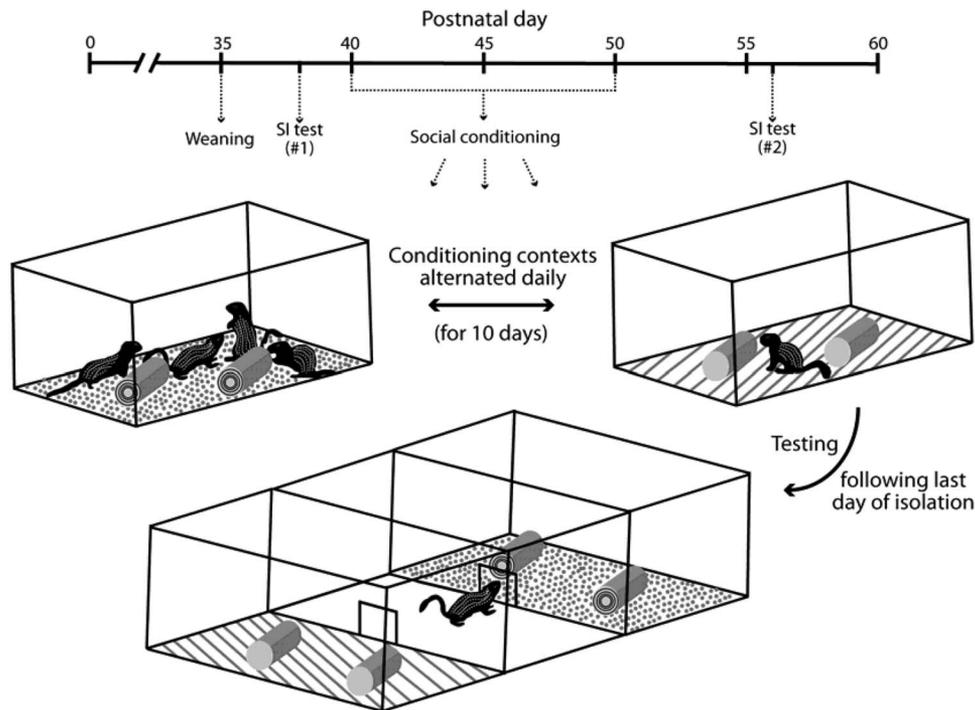

Figure 1. Developmental timeline of experimental procedures and illustration of social conditioned place preference. The top of the figure depicts the ages when postweanling squirrels were tested for social interaction, and conditioned and tested for social conditioned place preference, respectively. Grouped squirrels, on the left side in the bedding represented by gray dots, are undergoing social conditioning in a "corncob" environment with tubes threaded within the interior. The isolated squirrel, on the right side in the bedding represented by diagonal lines, is undergoing conditioning alone in a "paper" environment with tubes containing a smooth interior. The social conditioned place preference testing arena is illustrated at the bottom.

consisted of placing a stimulus squirrel into the home cage of a test squirrel from the same social group. Behaviors of the test and stimulus squirrels were video-recorded with an overhead camcorder (Sony, DCR-VX2100) for a 5-min period. Following each test, squirrels were returned to their original social group in a fresh cage. Testing was conducted in the afternoon from 1200 to 1900 hr. All laboratory procedures were conducted from June 20, 2007 (sunrise 0511 hr, sunset 2040 hr), to July 7, 2007 (sunrise 0518 hr, sunset 2040 hr). Following completion of the first SI test, squirrels were returned to housing in their respective social groups.

Three categories of behavior were assessed during the SI test; social play (Nunes et al., 1999; Pasztor, Smith, MacDonald, Michener, & Pellis, 2001; Pellis, MacDonald, & Michener, 1996), social investigation (Grubitz, 1963; Michener & Sheppard, 1972; Panksepp et al., 2007), and vigilant behavior (Arenz & Leger, 1999). See Table 1 for descriptions of each behavioral category. Behaviors were analyzed with computer-assisted analysis software (ButtonBox v5.0, Behavioral Research Solutions, Madison, WI) by two independent observers, and all presented data and statistical analyses are based on an average of these two measurements (interrater reliability, Pearson's correlation coefficient for social investigation, $r = .90$; play behavior, $r = .98$; and vigilance, $r = .80$). A total of 44 squirrels were tested for social interaction.

Social Conditioned Place Preference Test

After 24 hr in their home cage, on PD 39/40, social groups were housed together in a fresh cage containing one of two novel conditioning environments. The conditioning environments were composed of either "paper" (soft paper bedding; Cellu-Dri Soft, Shepherd Specialty Papers, Richland, MI) and two smooth 1.5-in. PVC couplers or "corncob" (0.25-in. grain-size corncob bedding; Harlan, Teklad, Indianapolis, IN) and two threaded 1.5-in. PVC couplers (see Panksepp & Lahvis, 2007, for additional background on Social Conditioned Place Preference [SCPP] experiments). After the first 24-hr social conditioning period, squirrels were separated and isolated into a fresh cage containing the alternate conditioning environment. Social and isolate housing contexts and their respective conditioning environments were alternated every 24 hr over a 10-day period (see Figure 1). In both environments, squirrels were provided with ad libitum dog chow and water. To assess whether squirrels had a natural preference for either of the two conditioning environments, a control group ($n = 15$) was alternated daily as a social unit between the two environments (i.e., no conditioning).

Habituation to the testing situation entailed placing squirrels individually into a CPP testing apparatus after conditioning on Days 8 and 9, where they were allowed to move freely about in the absence of conditioning environments for 20 min under dim fluorescent lighting. The CPP apparatus, constructed of acrylonitrile-butadiene-styrene (ABS) plastic, consisted of three adjacent compartments (each 480 mm $\times$ 247 mm $\times$ 200 mm) accessible to the test squirrel via square openings (76 $\times$ 76 mm). On the test day (PD 49/50), the paper and corncob environments were assembled in the peripheral compartments. An individual test squirrel was placed in the central compartment (exposed polycarbonate floor) and the apparatus was covered with a clear acrylic glass top. Movement between compartments was recorded from an overhead video camera for 30 min under dim lighting. Following the SCPP test, squirrels were returned to their original social groups in a fresh cage. Two independent observers analyzed $\approx$40% ($n = 20$) of the SCPP tests (interrater reliability, Pearson's correlation coefficient, $r = .99$). Data from a small subset of ground squirrels ($n = 7$) was excluded from statistical analyses because these squirrels failed to explore the conditioning environments during testing, defined as remaining motionless for $\geq$15 min of the 30-min test or repeatedly attempting to escape from the testing arena. Six additional squirrels were excluded due to technician error during the conditioning procedure. A total of 41 squirrels were evaluated for SCPP. The University of Wisconsin–Oshkosh Institutional Animal Care and Use Committee approved all experimental procedures (protocol number: 0–03-0026–000188-5–18-07).

Field Observations

The University of Wisconsin–Madison permitted us to observe and video-record 13-lined ground squirrels at a field site (43.06° N, 89.52° W) maintained by the Agriculture Research Station (ARS), approximately 90 miles from Oshkosh, where the laboratory studies were conducted. The site consisted of over 60% perennial ryegrass (*Lolium perenne*), along with tall fescue (*Festuca arundinacea*) and common meadow grass (*Poa pratensis*). Field observations were conducted at various time points between 1530 and 1900 hr over a 5-week period between June 26 and August 2, 2007. The length of each observation period varied with levels of squirrel activity, weather conditions and use of the field by ARS staff. Observation periods occurred 3–4 days/week and each period lasted 107 $\pm$ 28 min/day (mean $\pm$ *SD*). ARS staff avoided the field site during observation periods. Ground squirrel activity was monitored for the occurrence of interactions between two or more squirrels within an area of approximately 400 m^2 and

Table 1

Ethogram of Social Interaction Test in the Laboratory

Category	Behavior	Description
Social investigation	Grasping	Placing both forepaws on back of other juvenile without lower body "mounting"
	Allo-grooming	Grooming or licking any area of other juvenile
	Sniffing and pursuit	Sniffing or snout contact of head, flank or anogenital areas, pursuit within one body length
Social play	Wrestling	Facing other juvenile, often in a face-to-face clench or embrace, pecking at juvenile's neck, throat, cheeks, chest or abdomen, without inflicting bite wounds
	Boxing	Batting other juvenile with forepaws, usually reciprocated
	Mounting	Climbing on other juvenile's back, wrapping forepaws around chest or abdomen, then attempting intromission
Vigilance	Stand/freeze	Rearing onto hind feet and maintaining upright posture

ranging from approximately 10 to 30 m from the observer. Social interactions observed on the site were video-recorded with a professional video camera under $20\times$ optical zoom (XL2 $20\times$ IS zoom, Canon, Tokyo, Japan). Once a social interaction began, recording continued until the interacting ground squirrels dispersed, entering a burrow or disappearing into taller grass.

To quantify field observations of wild squirrels, an observer first evaluated behaviors while watching video recordings in real time. Beginning anytime two or more squirrels entered the camera's field of view, the observer noted the time stamp and then classified behaviors according to an ethogram (see Table 2). In subsequent analysis, the observer returned to each time stamp and conducted frame-by-frame analysis of the video recordings at 30-fold slower speed via iMovie (Apple, Cupertino, CA). In cases where there were questions about a particular behavior, a second rater was consulted. A second independent observer analyzed $\approx$50% ($n = 122$) of the field behaviors to assess reliability between observers (interrater agreement, Cohen's kappa coefficient, $\kappa = 0.72$). Social behaviors were classified according to an ethogram similar to the one used for captive squirrel behavior that included the affiliative behaviors observed in captivity along with spatial proximity and chasing behaviors observed in the wild (Betts, 1976; Michener & Sheppard, 1972). See Table 2 for descriptions of each behavioral category. Frequencies of each behavior were tallied for each week of observation.

Statistical Analyses

A 2×4 analysis of variance (ANOVA) was used to evaluate effects of age and the sex pairing of squirrels on play behavior, social investigation and vigilance, respectively, during the SI tests. For SCPP testing, a 2×3 ANOVA was utilized, with sex and experimental group as between-subjects factors. Specific comparisons between groups were conducted with orthogonal contrasts (which include averaged error variance across all experimental groups). Data from the field are presented as descriptive statistics for weekly totals of individual types of social behavior (e.g., approach, play, etc.). "Expected" weekly counts for the combined social behavior measure were generated assuming an equal distribution over the 5-week observation period from late June to early August and are represented in Figure 4. Expected values for each specific type of social behavior were adjusted for observed changes in social behavior (combined) across the 5-week period. All analyses were performed using JMP v8.0.1 (SAS Institute Inc., Cary, NC). Statistical significance was set at $p < .05$.

Results

Social Interaction Test

Adolescent squirrels engaged in more play behavior during the first SI test (on PD 38/39) than the second test (PD 56/57) occurring about 20 days later (Figure 2A): main effect of age, $F(1, 42) = 5.2$, $p = .03$, $R^2 = 0.08$. Play behavior was also sensitive to the sex of the interacting squirrels (Figure 2A'): main effect of sex, $F(3, 40) = 5.6$, $p = .003$, $R^2 = 0.27$, with the highest level of play observed during interactions between two males: orthogonal contrast, male-male pairs versus all other groups, $F(1, 36) = 15.9$, $p = .0003$. The influence of sex on social play did not differ between the first and second SI tests: Age $\times$ Sex interaction, $F(3, 40) = 1.7$, $p = .19$.

Social investigation declined between PD 38 and PD 56 (Figure 2B): main effect of age, $F(1, 42) = 4.4$, $p = .04$, $R^2 = 0.08$, and was dependent on the sex of both the test and stimulus animal (Figure 2B'): main effect of sex, $F(3, 40) = 5.2$, $p = .004$, $R^2 = 0.26$. Social investigation was highest during female-female interactions compared to all other sex pairings: orthogonal contrast, female-female pairs versus all other groups, $F(1, 36) = 15.1$, $p = .0004$. The influence of sex on social investigation did not vary with age: Age $\times$ Sex interaction, $F(3, 40) = 0.9$, $p = .44$.

The expression of vigilant behavior was greater during the second SI test than during the first test (Figure 2C): main effect of age, $F(1, 42) = 13$, $p = .0009$, $R^2 = 0.24$. Vigilant behavior was not dependent on the sex of the interacting squirrels (Figure 2C'): main effect of sex, $F(3, 40) = 0.4$, $p = .73$. Moreover, vigilance during social interactions was negatively correlated with social play (Pearson's correlation coefficient, $r = -0.38$, $df = 43$, $p = .009$) and with social investigation ($r = -0.24$, $df = 43$, $p = .12$), although the latter relationship failed to reach statistical significance. There was no relationship between play behavior and social investigation ($r = -0.05$, $df = 43$, $p = .76$). During SI testing, the time that elapsed while squirrels were engaged in social investigation, play and vigilance accounted for less than half of the 5-min testing session (Figure 2D), indicating that the associations between these behaviors were not impacted by the duration of the testing period.

Social Conditioned Place Preference Test

Social conditioning altered the time spent in each of the peripheral compartments of the CPP arena (Figure 3): main effect of conditioning, $F(2, 38) = 11.8$, $p < .0001$, $R^2 = 0.38$. Although

Table 2
Ethogram of Social Behaviors in the Field

| Category	Behavior	Description
Affiliative	Approach	Approaching a stationary squirrel or following a moving squirrel without threat posture characteristic of chase behaviors
	Social investigation	See "Social investigation" category in Table 1
	Play	Two squirrels engaging in any nonsnout-directed physical contact, including mounting, boxing, and wrestling
	Proximity	Within two body lengths of another squirrel but not engaging in other social behavior
Agonistic	Chasing	Aggressive pursuit of another squirrel, typically preceded or followed by a threatening posture involving an upright stance and tail flicking
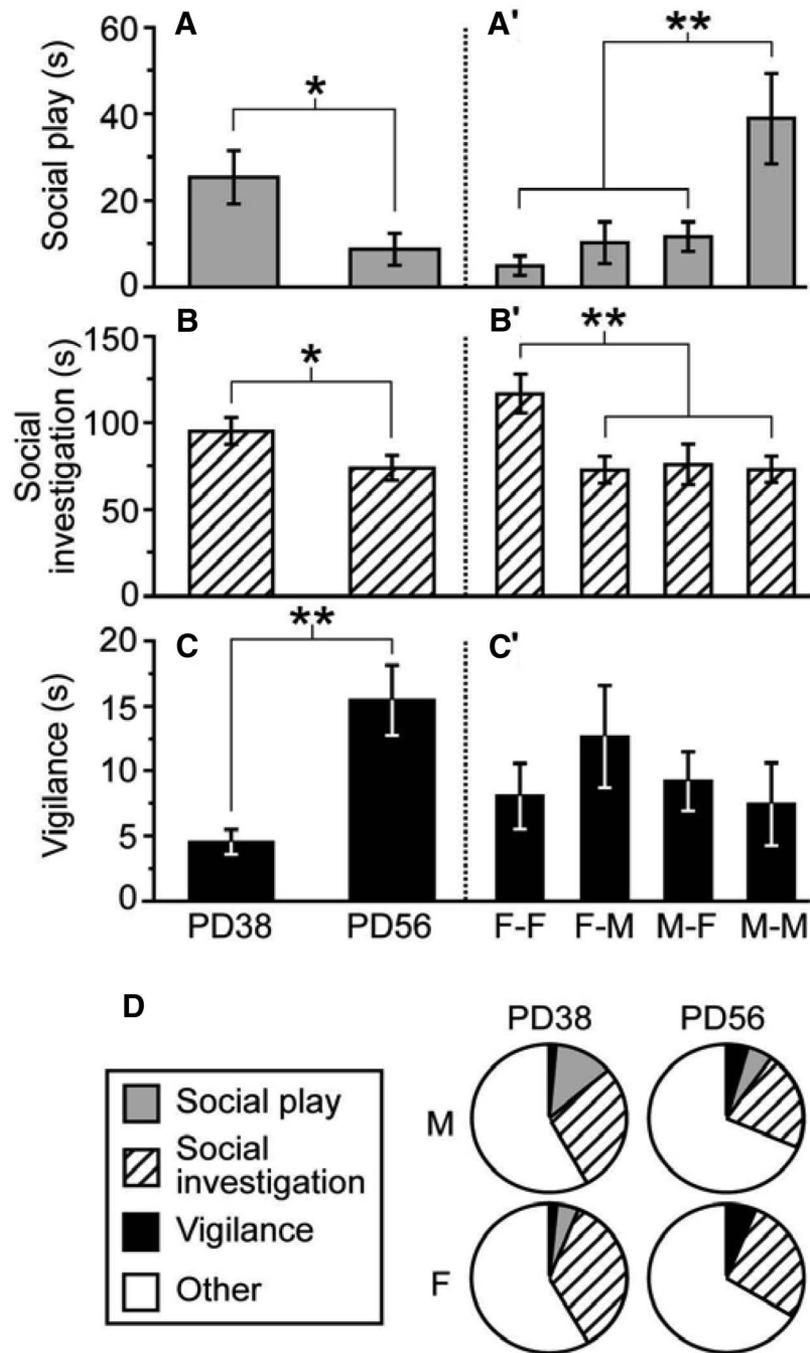
Figure 2. Behavioral differences between early and late adolescent ground squirrels in the laboratory. Labels on the abscissa indicate the age at testing and the sex of the interacting squirrels (test-stimulus combinations). (A) Play behavior of test squirrels diminished across adolescent development from PD38 to PD56 and (A′) was most highly expressed in male-male pairs. (B) Social investigation was greater at PD38 versus PD56 and (B′) was expressed more within female-female pairs. (C) Vigilance behavior was higher on PD56 versus PD38 and (C′) did not differ between the sex-pairings of squirrels. (D) "Time budgets" for squirrels during the 5-min test period demonstrated that a majority of time was spent engaged in behaviors that were not social in nature. Ns = 10–12 squirrels per sex combination. PD = postnatal day. All data are presented as the mean ± standard error. $^{*} p < .05.$ $^{**} p < .001.$
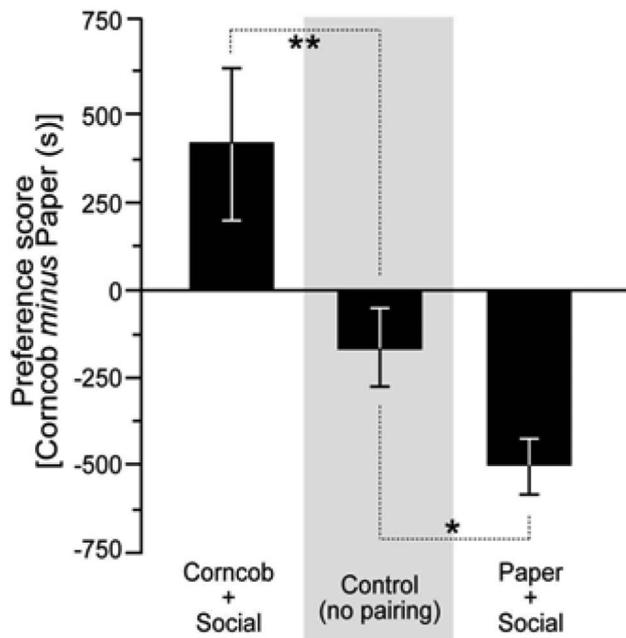
Figure 3. Social conditioned place preference in adolescent ground squirrels. Preference scores were generated by subtracting the amount of time each squirrel spent in the "paper" environment from the amount of time in the "corncob" environment. A positive preference score therefore indicates an individual spent more time in the corncob environment during the 30-min trial whereas a negative score indicates more time spent in the paper environment. Ns = 6–18 squirrels per group. All data are presented as the mean ± standard error. * $p < .05$. ** $p < .001$.

unconditioned squirrels expressed a modest preference for the paper bedding ($t = -1.44$, $df = 15$, $p = .08$), association of the paper environment with social housing resulted in a CPP relative to this control group: orthogonal contrast, $F(1, 34) = 4.9$, $p = .03$. When social exposure was paired with the corncob bedding, social conditioning resulted in an increased duration spent in the corncob bedding: orthogonal contrast, $F(1, 34) = 10.3$, $p = .003$. There was no effect of the sex of test animals on social conditioning: main effect of sex, $F(1, 39) = 1.4$, $p = .25$, or an interaction: Sex × Conditioning interaction, $F(2, 38) = 1.5$, $p = .24$.

Field Observations

Wild 13-lined ground squirrels expressed both social play and social investigation behaviors. In all, 228 instances of social behavior were observed during ≈30 h of video recordings collected in the field over a 5-week period when ground squirrels were visible. In the field, proximity (two or more ground squirrels within two body lengths of each other) accounted for 60% of all social behaviors. Chasing accounted for 15% of the total social behaviors observed, followed by approach, play and investigation, which each contributed 7–8%.

The total number of social behaviors observed each week varied substantially (Figure 4A), but generally decreased over the 5-week period (95 social behaviors were observed in Week 1 vs. 12 social behaviors during Week 5). Affiliative social behaviors were more typical during the first few weeks of observation, whereas agonis-

tic social behaviors were common at later time points. Behaviors categorized as approach, investigation, play and proximity (Figure 4B–E) were predominantly observed in late June to mid-July (Weeks 1–3), but rarely from late July to early August (Weeks 4–5). By contrast, chasing behavior (Figure 4F) occurred most frequently during mid-July and August (Weeks 3–5).

Though we observed 228 distinct bouts of ground squirrel social behavior, we were unable to count individuals since we did not capture or tag them. Over our 5 weeks of field observations, we video recorded four or more individuals in close proximity on three separate occasions. Considering the low frequency of interactions known to occur between adults, these observations suggest an absolute lower boundary on the number of juveniles sampled in the colony. However, we estimate a considerably larger number of juveniles. For 13-lined ground squirrels, 100 burrow entrances correspond to approximately 14 nesting burrows (Desha, 1966). Our estimate of 50 to 100 burrow entrances at the colony indicates seven to 14 nesting burrows. Each nesting burrow supports on average eight pups per litter (Rongstad, 1965; Streubel & Fitzgerald, 1978), so we estimate 56–112 juveniles in our colony. The video camera did not provide sufficient field-of-view to record all social interactions observed, so some of the residing juveniles may not have been sampled.

Discussion

At a juvenile age corresponding to emergence from the burrow (PD 38/39), captive 13-lined ground squirrels engaged in vigorous social interactions that differed between sexes: male dyads more typically in bouts of play-fighting and females in social investigation. These sex-dependent social patterns were maintained, albeit at lower overall levels, when squirrels were tested as older adolescents (PD 56/57). Our results mirror an early study of captive *S. richardsonii,* showing that nonsibling females preferentially interacted with "recognitive" behaviors (Sheppard & Yoshida, 1971), akin to social investigation, whereas male dyads engaged in "agonistic" behaviors, which included rough-and-tumble behaviors that were *not* deemed to be playful. These contrasting interpretations may reflect differences in squirrel rearing environment; most juveniles in the Sheppard and Yoshida study were trapped in wild environments that afforded social refuge so subsequent confinement may have fostered aggression. A different explanation is that Sheppard and Yoshida may have misinterpreted play-fighting as aggression. The first report of play-fighting among ground squirrels was published in the same year (Steiner, 1971) and only much later systematically dissociated from agonistic behavior (Pellis & Pellis, 1987; Pellis, 1988). More recently, free-living male juveniles of *S. richardsonii* and *S. beldingi* were shown to engage in more play-fighting than females (Nunes et al., 1999; Pasztor et al., 2001). This sexually dimorphic pattern is also expressed by juvenile laboratory rats (Olioff & Stewart, 1978; Pellis, 2002). Among rats, play-fighting was found to be rewarding, as revealed by CPP experiments (Calcagnetti & Schechter, 1992; Siviy, 1998). Physiological studies show that play-fighting is influenced by activation of perinatal steroid receptors (Meaney & Stewart, 1981; Olesen, Jessen, Auger, & Auger, 2005) and supported by reward circuits (Burgdorf, Panksepp, Beinfeld, Kroes, & Moskal, 2006; Siviy, Fleischhauer, Kerrigan, & Kuhlman, 1996; Trezza & Vanderschuren, 2008). Environmental harshness may influence the timing of play and dispersal via its effects on calorie intake, a
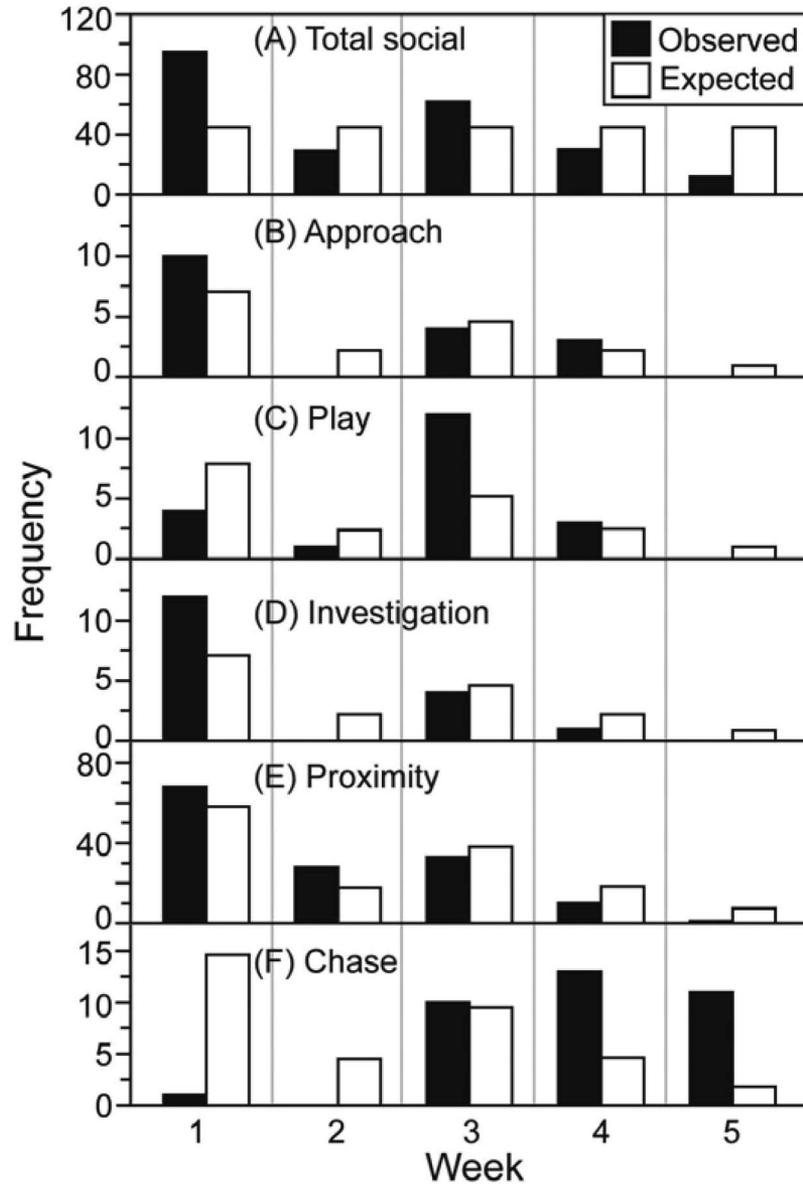
Figure 4. Social behaviors of adolescent ground squirrels in the field. Expected weekly counts of social behaviors were generated assuming an equal distribution of the total social behaviors ($n = 228$) over the 5-week observation period from late June and early August. (A) The number of social behaviors observed each week deviated from the expected values and generally decreased over the 5-week period. Social behaviors were categorized as approach, play, investigation, maintaining proximity or chasing (see Table 2), and total counts were tallied for each week. Observed behaviors in the field varied significantly from the expected values (B) approach, (C) play, (D) investigation, and (E) proximity were predominantly observed during Weeks 1–3, while (F) agonistic chasing became more frequent in Weeks 3–5. Total minutes of observation: Week 1 = 291 min, Week 2 = 357 min, Week 3 = 551 min, Week 4 = 298 min, Week 5 = 321 min. All data are presented as frequencies of occurrence.

factor that moderates play behavior in laboratory rats (Siviy & Panksepp, 1985) and free-living squirrels (Nunes et al., 1999) and can influence reward circuits (Alamy & Bengelloun, 2012; Vucetic et al., 2010).

Captive and wild squirrels expressed analogous declines in affiliative social behaviors over the summer season despite profound differences in their rearing environments. Unlike their wild counterparts, captive squirrels were provisioned with predictable food availability and quality. To maintain defined levels of social motivation during SI testing (Panksepp et al., 2008), we exposed captive squirrels to specific durations of social isolation. To eliminate possible influences of changing

adult sociality on adolescent behavior, we restricted the social environment of captive juveniles to interactions their peers. Were adults to become aggressive in response to more fully grown adolescents, these behaviors might, in turn, have promoted a shift from affiliative to agnostic behaviors among juveniles. Confining captive squirrels to "adult-free" housing, we imposed a less variable, more unnatural, social environment. In light of these considerable differences, the similar seasonal decline in affiliative social behaviors of captive and wild squirrels underscores common developmental changes. Taken together, this comparison supports the idea that factors intrinsic to the maturing 13-line ground squirrel moderate sociality, akin to the idea that an "ontogenetic switch" within the adolescent's "internal milieu" influences social development (Holekamp, 1984a, 1984b).

We also urge caution in relating social behaviors expressed in nature with those expressed in the lab. Comparative field studies indicate 13-lined ground squirrels are among the least social of the ground squirrel species (Rayor & Armitage, 1991; Wistrand, 1974). Some studies consider *I. tridecemlineatus* to be "asocial" (McCarley, 1966; and see also Luna & Baird, 2004), forming colonies not out of social attraction, rather because they share a preference for complex habitats that *reduce* social contact (McCarley, 1966). By contrast, we were impressed by the high levels of social investigation expressed by captive squirrels when contrasted with our own experience with laboratory mice. Captive squirrels spent 25–40% of a 5-min behavioral test interacting socially (see Figure 2d). This level of social investigation lies between levels expressed by a gregarious inbred laboratory mouse strain (~60%) and those of a less social strain (~20%; Fairless et al., 2008; Panksepp et al., 2007; Sankoorikal, Kaercher, Boon, Lee, & Brodkin, 2006), a comparison that highlights the confusing species- and context-specific connotations of the term "sociality." The labels "social" and "asocial" reference the overall social nature of a species but are, in fact, based upon single social phenotypes under specific environmental conditions, classifications applied to monogamous versus polygamous voles (McGraw & Young, 2010), to mouse strains expressing different levels of social approach (Moy et al., 2007), and to ground squirrels living in overlapping versus discontinuous territories. Such labels become confusing when considered in juxtaposition. Our finding of a robust expression of social reward by *I. tridecemlineatus* suggests that a comprehensive study of social CPP responses across ground squirrel species would improve our understanding of the putative relationship between social motivation and patterns of play and dispersal.

Field and laboratory studies also impose their own limitations to how we measure sociality. For instance, ultrasonic vocalizations are difficult to record in the field. By contrast, laboratory studies offer limited options for rodents to make complex social decisions and confinement to a cage can alter some (Holmes & Mateo, 2007), but not all (Mateo & Holmes, 1999a, 1999b) social phenotypes. The necessary integration of field and laboratory nomenclature for rodent social behavior (Blumstein et al., 2010; Cacioppo & Berntson, 2005) will be facilitated by replacing general terms like "social" and "asocial" with ethological terms that describe social behaviors within specific experimental, environmental and social contexts. Such refinements are critical as neuroscience focuses on viable alternatives to caged animals, constrained as they are by a poverty of affective experience and lacking the rich spatial and temporal variation essential for normal development and afforded by natural environments (Taborsky et al., 2015; Lahvis, in press).

This study provides the first demonstration of social CPP in an undomesticated rodent. When laboratory rodents prefer an environment paired with social housing, a common interpretation is that they derive a pleasurable experience from social interaction (and/or discomfort from social deprivation (Panksepp et al., 2007). Use of the CPP test has demonstrated that positive affective experiences occur during juvenile social interactions (Calcagnetti & Schechter, 1992; Douglas et al., 2004), mating opportunities (Camacho, Sandoval, & Paredes, 2004; Jenkins & Becker, 2003), access to offspring (Mattson, Williams, Rosenblatt, & Morrell, 2001), and even aggressive social interactions (Martínez, Guillen-Salazar, Salvador, & Simon, 1995; Tzschentke, 2007). Importantly, domestication removes some sources of natural selection faced by wild ancestors and can alter the social phenotypes of rodents (Künzl, Kaiser, Meier, & Sachser, 2003). Tame animals tend to be less aggressive toward conspecifics (Boreman & Price, 1972; Ebert, 1976) and more readily engage in mating opportunities without requiring mate choice (Drickamer, Gowaty, & Holmes, 2000; Manning, Potts, Wakeland, & Dewsbury, 1992; Penn & Potts, 1999). Thus, it is conceivable that responses of laboratory mice and rats to social CPP tests reflect the artificial selection and/or genetic fixation associated with domestication. Our present finding of social CPP in captive 13-lined ground squirrels argues against this, demonstrating social experience is similarly rewarding to wild-derived animals with minimal domestication—for a species not thought to be particularly social in the wild.

The possibility that positive emotions accompany social interactions in the wild may be central to answering questions about the selective advantages of animal play (Allen & Bekoff, 2005) and altruistic social behavior (Hui & Deacon, 2009; Roberts, 2005). Social isolation impairs brain development (Black & Greenough, 1998; Champagne & Curley, 2005; Wiedenmayer, 2009), immune reactivity (Boissy et al., 2007; Shanks, Renton, Zalcman, & Anisman, 1994; Tuchscherer, Kanitz, Puppe, & Tuchscherer, 2010), burn and wound healing (Detillion, Craft, Glasper, Prendergast, & DeVries, 2004; İşeri et al., 2010), response to ischemia (Norman et al., 2010), recovery from social defeat (Ruis et al., 1999), resiliency to metastasis (Wu et al., 2000), and competence in social hierarchies (van den Berg et al., 1999). These studies suggest that psychological responses to social context can moderate systemic physiology (e.g., circulating corticosteroids and proinflammatory cytokines) and influence survivability. The experience of social reward may thereby serve as a proximate substrate for cooperation among animal societies, as sufficient interdependence or a "stake" among its individuals (Roberts, 2005), one that exists irrespective of the classical requirements for the evolution of altruism (i.e., repeated interaction, recognition, and "active" reciprocity). Positive affective experiences associated with social reunion could be supportive mechanisms in this regard (Hui & Deacon, 2009), and our present study demonstrates that social reward is operational in an outbred, minimally domesticated rodent. Thus, our findings suggest that the psychological concept of social reward is deserving of ample consideration in interpreting the behavioral features of

animal societies in the wild, a "camaraderie effect," if you will, that promotes social cooperation and altruism.

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