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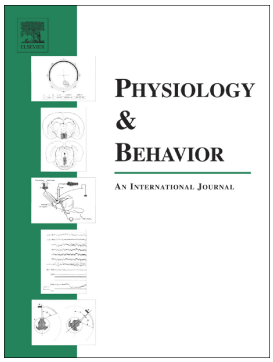

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Inhibition of lysine-specific demethylase enzyme disrupts sexually conditioned mate guarding in the female rat.

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Abstract

Although female rats are typically described as having a promiscuous mating strategy, if sexually naïve females have their formative sexually rewarding experiences paired with the same male, they will recognize that male and display mate-guarding behavior toward him in the presence of a female competitor. Female rats that display mate guarding behavior also show enhanced activation of oxytocin and vasopressin neurons in the supraoptic and paraventricular hypothalamic nucleus. Here, we examined the potential role that histone demethylation might have in establishing this pair-bonded behavior, and whether the corresponding changes in oxytocin and vasopressin neuronal activation depended on demethylation. To accomplish this, we examined the effect of a lysine-specific demethylase-1 inhibitor to block the action of demethylase enzymes and maintain the methylation state of corresponding genes. Female rats treated with the demethylase inhibitor failed to show any measure of mate guarding, whereas females treated with vehicle displayed mate guarding behavior. Demethylase inhibitor treatment also blocked the ability of familiar male cues to activate oxytocin and vasopressin neurons, whereas vehicle-treated females showed this enhanced activation. These data indicate that histone demethylation is a crucial component in the epigenetic modification of neural circuitry that underlies conditioned mate guarding in female rats. These results are the first to demonstrate the role of histone demethylation underlying changes in mating strategy.

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Introduction

Individual differences in how animals approach biologically significant situations such as mating are acquired through learning and experience. This is essential for adaptability and survival in a complex environment. In the context of mating, much work has been devoted into identifying how excitatory and inhibitory brain systems mediate "normal" mating patterns in a variety of species (McClintock 1982, Pfaus, 2009; Pfaus et al 2012; Young et al., 2011). However, how the brain produces flexible patterns of mating that result in individual patterns of behavior is less explored, especially in the context of possible epigenetic modifications that underlie alterations in gene expression and protein availability.

Although rats are described as having a promiscuous mating strategy (McClintock, 1978), they acquire conditioned mate preferences for a familiar partner through Pavlovian association of critical partner cues with sexual reward (Pfaus et al., 2012). If female rats have their formative sexual experiences paired with the same male, they will mate guard that male in the presence of a competitor female (Getz et al., 1992; Chuard et al., 2016; Holley et al., 2014; 2015). In addition to increased sexual solicitations of the male, this conditioned mate-guarding behavior consists of hovering next to the male and presenting a pre-lordosis crouch (PRES), positioning her body between the male and the approaching competitor to block any competitive solicitation (Bet), and female-female mounting (FFM) of the competitor should she actually solicit the male (Holley et al., 2015; 2015; Pfaus et al., 2015). With increasing experience, FFM sensitizes and becomes the dominant form of the behavior, initiated very quickly after the competitor is introduced into the open field. As in prairie voles, this conditioned mate-

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guarding behavior depends critically on the activation of bonding circuits in the brain, including oxytocin (OT) and vasopressin (AVP) neurons within supraoptic (SON) and paraventricular (PVN) hypothalamic nuclei, by their preferred male, but not by a novel male. Control females that were given unrestricted access to several males during their formative sexual experiences also did not display any mate guarding behavior, nor did they show the activation patterns of hypothalamic OT or AVP neurons (Holley et al., 2015). Peripheral administration of OT or AVP during their first sexual experience facilitated PRES and RI, respectively, further demonstrating the critical nature of OT and AVP in the expression of this behavior (Holley et al., 2015). Thus, conditioned sexual reward is a powerful mechanism capable of influencing the mating strategy of female rats to shift from promiscuity towards monogamy.

Memory formation requires gene transcription, and epigenetic modifications to chromatin structure are vital in regulating such gene expression, especially for long-term memory formation (Chwang et al., 2006; Levenson et al., 2004; Gupta et al., 2010; Gupta-Agarwal et al., 2012; Reik et al., 2007; for review see: Kramer et al., 2013). Conditioned learning underlies the pair bonding, mate choice, and mate-guarding behavior we have observed in female rats (reviewed in Pfaus et al., 2012), and histone modifications have been shown to play a defining role in naturalistic bonding behavior observed in monogamous prairie voles.

Wang and colleagues (2013) demonstrated a role of histone modifications in pair bonding in prairie voles by threating sexually naïve females with histone deacetylase inhibitors (HDACi) prior to their induction into the pair bonding paradigm. Typically, in a pair bonding paradigm, female voles are allowed a 6h cohabitation with the male and

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allowed to mate with him, if not allowed to mate this condition will not produce a pair bond. However, when treated with the HDACi, a 6h cohabitation without mating was sufficient to induce pair bonding (Wang et al., 2013). The facilitated establishment of the pair bond in a presence of the HDACi lead to an up-regulation of OT receptor and vasopressin 1a receptor (V1aR), both of which have been shown to be absolutely essential for pair bonding, in the nucleus accumbens (Insel et al., 1992; Insel et al., 1994; Wang et al., 2013). Histone deacetylase inhibitors prevent the removal of acetyl groups from histone tails which promotes a more relaxed confirmation of the histones. This confirmation leads to DNA being more accessible and is therefore associated with an upregulation in gene expression. Therefore, it is consistent that treatment with an HDACi would facilitate the formation of pair bonding and the expression of pair bonding relevant neuropeptides. Although this line of work only explored one type of epigenetic histone modification, it demonstrates a strong role of epigenetic modification on the effects of early sexual experience that augment the activation of OT and AVP neuronal systems to underlie pair-bond formation.

Although a role of histone deactylation has been demonstrated, epigenetic modifications do not act alone, but rather, in combination to influence gene expression. Therefore, the present study examined whether histone demethylation (Sun et al., 2010; Metzger et al., 2005) is critical in the formation of conditoned mate-guarding in female rats. To do this, a lysine specific demethylase 1 (LSD-1) inhibitor (Culhane et al., 2006) was used to disrupt the demethylation process during conditioning. LSD1 removed methyl groups from mono and demethylated lysine 4 or 9 residues of H3 histone tails (Neelamegam et al., 2012). If histone demethylation is important in conditioned mate

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guarding, then the maintenance of methylated states by the inhibitor should disrupt the development of this behavior and its neural correlates, in particular the activation of OT and AVP neurons by familiar, partner-related cues.

Materials and methods

Animals and surgery

Sexually naïve Long-Evans female rats (200-250g) were obtained from Charles River Canada (St-Constant, QC, Canada). Animals were housed in shoebox cages in groups of two in a colony room on a reversed 12:12h light/dark cycle at approximately 21 °C and given free access to food and water. Female rats were ovariectomized (OVX) via bilateral lumbar incision. Proceeding surgery, female rats were anesthetized with 1ml/kg of ketamine hydrochloride (50mg/ml) and xylazine hydrochloride (4ml/kg), mixed in a ratio of 4:3 respectively, administered via intraperitoneal injection. Female rats were given 1 week to recover from surgery prior to the conditioning trials. Throughout the duration of the experiment, female rats were maintained on hormone replacement by subcutaneous injections of estradiol benzoate (EB; 10 µg in 0.1 ml of sesame oil) 48h prior to testing, and progesterone (P; 500 µg in 0.1 ml of sesame oil) 4h prior to testing.

Sexually naïve male rats (300-350g) were also obtained from Charles River Canada (St-Constant, QC, Canada). They were housed in group cages (4 animals per cage) and housed under conditions identical to those of the female rats.

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All animal procedures complied with the guidelines of the Canadian Council on Animal Care and were approved by the Concordia University Animal Research Ethics Committee.

Conditioning apparatus

Conditioning occurred in unilevel Plexiglas pacing chambers (38cm H x 60cm W x 38cm deep) with wire-mesh floors covering a layer of bedding. Chambers were bisected by a Plexiglas divider with four holes cut into the bottom which were large enough for the female to crawl through but too small for the male to crawl through [6,17,18]. These chambers are designed in a manner that optimizes the ability of the female to associate her paired male with a sexual reward state. The conditioning, testing, and behavioral paradigm follows our previous work on mate-guarding behavior (Holley et al., 2014; Holley et al., 2015).

Drug Preparation

The LSD1 demethylase inhibitor (LSD1) was purchased from Calbiochem (LSD1 inhibitor IV; RN-1, HCL; cat# 489479). 10mg of drug was dissolved in 2mL of saline to obtain a concentration of 5 mg/ml, aliquoted into Eppendorf tubes, and stored at -20^{o}C (Neelamegam et al., 2012).

Conditioning procedure

Conditioning sessions occurred at 4-day intervals, 4h after P injections, during the middle third of the rats' circadian cycle (lights off at 08:00). Females were assigned randomly to one of 3 groups. Paired females treated with LSD1, paired females treated with vehicle alone, or unpaired females that received no treatment (N=12/group). All paired females copulated with the same male across all trials, whereas unpaired females

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copulated with a variety of males across all trials. 1h prior to testing, paired females were given an intraperitoneal injection of either LSD1 (10mg/kg) or vehicle (0.9% saline). The chosen dose and 1h time frame was chosen based off of microdialysis data demonstrating that 1h is sufficient time for LSD1 to cross the blood brain barrier (Neelamegam et al., 2012). Males were placed onto one side of the conditioning chambers and allowed to habituate for 5-minutes before each trial. Females were then placed into the opposite side of the conditioning chamber and allowed to have paced sex with the male for 20 minutes. Each group received 10 trials, which were all recorded on video.

Throughout the course of this study, the health of the animals was assessed daily. Rats eating and drinking was monitored and body weights were taken bi-daily to ensure drug treatment did not impair the health of the animals. This was done to ensure the overall health and comfort of the rats, and also, to ensure that any effects were due to the action of the drug and not to failing health or abnormal behavior.

Mate-guarding test

Four days after the final conditioning trial mate guarding was assessed using an open-field (123cm x 123cm x 46cm) with a thin layer of bedding (Coria-Avila et al., 2005). The open-field apparatus allows enough space for the agonistic interactions between females, and for the display of mate-guarding behavior as observed in our previous studies (Holley et al., 2014; Holley et al., 2015). Each open-field contained a paired female, from either LSD-1 or vehicle groups, along with her corresponding male and an unpaired competitor female. Before the test, males were placed into the open field for a 5-minute habituation period, after which both the paired and unpaired females were placed into the open-field at 2 diagonal corners. Rats were allowed to copulate freely for

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a 1-hr period. After the open-field test female rats were perfused and their brains were collected to examine Fos induction within OT and AVP neurons.

All open-field tests were recorded on video and scored afterward using a computerized event recorder customized for rat sexual behavior in an open-field [Cabilio]. Mate guarding was defined operationally as any behavior engaged in by the female that took away the male's ability to mate with the competitor female. Although this can be achieved through a variety of aggressive, dominant, sexual, competitive, and social behaviors, and consisted of one or more of the following: 1) presenting behavior (PRES) occurred when one female hovered in a receptive position (lordosis or pre-lordosis crouch) after the male had mounted or intromitted, which would more normally be followed by the female hopping and darting away from the male; 2) interference behavior (Bet), characterized by a female positioning her body between the male and the other female in an attempt to block her access to the male. Interference behavior typically preceded 3) female-female mounting (FFM) which occurred when one female mounted the other, typically until the mounted female left the vicinity of the male. We also took into account the number of times female rats initiated body contact with the male (init) and the total amount of time spent in the vicinity of the male (time spent). Similar measures are used commonly to determine pair-bond formation in prairie voles (Gubernick, 1994). We also scored typical measures of appetitive and consummatory female sexual behaviors, including 1) solicitations (sol), which were displayed as a headwise orientation to the male followed by turning, darting away, or a full runaway (as in McClintock, 1984); 2) hops and darts, and 3) lordosis. Instances of aggression were quantified when one rat attacked another and the victim rat displayed signs of distress and

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vocalizations. Finally, we assessed the number of intromissions (Intro) and ejaculations (Ejac) received by each of the females on the final open-field test.

Perfusions

Animals were deeply anesthetized with an overdose of sodium pentobarbital (120mg/kg) administered via intraperitoneal injection. They were then perfused intracardially with 250ml of ice-cold phosphate-buffered saline (PBS) followed by 250ml of ice-cold 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde for 4 hours at 4^{o}C, then placed into a 30% sucrose solution for 48 hours at 4^{o}C, after which they were flash frozen in crushed dry ice, covered with aluminum foil, and stored at -80°C until slicing.

Tissue preparation and immunocytochemistry

Since no animals were disqualified from behavioral testing due to being outliers, brains were chosen at random from paired and unpaired females (N=5 brains/group) were cut coronally at 30μm on a freezing microtome and divided into three series, one of which was used for double immunohistochemistry to label for Fos and OT, the second was used to label for Fos and AVP, and the third was stored as a back up. In the primary phase, sections were washed in cold Tris-buffered saline (TBS) and incubated first with 30% H_2O_2 in TBS for 30 min at room temperature followed by 3% Normal goat serum (NGS) in 0.05% Triton-TBS for 90 min at 4°C, with rabbit polyclonal anti-Fos (Fos ab5, Calbiochem, Mississauga, ON; diluted 1:40,000) in 0.05% Triton-TBS with 3% NGS for 72 h at 4°C. In the secondary phase, sections are incubated in biotinylated goat anti-rabbit IgG (Vector Laboratories Canada, Burlington, ON; 1:200), in 3% NGS, and .2% Triton TBS for 1 hour. Following this, sections enter a tertiary phase, where sections are

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incubated in avidin–biotinylated–peroxidase complex (Vectastain *ELITE*® ABC KIT, Vector Laboratories Canada; diluted 1:55) in 3% NGS, and .05% Triton TBS for 2 h at 4°C. Sections were washed in TBS (3 × 5 min) between each incubation.

Immunoreactions were stained by sequential treatments with 50 mM Tris for 10 min, 3,3′-diaminobenzidine (DAB) in 50 mM Tris (0.1 ml of DAB/Tris buffer, pH 7.8) for 10 min, and 8% nickel chloride (400 µl per 100 ml of DAB/Tris buffer + H2O2) all at room temperature. Reaction was stopped by rinsing (3 x 10 min) in PBS. Sections were then transferred into the second primary antibody, either rabbit polyclonal anti-oxytocin (Chemicon, AB911, diluted 1:10,000) or rabbit polyclonal anti-vasopressin (Millipore, AB1565, diluted 1:10,000) in 0.05% Triton-TBS with 3% NGS, and incubated for 72h at 4 °C. After completion of this incubation, secondary and tertiary phases are repeated. However, no nickel chloride was added to the final DAB wash in order to create a light brown cytoplasmic stain.

Following staining, sections were mounted onto gel-coated slides and cover slipped following immunohistochemistry. Cover slipping procedure started with sequential washes of distilled water followed by washes in 70%, 90%, and 100% alcohol each at room temperature for 1min duration each. Slides were then dipped 50 times in Citrisolve at room temperature after which they were cover slipped using Permount solution. Slides were set aside for a week to allow for the Permount to dry and then cleaned and coded so that all analysis would be conducted blind.

Sections were examined using an Olympus light microscope at 400x magnification. Pictures were taken at 200x and 1000x using Q-Capture pro software. Double-labeled cells were identified by eye as any cell having a dark black nucleus (Fos

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positive) and a light brown cytoplasm (OT or AVP positive). Brain regions were defined using the atlas of Paxinos and Watson (2006). Double-labeled cells were counted within the supraoptic nucleus (SON, Plates 21-25), and paraventricular nucleus (PVN, Plates 25-26) as these two regions are where OT and AVP neuronal bodies reside. Five slices were counted per animal. These slices represented each area rostrally to caudally and were matched among animals to ensure accuracy. Counts were done bilaterally in a total of 5 animals per group. Total number of double-labeled cells was counted in each section, which were then used to derive the mean number of double-labeled cells within the region.

Statistical Analyses

Mate-guarding behaviors in the open-field were assessed using independent samples t-tests, since we had planned comparisons ahead of time, described below in the results section. In short; only animals that behaved within the same open field test were measured together for statistical purposes. For example, the behavior of LSD1 treated females was compared to the behavior of saline treated females that served as competitor females in their set of open fields (LSD1 unpaired). Likewise, the behavior of untreated paired females was compared to the behavior of the saline treated animals that served as competitors in their set of open fields (Saline unpaired). This design rendered us with two groups and one level of comparison, which made independent samples t-tests appropriate statistical tests for this study. Each female in the 4 groups was placed together with an unpaired, saline-treated competitor female. Planned comparisons were made between the subject female and the competitor female and summed into means for each of the four subject treatment groups. Cohen's d was used to assess effect size. The data collected

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from Fos-IR cells were analyzed using a one-way analysis of variance (ANOVA) with post-hoc t-tests between individual groups corrected for elevated experiment-wise error using the Bonferroni method. Eta squared was used as a measure of effect size.

Results

Mate guarding behavior in the open field

During the final open-field test, paired females treated with the vehicle initiated more contacts with the male (INIT) than did the unpaired females they were tested with $t(10) = 2.791$, $p = .028$, d=1.609 (Figure 1), but did not display more FFMs ($t(10) = .422$, $p = .683$), Sol ($t(10) = .114$, $p = .115$), Pres ($t(10) = .439$, $p = .672$), Time Spent ($t(10) = 1.268$, $p = .234$) or RI ($t(10) = .073$, $p = .838$). In contrast, paired females treated with LSD-1 inhibitor failed to show any measure of mate guarding behavior and did not differ behaviorally from unpaired, vehicle-treated females. During the open field test, LSD-1 inhibitor treated females did not react protectively towards their partner male, and passively allowed the conspecific to approach and mate with the male. Control behaviors, such as hops and darts (HOP), were assessed in order to evaluate sexual proceptivity to ensure all animals were sexually receptive and behaving normally. No statistical differences were found in HOP behavior between groups indicating that all animals were sexually receptive and behaving in a comparable manner ($t(10) = -1.040$, $p=.561$).

Double-labelled cell counts

A one-way ANOVA was used to examine differences in Fos induction within OT and AVP neurons between paired LSD-1, paired vehicle, and unpaired untreated females.

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Vehicle treated females had significantly more double-labelled Fos/OT neurons within both the SON, $F(2,12) = 97.063$, $p=.000$, $\eta^2=.942$, and PVN, $F(2,12) = 70.903$, $p=.000$, $\eta^2=.922$, compared to females treated with LSD-1 or controls (Figure 2). Paired vehicle treated females also had more double-labelled Fos/AVP neurons within both the SON, $F(2,12) = 25.895$, $p=.000$, $\eta^2=.812$, and PVN, $F(2,12) = 10.836$, $p=.002$, $\eta^2=.644$ compared to treated with LSD-1 and controls (Figure 3). Fos induction within OT and AVP neurons did not differ between females treated with LSD-1 and controls (Figures 2 and 3), $p>.5$ for all measures. Since double-labeling data from the LSD1-unpaired and saline-unpaired groups did not differ, we paired their brains for analysis.

Discussion

These data demonstrate that conditioned mate guarding behaviors in female rats, along with the activation of at least two important neurochemical mediators, are regulated epigenetically by lysine-specific histone demethylation during a female rat's first experiences of sexual reward. Blockade of the lysine-specific demethylase-1 enzyme blocked the development of conditioned mate guarding behavior in female rats, and blocked the corresponding increase in activation of OT and AVP neurons that underlies different aspects of this behavior. However, this treatment did not block the activation of solicitations or lordosis by estradiol and progesterone, indicating that the effect was specific to the formation of conditioned sexual partner preferences and not to the general display of appetitive or consummatory sexual behaviors. Taken together, these data provide evidence that the conditioned shift in mating strategy from promiscuity towards monogamy that we have observed in female rats in response to sexual conditioning is

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dependent, in part, on an epigenetically-regulated increase in activation of OT and AVP neurons by individual cues of the familiar male.

The LSD-1 demethylase inhibitor used here has been shown to cross the blood brain barrier in under and hour, and has also been shown to disrupt central processes such as long-term memory formation. Specifically, mice that were given LSD1 failed a novel object recognition task (Neelamegam et al., 2012). Although LSD-1 demethylase enzymes have been shown to act centrally they also can act peripherally, so it could be that the effect we observed here is due to the blockade of LSD-1 demethylase enzyme function in a peripheral location (Neelamegam et al., 2012; Metzger 2005, Yang et al., 2007). However, because LSD-1 inhibitor treatment did not create any symptoms of sickness in treated animals (no visible weight loss, tumors, loss of appetite, failure to groom, discoloration of paws), did not disrupt appetitive or consummatory behaviors in general, and blocked the activation of Fos within OT and AVP neurons in both the PVN and SON, the effect on behavior is likely mediated by central actions. Further work is warranted in order to investigate the role of central vs. peripheral contributions.

Neuroanatomical and pharmacological studies have provided strong evidence that both OT and AVP are crucial for bonding in prairie voles (Ross et al., 2009; Wang et al., 1995; Insel et al., 1994; Smeltzer et al., 2006; Insel et al., 1992; Winslow et al., 1995). Previously, we demonstrated that female rats conditioned to display monogamous behaviors showed an increased activation of OT neurons within the PVN and SON, and increased activation of AVP neurons in the SON, when exposed to their individual partnered males (Holley et al., 2015). Control groups of female rats, with the same amount of sexual experience, but with a variety of partners do not show this increase in

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OT and AVP neuronal activation in response to exposure to potential mate (Holley et al., 2015). In the second part of that study, peripheral administration of OT or AVP prior to a female's first paced copulation conditioning trial with a male facilitated the display of presenting or interference behavior, respectively, on the second trial with that male, whereas saline injection did not (Holley et al., 2015). Thus, the conditional activation of OT and AVP shapes the natural display of these two aspects of mate guarding in female rats.

That both mate guarding behaviors and the activation of OT and AVP neurons were blocked by LSD-1 demethylase inhibitor treatment in the present study indicates that histone demethylation is an essential mechanistic component that enhances the activation of OT and AVP neurons in response to cues of the partner male, leading to the crystallization of a monogamous-like partner preference and mate-guarding behavior. Although we have not identified the precise mechanism, it is likely that the disruption in histone demethylation by LSD1 is occurring within cells that make up the OT/AVP neural circuitry.

Histone methylation/demethylation does not have straightforward transcriptional outcomes as do other histone modifications, like acetylation, which is almost invariably associated with transcriptional activation (For review: Kouzarides et al., 2007). What is of vital importance in regards to histone methylation/demethylation is the pattern of methylation that is established. Patterns of methylation incorporate both methylated and demethylated residues, and are what ultimately play a role in transcriptional outcomes. Patterns of histone methylation can act bidirectionally, meaning that some patterns result in transcriptional activation, whereas others result in transcriptional repression, and both

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repressive and activational patterns can occur simultaneously (Kouzarides et al., 2007; Suganuma et al., 2002). In the present study, inhibiting LSD1 demethylase enzymes disrupted the ability of cells to properly establish histone methylation/demethylation patterns, thus creating a deficit in the cells' ability to transcribe the gene products necessary for the enhanced induction of OT, AVP, and the subsequent mate-guarding behaviors we observed. It could also be that the disruption in histone demethylation within OT and AVP circuitry is altering the expression of upstream neurochemical inputs that drive the activation of OT or AVP neurons, and/or of other neurochemical pathways that are themselves driven by OT and AVP action or influence downstream OT and AVP action in the brain during a female rat's first rewarding sexual experiences.

In summary, this study is the first to demonstrate a definitive role of epigenetic histone modifications in a conditioned sexual response. Histone modifications are a key element in gene regulation through chromatin remodeling. LSD-1 demethylases have been shown to demethylate repressive histone markers thus leading to transcriptional activation (Metzger et al., 2005). The up-regulation of OT and AVP neuronal activation in response to monogamous conditioning would require changes in gene expression. Although the data from this study strongly support the role of LSD-1 histone demethylases in this process, further work is required to explore the contribution of other histone modifications. Epigenetic regulation requires the interplay of processes such as methylation, acetylation, and phosphorylation (Kouzarides et al., 2007). It is therefore highly unlikely that only one of these processes works alone to establish the changes to neural circuitry that underlie conditioned mate guarding. Indeed, it has already been established that histone acetylation plays an important role in naturally occurring pair

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bonding in prairie voles (Wang et al., 2013). It would therefore be important to explore the role of other histone modifications in conditioned mate guarding.

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Highlights

- Female rats given all of their sexual experience with one male will mate guard that male in the presence of a female competitor
- Females that mate guard show enhanced oxytocin and vasopressin activation within the supraoptic and paraventricular nuclei
- Inhibition of lysine specific demethylase enzymes prevented the establishment of mate guarding behavior in drug treated rats whereas vehicle injection did not
- Inhibiting LSD1 also prevented the enhancement of oxytocin and vasopressin previously seen, whereas saline did not.

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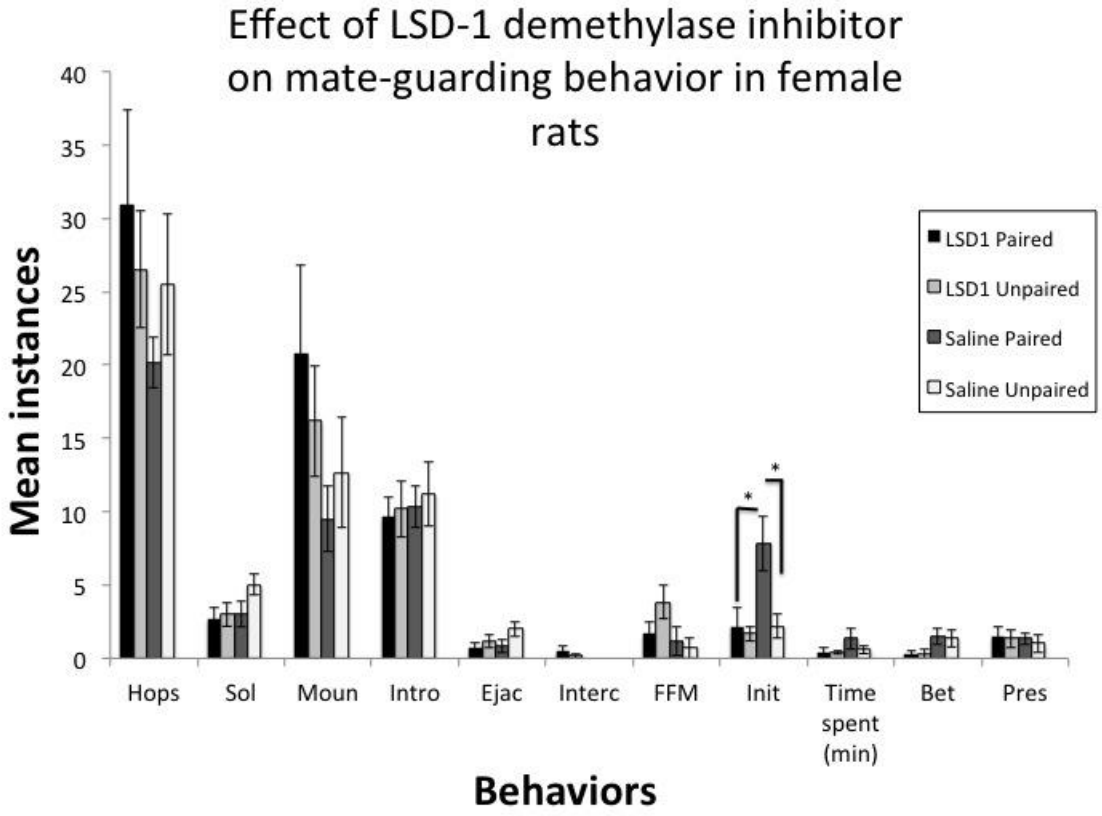

Figure 1:

Mate guarding behavior displayed in open field by paired female rats that were treated with either LSD1 or Saline. Behaviors include hops, solicitations (Sol), mounts received (moun), intromissions received (intro), ejaculations received (ejac), interceptions (interc), female-female mounting (FFM), number of times initiating closeness to the male (Init), time spent in proximity to the male (TimeSpent), interference (RI), and presenting (pres). Data are means ± SEM. * $p < 0.05$, ** $p < .01$

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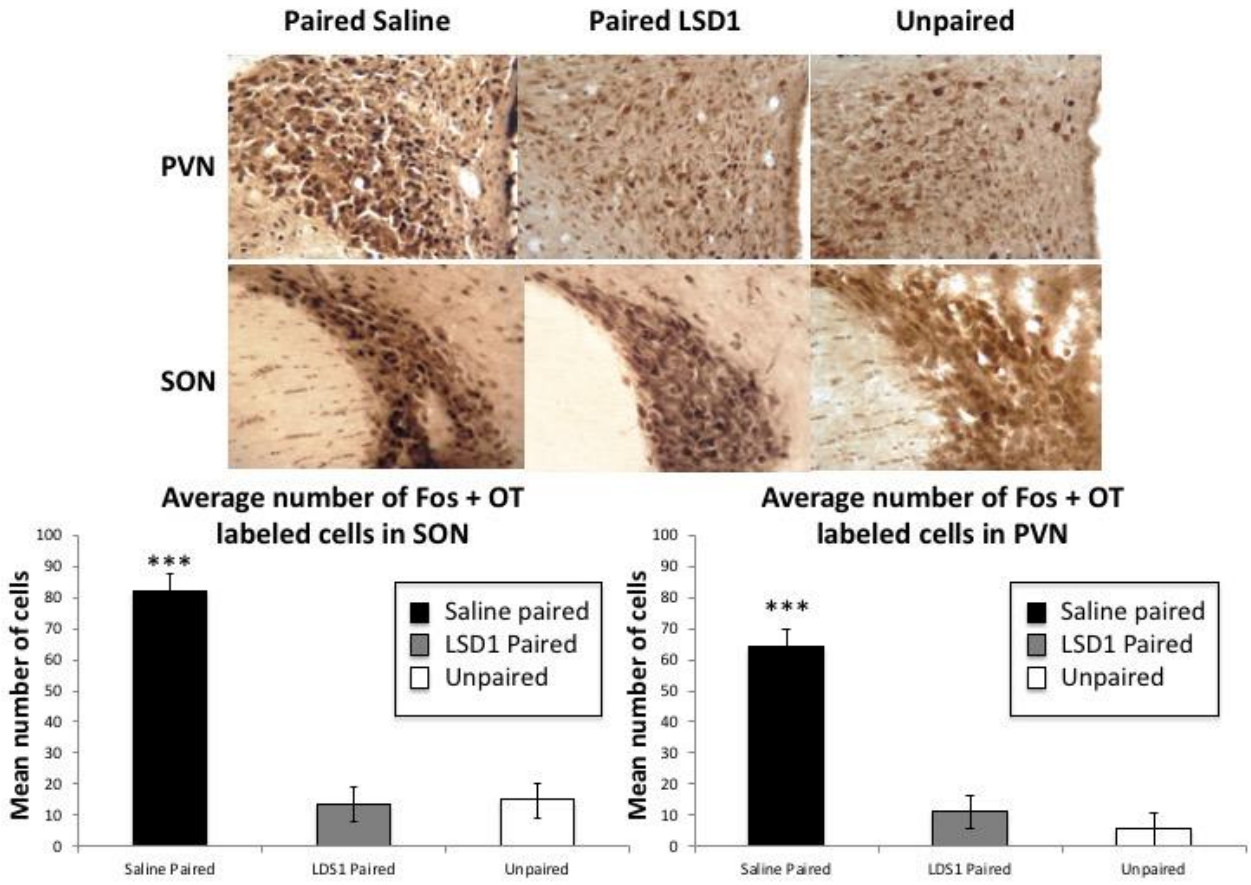

Figure 2:

Top: Photomicrographs (40x) showing OT+Fos-IR in the SON and PVN following the open-field test. Cells positive for both markers have a light brown cytoplasmic stain (OT) with a dark black nucleus (Fos). Magnified inserts taken of selected regions at 100x to more clearly demonstrate double labeled v. single labeled cells. Bottom: OT+Fos positive cells in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) from saline paired (black), LSD1 paired (grey), or unpaired (white) groups. Data are means ± SEM. * $p < .05$, ** $p < .01$; *** $p < .001$.

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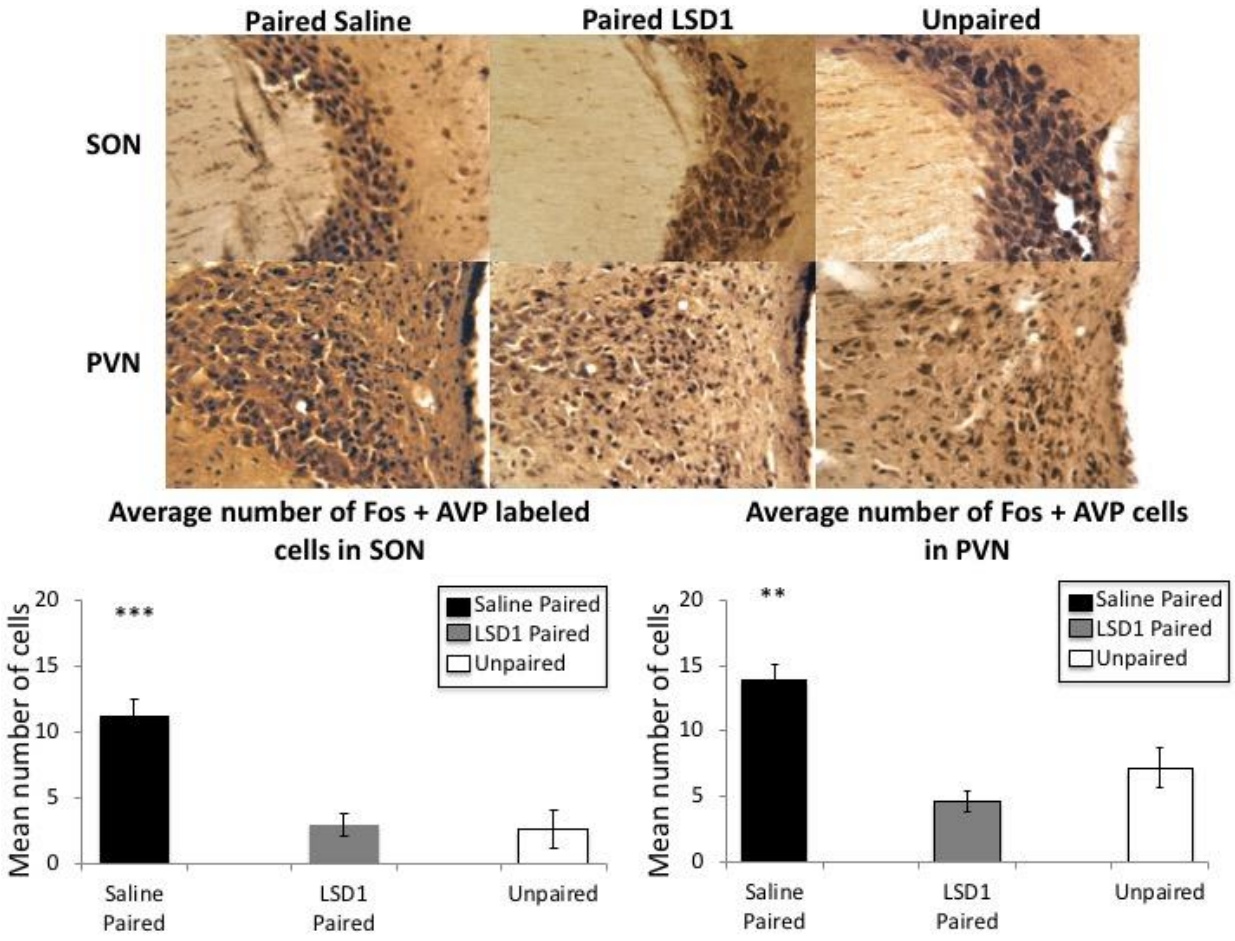


Figure 3:

Top: Photomicrographs (40x) showing AVP+Fos-IR in the SON and PVN following the open-field test. Cells positive for both markers have a light brown cytoplasmic stain (AVP) with a dark black nucleus (Fos). Magnified inserts taken of selected regions at 100x to more clearly demonstrate double labeled v. single labeled cells. Bottom: AVP+Fos positive cells in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) from saline paired (black), LSD1 paired (grey), or unpaired (white) groups. Data are means ± SEM. * $p < .05$, ** $p < .01$; *** $p < .001$.

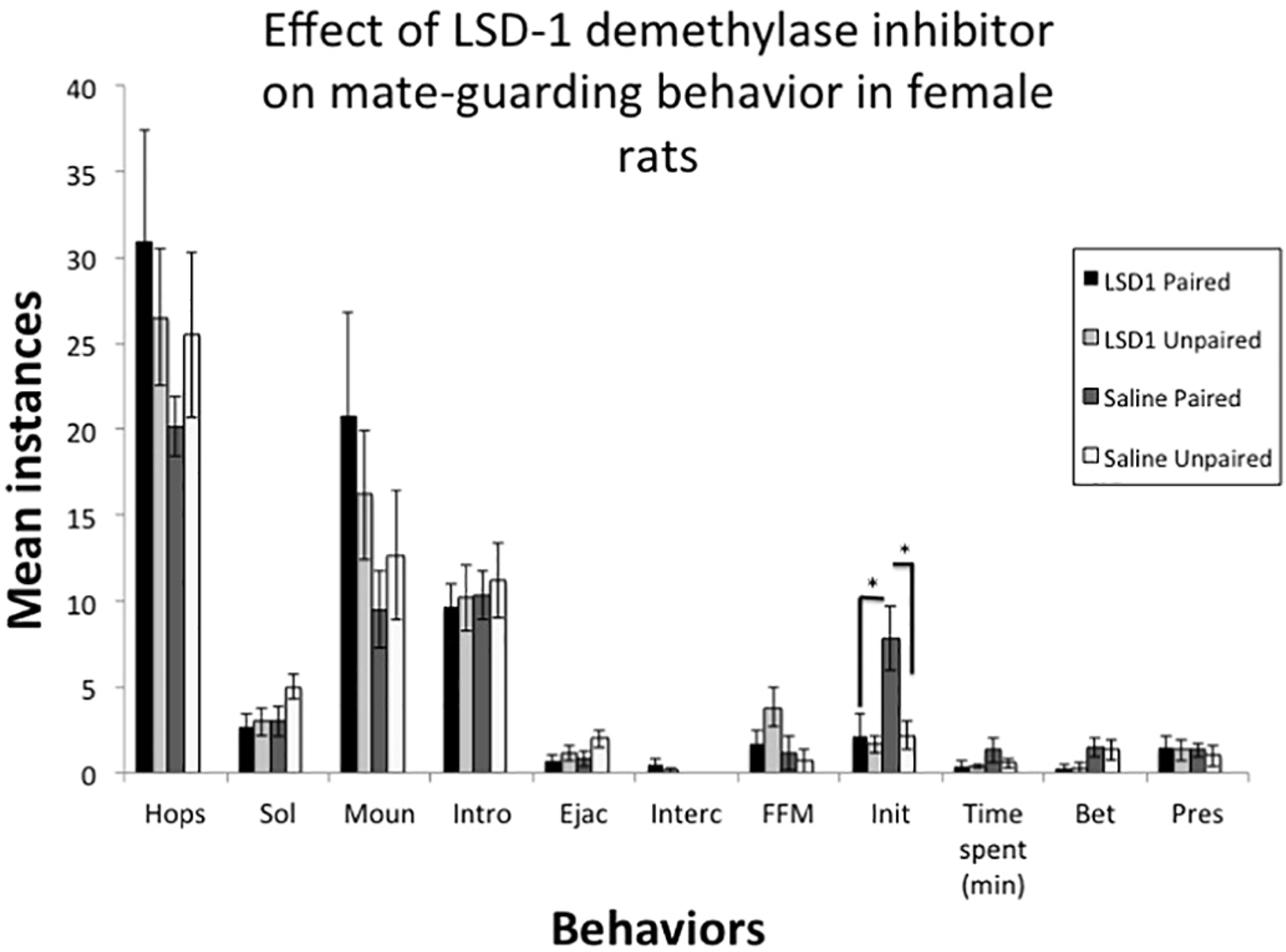

Effect of LSD-1 demethylase inhibitor on mate-guarding behavior in female rats
Mean instances
40
35
30
25
20
15
10
5
0
LSD1 Paired
LSD1 Unpaired
Saline Paired
Saline Unpaired
*
*
Hops
Sol
Moun
Intro
Ejac
Interc
FFM
Init
Time spent (min)
Bet
Pres
Behaviors

Figure 1

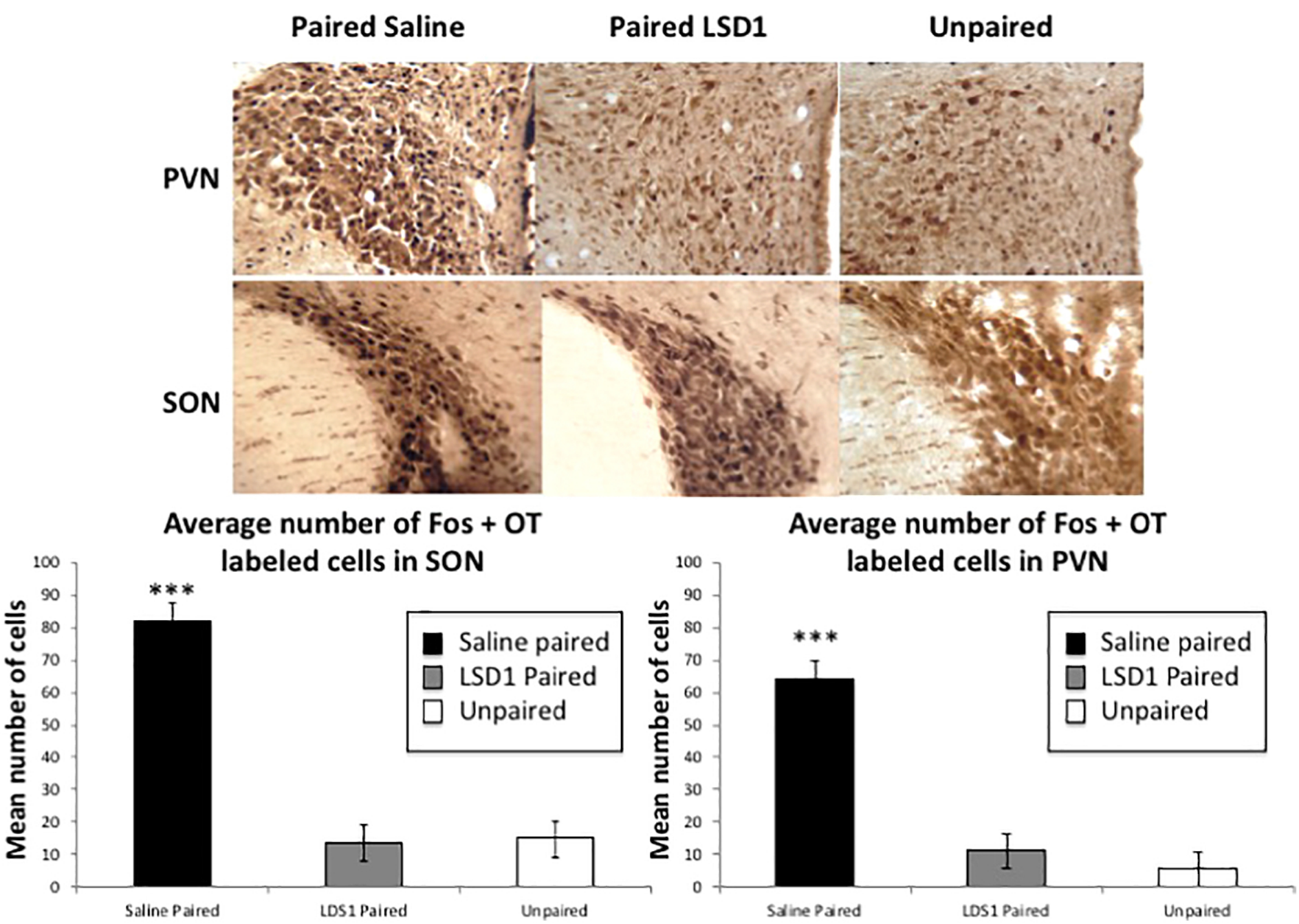
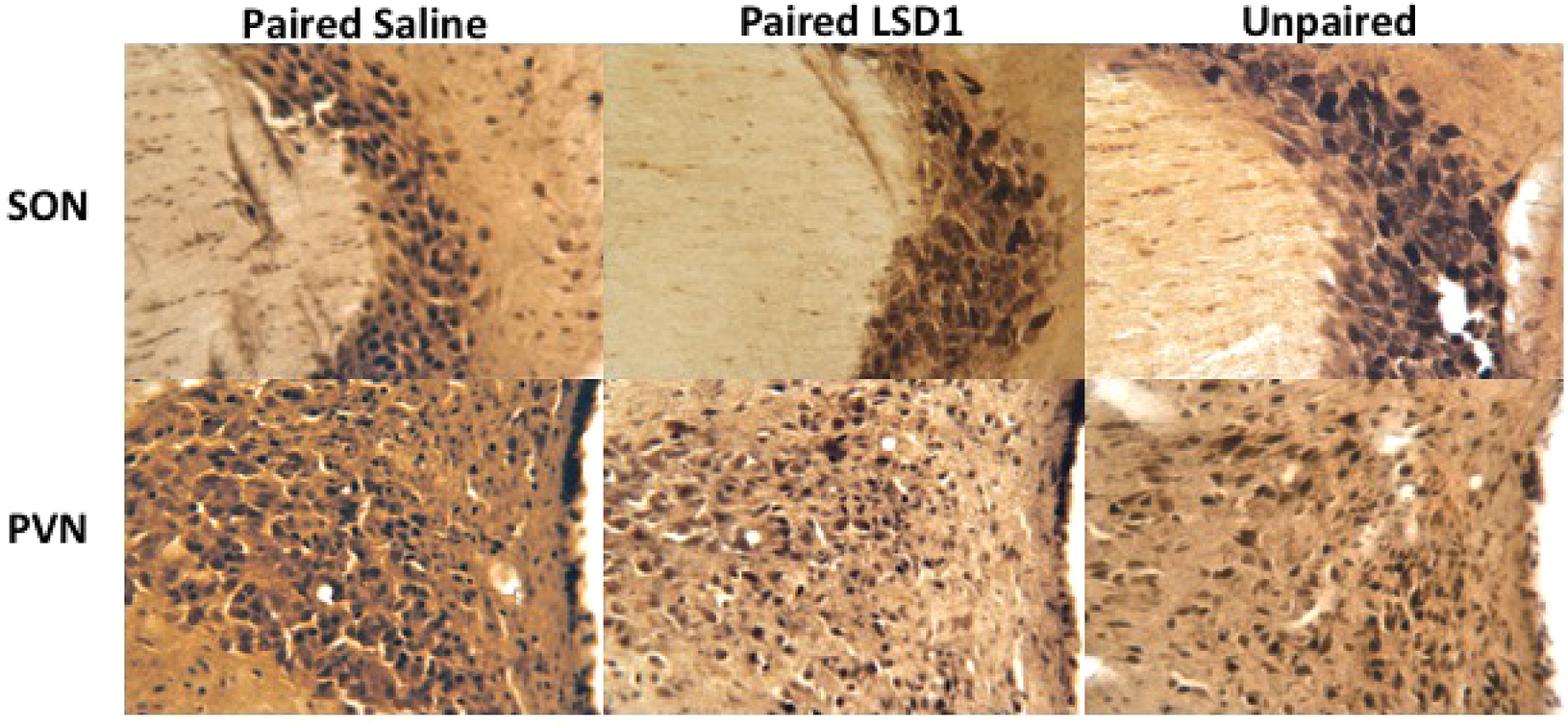
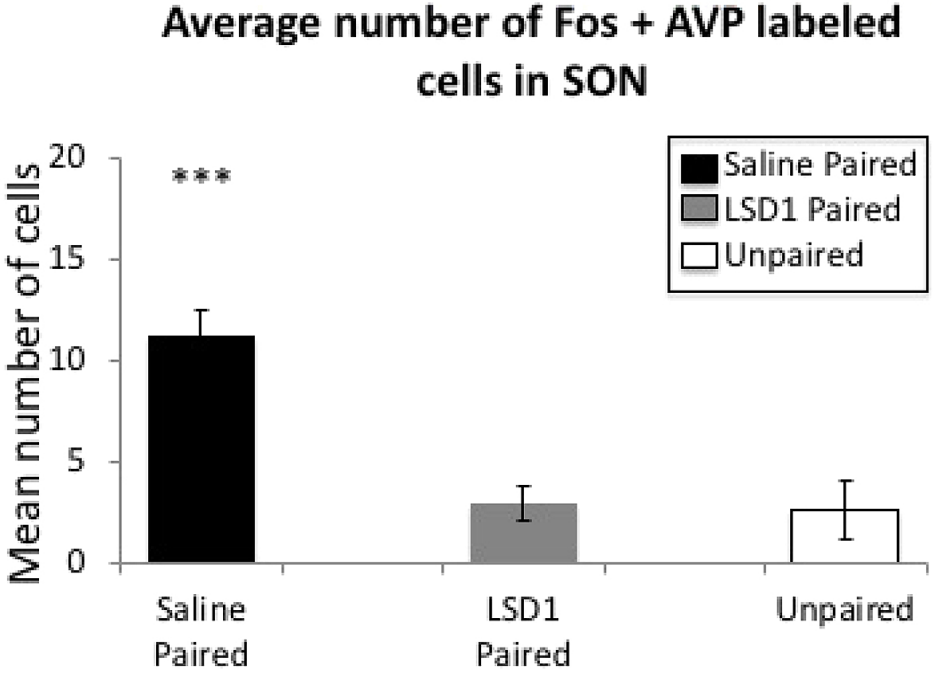

Paired Saline
Paired LSD1
Unpaired
PVN
SON
Average number of Fos + OT labeled cells in SON
Mean number of cells
***
Saline paired
LSD1 Paired
Unpaired
Saline Paired
LDS1 Paired
Unpaired
Average number of Fos + OT labeled cells in PVN
Mean number of cells
***
Saline paired
LSD1 Paired
Unpaired
Saline Paired
LDS1 Paired
Unpaired

Figure 2


Paired Saline
Paired LSD1
Unpaired
SON
PVN


Average number of Fos + AVP labeled cells in SON
Mean number of cells
20
15
10
5
0
***
Saline Paired
LSD1 Paired
Unpaired
Saline Paired
LSD1 Paired
Unpaired

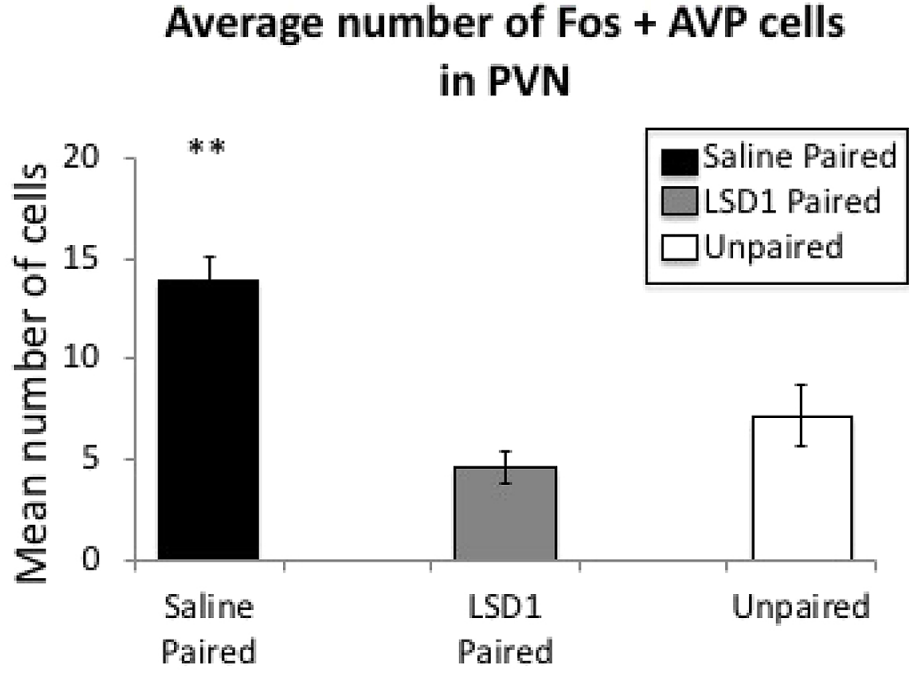

Average number of Fos + AVP cells in PVN
Mean number of cells
20
15
10
5
0
**
Saline Paired
LSD1 Paired
Unpaired
Saline Paired
LSD1 Paired
Unpaired

Figure 3