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PROSOCIAL EFFECTS OF PROLACTIN IN MALE RATS: SOCIAL RECOGNITION, SOCIAL APPROACH AND SOCIAL LEARNING

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Abstract

Prolactin (PRL) and oxytocin (OT) are pituitary hormones essential for lactation, but also promote sexual behavior. OT stimulates social behaviors, such as recognition, approach, and learning, but less is known about PRL in these behaviors. Since PRL and OT have complementary functions in reproduction, we hypothesized that PRL increases social recognition, approach, and learning. Male Long-Evans rats received ovine PRL (oPRL; 0.5, 2.0 or 5.0 mg/kg), the PRL antagonist bromocriptine (0.1, 3.0 or 5.0 mg/kg) or saline 20 mins before testing for recognition of familiar vs. unfamiliar stimulus males. Saline controls preferred the unfamiliar male ($p < 0.05$), while bromocriptine blocked this preference. oPRL did not increase preference. To measure social approach, we determined if PRL restores approach 2 hours after defeat by an aggressive male. Defeated rats avoided the aggressive male. 2 mg/kg oPRL, before or after defeat, restored approach towards the aggressive male ($p < 0.05$). In non-defeated rats, oPRL or 3 mg/kg bromocriptine had no effect. To determine if PRL increases social learning, we tested social transmission of food preference. Rats choose between two unfamiliar flavors, one of which they have previously been exposed to through interaction with a demonstrator rat. Vehicle controls preferred chow with the demonstrated flavor over the novel flavor. oPRL-treated rats were similar. Bromocriptine-treated rats failed to show a preference. When tested one week later, only oPRL-treated rats preferred the demonstrated flavor. The results suggest that PRL is required for social recognition and learning, and that increasing PRL enhances social memory and approach, similar to OT.

Keywords

Prolactin; Social behavior; Oxytocin; Bromocriptine; Rats

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INTRODUCTION

Prolactin (PRL) and oxytocin (OT) are pituitary hormones essential for lactation [reviewed in (Crowley, 2015)]. PRL promotes milk synthesis (Koprowski and Tucker, 1973), and its release from the anterior pituitary is regulated by tonic dopamine inhibition [reviewed in Ben-Jonathan and Hnasko (2001)]. OT is released from the posterior pituitary to control milk let-down (Nickerson et al., 1954). Although lactation is limited to females, PRL and OT also function as neuromodulators in both males and females. PRL and OT increase social behaviors related to reproduction, including maternal behavior (Bridges et al., 1990; Pedersen and Prange, 1979) and lordosis in female rats (Arletti and Bertolini, 1985; Drago and Lissandrello, 2000), penile erections in male rats (Argiolas et al., 1986; Drago and Lissandrello, 2000), and pair-bonding in monogamous tamarin monkeys (Snowdon et al., 2010; Snowdon and Ziegler, 2015). Furthermore, OT has also been shown to increase non-sexual social behavior in male rats, such as social recognition (Ferguson et al., 2001; Popik et al., 1992), social approach (Lukas et al., 2011), and social learning (Popik and Van Ree, 1993). Since PRL and OT have complementary peripheral and central functions in reproduction, it is reasonable to expect that they may have complementary functions in social behavior in both males and females.

Recent studies have shown that OT increases social behaviors in rodents and humans. OT restores social approach following a social defeat in male rats (Lukas et al., 2011), and increases social learning in rats during the social transmission of food preference task (STFP, Popik and Van Ree, 1993). Likewise, OT knockout mice have social memory impairments, which can be restored by OT infusion into the medial amygdala (Ferguson et al., 2001). In humans, intranasal OT increases social risk-taking, such as cooperation (Declerck et al., 2010), and trust (Kosfeld et al., 2005) regardless of deceit (Baumgartner et al., 2008). However, according to the social salience hypothesis of Shamay-Tsoory and Abu-Akel (2016), the effects of OT on social behavior depend on context, and can enhance negative responses toward out-group members. Moreover, OT is rewarding even in the absence of social stimuli as indicated by self-administration (Donhoffner et al., 2016) and conditioned place preference (CPP, Kent et al., 2013; Liberzon et al., 1997).

PRL is a pleiotropic hormone with over 300 functions [reviewed in Grattan (2015)], including both peripheral actions (e.g. immune function, salt and water balance) and central effects (e.g. food intake, neurogenesis). PRL release changes with stress (Euker et al., 1975), seasonality [reviewed in Curlewis (1992)] and circadian rhythms (Bertani et al., 2010). Moreover, PRL modulates reproduction even in species that do not lactate, such as fish [reviewed in Whittington and Wilson (2013)]. In birds, fish and mammals PRL promotes parental behavior, including olfactory recognition of offspring (Larsen and Grattan, 2010; Mak and Weiss, 2010) and paternal behavior [reviewed in Schradin and Anzenberger (1999)]. PRL also enhances opposite-sex odor preferences and attractiveness in meadow voles (Ferkin et al, 1997; Leonard and Ferkin, 1999).

We hypothesize that PRL and OT have similar central effects on non-sexual social behavior. If so, PRL should promote social recognition, social approach, social learning, and reward,

similar to OT. The present study tested the effects of PRL on social behavior in male rats. While basal PRL levels in females are approximately double those in males (Kinsley et al, 1989), PRL has biological relevance in both males and females (Drago and Lissandrello, 2000). Furthermore, OT promotes social behavior in males. Rats were treated either with exogenous PRL to raise PRL levels in circulation, or with the dopamine D2 receptor agonist bromocriptine to block PRL release.

MATERIALS AND METHODS

Animals and housing

Male Long-Evans rats (ca. 225g at arrival; Charles River Laboratories, Wilmington, MA) were used to test social recognition (n=24), social approach (n=40), social learning (n=24) and CPP (n=24) to measure reward. They were pair-housed, and maintained in a temperature- and humidity-controlled room on a reversed 14:10 light/dark cycle (lights off at 9 a.m.). Food and water were available *ad libitum*, except during behavioral testing. All experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (Council, 2011) and with the approval of the University of Southern California Institutional Animal Care and Use Committee (IACUC).

Experimental design

To determine if PRL promotes social behavior, rats received ovine PRL (oPRL; Sigma-Aldrich, St. Louis, MO), the PRL antagonist bromocriptine (Sigma-Aldrich) or saline vehicle 20 min before behavioral testing. Separate groups of rats were tested for social recognition, social approach, social learning, and CPP to measure reward. Initially, to establish behaviorally-effective doses of oPRL and bromocriptine, rats were tested for social recognition in response to oPRL at 0.5, 2.0, and 5.0 mg/kg, or to bromocriptine at 0.1, 3.0, and 5.0 mg/kg. Subsequently, to determine whether PRL promotes social approach, social learning, and CPP, rats received oPRL at 2.0 mg/kg or bromocriptine at 3.0 mg/kg, based on behaviorally-effective doses from the social recognition test. While we cannot rule out the possibility that different social behaviors respond to different doses of oPRL or bromocriptine, the remaining behavioral assays in this study are not amenable to repeated testing at different drug doses. All behavioral tests were videotaped and scored by an observer blind to the treatment groups.

oPRL was delivered by ip injection. While PRL synthesis can occur centrally in the hypothalamus, cerebral cortex and hippocampus [reviewed in Freeman et al. (2000)], the majority of PRL in the rat brain originates from anterior pituitary release (Ben-Jonathan et al., 2008). PRL crosses into the cerebral spinal fluid via the choroid plexus [reviewed in Freeman et al. (2000)]. Doses of oPRL were based on previous rodent studies. At 2.0 mg/kg, oPRL inhibits gastric emptying and increases plasma cholecystokinin, whereas a 0.5 mg/kg dose has no effect (Chang et al., 2012). At 5.0 mg/kg, oPRL decreases anxiety in female rats on the elevated plus maze (Torner et al., 2001).

Bromocriptine is a dopamine D2 receptor agonist with low blood brain barrier permeability (Markey et al., 1979), which has been used extensively as a PRL antagonist [reviewed in

Horrobin (1973)]. Following iv injection, bromocriptine concentrations are 10-fold higher in the pituitary than in the striatum (Granveau-Renouf et al., 2000). Bromocriptine binds to D2 receptors on lactotrophs to inhibit PRL synthesis and release from the anterior pituitary (Israel et al., 1985). At 0.1 mg/kg, bromocriptine reduces serum PRL selectively in older female rats, but is ineffective in younger females (Cocchi et al., 1984). At 3.0 mg/kg, bromocriptine decreases serum PRL in male and female rats within 30 min of injection (Atterwill et al., 1989). At 5.0 mg/kg, bromocriptine reduces PRL and corticosterone in male rats (Kan et al., 2003).

PRL effects on social behaviors

Social recognition—To determine if PRL promotes social memory, we tested rats for social recognition as adapted from Engelmann et al. (2011). Rats prefer a novel stimulus rat over a familiar one, and this preference lasts for approximately 45 min (Noack et al., 2010). After 120 min, test rats fail to show a preference for the novel stimulus rat. In the present study, each test rat was isolated in a clean cage for 2h. Twenty minutes before the first encounter with an unfamiliar stimulus rat (ovariectomized female), test rats received an injection of vehicle, oPRL, or bromocriptine (n=8/group). Time spent investigating the stimulus rat was recorded, where investigation was defined when the test rat had its nose within 1 cm of the stimulus rat. After 4 min, the stimulus rat was removed. After 45 min (short-term) or 120 min (long-term), the test rat was exposed to the same stimulus rat and a novel stimulus rat for 4 min, and investigation of each stimulus rat was recorded. Each test rat was evaluated for short-term and long-term social recognition on 3 occasions with the same treatment (vehicle, oPRL or bromocriptine) at increasing doses of oPRL or bromocriptine. Data from individual vehicle-treated rats were averaged over the 3 tests. At least 2 days elapsed between successive tests, and stimulus rats were rotated to avoid familiarity.

Social approach—To determine if PRL promotes social approach, social approach following social defeat was evaluated according to Lukas et al. (2011). Initially, the test rat was placed in the home cage (43 × 24.5 × 20 cm) of a larger, aggressive male rat (defeater) for 30 min. Defeater rats were prescreened for aggressive behavior towards an intruder. Test rats showed freezing behavior during the first 10 minutes with the defeater for an average of 188.4±32.0 sec. Physical interaction with the defeater rat was terminated after 10 min by introduction of a wire-mesh screen to bisect the cage. After 20 minutes, test rats were removed to a clean cage. 90 min later, test rats were tested for social approach in a novel arena (81 × 81 × 46 cm). Initially, rats had 4 min with an empty enclosure (17 × 17 × 17 cm; object approach), followed by 4 min with an identical enclosure containing the defeater rat (social approach). Investigation was defined as the test rat being within 4 cm of the enclosure. The chamber was cleaned between trials. All test rats received 2 injections: 1 injection 20 min before defeat, and a second injection 20 min before exposure to the novel arena. Vehicle-treated rats (n=8/group) received 2 injections of saline. Separate groups of rats (n=8 each) received an injection of 2.0 mg/kg oPRL, either before defeat or before the novel arena, and a second injection of saline.

To determine if PRL promotes social approach in the absence of defeat, test rats were placed in a clean cage for 30 min. 90 minutes later, they were exposed to the novel arena as described above. 8 rats received injections of 2.0 mg/kg oPRL before the clean cage and before the novel arena. 8 additional rats received 3.0 mg/kg bromocriptine before the clean cage to determine if PRL is required for social approach. Because we hypothesized that PRL would increase social approach following defeat, bromocriptine was not tested following social defeat.

Social learning—To determine if PRL facilitates social learning, rats were tested with STFP according to Galef (2003). STFP measures if rats overcome food neophobia when they detect odors of unfamiliar food consumed by a demonstrator rat. Demonstrators (castrated males) and test rats were food-restricted for 24h before STFP testing. The next day, demonstrator rats were put in a clean cage and allowed 1h to eat flavored chow (crushed rat chow containing either 2% cinnamon or 4% cocoa). The flavored chow was removed, and test rats were exposed to the demonstrator for 30 min. Immediately afterwards (short-term), test rats were moved to a clean cage with jars of cinnamon- and cocoa-flavored chow for 1h. Food consumption was determined by weight. 20 min before pairing with the demonstrator, test rats received an injection of vehicle, 2.0 mg/kg oPRL, or 3.0 mg/kg bromocriptine (n=8/group).

One month later, the STFP test was repeated. Demonstrators were exposed to chow flavored with 0.4% clove or 0.5% cumin, and test rats received the same drug or vehicle injection as above. To evaluate long-term retention of social learning, test rats were offered clove- and cumin-flavored chows 1 week later (long-term).

PRL effects on non-social behaviors

Elevated plus maze—As a control for potential anxiolytic effects of oPRL on social approach, rats were tested on the elevated plus maze (File et al., 2004) to evaluate anxiety. Behavior in the elevated plus maze was evaluated one week after social approach testing. The apparatus consisted of a plus-shaped maze with two arms (50 × 10 cm) enclosed by opaque plastic sidewalls and two arms without walls. The maze was located 50 cm above the floor and visually isolated by a curtain enclosure. Twenty min before testing, rats received the same drug or vehicle treatment as with the social approach test, and were placed in the center of the maze facing an open arm. They were allowed to explore freely for 5 min. The number of closed arm entries was scored as a measure of overall locomotor activity, and the percentage of time spent in the open arms was used as a measure of anxiety. An entry was recorded when all four paws entered the arm.

Conditioned odor aversion—As a control for potential bromocriptine-induced disruption of olfactory function that could interfere with the STFP test, rats were tested for conditioned odor aversion according to methods adapted from our previous study (Vuckovic et al., 2008). Rats were deprived of food and water for 24h, and were singly housed for 1h before testing. There were four 10-min trials, with 30 min between trials. Test rats used to measure STFP received an injection of vehicle (n=6) or 3.0 mg/kg bromocriptine (n=8) 20 min before the first trial. In the first 2 trials, rats were allowed to drink water containing

0.1% isoamyl acetate (artificial banana) and 0.5% quinine hydrochloride (bitter tastant). In the third trial, rats drank water with isoamyl acetate, but no quinine hydrochloride. For the final trial, rats were tested with plain water. Fluid consumption was determined by weight, and a preference ratio for isoamyl acetate was determined from the last two trials: isoamyl acetate water / isoamyl acetate water + plain water. A preference ratio below 50% indicated aversion.

Conditioned place preference—To determine whether PRL promotes reward, rats were tested for CPP in a 3-chamber apparatus based on Kent et al (2013). The two chambers (each 35 × 35 × 35 cm) were differentiated by visual and tactile cues: one with white walls and Sani-Chips® (PJ Murphy Forest Products Corp., Montville, NJ) bedding, whereas the other had black-and-white patterned walls and a black rubber mat floor. The neutral chamber (18 × 35 × 35 cm) was only available during habituation, pre-testing and CPP-testing. The apparatus was wiped down between tests.

Initially, rats were habituated to the entire apparatus for 10 min. The next day, rats were tested for pre-test preference: rats were placed into the neutral chamber and allowed to explore the apparatus for 10 mins. For each rat, the less-preferred chamber was designated as the CS+, and the preferred side was the CS-. Pairing began 24h after the pre-test. Rats received drug injections (saline, oPRL or bromocriptine) and were paired in the CS+ chamber 30 min/day for 4 days. On alternate days, they received saline and were paired in the CS- chamber (8 days total). Vehicle controls received saline in both CS+ and CS- chambers. CPP testing, identical to the pre-test, was conducted the day after the final pairing. Time spent in each chamber during pre-test and CPP-testing was recorded by video camera and analyzed by an experimenter blind to the treatment groups. Entry into a chamber counted when all four paws were inside. Preference during pre-test and CPP for each rat was calculated as time (in sec) in the CS+ chamber minus time in the CS-. Next, we determined the change in preference score for each rat (CPP preference minus pre-test preference), and averaged data from all rats in each experimental group.

Data analysis

Statistical analyses were completed using JMP Pro 12 (SAS Institute Inc., Cary, NC). Data are presented as mean±SEM. Cohen's d effect size was calculated using the Campbell Collaboration Effect Size Calculator. For ANOVAs, eta-squared (η^2) was calculated as the $SS_{\text{effect}} / SS_{\text{total}}$, and partial eta-squared ($p\eta^2$) was calculated as the $SS_{\text{effect}} / (SS_{\text{effect}} + SS_{\text{error}})$. Differences were considered significant at $p < 0.05$.

PRL effects on social behaviors—For social recognition, the effect of drug (vehicle, oPRL, bromocriptine) and dose (low, medium, high) on time spent investigating the stimulus rat during the first encounter was compared by ANOVA with post-hoc comparison by Dunnett's test. The time investigating same vs. novel stimulus rats after short-term and long-term delay was analyzed for drug and dose by repeated measures ANOVA (RM-ANOVA) with the two stimulus rats (same vs. novel) and time as the repeated measures.

For social approach in non-defeated rats, the effect of drug (vehicle, oPRL, bromocriptine) on investigation of the object stimulus vs. social stimulus was analyzed by RM-ANOVA

with the two stimuli (object vs. social) as the repeated measure. The effect of oPRL on social avoidance in defeated rats was analyzed similarly.

For the STFP task, the effect of drug (vehicle, oPRL, bromocriptine) on consumption of novel vs. demonstrated chow after short-term and long-term delay was determined by RM-ANOVA, with flavor (novel vs. demonstrated) as the repeated measure. To ensure that oPRL or bromocriptine did not alter food intake, total chow consumed for each drug treatment was analyzed by ANOVA.

PRL effects on non-social behaviors—For the elevated plus maze, the effect of drug (vehicle, oPRL, bromocriptine) on entries into closed arms and percentage of time spent on the open arms were analyzed by ANOVA with Dunnett's post-hoc test. For conditioned odor aversion, preference ratios for isoamyl acetate were compared by t-test in vehicle- and bromocriptine-treated rats. For conditioned place preference, the effect of drug (vehicle, oPRL, bromocriptine) on preference score was compared by RM-ANOVA with time (pre-test and CPP) as the repeated measure. Initial preference scores during pre-test were also compared across experimental groups by ANOVA.

RESULTS

PRL effects on social behaviors

Social recognition—Table 1 presents investigation of the two stimulus rats during social recognition testing. During the 1st encounter with a stimulus rat, there was a significant difference between groups in investigation time ($F_{5,143}=2.58$, $p<0.05$, $\eta^2=0.09$). In particular, oPRL at 5 mg/kg reduced investigation of the stimulus rat during the 1st encounter with short-term social recognition testing, compared to vehicle controls ($p<0.05$, $d=1.8$). However, in repeated testing with the same dose of oPRL or bromocriptine (short-term and long-term recognition), there was no difference in investigation time during the 1st encounter.

By RM-ANOVA, there was a significant effect of stimulus rat (same vs. novel) on investigation time ($F_{1,138}=37.9$, $p<0.05$), and a significant effect of drug dose (low, medium, high; $F_{2,138}=3.96$, $p<0.05$). There was also a significant interaction of stimulus rat by time (short vs. long-term; $F_{1,138}=7.83$, $p<0.05$). As in previous studies with a short-term (45-min) inter-exposure interval (Markham and Juraska, 2007), vehicle-treated rats spent 50% longer investigating the novel stimulus rat (75.3 ± 4.6 sec/4 min) than the same stimulus rat (48.0 ± 1.3 sec/4 min; $d=1.6$). After a 2h (long-term) delay, vehicle-treated rats no longer showed a preference for the novel rat (65.1 ± 6.0 sec vs. 64.5 ± 4.1 sec for the same stimulus rat). Treatment with oPRL at 0.5, 2.0 or 5.0 mg/kg had no effect: preference for the novel stimulus rat was not increased after a short delay, and was not prolonged after a longer delay. However, treatment with bromocriptine at 0.1, 3.0 and 5.0 mg/kg eliminated preference for investigation of the novel stimulus rat after a short-term or long-term delay.

Social approach—1 vehicle-treated defeated rat was excluded from analysis, because it had been accidentally exposed to its defeater rat prior to testing. Figure 1 shows investigation of object (empty enclosure) and social (defeater rat) stimuli over 4 min in previously-

defeated rats and in non-defeated controls. By RM-ANOVA with stimulus (object vs. social) as the repeated measure, non-defeated rats showed a significant preference for investigation of the social stimulus ($F_{1,21}=43.09$, $p<0.05$, $\eta^2=0.66$). As in Lukas et al. (2011), vehicle-treated controls spent nearly twice as much time with the (unfamiliar) defeater rat (146.8 ± 17.1 sec/4min) over the empty cage (84.6 ± 9.3 sec/4min; $d=1.7$). PRL had no effect on social approach in the absence of defeat. In non-defeated rats, there was no effect of drug (oPRL or bromocriptine) on preference for the social stimulus over the object stimulus ($F_{2,21}=0.08$, N.S.) and no interaction ($F_{2,21}=0.50$, N.S.).

For defeated rats, there was a significant effect of drug ($F_{2,20}=4.04$, $p<0.05$, $\eta^2=0.40$), stimulus ($F_{1,20}=113.39$, $p<0.05$, $\eta^2=0.72$), and a drug $\times$ stimulus interaction ($F_{2,20}=13.28$, $p<0.05$, $\eta^2=0.16$). In vehicle-treated rats, defeat abolished preference for the social stimulus (107.3 ± 6.6 sec/4min) over the object (89.6 ± 8.5 sec/4min), reflecting social avoidance (Lukas et al., 2011). By contrast, rats treated with 2.0 mg/kg oPRL either before or after social defeat retained a preference for the social stimulus over the object stimulus ($p<0.05$; $d=4.2$).

Social learning—2 rats (1 vehicle, 1 bromocriptine) knocked over a food jar during short-term testing, and were excluded from analysis. By RM-ANOVA, there were significant effects of flavor ($F_{1,40}=22.75$, $p<0.05$, $\eta^2=0.32$), time ($F_{1,40}=5.98$, $p<0.05$, $\eta^2=0.33$) and a flavor $\times$ drug interaction ($F_{2,40}=3.92$, $p<0.05$, $\eta^2=0.39$) on chow consumption. However, there was no effect of drug ($F_{2,40}=0.17$, N.S.). With a short-term delay (Fig. 2A), vehicle-treated rats ate 3/4 of their total food consumption from the demonstrated chow (6.8 ± 1.0 g/h vs. 2.5 ± 1.0 g/h of novel chow); food preference in oPRL-treated rats was similar. By contrast, bromocriptine-treated rats consumed similar amounts of demonstrated (3.2 ± 0.9 g/h) and novel chow (3.8 ± 1.3 g/h). When tested after a long-term delay (1 week, Fig. 2B), vehicle-treated rats no longer showed a preference for the demonstrated flavor (6.6 ± 1.5 g/h) over the novel flavor (4.4 ± 1.2 g/h). Food preference in bromocriptine-treated rats was similar. However, rats treated with oPRL maintained a substantial preference for the demonstrated chow (78.7% of total food intake). For both short-term and long-term delays, there was no effect of drug on total chow consumed.

PRL effects on non-social behaviors

Elevated plus maze—oPRL and bromocriptine had no effect on anxiety behavior on the elevated plus maze (Fig. 3A). Rats treated with vehicle spent $4.1\pm 2.1\%$ of the 5-min test in the open arms, and oPRL and bromocriptine had no effect on this measure of anxiety ($F_{2,21}=0.31$, N.S.). However, there was a significant effect of drug on locomotor activity as reflected by the number of closed arm entries ($F_{2,21}=3.58$, $p<0.05$, $\eta^2=0.25$; Fig. 3B). Rats treated with oPRL made significantly fewer closed arm entries/5 min (9.3 ± 1.3) than vehicle-treated rats (12.0 ± 0.5 entries/5 min; $p<0.05$, $d=1.2$).

Conditioned odor aversion—Bromocriptine had no effect olfactory function as measured by the conditioned odor aversion test (data not shown). After exposure to water containing isoamyl acetate and quinine, both vehicle- and bromocriptine-treated rats avoided

water scented with isoamyl acetate alone (preference ratio for vehicle: 0.32 ± 0.08 ; bromocriptine 0.40 ± 0.05 , N.S.).

Conditioned place preference—Figure 4 shows CPP preference scores for rats treated with vehicle, oPRL, or bromocriptine. Pre-test preference scores for all rats averaged -179.2 ± 20.1 sec, and there was no difference between groups in pre-test preference by ANOVA ($F_{2,21}=0.4$, N.S.). By RM-ANOVA, there was no effect of drug ($F_{2,21}=0.86$, N.S.), time (pre-test vs. CPP; $F_{1,21}=1.61$, N.S.) and no time $\times$ drug interaction ($F_{2,21}=0.40$, N.S.). Vehicle-treated rats showed no significant change in preference score from pre-test (-153.0 ± 33.4 sec) to CPP (-59.3 ± 73.7 sec; N.S.). oPRL- and bromocriptine-treated rats were similar (N.S.).

DISCUSSION

The present study shows that PRL is required for social recognition and social learning in male rats. Bromocriptine-induced inhibition of PRL release impairs social recognition measured by preference for a novel stimulus rat. In the STFP test to measure social learning, bromocriptine-treated rats fail to show a preference for a novel flavor they had previously encountered through an unfamiliar demonstrator rat. Our study also demonstrates that increasing PRL improves social learning in the STFP test, and restores social approach after social defeat. These results suggest that prolactin has pro-social effects on behavior, similar to the effects of OT.

As demonstrated from measurement of PRL in peripheral blood samples (Atterwill et al., 1989), bromocriptine causes a sustained suppression of circulating PRL concentrations. The effects of bromocriptine to inhibit social recognition and social learning suggest that PRL is important for aspects of learning and memory relevant to social cues. Bromocriptine binds to D2 receptors on lactotrophs to inhibit PRL synthesis and release from the anterior pituitary (Israel et al., 1985). It has been used extensively as a PRL antagonist in animal studies, and as a clinical therapy for treatment of lactotroph adenoma [reviewed in Molitch (2017)]. However, one potential caveat is that bromocriptine could act centrally as a D2-receptor agonist, and thereby affect behavior. This is unlikely for several reasons. First, bromocriptine has low blood-brain barrier permeability (Markey et al., 1979). Following an iv injection, bromocriptine concentrations are 10-fold higher in the pituitary than in the striatum (Granveau-Renouf et al., 2000). Furthermore, while central D2-receptor activation has a bi-phasic effect on locomotor activity (Hoffman and Wise, 1992; Van Hartesveldt, 1997), we found no effect of bromocriptine on closed arm entries on the elevated plus maze. Instead, oPRL decreased locomotion on the elevated plus maze, as reported previously (Torner et al., 2001). Lastly, bromocriptine and exogenous PRL have opposite effects on the STFP test in the present study, and on maternal behavior in female rats (Bridges et al., 1990). Together, these results suggest that behaviors inhibited by bromocriptine in the present study reflect the inhibition of PRL release.

Although oPRL inhibited closed arm entries on the elevated plus maze, it is unlikely that this reduction in locomotor activity accounted for the behavioral results of the present study. At 2 mg/kg, oPRL increased investigation following social defeat, compared with vehicle

controls. At 5 mg/kg, oPRL reduced investigation of a stimulus rat during the initial encounter in the social recognition test. Nonetheless, these rats still showed a preference for a novel stimulus rat during the second encounter 45 minutes later. As with locomotor activity, olfactory function and food intake were unaffected by bromocriptine in the present study. Since rats rely on olfaction for social recognition and social learning [reviewed in Garipey et al. (2014)], it was important to verify that bromocriptine did not impair olfactory function as measured by conditioned odor aversion. Our findings were similar to previous studies demonstrating normal olfactory function in rodents with social deficits due to disruption of OT signaling, either treatment with an OT antagonist (Nelson and Panksepp, 1996) or lack of OT receptors (Kavaliers et al., 2003). Likewise, neither bromocriptine nor oPRL altered total food intake in the STFP test, even though oPRL increased long-term social learning. In this regard, PRL stimulates feeding behavior in a dose-dependent manner in female rats (Gerardo-Gettens et al., 1989), but has no effect in males (Heil, 1999). Similarly, bromocriptine reduces chow consumption in male rats at a dose of 10 mg/kg, but not at 1 mg/kg (Thanos et al, 2011).

Although the present study used male rats, it is important to note that there are sex differences in pituitary PRL release and PRL receptor (PRL-R) density in the brain. In rats, basal PRL levels in females are approximately double those in males (Kinsley et al., 1989). Using receptor-binding and immunocytochemistry, PRL-Rs are widely distributed throughout the brain in females, including in hippocampus, amygdala, and hypothalamus (Muccioli et al., 1991; Roky et al., 1996). However, reports of PRL-R in male rat brain are mixed. Muccioli et al (1991) detected radiolabeled PRL binding in the same brain areas of males and females (e.g. hypothalamus, substantia nigra, striatum, cerebral cortex), but binding was higher in the hypothalamus of females. Using immunohistochemistry, Pi and Grattan (1998) detected much lower levels of PRL-R staining in males compared to females, with only weak staining in the choroid plexus, arcuate nucleus, and periventricular hypothalamic nucleus. Sex differences in PRL release and receptor density could be due to estrogen, which is a positive regulator of PRL [reviewed in Grattan (2015)]. Estradiol treatment increases serum PRL levels (Gudelsky et al., 1981). Ovariectomy decreases PRL-Rs in the brain, and administration of estradiol restores PRL-R expression (Muccioli et al., 1991; Mustafa et al., 1995; Shamgochian et al., 1995). While males and females are sensitive to the central (Torner et al., 2001) and peripheral (Drago and Lissandrello, 2000) actions of PRL, sex differences in release and receptor density may modulate response to oPRL and bromocriptine in males and females.

PRL and OT positively regulate each other, and there is considerable overlap in the distribution of PRL-R and OT receptors (OT-R) in the brain (OT-R; Elands et al., 1988; Yoshimura et al., 1993). PRL-R are present on magnocellular neurons that release OT (Kokay et al, 2006), and PRL infusion i.v. to lactating dams increases OT release (Parker et al, 1991). Conversely, OT-R are present on the lactotrophs which synthesize PRL (Breton et al, 1985). OT stimulates PRL release in ovariectomized female rats (Egli et al, 2006), and from male rat pituitary cells in vitro (Lumpkin et al, 1983). In the amygdala and the hippocampus, PRL-R and OT-R presumably contribute to social recognition and social learning. With regard to learning, PRL-R is a cytokine receptor activating MAPK and PI3K/AKT signaling pathways [reviewed in Gass et al. (2003)] implicated in learning and

memory [reviewed in Giese and Mizuno (2013)]. Furthermore, PRL increases hippocampal neurons in culture (Walker et al., 2012). As with bromocriptine treatment in the present study, OT knockout mice have impaired social recognition (Ferguson et al., 2000; 2001), and infusion of OT into the medial amygdala restores this deficit (Ferguson et al., 2001). Likewise, PRL-R in the amygdala is likely to be involved in the oPRL facilitation of social approach following social defeat shown in the present study. In this regard, PRL infusion i.c.v. reduces Fos mRNA induced by restraint stress in the central nucleus of the amygdala and paraventricular nucleus of the hypothalamus (Donner et al., 2007).

Lastly, PRL-R in the hypothalamus, and PRL links with dopaminergic systems, may relate to rewarding aspects of social behavior. PRL release from the anterior pituitary is tonically inhibited by tuberoinfundibular dopaminergic (TIDA) neurons [reviewed in (Reymond and Porter, 1985)], and PRL has a short-feedback loop to regulate its own release by increasing dopamine from TIDA neurons [reviewed in (Ben-Jonathan and Hnasko, 2001)]. However, oPRL also increases extracellular levels of dopamine and its metabolites in the nucleus accumbens in male rats (Gonzalez-Mora et al., 1990). Dopamine in the nucleus accumbens is implicated in response to both natural rewards, such as food (Hernandez and Hoebel, 1988) and sex (Damsma et al., 1992), and to drugs of abuse (Di Chiara and Imperato, 1988). In this regard, OT and PRL are each released during orgasm (Brody and Kruger, 2006; Carmichael et al., 1987), and PRL levels are increased approximately 300% following chronic cocaine use in rhesus monkeys (Mello et al., 1994). It is thought that OT promotes rewarding social behaviors via interactions with dopamine [reviewed in Young et al. (2001)]. Furthermore, OT is itself rewarding, as demonstrated by CPP (Kent et al., 2013; Liberzon et al., 1997) and self-administration (Donhoffner et al., 2016). Therefore, we were somewhat surprised that PRL failed to induce CPP in the present study. There is the additional possibility that PRL may be rewarding when rats are tested in the presence of a social partner, as has been demonstrated for OT (Kent et al., 2013).

Since the majority of our data showed an effect from loss of PRL as opposed to addition of PRL, this suggests that PRL functions primarily as a permissive signal to modulate behavior. Furthermore, it is likely that prosocial effects of PRL are specific to social behavior rather than a generalized anxiolytic action of PRL, based on the lack of effect of either bromocriptine or oPRL on anxiety-related behavior in the elevated plus maze. Bromocriptine decreased social recognition, and social learning, whereas PRL administration increased social learning. Social behavior is important for survival, but animals expose themselves to risks when seeking social partners, such as disease (Kappeler et al., 2015). Considering the diverse actions of PRL on physiology and behavior, increased social interaction in response to elevated PRL release could potentially be maladaptive.

PRL's effects on male rat social behavior in the present study parallels studies of PRL and alloparental care in cooperatively breeding animals, including woodpeckers (Khan et al., 2001), meerkats (Carlson et al., 2006), and marmosets (da Silva Mota et al., 2006). In these species, PRL levels increase in male helpers who assist in caring for dependent young. In meerkats, the increase in PRL precedes the helping behavior (Carlson et al., 2006). In marmosets, PRL levels correlate with the number of infants being carried (da Silva Mota et al., 2006), and bromocriptine reduces infant retrieval and carrying duration (Roberts et al.,

2001). These findings suggest that PRL levels are causally related to prosocial caregiving behavior towards immature conspecifics, even in non-parents. One implication is that PRL could have potential therapeutic applications for human social deficits associated with neuropsychiatric diseases, similar to use of oxytocin as a treatment for autism spectrum disorders (ASD; Yamasue, 2016). In this regard, PRL has been identified as a possible genetic link for ASD (Yrigollen et al., 2008). PRL's permeability across the blood brain barrier with peripheral administration [reviewed in Freeman et al. (2000)] offers an advantage over intranasal oxytocin administration (Leng and Ludwig, 2016) as a potential candidate drug to treat social deficit symptoms.

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HIGHLIGHTS

- Prolactin (PRL) and oxytocin (OT) are pituitary hormones essential for lactation, but also promote sexual behavior.
- While OT is known to promote social recognition, approach and learning, the role of PRL in rodent social behavior remains unclear.
- Treatment with the PRL antagonist, bromocriptine, decreased social recognition, and learning, whereas ovine PRL-treatment increased approach following social defeat and social memory in male rats.
- These results suggest that PRL is required for social recognition, and it that it promotes social approach and social memory, similar to OT.

Fig. 1. Social approach. Investigation time in sec (mean $\pm$ SEM) of object (open bars) and social (closed bars) stimuli over 4 min in non-defeated controls (A) and socially-defeated rats (B). See text for statistical significance.

Fig. 2. Social transmission of food preference. Food consumption (g; mean $\pm$ SEM) of novel (open bars) and demonstrated (closed bars) chow following short-term (A) or long-term (B) delay. See text for statistical significance.

Fig. 3. Elevated plus maze. (A) Percentage of time (mean±SEM) on the open arms, and (B) number of closed arm entries in a 5 min test. *Asterisk* indicates significant difference between vehicle- vs oPRL-treated rats ($p<0.05$).

Fig. 4. Conditioned place preference. Preference score (time in sec in the CS+ chamber minus the CS- over 10-min; mean $\pm$ SEM) for pre-test and CPP.

Table 1

Social recognition. Investigation time in sec (mean $\pm$ SEM) during a 4 min exposure to a previously-unfamiliar stimulus rat (1st encounter). Following a short-term (45 min) or long-term delay (2h), test rats were exposed to the same rat and a novel stimulus rat. #Data from vehicle-treated rats were averaged over 3 occasions of repeated testing. See text for statistical significance.

Drug	Dose (mg/kg)	Short-term			Long-term		
		1st encounter	Familiar	Novel	1st encounter	Familiar	Novel
#Vehicle	0.0	142.3 $\pm$ 5.5	48.0 $\pm$ 1.3	75.3 $\pm$ 4.6	127.5 $\pm$ 6.5	64.5 $\pm$ 4.1	65.1 $\pm$ 6.0
oPRL	0.5	145.8 $\pm$ 12.7	44.3 $\pm$ 5.5	71.4 $\pm$ 4.9	134.9 $\pm$ 14.2	53.9 $\pm$ 4.9	65.4 $\pm$ 7.1
oPRL	2.0	136.6 $\pm$ 10.2	43.3 $\pm$ 4.4	68.3 $\pm$ 5.0	108.6 $\pm$ 17.9	56.1 $\pm$ 6.3	54.3 $\pm$ 9.3
oPRL	5.0	86.3 $\pm$ 17.2	52.0 $\pm$ 3.6	69.4 $\pm$ 7.0	118.3 $\pm$ 8.3	69.3 $\pm$ 6.3	90.9 $\pm$ 7.1
Bromocriptine	0.1	144.9 $\pm$ 7.9	50.8 $\pm$ 10.3	72.9 $\pm$ 7.2	120.6 $\pm$ 11.2	44.9 $\pm$ 6.7	59.9 $\pm$ 10.1
Bromocriptine	3.0	148.9 $\pm$ 11.3	55.6 $\pm$ 10.7	66.9 $\pm$ 7.6	138.5 $\pm$ 12.0	45.6 $\pm$ 4.0	62.3 $\pm$ 6.9
Bromocriptine	5.0	151.5 $\pm$ 7.9	55.0 $\pm$ 6.7	75.4 $\pm$ 11.6	148.5 $\pm$ 7.5	53.4 $\pm$ 6.5	65.9 $\pm$ 5.2