Hormones and Behavior 58 (2010) 193–199

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Estrous phase alters social behavior in a polygynous but not a monogamous *Peromyscus* species

Kate Karelina [a,*], James C. Walton [a], Zachary M. Weil [a,1], Greg J. Norman [b], Randy J. Nelson [a,b,c], A. Courtney DeVries [a,b,c]

[a] Department of Neuroscience, Ohio State University, Columbus, OH 43210, USA
[b] Department of Psychology, Ohio State University, Columbus, OH 43210, USA
[c] Institute for Behavioral Medicine Research, Ohio State University, Columbus, OH 43210, USA

ARTICLE INFO

Article history:
Received 14 January 2010
Revised 20 March 2010
Accepted 31 March 2010
Available online 9 April 2010

Keywords:
Peromyscus californicus
Peromyscus leucopus
Social behavior
Oxytocin
Corticosterone

ABSTRACT

The social organization of rodent species determines behavioral patterns for both affiliative and agonistic encounters. The neuropeptide oxytocin has been implicated in the mediation of social behavior; however, variability in both neuropeptide expression and social behavior within a single species indicates an additional mediating factor. The purpose of the present comparative study was to investigate social behaviors in naïve mixed-sex pairs of monogamous *Peromyscus californicus* and polygynous *Peromyscus leucopus*. We identified substantial inter- and intra-specific variability in the expression of affiliative and agonistic behaviors. Although all *P. californicus* tested engaged in frequent and prolonged intervals of social contact and rarely engaged in aggressive behaviors, *P. leucopus* exhibited significant variability in both measures of social behaviors. The naturally occurring differences in social behavior displayed by *P. leucopus* vary across the estrous cycle, and correspond to hypothalamic oxytocin, as well as circulating oxytocin and glucocorticoid concentrations. These results provide evidence for a rhythm in social behavior across the estrous cycle in polygynous, but not monogamous, *Peromyscus* species.

© 2010 Elsevier Inc. All rights reserved.

Introduction

Mammals display a variety of social systems, ranging from the monogamous to highly promiscuous. Although the majority of mammalian species exhibit a polygynous social system, a substantial minority of socially monogamous mammals have been identified (Donaldson and Young, 2008). Extensive insight into the neurobiological correlates of social behavior has been made possible through comparison of closely related rodent species that exhibit different social systems (Carter et al., 1995; DeVries et al., 1995; Young, 2002; Young et al., 2001). For example, socially monogamous California mice (*Peromyscus californicus*) form long-lasting pair-bonds, engage in biparental behaviors, and exhibit aggressive behavior toward unknown same-sex conspecifics (Gubernick and Alberts, 1987). In contrast, closely related but socially polygynous white-footed mice (*Peromyscus leucopus*) typically do not form pair bonds, exhibit mostly uniparental behavior, and are less likely to engage in territorial or conspecific aggression (Bester-Meredith et al., 1999).

Studies of closely related rodent species have demonstrated a role for the neuropeptide oxytocin (OT) as an important influence in the development and maintenance of social behaviors. There are distinct differences in OT receptor (OTR) distribution between socially monogamous and polygynous species (Insel and Shapiro, 1992). Indeed, OTR density is elevated in the nucleus accumbens, lateral septum, and hypothalamus of monogamous vole species and correlates with affiliative behaviors in general and parental behaviors in particular (Francis et al., 2002; Insel and Shapiro, 1992; Ross et al., 2009). Early studies indicated that intracerebroventricular administration of OT facilitates the onset of affiliative social behaviors, including female sexual receptivity (Greer et al., 1986), parental behavior (Pedersen and Prange, 1979), and pair-bonding (Cho et al., 1999; Williams et al., 1992). In contrast, treatment with an OT receptor antagonist or antisense oligodeoxynucleotides decreases or eliminates these behaviors (McCarthy et al., 1994; Young et al., 2001), indicating a central role of OT in affiliative behavior. Converging evidence from OT knockout studies in mice provides further support for a role of OT in maintaining social behaviors; OT knockout mice fail to develop social memory, but this deficit is rescued by administration of OT (Ferguson et al., 2000).

Several of the behavioral effects of OT are modulated by ovarian steroids (Cushing and Kramer, 2005; McCarthy et al., 1994; Yamamoto et al., 2006). The expression of maternal behavior, including maternal aggression, following OT administration in rodents is facilitated by

* Corresponding author. Department of Neuroscience, Ohio State University, 750 Biomedical Research Tower, 460 W 12th Ave., Columbus, OH 43210, USA. Fax: +1 614 292 3464.
E-mail address: karelina.1@osu.edu (K. Karelina).
[1] Current Address: Laboratory of Neurobiology and Behavior and Laboratory of Neuroendocrinology, Rockefeller University, New York, NY 10065, USA.

0018-506X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2010.03.022

estrogen priming (Pedersen and Prange, 1979; Pederson, 1999), and sexual receptivity requires both estrogen and progesterone priming (Greer et al., 1986; Schumacher et al., 1990). In addition, expression of OTR mRNA and protein is regulated by estrogen, and exhibits a cyclic pattern across the rodent estrous cycle: OTR expression in the hypothalamus and uterus increases during late proestrus, coincident with peak estrogen levels (Bale et al., 1995; Larcher et al., 1995). This cyclic pattern of OTR expression is a likely mediator of social behaviors in female free-cycling rodents; for example, *P. californicus* females reduce inter-female aggression during proestrus and estrus; (Davis and Marler, 2004).

OT also suppresses the hypothalamic–pituitary–adrenal axis (Windle et al., 1997), which in turn facilitates social behavior (DeVries et al., 2007; DeVries et al., 1995). Importantly, glucocorticoid concentrations differ substantially between monogamous and polygynous vole species and play a role in pair-bond formation (DeVries et al., 2007). Corticosterone concentrations are lower in *P. leucopus* relative to *P. californicus* (Glasper and Devries, 2005), however, the role of glucocorticoids in *Peromyscus* social organization requires further clarification.

In the current comparative study, we examined the endocrine correlates of affiliative and agonistic social behaviors in polygynous *P. leucopus* and monogamous *P. californicus*. In a behavioral analysis of social contact between mixed-sex pairs, we identified substantial intra-specific variability among *P. leucopus*, but not *P. californicus,* on both affiliative and agonistic measures, as well as the relationships between OT, corticosterone and behavior. Further assessment revealed a significant influence of estrous phase on *P. leucopus* behavior.

Methods

Animals

Adult male and female *P. californicus* and *P. leucopus* (60–70 days old) were bred from stock received from the *Peromyscus* Genetic Stock Center (University of South Carolina, Columbia, SC). The mice were maintained on a 14:10 light/dark cycle in a temperature and humidity-controlled vivarium with *ad libitum* access to food and water. Following weaning, all mice were individually housed until initiation of the study. This study and animal care was conducted in accordance with National Institutes of Health guidelines for the care and use of animals and under protocols approved by the Ohio State University institutional animal care and use committee.

Experimental procedures

In both experiments social behaviors were measured and compared to oxytocin immunoreactivity (OT-ir), as well as circulating OT and corticosterone (CORT) concentrations. In Experiment 1, mice were paired (*P. leucopus*, $n=10$ pairs; *P. californicus,* $n=5$ pairs) in a neutral cage and videotaped for 3 h. An additional cohort of male and female mice of both species were placed individually (control group) in a neutral cage for 3 h, as a means of controlling for handling and exposure to a novel cage (*P. leucopus,* $n=6$ males, $n=7$ females; *P. californicus,* $n=7$ males, $n=7$ females). In Experiment 2, phase of the estrous cycle was determined for a period of 7 days prior to pairing for 3 h (*P. leucopus,* $n=35$ pairs; *P. californicus,* $n=20$ pairs). Hormone analysis was only conducted in females in Experiment 2.

Behavioral testing

Social interactions were assessed in naïve mixed-sex pairs of both species of *Peromyscus*. Pairs were placed in a clean mouse cage, and videotaped for 3 h. Latency to first contact, total duration of side-by-side contact, frequency of contact, and number of aggressive bouts were hand scored for each pair by experimentally uninformed observers. Side-by-side contact was scored as physical contact, and thus excluded investigation behaviors.

Estrous cycle determination

Female mice were assessed for estrous cycle phase via daily vaginal lavage for 7 consecutive days prior to behavioral analysis. Multiple lavages were necessary for accurate assessment of estrous phase on the day of testing. A final lavage was performed immediately prior to tissue collection. Briefly, vaginal lavage was performed by using 50 μL of sterile saline which was dispensed into the vagina using a sterile plastic round-tipped pipette. The vaginal wall was washed and saline collected on slides for analysis. Collected cells were stained with hemotoxylin and eosin, dehydrated and coverslipped. Estrous phase was determined by leukocyte and epithelial cell number and morphology as previously described (Goldman et al., 2007).

Tissue collection

Immediately following behavioral testing, mice were treated with an overdose of sodium pentobarbital; once non-responsive to tactile stimuli, a blood sample was collected from the retro-orbital sinus. Blood sampling was completed within 2 min of handling. The mice were then transcardially perfused with 0.1 M phosphate buffered saline (PBS) followed by 4% paraformaldehyde in 0.1M PBS. Brains were post-fixed for 4 h in 4% paraformaldehyde and cryoprotected in 30% sucrose in 0.2 M phosphate buffer. Brains were frozen on crushed dry ice, embedded in mounting medium (Electron Microscopy Sciences, Hatfiled, PA), and 14 μM sections through the hypothalamus including the paraventricular (PVN) and supraoptic nucleus (SON) were cut and thaw mounted onto slides.

Histology

After drying at room temperature, slides were treated with proteinase K (20 μg/mL) for 10 min at 37 °C for antigen retrieval. Slides were then quenched in 3% hydrogen peroxide, rinsed in 0.1M PBS, and incubated in 0.5% Triton-X and 4% normal goat serum in 0.1 M PBS. Rabbit anti-oxytocin antibody (1:5000; Millipore, Temecula, CA) was then added to the slides and incubated overnight at room temperature. The following day, slides were rinsed in 0.1 M PBS and incubated in goat anti-rabbit secondary antibody (1:500, company, place) for 2 h at room temperature. Slides were then rinsed, incubated in ABC (Vector labs, Burlingame, CA) and visualized with DAB. Finally, slides were dehydrated through a series of graded ethanol solutions followed by xylene and then coverslipped using Permount. Control sections, in which primary antibody was omitted, showed no staining. Oxytocin-positive cells were counted in one hemisphere and averaged through the two most abundantly stained sections of the PVN and SON (at least 50 μm apart). (see Fig. 1)

Preparation of blood samples for oxytocin and corticosterone radioimmunoassays

Blood samples were collected from the retro-orbital sinus into microcentrifuge tubes. The samples were centrifuged at 6000 rpm for 30 min at 4 °C; sera were stored at −80 °C until assayed. Serum OT concentrations were determined by using an I^{125} OT kit (Phoenix Pharmaceuticals, Inc, Burlingame, CA) according to kit instructions. The standard curve and samples were run in duplicate. The cross reactivity of the assay is 100% for oxytocin and 0% for [Arg^8]-Vasopressin. All samples within an experiment were run in a single assay, the intra-assay variability was 2.48%.

Corticosterone concentrations were determined by using an I^{125} CORT kit (MP Biomedical, Solon, OH) according to kit instructions. The standard curve was run in triplicate and samples were run in

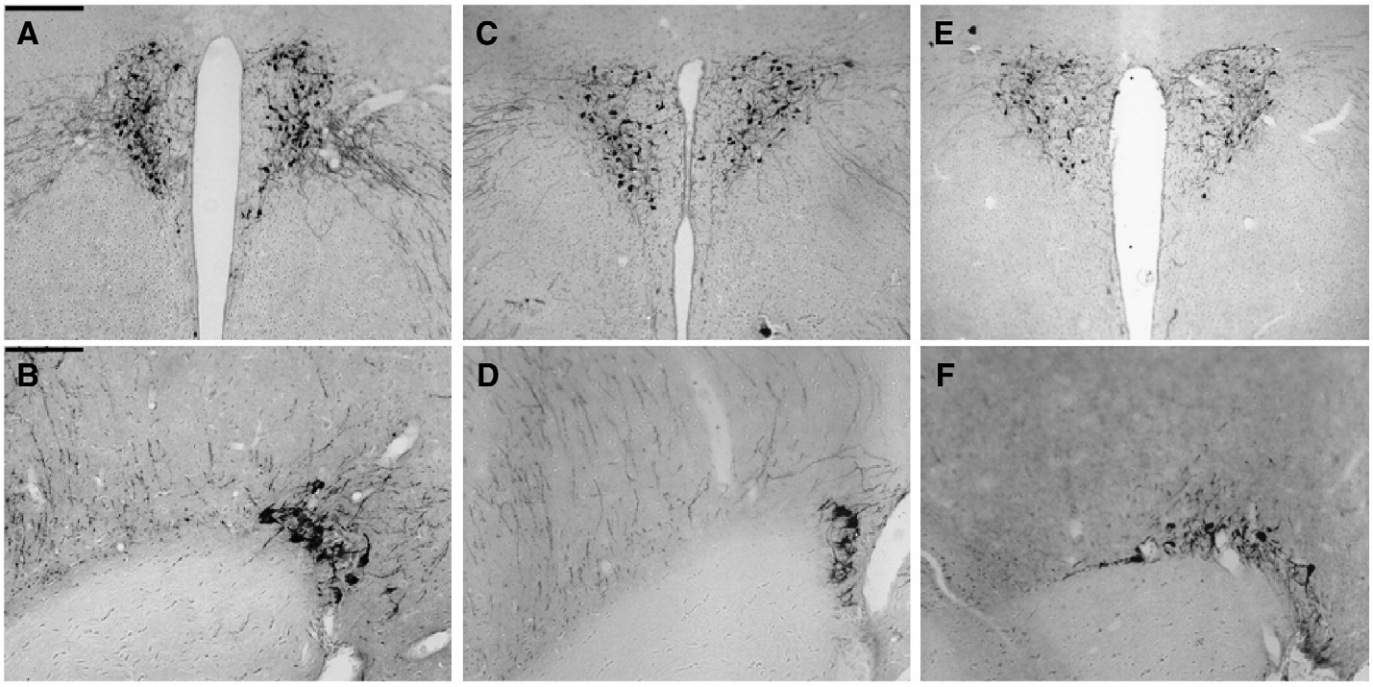

Fig. 1. *Oxytocin immunohistochemistry.* Representative photomicrographs of OT-ir cells in the PVN (top row) and SON (bottom row) of *P. leucopus* (A-D) and *P. californicus* (E, F) females. *P. leucopus* panels are representative of OT-ir in females of a pair that exhibited prolonged duration of social contact (A-B) and a brief duration of social contact (C, D). Scale bars: A = 200 μm, in B = 100 μm.

duplicate. All samples in an experiment were run in a single assay, the intra-assay variability was 7.29%.

Data analysis

In Experiment 1, behavioral data were compared using a 1-way ANOVA (factor was species) and endocrine data were analyzed using a 2-way ANOVA [factors were species and social factor (control vs. paired)]. Pearson's rank correlations were also performed within each species for analysis of the relationship between behavioral and endocrine endpoints. 2-way ANOVA analyses (factors were species and estrous phase) were conducted on behavioral data in Experiment 2. Endocrine data were compared using 1-way ANOVAs both between (factor was species) and within species (factor was estrous phase). Significant overall ANOVA results ($p<0.05$) were followed by a Tukey HSD post hoc test. Group differences were considered statistically significant at $p<0.05$.

Results

Experiment 1

Behavioral assessment

A direct comparison of social behaviors (latency to first approach, number of approaches, duration of side-by-side contact, and number of aggressive bouts) did not reveal significant main effect of species (all $p>0.05$); however, these results are most likely accounted for by the substantial variability within and between species. During the 3-h social interaction test, *P. californicus* pairs remained in physical contact for 125.50 min (median), and engaged in few aggressive bouts. In contrast, *P. leucopus* pairs exhibited substantial variability in both measures of social behaviors (Fig. 2A–B). A Pearson's correlation analysis was performed as a post hoc analysis because of the degree of variability found in *P. leucopus* behavior and revealed that duration of contact was related to other measures of social behaviors in *P. leucopus*, including number of approaches ($r=0.946$, $p<0.01$) and number of aggressive bouts ($r=-0.738$, $p<0.05$), but not approach latency ($p>0.05$). Interestingly, *P. californicus* do not display substantial variability in these measures of social behavior and thus duration of contact was not significantly correlated with any of these measures of social behavior (all $p>0.05$).

Endocrine analysis

Hypothalamic OT, as well as circulating OT and CORT, concentrations were assessed in male and female *P. leucopus* and *P. californicus* (Table 1). A 2-way ANOVA (species by social factor) revealed a significant main effect of species for both OT and CORT. Circulating OT concentrations were significantly elevated in *P. leucopus* females ($F_{1,23}=8.249$, $p<0.05$) and males ($F_{1,26}=12.956$, $p<0.01$) compared to *P. californicus*. Serum CORT concentrations were significantly lower in *P. leucopus* in both females ($F_{1,24}=10.596$, $p<0.01$) and males ($F_{1,23}=12.388$, $p<0.01$) compared to *P. californicus*. In the hypothalamus, the number of OT-positive cells in the SON was substantially increased in female ($F_{1,28}=5.375$, $p<0.05$) but not male *P. leucopus* compared to *P. californicus*. There were no species differences in number of PVN OT-ir cells (all $p>0.05$). There was also no main effect of social factor (pairing vs. control) on any of the endocrine measures (all $p>0.05$).

Finally, a Pearson's correlation analysis was performed to assess endocrine-behavioral correlates within each species. Among *P. leucopus* females, behavioral measures significantly correlated with circulating CORT and hypothalamic OT. Specifically, the number of SON OT-ir cells in female *P. leucopus* significantly correlated to number of approaches ($r=0.760$, $p<0.05$) during 3 h of pairing. Further, there was a negative correlation between SON OT-ir and circulating CORT of female *P. leucopus* ($r=-0.567$, $p<0.05$), thus it is unsurprising that circulating CORT in turn correlates to approach behavior. Specifically, increasing concentrations of circulating CORT are related to increased latency to first approach ($r=0.863$, $p<0.05$) among *P. leucopus*. Interestingly, endocrine measures in male *P. leucopus* did not relate significantly to any of the observed behaviors (all $p>0.05$). Behavioral assessment in *P. californicus* did not reveal significant correlations with any of the endocrine measures (all $p>0.05$).

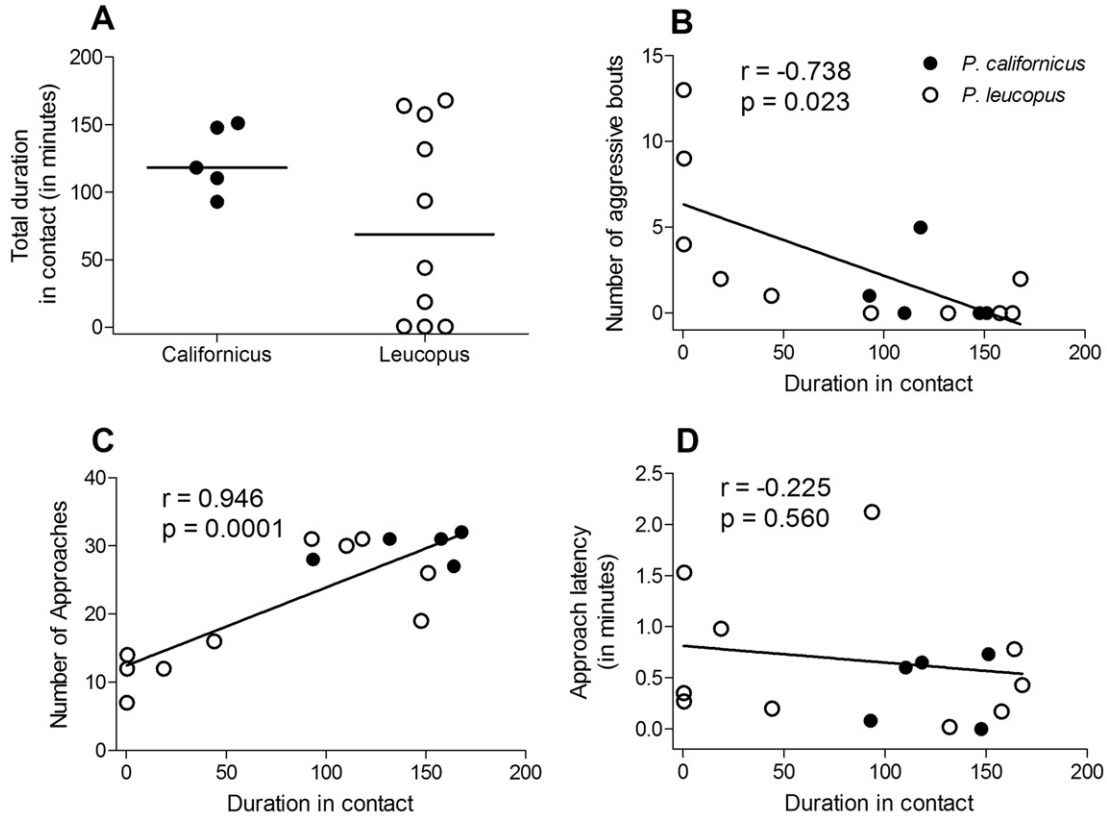

Fig. 2. Social behaviors and endocrine measures among naïve mixed-sex *P. leucopus* and *P. californicus* pairs. *P. leucopus* and *P. californicus* exhibit marked differences in total duration of side-by-side contact in a 3-h period; (A) *P. californicus* pairs cluster around the median and *P. leucopus* exhibit substantial variability, the line indicates median duration in contact for each species. Further, while *P. californicus* exhibit little variability in social behaviors, *P. leucopus* show a range of aggressive (B), and approach (C) behaviors which correlate with duration of contact. Approach latency (D) does not correlate to duration of contact in either species. Correlation and *p*-values are shown for *P. leucopus*.

Experiment 2

Behavioral assessment

Experiment 2 was designed to test the hypothesis that the variability in social behaviors among the polygynous *P. leucopus* varies across the estrous cycle (Fig. 3). An overall 2-way ANOVA revealed a main effect of species for frequency and duration of side-by-side contact, as well as number of aggressive bouts. Specifically, overall *P. leucopus* exhibited fewer approaches ($F_{1,55} = 30.643$, $p < 0.001$), maintained a significantly shorter duration of side-by-side contact ($F_{1,56} = 70.353$, $p < 0.001$), and more frequently engaged in aggressive bouts ($F_{1,53} = 21.110$, $p < 0.05$), compared to *P. californicus.* Duration of side-by-side contact was also assessed across each 30-min bin over the 3-h period of social interaction. Duration of contact among *P. californicus* did not vary throughout the estrous cycle at any time point (all $p > 0.05$), in fact, *P. californicus* exhibited very little variance in total duration of contact in each estrous phase ($p = 0.926$). In contrast, *P. leucopus* pairs exhibited an increase in affiliative social behaviors during diestrus and proestrus, but engaged in limited social contact during estrus and metestrus. These estrous cycle-mediated differences in social behavior among *P. leucopus* emerge by 150 min ($F_{3,29} = 3.444$, $p < 0.05$) and remain evident through 180 min ($F_{3,29} = 4.039$, $p < 0.05$) of pairing. Although there was not a main effect of estrous phase on social behaviors, duration of contact in the last 30 min of the 3-h social interaction test revealed an interaction of species by estrous phase ($F_{3,48} = 2.901$, $p < 0.05$), whereby *P. leucopus* in estrus or metestrus engaged in the shortest duration of contact compared to all other pairs tested (Tukey post-hoc, $p < 0.05$).

Table 1
Endocrine correlates of social behavior in *P. californicus* and *P. leucopus*.

	SON OT-ir cells	PVN OT-ir cells	Serum OT (pg/mL)	Serum CORT (pg/mL)
Control groups				
Californicus F	1.86 (0.86)	14.93 (2.64)	67.84 (59.15)	1337.81 (174.65)
Californicus M	4.25 (1.18)	15.92 (2.51)	10.74 (5.52)	1293.49 (306.63)
Leucopus F	1.57 (0.30)	13.36 (2.13)	181.96 (43.81)*	643.33 (141.52)*
Leucopus M	1.30 (0.73)	14.50 (2.09)	284.90 (44.72)*	477.55 (63.51)*
Paired groups				
Californicus F	1.30 (0.66)	16.80 (2.54)	8.95 (3.63)	1047.75 (198.52)
Californicus M	1.86 (1.09)	17.13 (2.79)	19.20 (7.22)	1190.04 (304.145)
Leucopus F	4.75 (1.02)*	14.63 (1.26)	129.05 (31.76)*	677.97 (107.51)*
Leucopus M	3.05 (1.15)	14.33 (1.20)	161.03 (44.20)*	442.24 (52.07)*

Shown are number of OT-ir cells in supraoptic (SON) and paraventricular (PVN) nuclei, as well as serum OT and CORT concentrations in male (M) and female (F) *P. californicus* and *P. leucopus* that are either individually housed (control) or pair housed with an animal of the opposite sex. The data are presented as mean (SEM). An asterisk (*) indicates that the group is significantly different from *P. californicus*, $p < 0.05$.

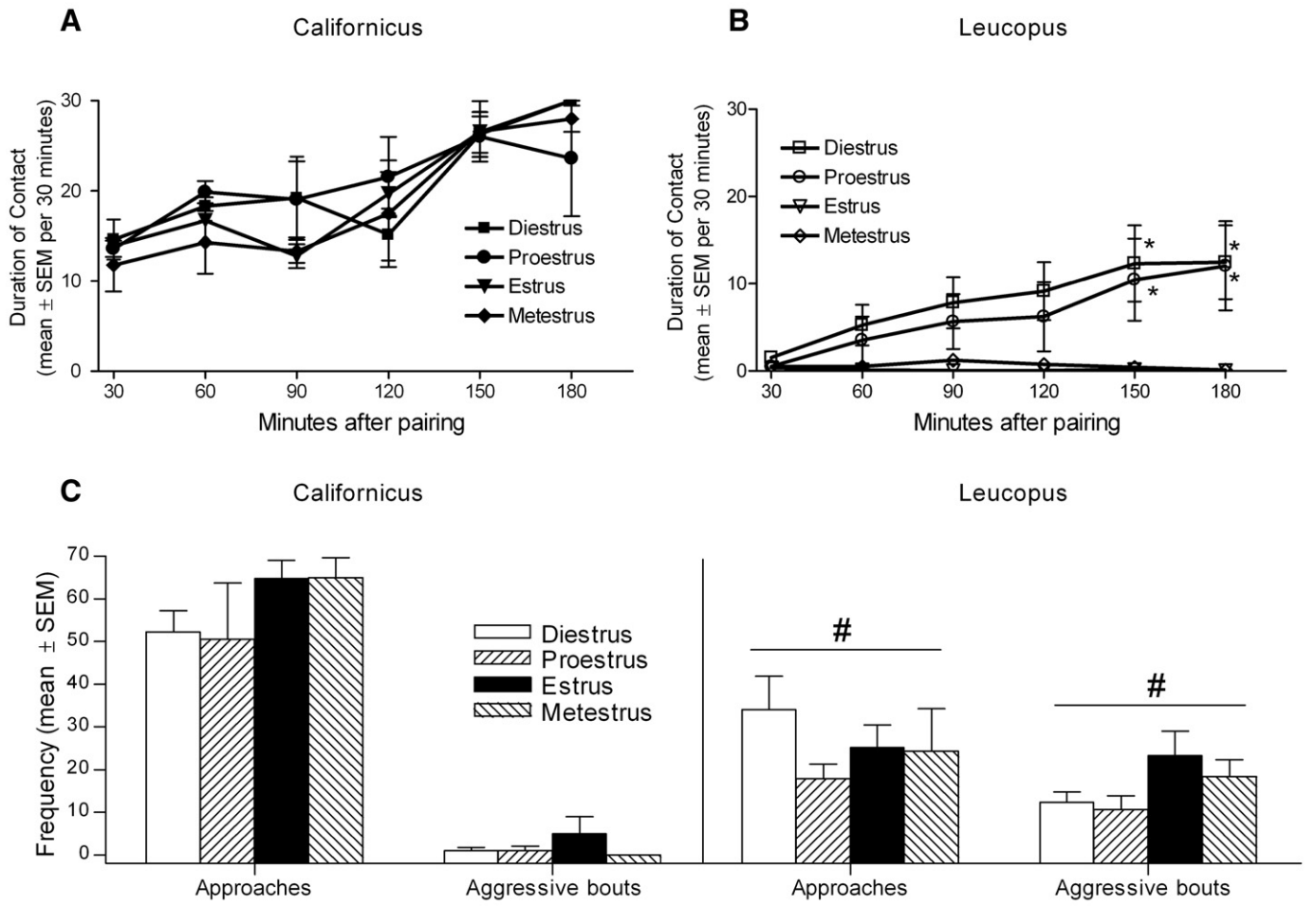

Fig. 3. *Social behaviors of P. leucopus and P. californicus across the estrous cycle.* A) Duration of contact does not vary across the estrous cycle in *P. californicus*, B) but differs throughout the estrous cycle of *P. leucopus.* C) Frequency of approach and aggressive bouts do not vary across the estrous cycle in either species. An asterisk (*) indicates that the group is significantly different from *P. leucopus* in estrus and metestrus as well as all *P. californicus* groups , a pound sign (#) indicates that the group is different from *P. californicus* on each measure as indicated; $p<0.05$.

Endocrine analysis

There were no significant group differences in PVN OT-ir, however, SON OT-ir varied significantly across the estrous cycle in both *P. leucopus* ($F_{3,29}=3.359$, $p<0.05$) and *P. californicus* ($F_{3,19}=4.764$, $p<0.05$); relative to metestrus, OT-ir was significantly elevated during proestrus among *P. californicus* and during diestrus among *P. leucopus*. As in Experiment 1, serum OT concentrations were elevated among *P. leucopus* compared to *P. californicus* ($F_{1,49}=35.450$, $p<0.001$). Serum CORT concentrations were lower among *P. leucopus* compared to *P. californicus* ($F_{1,44}=54.694$, $p<0.001$), however, there were no significant differences in serum CORT concentrations across the estrous cycle in either species ($p>0.05$). (See Table 2).

Discussion

Members of the genus *Peromyscus* provide a valuable model for the study of affiliative and agonistic social behaviors. Although closely related, several of the *Peromyscus* species display marked differences

Table 2
Endocrine correlates of social behavior across the estrous cycle.

	SON OT-ir cells	PVN OT-ir cells	Serum OT (pg/mL)	Serum CORT (pg/mL)
P. californicus				
Diestrus	2.13 (0.31)	13.63 (3.20)	61.77 (35.99)†	1396.18 (296.86)†
Proestrus	7.66 (1.59)*	10.75 (6.75)	14.10 (3.46)†	1670.50 (106.44)†
Estrus	1.50 (.50)	29.75 (7.75)	46.93 (14.16)†	1712.12 (72.14)†
Metestrus	2.67 (1.92)	23.00 (3.50)	85.30 (57.62)†	1891.69 (216.43)†
P. leucopus				
Diestrus	6.81 (0.92) #	16.55 (1.84)	845.87 (125.19)	710.66 (34.76)
Proestrus	6.21 (1.01)	13.89 (2.07)	360.73 (105.95)	613.04 (117.64)
Estrus	4.25 (1.18)	13.30 (1.18)	687.05 (278.29)	908.62 (229.65)
Metestrus	2.38 (0.94)	15.75 (4.83)	581.87 (106.12)	700.37 (143.01)

Shown are number of OT-ir cells in supraoptic (SON) and paraventricular (PVN) nuclei, as well as serum OT and CORT concentrations across the estrous cycle in *P. californicus* and *P. leucopus*. An asterisk (*) indicates that the group is significantly different from *P. californicus* in diestrus; a dagger (†) indicates that the group is significantly different from all P. leucopus; a pound sign (#) indicates that the group is significantly different from *P. leucopus* in metestrus, $p<0.05$. The data are presented as mean (SEM).

in social structure (Bester-Meredith et al., 2005; Bester-Meredith et al., 1999). The current study provides further evidence for social behavior differences between *P. californicus* and *P. leucopus*, as well as marked variability within *P. leucopus.* This study confirms the pro-social phenotype of monogamous mammalian species (Shapiro and Dewsbury, 1990), whereby mixed-sex *P. californicus* pairs engage in frequent and prolonged affiliative contact and infrequent bouts of aggression with minimal variability. In contrast, *P. leucopus* display substantial variability in patterns of social behavior. Indeed, during a 3-h period of social interaction individual *P. leucopus* vary between spending 0 to 167 min of side-by-side contact. Moreover, duration in contact significantly correlated to other social measures, including number of approaches and aggressive bouts among *P. leucopus* but not *P. californicus* pairs.

The disparate behavioral response of *P. leucopus* to social cues may be related to cyclic hormonal fluctuations. Hypothalamic OT in female *P. leucopus* (particularly in the SON) correlated significantly to number of approaches exhibited during the 3-h social interaction. This relationship may represent a surge in OT in response to social cues; however, it is also possible that these data reflect a natural variation of OT, which then drives the behavioral response. The high variability of SON OT-ir in unpaired, control *P. leucopus* females indicates a substantial fluctuation of OT in the absence of social cues. Elevated central OT (i.e. following exogenous administration of OT) induces a pro-social behavioral phenotype (Cho et al., 1999; Williams et al., 1992; Witt et al., 1992), thus, elevated endogenous OT prior to and during social interaction potentially contributed to increased affiliative social contact in *P. leucopus.*

One factor that likely accounts for the high variability of OT is that synthesis and release of OT fluctuates with the estrous cycle, as central OT concentrations increase with increasing estrogen and progesterone during proestrus (Greer et al., 1986; Van et al., 1988). Indeed, data in the current study support the assertion that the estrous cycle influences social behavior in *P. leucopus*. *Peromyscus leucopus* pairs were more likely to engage in affiliative contact when the female was in diestrus or proestrus, and engaged in minimum affiliative contact during estrus and metestrus (Fig. 3B). This behavioral pattern is consistent with previous reports, whereby increased display of affiliative social behaviors coincides with increasing estrogen and progesterone during behavioral estrus (proestrus; Root, 2005). Additionally, the peak of hypothalamic OT occurred during diestrus in *P. leucopus*. Estrogenic control of social behaviors is likely mediated at least in part via regulation of OT gene expression. Estrogen administration induces OT production and increases OTR transcription in the hypothalamus and amygdala, regions that play an important role in social behaviors including reproductive (Lee and Pfaff, 2008) and maternal (Numan, 2007) behaviors, as well as social recognition (Ferguson et al., 2000). Indeed, the social phenotypes of estrogen receptor-α and -β knockout mice and OT knockout mice are similar (Choleris et al., 2006). Furthermore, the effect of OT on inducing social behaviors is abolished by ovariectomy and reinstated by estrogen treatment (Pedersen and Prange, 1979).

In contrast to *P. leucopus*, *P. californicus* did not exhibit any observable variability in affiliative behaviors throughout the estrous cycle (Fig. 3A, C). It is relatively clear that natural selection has favored a coordination of reproductive neuroendocrine systems with social behavior in female *P. leucopus* such that close social contact is only tolerated when the animals are capable of being impregnated. On the other hand, *P. californicus* exhibit high levels of social contact across the estrous cycle, which is consistent with reports that ovarian steroids do not appear to be essential for social behaviors in monogamous species (Cushing and Carter, 1999; Dluzen and Carter, 1979). For example, unlike rats and mice (reviewed in Blaustein, 2008), prairie vole OTR distribution does not appear to be sensitive to ovarian steroids (Witt et al., 1991) and OT-mediated induction of sexual receptivity in prairie voles is not eliminated by ovariectomy (Cushing and Carter, 1999). These data suggest that among pair bonding species there may be advantages to engaging in close social contact, even when breeding on that day is not possible. Future studies are underway to determine the specific role of ovarian steroids in estrous-mediated behavioral fluctuations in *Peromyscus* species.

In both experiments, *P. californicus* consistently had lower serum OT concentrations relative to *P. leucopus* (Tables 1 and 2). Although central OT signaling is necessary for the formation of pair bonding in female voles, among other species the relationship between mating system and circulating OT concentrations is not always consistent. For instance, circulating OT concentrations are much higher in socially monogamous prairie voles than in polygynous Sprague–Dawley rats (Cushing and Kramer, 2005), but OT concentrations are higher in socially isolated prairie voles as compared to those that have been pair housed (Grippo et al., 2007). In the current study, lower serum OT in monogamous *P. californicus* may reflect OTR distribution differences as have been reported between monogamous and polygynous species (Hammock and Young, 2006; Insel and Shapiro, 1992; Witt et al., 1991). Further, profound differences in sensitivity to OT have been reported, whereby unlike monogamous prairie voles, polygynous motane voles do not increase affiliative behaviors following OT infusion, even at high doses (Winslow et al., 1993). These data demonstrate that OTR sensitivity and ability to respond to OT can differ profoundly among species. In any case, circulating OT provides only an indirect index of central OT signaling and sensitivity to central oxytocinergic ligands (Ludwig and Leng, 2006). Therefore, the lower circulating OT in *P. californicus* does not necessarily conflict with a causal role for OT signaling in mediating species differences in social behaviors.

Further, CORT may represent an additional proximate mediator of social behavior in this species. OT is well known for its capacity as an anxiolytic neuropeptide by attenuation of corticotrophin-releasing hormone-mediated adrenocorticotropin hormone release from the pituitary (Neumann et al., 2000). This may represent a causal relationship whereby OT influences social behaviors via suppression of circulating glucocorticoids. Indeed, serum CORT concentrations were negatively correlated with SON OT among *P. leucopus*, and in turn attenuated levels of circulating CORT were related to shorter latencies to first approach during the social interaction task. In contrast, CORT concentrations were consistently higher in *P. californicus* relative to *P. leucopus*. Glucocorticoid concentrations are similarly elevated in monogamous prairie voles relative to polygynous montane voles and rats (Taymans et al., 1997) and play a causal role in the formation of pair bonds (DeVries, 2002; DeVries et al., 1995). The current data provide further evidence of the involvement of the HPA axis in mediating social behavior.

Taken together these data provide strong evidence for a rhythm in social behavior across the estrous cycle in female *P. leucopus* but not *P. californicus.* Further, a role for ovarian hormones in the regulation of central oxytocin signaling is implicated as a potential mediator of this phenomenon. Future studies will examine the ultimate consequences of these behaviors and further identify the proximate mediators that subserve this rhythm.

Acknowledgments

The authors thank Jackie Wells and Megan Cochran for technical support.

References

Bale, T.L., Dorsa, D.M., Johnston, C.A., 1995. Oxytocin receptor mRNA expression in the ventromedial hypothalamus during the estrous cycle. J. Neurosci. 15 (7 Pt. 1), 5058–5064.

Bester-Meredith, J.K., Martin, P.A., Marler, C.A., 2005. Manipulations of vasopressin alter aggression differently across testing conditions in monogamous and non-monogamous Peromyscus mice. Aggress. Behav. 31 (2), 189–199.

Bester-Meredith, J.K., Young, L.J., Marler, C.A., 1999. Species differences in paternal behavior and aggression in Peromyscus and their associations with vasopressin immunoreactivity and receptors. Horm. Behav. 36 (1), 25–38.
Blaustein, J.D., 2008. Neuroendocrine regulation of feminine sexual behavior: lessons from rodent models and thoughts about humans. Annu. Rev. Psychol. 59, 93–118.
Carter, C.S., DeVries, A.C., Getz, L.L., 1995. Physiological substrates of mammalian monogamy: the prairie vole model. Neurosci. Biobehav. Rev. 19 (2), 303–314.
Cho, M.M., DeVries, A.C., Williams, J.R., Carter, C.S., 1999. The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (*Microtus ochrogaster*). Behav. Neurosci. 113 (5), 1071–1079.
Choleris, E., Ogawa, S., Kavaliers, M., Gustafsson, J.A., Korach, K.S., Muglia, L.J., Pfaff, D.W., 2006. Involvement of estrogen receptor alpha, beta and oxytocin in social discrimination: a detailed behavioral analysis with knockout female mice. Genes Brain Behav. 5 (7), 528–539.
Cushing, B.S., Carter, C.S., 1999. Prior exposure to oxytocin mimics the effects of social contact and facilitates sexual behaviour in females. J. Neuroendocrinol. 11 (10), 765–769.
Cushing, B.S., Kramer, K.M., 2005. Mechanisms underlying epigenetic effects of early social experience: the role of neuropeptides and steroids. Neurosci. Biobehav. Rev. 29 (7), 1089–1105.
Davis, E.S., Marler, C.A., 2004. c-fos changes following an aggressive encounter in female California mice: a synthesis of behavior, hormone changes and neural activity. Neuroscience 127 (3), 611–624.
DeVries, A., 2002. Interaction among social environment, the hypothalamic–pituitary–adrenal axis, and behavior. Horm. Behav. 41 (4), 405–413.
DeVries, A.C., DeVries, M.B., Taymans, S., Carter, C.S., 1995. Modulation of pair bonding in female prairie voles (Microtus ochrogaster) by corticosterone. Proc. Natl. Acad. Sci. U. S. A. 92 (17), 7744–7748.
DeVries, A.C., Craft, T.K., Glasper, E.R., Neigh, G.N., Alexander, J.K., 2007. 2006 Curt P. Richter award winner: Social influences on stress responses and health. Psychoneuroendocrinology 32 (6), 587–603.
Dluzen, D.E., Carter, C.S., 1979. Ovarian hormones regulating sexual and social behaviors in female prairie voles, Microtus ochrogaster. Physiol. Behav. 23 (3), 597–600.
Donaldson, Z.R., Young, L.J., 2008. Oxytocin, vasopressin, and the neurogenetics of sociality. Science 322 (5903), 900–904.
Ferguson, J.N., Young, L.J., Hearn, E.F., Matzuk, M.M., Insel, T.R., Winslow, J.T., 2000. Social amnesia in mice lacking the oxytocin gene. Nat. Genet. 25 (3), 284–288.
Francis, D.D., Young, L.J., Meaney, M.J., Insel, T.R., 2002. Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: gender differences. J. Neuroendocrinol. 14 (5), 349–353.
Glasper, E.R., Devries, A.C., 2005. Social structure influences effects of pair-housing on wound healing. Brain Behav. Immun. 19 (1), 61–68.
Goldman, J.M., Murr, A.S., Cooper, R.L., 2007. The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies. Birth Defects Res. B. Dev. Reprod. Toxicol. 80 (2), 84–97.
Greer, E.R., Caldwell, J.D., Johnson, M.F., Prange, A.J., Pedersen, C.A., 1986. Variations in concentration of oxytocin and vasopressin in the paraventricular nucleus of the hypothalamus during the estrous cycle in rats. Life Sci. 38 (25), 2311–2318.
Grippo, A.J., Gerena, D., Huang, J., Kumar, N., Shah, M., Ughreja, R., Sue, C.S., 2007. Social isolation induces behavioral and neuroendocrine disturbances relevant to depression in female and male prairie voles. Psychoneuroendocrinology 32 (8-10), 966–980.
Gubernick, D.J., Alberts, J.R., 1987. The biparental care system of the California mouse, Peromyscus californicus. J. Comp. Psychol. 101 (2), 169–177.
Hammock, E.A., Young, L.J., 2006. Oxytocin, vasopressin and pair bonding: implications for autism. Philos. Trans. R Soc. Lond. B. Biol. Sci. 361 (1476), 2187–2198.
Insel, T.R., Shapiro, L.E., 1992. Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. Proc. Natl. Acad. Sci. U. S. A. 89 (13), 5981–5985.
Larcher, A., Neculcea, J., Breton, C., Arslan, A., Rozen, F., Russo, C., Zingg, H.H., 1995. Oxytocin receptor gene expression in the rat uterus during pregnancy and the estrous cycle and in response to gonadal steroid treatment. Endocrinology 136 (12), 5350–5356.
Lee, A.W., Pfaff, D.W., 2008. Hormone effects on specific and global brain functions. J. Physiol. Sci.: JPS 58 (4), 213–220.
Ludwig, M., Leng, G., 2006. Dendritic peptide release and peptide-dependent behaviours. Nat. Rev. Neurosci. 7 (2), 126–136.
McCarthy, M.M., Kleopoulos, S.P., Mobbs, C.V., Pfaff, D.W., 1994. Infusion of antisense oligodeoxynucleotides to the oxytocin receptor in the ventromedial hypothalamus reduces estrogen-induced sexual receptivity and oxytocin receptor binding in the female rat. Neuroendocrinology 59 (5), 432–440.
Neumann, I.D., Krömer, S.A., Toschi, N., Ebner, K., 2000. Brain oxytocin inhibits the(re) activity of the hypothalamo-pituitary-adrenal axis in male rats: involvement of hypothalamic and limbic brain regions. Regul.Pept. 96 (1-2), 31–38.
Numan, M., 2007. Motivational systems and the neural circuitry of maternal behavior in the rat. Dev. Psychobiol. 49 (1), 12–21.
Pederson, C.A., 1999. Oxytocin control of maternal behavior: regulation by sex steroids and offspring stimuli. In: Carter, S.C., Lederhendler, I.I., Kirkpatrick, B. (Eds.), The integrative neurobiology of affiliation. MIT Press, pp. 301–320.
Pedersen, C.A., Prange Jr, A.J., 1979. Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin. Proc. Natl. Acad. Sci. U. S. A. 76 (12), 6661–6665.
Root, K.M.V., 2005. Reproductive behavior of small animals. Theriogenology 64 (3), 734.
Ross, H.E., Freeman, S.M., Spiegel, L.L., Ren, X., Terwilliger, E.F., Young, L.J., 2009. Variation in oxytocin receptor density in the nucleus accumbens has differential effects on affiliative behaviors in monogamous and polygamous voles. J. Neurosci. 29 (5), 1312–1318.
Schumacher, M., Coirini, H., Pfaff, D.W., McEwen, B.S., 1990. Behavioral effects of progesterone associated with rapid modulation of oxytocin receptors. Science 250 (4981), 691–694.
Shapiro, L.E., Dewsbury, D.A., 1990. Differences in affiliative behavior, pair bonding, and vaginal cytology in two species of vole (*Microtus ochrogaster* and *M. montanus*). J. Comp. Psychol. 104 (3), 268–274.
Taymans, S.E., DeVries, A.C., DeVries, M.B., Nelson, R.J., Friedman, T.C., Castro, M., Detera-Wadleigh, S.D.W., Carter, C.S., Chrousos, G.P., 1997. The hypothalamic–pituitary–adrenal axis of prairie voles (Microtus ochrogaster): evidence for target tissue glucocorticoid resistance. Gen. Comp. Endocr. 106 (1), 48–61.
Van, T.O.L., Hm, H., Bolwerk, E.L.M., Liu, B.I.N., Burbach, J.P.H., 1988. Oxytocin and vasopressin gene expression in the hypothalamo-neurohypophyseal system of the rat during the estrous cycle, pregnancy, and lactation. Endocrinology 122 (3), 945–951.
Williams, J.R., Carter, C.S., Insel, T., 1992. Partner preference development in female prairie voles is facilitated by mating or the central infusion of oxytocin. Ann. N.Y. Acad. Sci. 652, 487–489.
Windle, R.J., Shanks, N., Lightman, S.L., Ingram, C.D., 1997. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. Endocrinology 138 (7), 2829–2834.
Winslow, J.T., Shapiro, L., Carter, C.S., Insel, T., 1993. Oxytocin and complex social behavior: species comparisons. Psychopharmacol Bull. 29, 409–414.
Witt, D.M., Carter, C.S., Insel, T.R., 1991. Oxytocin receptor binding in female prairie voles: endogenous and exogenous oestradiol stimulation. J. Neuroendocrinol. 3 (2), 155–161.
Witt, D.M., Winslow, J.T., Insel, T.R., 1992. Enhanced social interactions in rats following chronic, centrally infused oxytocin. Pharmacol. Biochem. Behav. 43 (3), 855–861.
Yamamoto, Y., Carter, C.S., Cushing, B.S., 2006. Neonatal manipulation of oxytocin affects expression of estrogen receptor alpha. Neuroscience 137 (1), 157–164.
Young, L.J., 2002. The neurobiology of social recognition, approach, and avoidance. Biol. Psychiatry 51 (1), 18–26.
Young, L.J., Lim, M.M., Gingrich, B., Insel, T.R., 2001. Cellular mechanisms of social attachment. Horm. Behav. 40 (2), 133–138.