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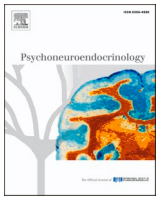

Mating-induced release of oxytocin in the mouse lateral septum: Implications for social fear extinction

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ABSTRACT

In mammals, some physiological conditions are associated with the high brain oxytocin (OXT) system activity. These include lactation in females and mating in males and females, both of which have been linked to reduced stress responsiveness and anxiolysis. Also, in a murine model of social fear conditioning (SFC), enhanced brain OXT signaling in lactating mice, specifically in the lateral septum (LS), was reported to underlie reduced social fear expression. Here, we studied the effects of mating in male mice on anxiety-related behaviour, social (and cued) fear expression and its extinction, and the activity of OXT neurons reflected by cFos expression and OXT release in the LS and amygdala. We further focused on the involvement of brain OXT in the mating-induced facilitation of social fear extinction. We could confirm the anxiolytic effect of mating in male mice irrespective of the occurrence of ejaculation. Further, we found that only successful mating resulting in ejaculation (Ej^+) facilitated social fear extinction, whereas mating without ejaculation (Ej^-) did not. In contrast, mating did not affect cues fear expression. Using the cellular activity markers cFos and pErk, we further identified the ventral LS (vLS) as a potential region participating in the effect of ejaculation on social fear extinction. In support, microdialysis experiments revealed a rise in OXT release within the LS, but not the amygdala, during mating. Finally, infusion of an OXT receptor antagonist into the LS before mating or into the lateral ventricle (icv) after mating demonstrated a significant role of brain OXT receptor-mediated signaling in the mating-induced facilitation of social fear extinction.

1. Introduction

Social anxiety disorder (SAD) is the second most common anxiety disorder and is characterized by persistent fear and avoidance of social situations. Traditionally, SAD is treated with a combination of cognitive-behavioral therapy and pharmacotherapy (Heimberg et al., 2014). However, SAD pharmacotherapy usually consists of treatment with benzodiazepines or selective serotonin reuptake inhibitors, both of which were initially designed to treat generalized anxiety or depression. The lack of more specific treatment regimens for SAD often leads to treatment resistance and relapse even after attaining complete remission (Blanco et al., 2013). We have previously generated an animal model of SAD, i.e., the social fear conditioning (SFC) paradigm based on operant conditioning principles (Toth et al., 2012b). Here, social fear conditioned (SFC^+) mice receive a mild foot shock when interacting with an unknown conspecific, which results in the robust and specific avoidance and fear of conspecifics. Using the SFC paradigm, we have started to reveal various neuronal and molecular mechanisms underlying the expression of social fear and its extinction (Bludau et al., 2023; Royer et al., 2022) with a focus on oxytocinergic signaling within the lateral septum (LS).

For example, we have shown that the neuropeptide oxytocin (OXT), which is produced in the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei and exerts prosocial (Menon and Neumann, 2023) and anxiolytic actions (Jurek and Neumann, 2018), acts within the LS to facilitate social fear extinction (Menon et al., 2018; Zoicas et al., 2014). While local OXT release was found to be blunted in SFC^+ mice, bilateral infusion of synthetic OXT into the LS reversed social fear in male and female SFC^+ mice (Menon et al., 2018; Zoicas et al., 2014). In support, lactating female mice, which are characterized by physiological activation of the brain OXT system, including increased expression of OXT and its receptor (OXTR) and elevated levels of release within the brain in

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response to the suckling stimulus (Neumann et al., 1996; Neumann and Landgraf, 1989; Slattery and Neumann, 2008), show reduced social fear in the SFC paradigm (Menon et al., 2018). This effect was found to be mediated by OXTergic projections to the LS originating mainly in the SON (Menon et al., 2018). Importantly, septal OXT signaling seems to be specifically involved in the extinction of social fear, but not cued fear, as studied in a model of cued fear conditioning (CFC) (Menon et al., 2018). Similar to lactation, sexual activity in males acutely activates hypothalamic OXT expression in rats (Witt and Insel, 1994), OXT secretion into blood in men (Carmichael et al., 1987; Kruger et al., 2003), and OXT release within the rat brain (Waldherr and Neumann, 2007). Moreover, in male rats, mating resulted in reduced anxiety-related behavior, an effect which was found to be mediated by centrally released OXT (Waldherr and Neumann, 2007).

Based on these findings, we investigated the effect of mating on anxiety-like behaviour, as well as on cued and social fear expression in respective models of fear conditioning in male mice. We further studied the involvement of OXT in mating-induced facilitation of social fear extinction. We show that mating reduces anxiety-related behaviour also in male mice and facilitates social fear extinction under specific mating conditions. Thus, only successful mating resulting in ejaculation (Ej^+) facilitated social fear extinction, whereas mating *per se*, i.e., without resulting in ejaculation (Ej^-), did not. Based on the expression of the neuronal activity markers cFos and pErk in response to mating and social fear extinction, respectively, we identified the LS as a potential region participating in the effect of ejaculation on social fear extinction. Microdialysis measurements confirmed the release of OXT also within the LS of male mice during mating. Finally, pharmacological blockage of OXTRs specifically within the LS before mating demonstrated a significant role of septal OXTR-mediated signaling in the mating-induced effect on social fear extinction.

2. Materials and methods

2.1. Animals

Male and female CD1 mice (8–12 weeks; Charles River, Sulzfeld, Germany) were kept under the standard laboratory conditions (12/12 h light/dark cycle, lights on at 06:00, 22°C, 60 humidity, food, and water *ad libitum*). Three days prior to each experiment, male mice were single-housed in observation cages (30×24×36cm). All experimental procedures were performed between 08:00 and 12:00 h in accordance with the Guide for the Care and Use of Laboratory Animals of the Government of Mittelfranken, the ARRIVE guidelines (Kilkenny et al., 2010), and with EU guideline 2010/63/EU.

2.2. Experimental protocols

2.2.1. Effects of mating on anxiety-like behavior, cued fear extinction, and social fear extinction

To evaluate the effect of mating on anxiety-like behavior, sexually trained (see below) male mice were allowed to mate for 1 h with a receptive female and subdivided into two groups, i.e., mice that mated without ejaculating (Ej^-) and those that mated and ejaculated (Ej^+). Non-mated males (NM) without contact with a female served as controls. Ten minutes after mating (Ej^- and Ej^+) or at matched time points (NM), anxiety-like behavior of male mice was tested either in the light-dark box (LDB) or the marbles burying test (Fig. 1A).

To evaluate the effect of mating on the extinction of either cued or social fear, separate cohorts of mice were exposed to the respective fear acquisition protocol of CFC or SFC on day 1. For CFC, all mice were conditioned, whereas for SFC, 2 groups were established, i.e., the conditioned (SFC^+) and unconditioned (SFC^-) groups. On day 2, mice were either allowed to mate in their home cage for 1 h prior to exposure to the extinction protocol of cued or social fear, or were not allowed to mate (NM; Figs. 1E and 1I). CFC mice were subdivided into NM, Ej^-, and Ej^+, while SFC mice were subdivided into SFC^-/NM controls and SFC^+ mice classified according to their mating behavior (SFC^+/NM, SFC^+/Ej^-, and SFC^+/Ej^+).

2.2.2. Effects of mating with and without ejaculation and social fear extinction on neuronal activity patterns

To study the underlying mechanisms of ejaculation-induced facilitation of social fear extinction, we analyzed neuronal activity, i.e., the expression of pERK and cFos, within the dorsal (dLS) and ventral (vLS) LS, the SON and PVN at distinct time points after mating/ejaculation and social fear extinction, respectively. Thus, SFC^+ mice were allowed to mate with a primed female, which was removed when ejaculation occurred (SFC^+/Ej^+) or at matched time points without ejaculation for control (SFC^+/Ej^-). Social fear extinction started 63 min after ejaculation (or after the removal of the primed female) and was stopped after exposure to the second social stimulus (i.e., after 27 min). This time point was chosen because SFC^+/Ej^+ mice displayed a significantly enhanced social contact in comparison to SFC^+/Ej^- mice during the exploration of the second social stimulus (see Fig. 1). This experimental setup also allowed us to analyze pErk expression as a marker of neuronal activity in response to social fear extinction, which peaks at around 10 min (Trainor et al., 2011), while cFos expression, which peaks at 90 min (63 min of mating and 27 min of extinction), served as a marker for ejaculation/mating (Fig. 2A).

2.2.3. Effects of mating on OXT release within the septum and amygdala

In the septum, five 30-min microdialysates were sampled, two under basal conditions (samples 1 and 2), two during (samples 3 and 4) and one after (sample 5) a 60-min mating period (Mating group) or during the presence of a non-receptive female (Contact group). In the amygdala region, six 30-min microdialysates were sampled, two under basal conditions (samples 1 and 2), three during (samples 3, 4, and 5) and one after (sample 6) a 90-min mating period (Mating group) or during the presence of a non-receptive female (Contact group). The duration of the sampling periods (30 min), however, did not allow to distinguish between the Ej^- and Ej^+ groups, as ejaculation is a relatively brief event (few seconds), which occurred either during the first (sample 3) or second (sample 4) 30 min of the 1-hour mating period.

2.2.4. Effects of pre-mating OXT receptor antagonist (OXTR-A) infusion into the LS on social fear extinction

In order to test whether OXT released within the septum during mating is involved in the facilitated social fear extinction, we administered either a specific OXT receptor antagonist (OXTR-A) or vehicle bilaterally into the LS of SFC^+ mice 30 min prior to the 1-h mating period and 80 min prior to social fear extinction (Fig. 5A).

2.2.5. Effects of post-mating icv OXTR-A administration on social fear extinction

In order to confirm the involvement of endogenous OXT in the process of social fear extinction (Menon et al., 2018; Toth et al., 2012b), we administered either a specific OXTR-A or vehicle into the left ventricle (icv) of SFC^+ mice immediately after 1 h of mating and 10 min prior to the start of social fear extinction (Fig. 6A).

2.3. Behavioral experiments

2.3.1. Mating procedure

Two days before the experiment, male mice were familiarized with the mating procedure by placing a primed and experienced female into their home cage for 30 min. Female mice were primed with estrogen (ß-estradiol 3-benzoate, 25 µg/0.05 ML of oil, sub-cutaneous.; Sigma-Aldrich) to increase sexual receptivity 38 h before the experiment, and were also adapted to the mating procedure by the presence of a sexually-experienced male for 30 min 18 h prior to the experiment. On the day of the mating experiment, the estrous cycle was assessed via vaginal smear

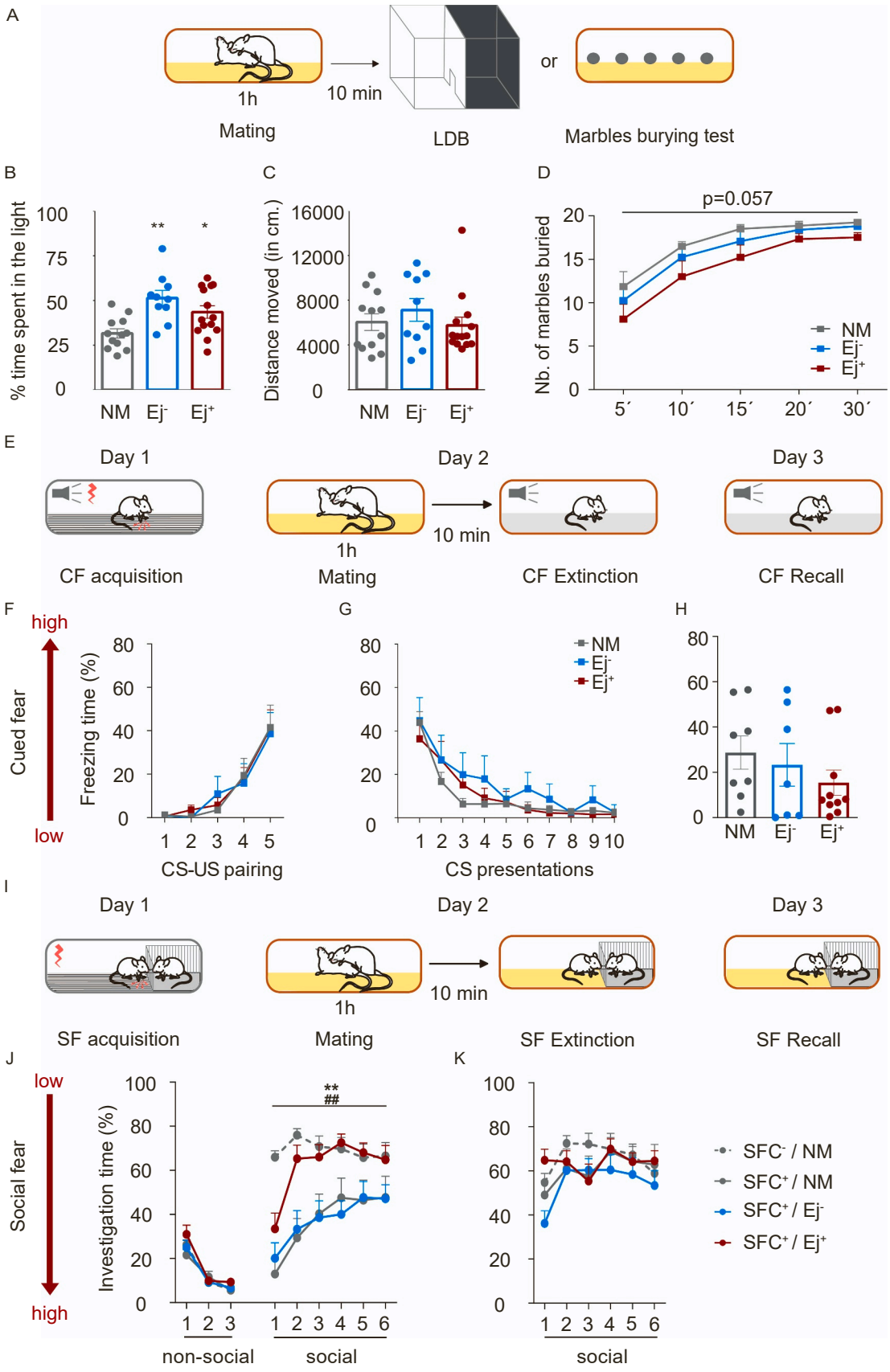

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Fig. 1. Mating differentially affects anxiety-like behavior, while ejaculation only facilitates social fear extinction. Schematic representation of the experimental design to test the effect of mating on anxiety-like behavior (A). Non-mated (NM) and mated mice (Ej^- = mating without ejaculation; Ej^+ = mating with ejaculation) were subjected to the LDB (n=10–14 mice/groups; B-C). The percentage of time spent in the light compartment (B) and the distance travelled (C) were monitored. Data are presented in mean ± SEM. * $p<0.05$ compared to NM, ** $p<0.01$ compared to NM. NM, Ej^- and Ej^+ mice were subjected to the marbles burying test (n=8–12 mice/groups; D). The number of marbles buried was evaluated after 5, 10, 15, 20, and 30 min. Data are presented in mean ± SEM. Schematic representation of the experimental design to test the effect of mating on cued fear extinction (E). Male mice were cued fear conditioned (day 1; F). 24 h later, mice were allowed to mate for 1 h 10 min prior cued fear extinction (n= 7–10 mice/group). Percentage of freezing of NM, Ej^-, and Ej^+ mice was monitored during cued fear extinction (day 2; G) and cued fear recall (day 3; H). Data are presented in mean ± SEM. Experimental design to test the effect of mating on social fear extinction (I). Male mice were social fear conditioned (SFC^+) or left unconditioned (SFC^-) (day 1; J-K). 24 h later, they were allowed to mate for 1 h 10 min prior social fear extinction (n=10–16 mice/groups). The percentage of investigation of three non-social (empty cage) and six social (cage with a conspecific) stimuli of NM, Ej^-, and Ej^+ mice was monitored during social fear extinction training (day 2; G) as well as six social stimuli during social fear extinction recall (day 3; H). Data are presented in mean ± SEM. ** $p<0.01$ SFC^+/NM vs. SFC^+/Ej^+; ## $p<0.01$ SFC^+/Ej^- vs. SFC^+/Ej^+. For detailed statistics, see S-Table 1.

to confirm pro-estrus or estrus, before the female was introduced into the home cage of the experimental male for 1 h. Non-mated (NM) control males were left single-housed for 1 h. All mating experiments were video-recorded for later analysis. The latencies of the first intromission and of ejaculation and the number of successful mountings and intromissions were also manually scored. After video analysis of the occurrence of ejaculation, the males of the mating group were separated into $Ej^-$and Ej^+. Males who did not successfully mount the female were excluded from the analysis.

2.3.2. Light-Dark Box (LDB)

The LDB was performed to test for the anxiolytic effect of mating as previously described (Bourin and Hascoet, 2003; Reber and Neumann, 2008). For details, see Supplementary Methods.

2.3.3. Marble burying test

The marble burying test was performed to check for the anxiolytic effect of mating as previously described (Deacon, 2006). For details, see Supplementary Methods.

2.3.4. Cued Fear Conditioning (CFC)

The CFC paradigm was performed as previously described (Toth et al., 2012a). For details, see Supplementary Methods.

2.3.5. Social fear conditioning (SFC)

The SFC paradigm was performed as previously described (Menon et al., 2018; Toth et al., 2012b). For details, see Supplementary Methods.

2.3.6. Social preference/social novelty test

The social preference/social novelty test was performed in a rectangular box divided by transparent plexiglas walls into three equal-sized chambers (each 40×20×20cm) connected via small rectangular openings allowing access to each chamber. One day prior to the test, each mouse was habituated to the empty box for 5 min before 2 identical non-social stimuli (small empty wire-mesh cages) were placed into the right and left chambers for 5 min. On the day of the experiment, the mouse was placed in the middle compartment and allowed to explore the empty three-chambered box for 5 min. After the habituation period, it was briefly removed from the apparatus, while a non-social (empty wire-mesh cage) and a social (wire mesh cage containing an unfamiliar conspecific) stimulus were placed in the left and right lateral chambers, respectively. The test mouse was reintroduced into the middle compartment and allowed to freely explore all 3 chambers for 5 min before returning to the home cage. Immediately thereafter, the non-social stimulus was replaced by a new social stimulus, and the test mouse was re-introduced into the middle compartment and could freely explore all 3 chambers for 5 min. The boxes were cleaned with detergent-containing water and dried prior to each test. To avoid a place preference bias, the compartments containing the non-social or the social stimuli were exchanged for each mouse. The sessions were video-recorded, and the time spent in each compartment was evaluated with the software Ethovision XT, a video tracking system (Noldus Information Technology, Wageningen, The Netherlands). Animals that did not enter all the compartments within the first 150 sec of habituation or of the test sessions were excluded from analysis.

2.4. Histological and immunohistochemical analyses

For histological analysis, brains were removed, directly frozen in dry ice-cold 2-methylbutane and conserved at −20°C. For immunohistochemical analysis, animals were intracardially perfused with paraformaldehyde (4% PFA, pH 7.4) after deep anesthesia and delicate opening of the thoracic cavity to reveal the heart. A 26 G needle connected to the perfusion pump was introduced into the right heart ventricle and, after starting the perfusion at a rate of 19 ML/min, the left atrium was opened. Perfusion started with a solution of 1 X PBS (pH 7.4) for about 2 min, before PFA was perfused for 2 min). Brains were stored in 4% PFA overnight to ensure complete tissue fixation and then transferred into a 30% sucrose solution for 48 h to remove remaining water molecules from the tissue before being flash-frozen in dry ice-cold 2-methylbutane. Brains were stored at –20°C.

2.5. Immunohistochemistry

Perfused brains were cryo-cut, and 30-μm slices (cryo Leica CM3050S; Leica Microsystems) were collected in series from the LS, PVN and SON, and co-stained with anti-cFos, anti-pErk and anti-OXT antibodies (Table1). In the LS, cFos- and pErk-positive neurons were analysed. In the PVN and SON, OXT, cFos and pErk -positive neurons were analyzed. Slices were washed thrice for 10 min in PBS, treated for 10 min in 100% ice-cold methanol, incubated for 20 min in a glycine buffer 0.1 M, and washed again thrice for 6 min in 1X PBS – 0,3% Triton before being incubated in a blocking solution (5% Normal Goat serum in 1X PBS – 0,3% Triton) for 1 h at room temperature. After blocking, slices were incubated with the primary antibodies diluted in blocking solution for 64 h at 4°C (see Table1 for dilution). The next steps were performed in the dark to preserve the fluorescence: The slices were washed 3 times 10 min in 1X PBS – 0,3% Triton to remove unbound primary antibodies before being incubated with the secondary antibodies coupled with fluorescent protein diluted in blocking solution for 2 h at room temperature (1:1000, conjugated with a CF dye 488, 561 or 647, Biotum). The slices were again washed thrice for 10 min, mounted on a slide, and covered.

2.6. Image analysis and cell quantification

Systematic random sampling was used through the different target regions by counting the cells on both hemispheres of each section in 1:4 series (40 μm apart). Pictures from the LS, PVN, and SON were obtained using a Leica THUNDER Imager Tissue upright microscope, 20x objective (Leica Microsystems). Cell quantification was performed using the image processing software package ImageJ (National Institute of Health, USA) (Schneider et al., 2012). Systematic random sampling was employed for cFos-positive cell quantifications in the LS. The unbiased Physical Paired Dissector method (Mayhew, 1992) was performed for unbiased stereological estimation of the number of each positive cell

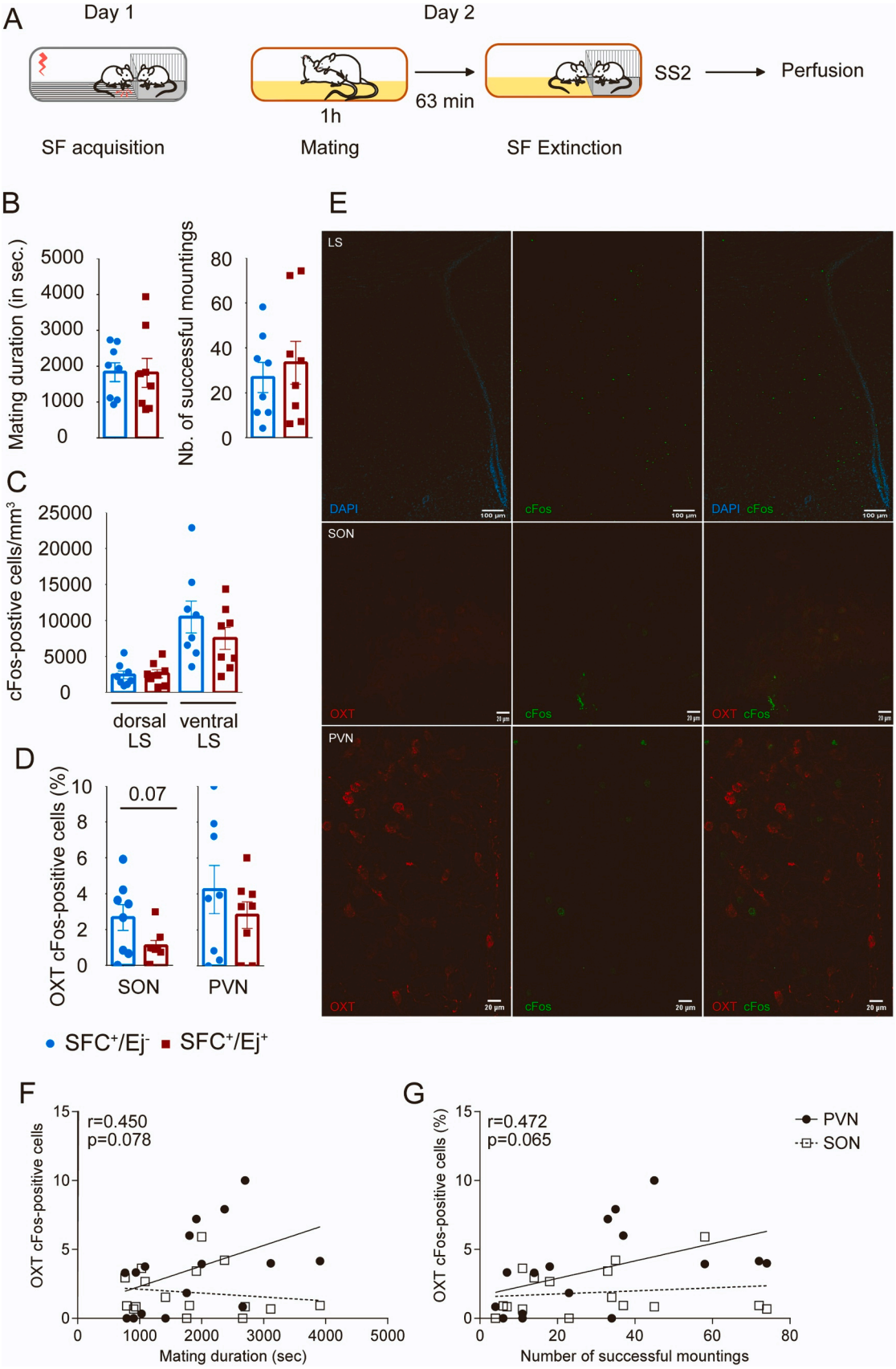

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Fig. 2. Neuronal activity in response to mating with and without ejaculation and to social fear extinction. Schematic representation of the experimental design (A). 24 h after acquisition, male social fear conditioned (SFC^+) mice were allowed to mate until ejaculation (SFC^+/Ej^+) or equivalent time point without ejaculation (SFC^+/Ej^-) (n=8 mice/group). The mating duration and number of successful mountings were monitored (B). 63 min after mating (independent of ejaculation), SFC^+/Ej^- and SFC^+/Ej^+ mice were subjected to the social fear extinction protocol (see Fig. 3) and immediately sacrificed after presentation of the second stimulus (SS2), i.e. 90 min after mating. Data represent mean of cFos-positive neurons /mm^3 in the dorsal (dLS) and ventral (vLS) lateral septum (LS) (C) and the percentage of OXT neurons expressing cFos within the SON and the PVN (D). Representative images of co-stained LS slices for DAPI (cyan) and cFos protein (green), and PVN and SON slices for OXT (red) and cFos protein (green) (E). Correlation between the percentage of OXT neurons expressing cFos within the SON and the PVN and mating parameters (mating duration and number of successful mounting) (F-G). Data are presented in mean ± SEM. For detailed statistics, see S-Table 2.

phenotype. The total number of both OXT neurons and OXT neurons expressing cFos was counted. All cell numbers are expressed as an average per section. For area quantification, region of interest was outlined manually and its area was calculated using the image processing software ImageJ.

2.7. Stereotaxic surgeries, intracerebral microdialysis and infusions

Stereotaxic implantations of guide cannulas or microdialysis probes were performed under semi-sterile conditions and deep isoflurane anesthesia (Forene, Abbott GmbH, Wiesbaden, Germany). Mice received ip injections of analgesic (Buprenovet, 300 µl/kg, Bayer, Germany) and antibiotics to avoid post-surgical infections (3 mg/30 µl Baytril, Bayer GmbH, Leverkusen, Germany).

For icv infusion the guide cannula (21 G, 8 mm length, Injecta GmbH, Germany) was placed 2 mm above the lateral ventricle (from Bregma: AP + 0.2 mm, ML + 1.0 mm, V: + 1.4 mm) (Franklin and Paxinos, 2019). For bilateral infusions into the LS the guide cannulas (23 G, 8 mm length, Injecta GmbH, Germany) were implanted 2 mm above the left or right LS (AP + 0.3 mm, ML ± 0.5 mm, V: + 1.6 mm). All guide cannulas were closed by a stylet.

For monitoring local OXT release a microdialysis probe (selfmade U-shaped probe with a 2 mm long semi-permeable membrane, molecular cut-off 10 kDa) (Zoicas et al., 2014) was implanted either into the septum (AP + 0.3 mm, ML ± 0.5 mm, V: + 3.8 mm) or the amygdala (AP −1.5 mm, ML −3.1 mm, DV −5.0 mm). After surgery, mice were repeatedly handled for 7 days (after implantation of guide cannulas) or for 2 days (microdialysis) to adapt them to the respective procedure. All intracerebral infusions were performed in awake, slightly restrained animals. The doses and the time of infusion were selected based on previous studies (Menon et al., 2018; Zoicas et al., 2014). The correct infusion site or placement of microdialysis probe were histologically verified.

Microdialysis: Two days after surgery, microdialysis probes were connected to the infusion pump via polyethylene tubing and rinsed for 2 h with sterile Ringer solution (pH 7.4, rate: 3.3 µl/min) without sampling to establish equilibrium between the inside and outside of the microdialysis membrane. In the septum, five 30-min microdialysis samples were collected, while in the amygdaloid region, six 30-min microdialysis samples were collected (for details see experimental procedures). In case of a delay of the mating behavior, i.e., occurrence of first successful mounting, e.g., due to surgical intervention within the amygdaloid region, mating was prolonged for 30 min. Experiments have been performed during the light phase and were repeated during the dark phase. As no statistical difference was found between OXT release during the light and the dark phase, data were pooled. Each dialysate was collected into a 200-µl microfuge tube containing 10 µl of 0.1 M HCl. Samples were immediately frozen and stored at −20°C before lyophilization and quantification by radioimmunoassay (sensitivity 0.3 pg/sample, cross-reactivity with related neuropeptides 0.7%; RIAgnosis, Germany) (Jong et al., 2015).

Icv infusions: For icv infusion, a stainless-steel cannula (26 G; 10 mm) was inserted into the implanted guide cannula and connected via polyethylene tubing to a Hamilton syringe (Hamilton Company, Bonaduz, Switzerland). Immediately after mating and 30 min prior to starting extinction training, animals received either a specific OXTR-A (desGly-NH2,d(CH2)5[Tyr(Me)2,Thr4]OVT; 2 µg/2 µl, kindly provided by Dr. Maurice Manning, Toledo, Ohio) or vehicle (sterile Ringer solution, 2 µl).

Local infusions: Bilateral infusions into the LS were performed using a glass fiber directly inserted into the previously implanted guide cannula and connected via polyethylene tubing to a Hamilton syringe (Hamilton Company, Bonaduz, Switzerland). The animals received either the OXTR-A (desGly-NH2,d(CH2)5[Tyr(Me)2,Thr4]OVT; 20 ng/0.2 µl/side) or a sterile Ringer solution (vehicle; 0.2 µl/side) 10 min before mating.

2.8. Statistics

Statistical analysis was performed using IBM SPSS Statistics 25 employing t-tests for independent samples, Pearsońs correlation, one-, two-way or mixed-model ANOVA with time as within-subjects factor, and mating and treatment as a between-subjects factor, where appropriate. Greenhouse-Geisser corrections were used on the p-value (the degrees of freedom within the groups and in between the groups, and the F value were not corrected), when the assumption of sphericity was violated according to Mauchly's test. In case of main effect or an interaction, Bonferroni pairwise comparisons were performed. Statistical significance was set at $p \leq 0.05$. All data are shown as mean ± SEM. For detailed statistical comparisons, see Supplementary Tables (S-Tab) 2–7, as indicated.

3. Results

3.1. Effects of mating on anxiety-like behavior, cued fear extinction and social fear extinction

Anxiety-related behaviour: LDB and marble burying test were performed to confirm the anxiolytic effect of mating observed in rats (Waldherr and Neumann, 2007) and to test whether such an anxiolytic effect could be the basis for altered social fear expression in the SFC paradigm. In the LDB, mating significantly affected the percentage (in LDB only time) of time spent in the light compartment (factor mating: $F(2,33)=7.61$, $p=0.002$, Fig. 1B). Bonferroni multiple comparisons test showed that both Ej^- ($p=0.002$) and Ej^+ ($p=0.05$) mice spent more time in the light compartment compared with the NM group, hence confirming the previously described anxiolytic effect of mating in male rats (Waldherr and Neumann, 2007) (Fig. 1B). However, mating (with or without ejaculation) did not affect locomotion, reflected by the distance traveled ($F(2,33)=0.73$, $p=0.50$; Fig. 1C; S-Table 1). Similarly, in the marbles burying test mating tended to affect the total number of marbles buried (factor mating: $F(2,27)=3.19$, $p=0.057$; Fig. 1D). Post hoc

Table 1
List of the primary antibodies.

Primary antibodies	Dilution
Rabbit anti-cFos (Abcam)	1:20 000, incubation 48 h 4°C
Mouse anti-neurophysin-OXT (p38), kindly provided Dr. Harold Gainer (NIH, Bethesda)	1:10 000, incubation 48 h 4°C
Rabbit anti-pERK	1:1000, incubation 64 h 4°C
Sheep anti-cFos (Abcam)	1:10 000, incubation 64 h 4°C

analysis revealed that Ej^+, but not Ej^- mice, buried a lower number of marbles (p=0.026 vs. NM; Fig. 1D; S-Table 2).

CFC: CFC was performed to assess the effect of mating on non-social fear. During acquisition of cued fear performed one day prior to mating, no group differences in the display of freezing were found (day 1: F(2,22)=0.009, p=0.99; Fig. 1F). Further, pre-extinction mating did affect the display of freezing neither during cued fear extinction (day 2; F(2,22)=0.57, p=0.57; Fig. 1G) nor during the recall (day 3; F(2,22)=0.93, p=0.41; Fig. 1H; S-Table 2). Thus, despite the general anxiolytic effect of mating, pre-extinction mating with or without ejaculation does not affect cued fear extinction.

SFC: A 1-hour mating period ending 10 min before social fear acquisition (day 1) did neither affect the number of non-conditioned stimuli (foot shocks) necessary to induce social fear (F(2,23)=1.66, p=0.21) nor the expression of social fear during extinction (day 2; F(2,24)=1.97, p=0.42; S-Table 7; Supplementary figure (S-Fig) 1). In contrast, 1 h of mating prior to social fear extinction (day 2) had an overall effect on the extinction of social fear, i.e., the time of social investigation across the presentation of 6 different social stimuli (F(2,38)=7.09, p=0.002). Specifically, we only found a higher percentage of social investigation in the SFC^+/Ej^+ group compared to both SFC^+/NM (p=0.013) as well as SFC^+/Ej^- (p=0.005) mice during social fear extinction. This indicates that only mating resulting in ejaculation facilitates social fear extinction (Fig. 1J). However, mating prior to social fear extinction did not alter the investigation of the 3 nonsocial stimuli (F(2,38)=1.18, p=0.32; Fig. 1J). Also, the social investigation time during recall performed on day 3 was not affected by mating prior to social fear extinction (F(2,38)=0.96; Fig. 1K; S-Table 1). To prove that mating did not accelerate social fear extinction by, for example, increasing social motivation, we further controlled for possible mating effects on general social behavior. However, neither naturally occurring social preference behaviour (F(2,21)=0.04, p=0.96) nor social novelty behaviour (F(2,25)=0.67, p=0.52) differed between mated Ej^+, mated Ej^- and NM groups (S-Fig. 2A; S-Table 7). Similarly, social contact with a non-primed female mouse (Ct) also did not alter social fear expression the SFC^+/Ct group expressed social fear at levels similar to that of the SFC^+/NM and SFC^+/Ej^- groups during social fear extinction (S-Fig. 3B).

Our data confirm the anxiolytic effect of mating recently described in male and female rats (Nyuyki et al., 2011; Waldherr and Neumann, 2007) also in male mice. Further, successful mating culminating in ejaculation was particularly effective to facilitate social fear extinction, whereas cued fear extinction was unaffected.

3.2. Effects of mating with and without ejaculation, and of social fear extinction on neuronal activity patterns

To study the mechanisms underlying the differential effects of mating with and without ejaculation on the facilitation of social fear extinction, we compared the activation patterns of neurons in the dorsal (dLS) and ventral (vLS) LS, and of OXT neurons in the PVN and SON, in response to mating and social fear extinction between SFC^+/Ej^- and SFC^+/Ej^+ mice. We used cFos and pErk as neuronal activity markers for mating and extinction, respectively (see experimental procedures). Except for the occurrence of ejaculation, the mating behavior of SFC^+/Ej^- and SFC^+/Ej^+ mice was similar (mating duration: t(14)=0.04, p=0.97; number of successful mountings: t(14)=-0.56, p=0.59; Fig. 2B). The number of cFos-positive cells did not differ between the SFC^+/Ej^- and SFC^+/Ej^+ groups either in the dLS (t(14)=-0.236, p=0.82) or vLS (t(14)=1.102, p=0.29; Fig. 2C). Moreover, the number of cFos-positive cells within the dLS or vLS did not correlate with any mating parameters (data not shown). In the SON, the percentage of cFos-positive OXT neurons tended to be lower in the SFC^+/Ej^+ compared to the SFC^+/Ej^- group (t(14)=2.02, p=0.072), but did not correlate with mating behaviors (Fig. 2F and G). In the PVN, no difference in the percentage of cFos-positive OXT neurons was found (t(14)=0.93, p=0.37; Fig. 2D), although the percentage of cFos-positive OXT neurons tended to correlate with mating parameters (duration: r=0.45, p=0.078; number of successful mounts: r=0.472, p=0.065; N=16) (Fig. 2F and G; S-Table 3).

The number of pErk-positive neurons in the vLS reflecting responses to social fear extinction were found to be lower in SFC^+/Ej^+ mice (t(14)=2.28, p=0.049 versus SFC^+/Ej^-; Fig. 3B), whereas no difference was found in the dLS (t(14)=0.68, p=0.51). However, the number of pErk-positive cells in the LS did not correlate with the investigation of the social stimulus during extinction (vLS: r=-0.303, N=16, p=0.25; dLS: r=-0.088, N=16, p=0.74, data not shown). However, we found a correlation between the expression of cFos and pErk both in the dLS (Fig. 3E, r=0.850, p=0.001) and the vLS (Fig. 3F, r=0.789, p=0.001). No group difference was found in the percentage of OXT neurons expressing pErk in the PVN (t(14)=0.166, p=0.87) and SON (t(14)=-0.515, p=0.61; Fig. 3C).

3.3. Effects of mating on OXT release within the septum and amygdala

Next, we monitored the mating-induced OXT release within the LS and the amygdala – regions, where the neuropeptide robustly facilitates either social fear extinction (LS) (Menon et al., 2018; Zoicas et al., 2014) or non-social fear expression and anxiety (amygdala) (Haubensak et al., 2010; Huber et al., 2005; Knobloch et al., 2012). In the septum, OXT content in microdialysates was found to be altered by mating (mating x time: F(8,64)=3.49, p=0.009; Fig. 4A). Posthoc analysis revealed an increase in OXT release during mating (sample 3 vs. sample 1: p=0.006) followed by a decline (sample 3 vs. sample 5: p=0.017). Also, OXT content in sample 3 tended to be higher in the Mating compared to the Contact group (p=0.074) further indicating a mating-induced increase in local OXT release (S-Table 5). In microdialysates sampled outside the LS (non-hit group) of mated or non-mated mice, a lower OXT release was detected during the two 30-min mating periods (sample 3: p=0.013; sample 4: p=0.041 vs. similar time points in the hit-mated group) (Fig. 4A). In contrast to the septum, OXT release within the amygdala was not affected by mating (F(1,29)=2.23, p=0.15), but generally changed over the sampling period (time: F(5145)=14.43, p=0.0001; Fig. 4C). Bonferroni posthoc analysis revealed an increased release both in the Contact group (p= 0.001) and by trend in the Mating group (p=0.053; S-Table 5) during the collection of sample 3 compared with sample 1 (basal), indicating that the release of OXT within the amygdala is rather stimulated by social interaction with the female than by mating.

3.4. Effects of administration of an OXT receptor antagonist (OXTR-A) into the LS before mating on social fear extinction

Intra-septal administration of an OXTR-A and blockade of local OXTR 30 min prior to mating did not affect mating behavior, as reflected by the latency to first successful mounting (Fig. 5B), number of successful mounting (Fig. 5C), or latency to ejaculate (Fig. 5D). Also, the OXTR-A did not alter the exploration of the 3 non-social stimuli presented prior to social fear extinction training (treatment: F(1,37)=0.11, p=0.92), but the occurrence of ejaculation affected this parameter (mating: F(1,37)=6.46 p=0.015; time x mating: F(2,74)=4.72, p=0.025): a higher investigation time of the first empty cage was found in Ej^+ compared to Ej^- mice (p=0.01) independent of treatment reflecting reduced anxiety- or fear-related behaviour.

OXTR-A treatment tended to affect social fear extinction (treatment: F(1,37)=3.24, p=0.08). Separate statistical analysis performed on the investigation times of the first social stimulus revealed an effect of treatment (F(1,37)=6.92, p=0.012) with reduced investigation in the OXTR-A compared with Veh groups, independent of the occurrence of ejaculation (F(1,37)=0.12, p=0.73; Fig. 5E). The OXTR-A and mating also affected social investigation during recall (treatment x time: F(5185)=3.56, p=0.034). Specifically, OXTR-A mice showed a longer investigation of the third (p=0.035) and fourth (p=0.021) social stimuli (Fig. 5F; S-Table 6) compared with Veh.

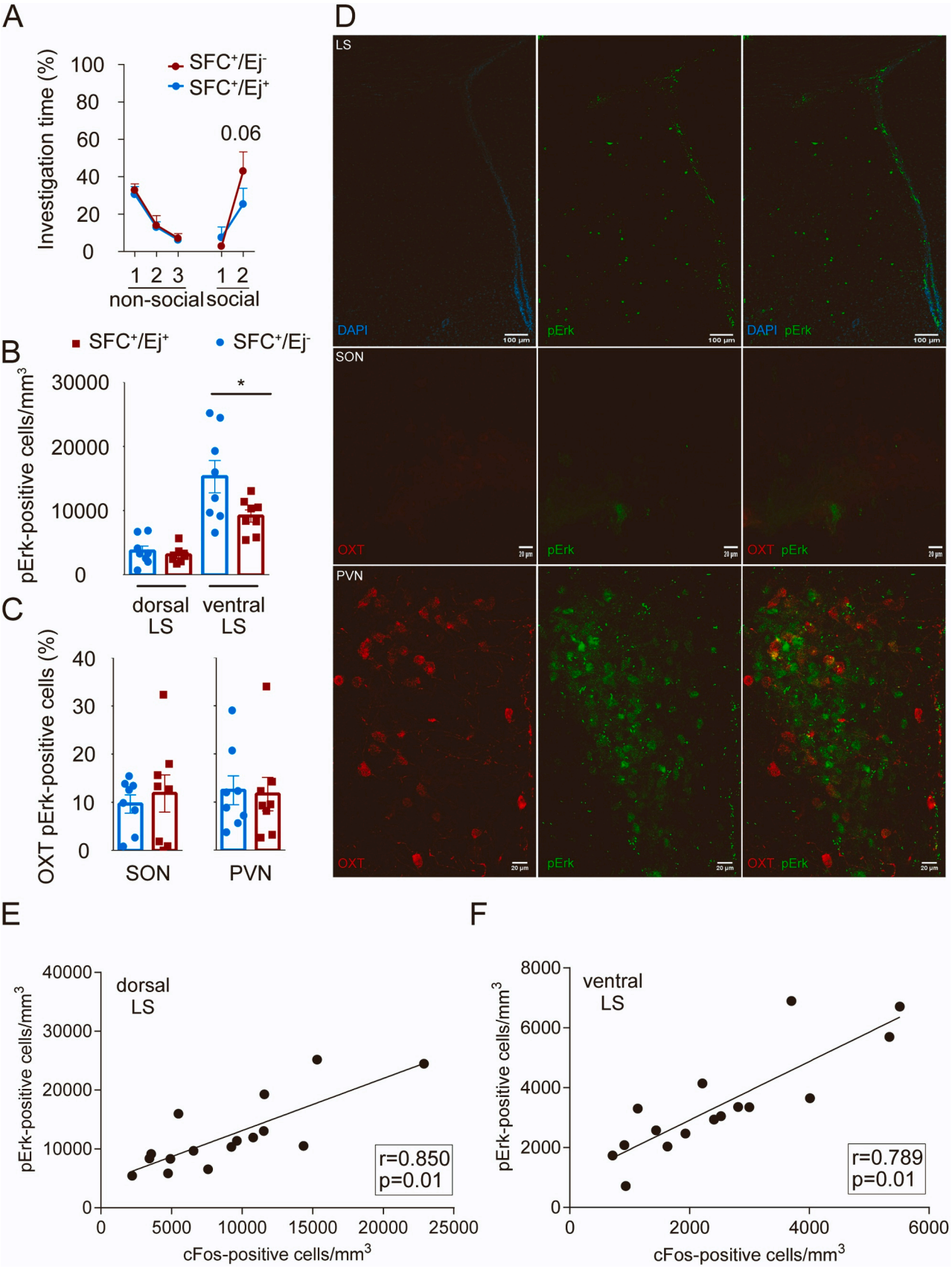

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Fig. 3. Neuronal activity during social fear extinction after mating. 24 h after social fear acquisition, male mice were allowed to mate until ejaculation (SFC^+/Ej^-) or equivalent time point (SFC^+/Ej^+) (n=8 mice/groups). 63 min after mating, SFC^+/Ej^- and SFC^+/Ej^+ mice were subjected to the social fear extinction and immediately sacrificed. Percentage of investigation of three non-social (empty cage) and two social (cage with a conspecific) stimuli of SFC^+/Ej^- and SFC^+/Ej^+ mice was monitored during social fear extinction training (day 2) (A). Data are presented in mean ± SEM. (B-C) Data represent mean of pErk positive neurons /mm^3 in the dorsal (dLS) and ventral (vLS) lateral septum (LS) (B) and the percentage of OXT neurons expressing pErk within the SON and the PVN (C). Data are presented in mean ± SEM. * $p<0.05$ SFC^+/Ej^- vs. SFC^+/Ej^+). Representative images of co-stained LS slices for DAPI (cyan) and pErk protein (green), and PVN and SON slices for OXT (red) and pErk protein (green) (D). Correlation between the mean of cFos positive neurons /mm^3 and the mean of pErk positive neurons /mm^3 in the dLS and vLS (F-G). Data are presented in mean ± SEM. For detailed statistics, see S-Table 2.

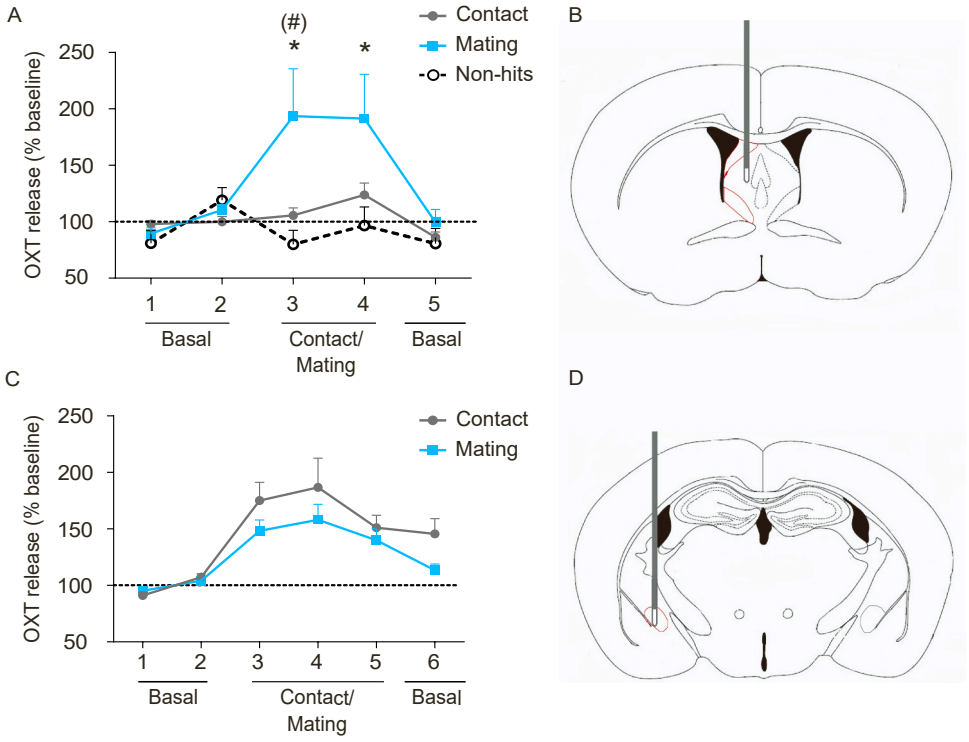

Fig. 4. Release of OXT within the septal and amygdaloid regions of male mice during mating or social interaction. In the Septum 30-min microdialysates were sampled before (samples 1 and 2; basal conditions), during (sample 3, presence of the female), and after (samples 4 and 5) mating or contact with a female conspecific (A). Non-mated (interaction with a non-receptive female; Contact) and mated (mating with a receptive female with or without ejaculation; Mating) mice received a female conspecific for 1 h (n=6–7 mice/groups). Data represent the percentage of mean basal values ± SEM. * $p<0.05$ Mating vs. Non-hits, (#) p=0.074 Mating vs. Contact. Schematic representation of the microdialysis probe implantation. The red line represents the lateral septum (B). OXT release within the amygdaloid region in response to mating (C). Contact and Mating mice received a female conspecific for 90 min (n=13–18 mice/groups). 30-min microdialysates were sampled within the amydaloid region before (samples 1 and 2; basal conditions), during (samples 3, 4, and 5; presence of the female), and after (sample 6, basal conditions) mating or contact with a female conspecific. Data represent the percentage of mean basal values ± SEM. Schematic representation of the microdialysis probe implantation (D). The red line represents the central amygdala. For detailed statistics, see S-Table 4.

To confirm the role of OXT in social fear extinction, we also infused the OXTR-A icv after mating (to prevent interference with mating) and before social fear extinction training, and this resulted in impaired extinction ($F(1,29)=7.33$, $p=0.011$; Fig. 6B) in both SFC^+/Ej^- and SFC^+/Ej^+ groups. Thus, brain OXTR-mediated actions are essential for successful social fear extinction after mating. However, the difference between SFC^+/Ej^- and SFC^+/Ej^+ groups could not be confirmed in these pharmacological experiments. The implantation of guide cannulas and local infusions into the LS have likely prevented the ejaculation-induced facilitation of social fear extinction.

4. Discussion

In this study, we combined behavioral, immunohistochemical, and pharmacological approaches to investigate whether mating and mating-induced release of OXT within the LS affect anxiety-like and fear behaviors in male mice. Extending our previous results in rats (Waldherr and Neumann, 2007), we show that mating also induces anxiolysis in male mice. Moreover, pre-extinction mating, specifically with ejaculation, facilitated social fear extinction but did not affect cued fear extinction. Activity within the LS induced by mating and ejaculation also correlated with the local cellular activity in response to social fear extinction, suggesting a priming effect of ejaculation on extinction. We also provide evidence for the involvement of OXTR-mediated signaling, specifically within the LS, in mating-facilitated extinction. A 1-hr mating period stimulated the release of OXT within the septum and pre-mating blockade of OXTR within the LS by local infusion of an OXTR-A impaired social fear extinction.

4.1. Anxiolytic effect of mating in mice

The anxiolytic effect of mating has repeatedly been shown in various tests in male (Fernandez-Guasti et al., 1989; Waldherr and Neumann, 2007) and female (Nyuyki et al., 2011) rats. In males, mating-induced anxiolysis was found to last for at least 4 hours and was mediated by OXT released within the PVN during mating (Waldherr and Neumann, 2007). Here, we can confirm and extend this finding to male mice, which displayed reduced anxiety-like behaviour in the LDB and the marble

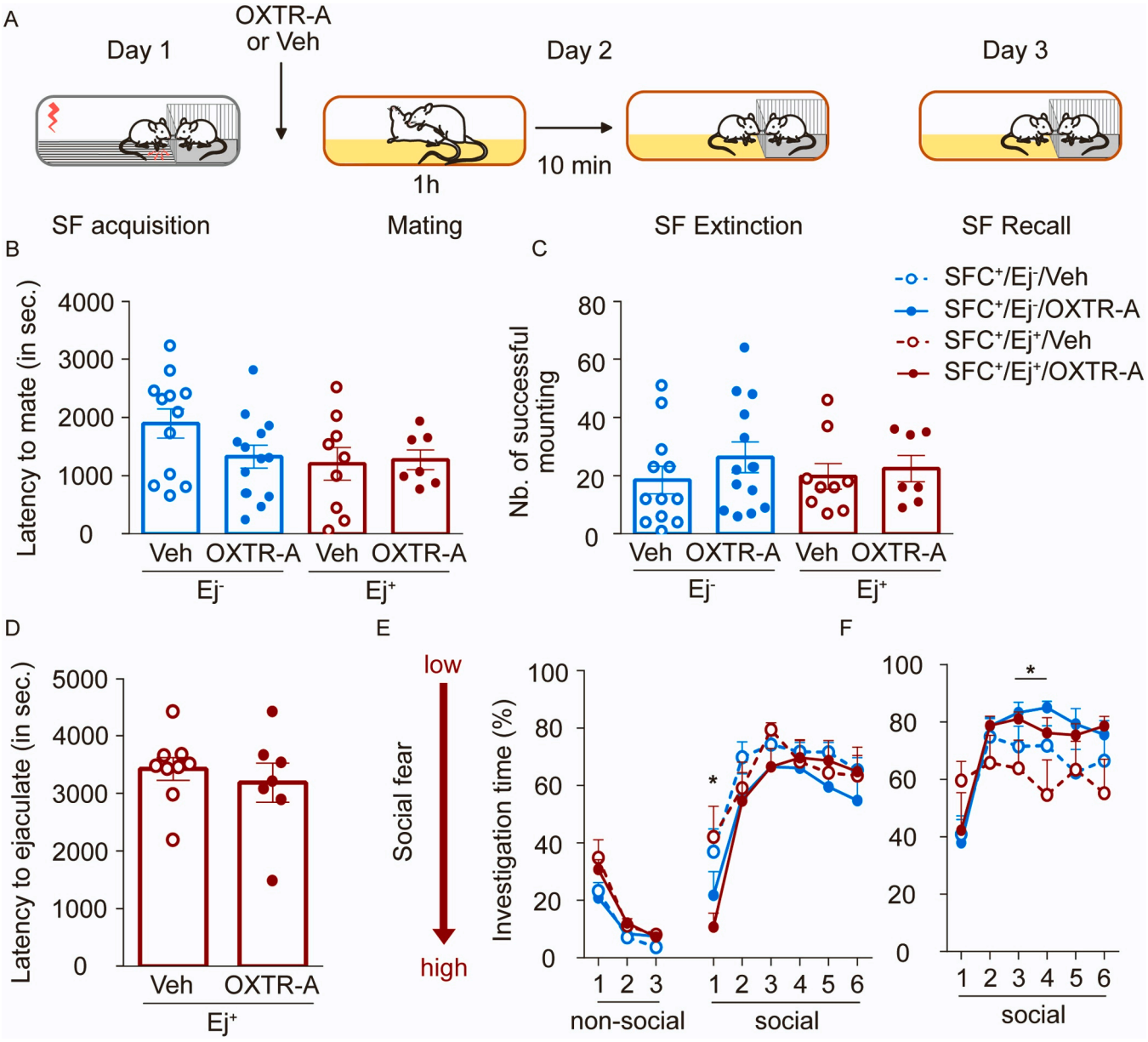

Fig. 5. Septal administration of an oxytocin receptor antagonist (OXTR-A) before mating slightly impairs social fear extinction. Schematic representation of the experimental design to test the role of OXT within the LS on ejaculation-induced facilitation of social fear extinction (A). One week after surgery, mice were conditioned (SFC^{+}). 24 h later, mice were bilaterally infused with Veh (Ringer/0.2 µl/side) or an OXTR-A (20 ng/0.2 µl/side) 10 min before mating. The effect of septal OXTR-A infusion on mating behavior was assessed by recording the latency to mate (B), the number of successful mounting (C), and the latency to ejaculate (D) of mated mice without ejaculation (Ej^{-}) and with ejaculation (Ej^{+}) (n= 7–13 mice/group). Data represents mean ± SEM. 10 min after mating, mice were subjected to social fear extinction. Percentage of investigation of three non-social (empty cage) and six social (cage with a conspecific) stimuli was monitored during social fear extinction training (E) as well as six social stimuli during social fear extinction recall (F). Data represents mean ± SEM. * $p<0.05$ Veh vs. OXTR-A. For detailed statistics, see S-Table 5.

burying test after one hour of mating, irrespective of ejaculation (Fig. 1B). The latter finding supports our previous observations in rats (Waldherr and Neumann, 2007), but is partly contrary to findings that the anxiolytic effect of mating is dependent on the occurrence of ejaculation as assessed in the conditioned defensive burying test (Fernandez-Guasti et al., 1989; Fernandez-Guasti and Saldivar, 1990; Rodriguez-Manzo et al., 1999). However, the above-mentioned test uses fear conditioning with electric shocks delivered by a novel object, which the subject later buries. Thus, differences in burying time can also be interpreted as a measure of fear.

4.2. *No effect of mating on cued fear extinction*

In male mice, mating for 1 hr immediately before cued fear extinction did not alter freezing behaviour during extinction (Fig. 1G). Previously, positive social encounters, such as interactions or co-housing with a same-sex conspecific or a mating partner, were shown to facilitate contextual fear extinction in rats and mice (Bai et al., 2009; Ferreira et al., 2019; Gao et al., 2020; Mikami et al., 2020; Yuan et al., 2018). However, these studies either examined the effects of social buffering provided by co-housing of the conditioned animal with a receptive female (Gao et al., 2020), a same-sex affiliative conspecific (Mikami et al., 2020), or social support during (Ferreira et al., 2019) or right after (Bai et al., 2009) contextual fear extinction. This suggests that long-lasting

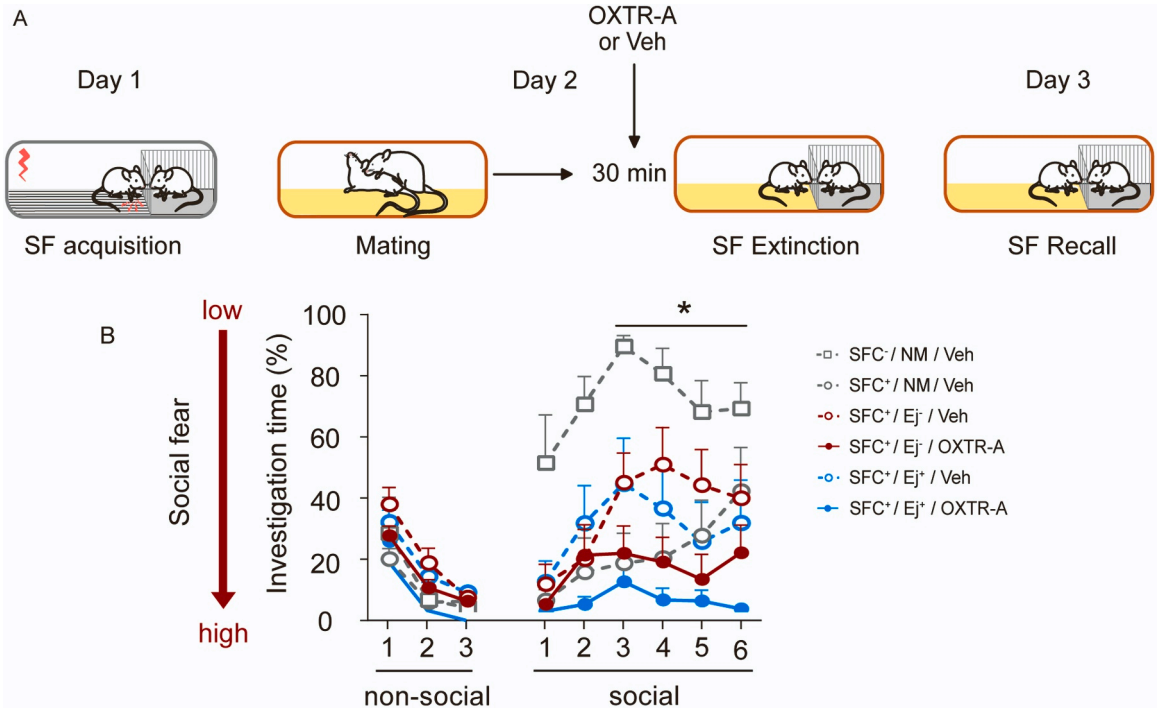

Fig. 6. Icv administration of an oxytocin receptor antagonist (OXTR-A) after mating and prior social fear extinction impairs social fear extinction. Schematic representation of the experimental design to test the role of OXT on social fear extinction (A). Three days after surgery, mice were conditioned (SFC^+) or left unconditioned (SFC^-). 24 h later, mice were allowed to mate for 1 h before receiving an icv infusion of Veh (Ringer/2 µl) or OXTR-A (2 µg/2 µl). 30 min after infusion, non-mated (NM) and mated mice (Ej^- = mating without ejaculation; Ej^+ = mating with ejaculation) underwent social fear extinction. Percentage of investigation of three non-social (empty cage) and six social (cage with a conspecific) stimuli was monitored during social fear extinction training (B). (n= 5–10 mice/group). Data presented in mean ± SEM. *p<0.05 in all SFC^+/Veh vs. all OXTR-A. For detailed statistics, see S-Table 6.

social support during co-housing versus an acute pre-extinction mating may differentially affect the formation, consolidation, and extinction of non-social, i.e., cued fear.

As mating did not affect cued fear expression in our study (Fig. 1G-H), we did not continue to study the potential involvement of OXT in cued fear extinction in this context. Interestingly, acute stimulation of OXT signaling within the central amygdala reduced non-social fear in rats (Knobloch et al., 2012). However, we could not identify an elevated neuropeptide release within the amygdala in response to mating, but rather during social contact with a receptive female, i.e., in both mated and non-mated male mice. Thus, the lack of significant mating-induced neuropeptide release in the amygdala (Fig. 4C) may also explain the absence of an effect of mating on cued fear extinction. Moreover, we have also previously shown that enhanced OXT signaling in female mice during lactation has no effect on cued fear extinction (Menon et al., 2018), which strengthens the idea that activation of endogenous OXT-signaling might have different effects on social versus non-social conditioned fear. Further studies need to reveal whether elevated levels of endogenous OXT in the amygdala, e.g., over an extended period during social support, can reduce fear behaviour.

4.3. *Mating facilitates social fear extinction in dependence on ejaculation*

As an important prerequisite for testing the hypothesis that mating facilitates social fear extinction, we had to exclude the possibility that sexual behaviour is substantially impaired 24 hrs after social fear acquisition. In fact, social fear-conditioning by coupling of social investigation of a male conspecific with a mild foot shock did not alter social interactions and mating behaviour with a female mouse on the next day, i.e., at a time point when male SFC^+ mice otherwise display strong signs of social fear and avoid unknown male stimulus mice in the beginning of the extinction training. Thus, remarkably, social fear acquisition induces a generalized, but sex-specific fear of conspecifics or, alternatively, sexual motivation acutely overcomes social fear in male mice.

In contrast to the general effect of mating on anxiety-related behaviour irrespective of the occurrence of ejaculation, a facilitatory mating effect on social fear extinction was only found in the Ej^+ group (Fig. 1J). This effect reiterates the requirement of ejaculation for reversing the effects of strong aversive stimuli generated by conditioning, which has been previously shown in the conditioned marble burying paradigm (Fernandez-Guasti and Saldivar, 1990; Ferreira et al., 2019; Rodriguez-Manzo et al., 1999). We, thus, conclude that the facilitation of extinction specifically after the occurrence of ejaculation is not a result of decreased anxiety. We can further exclude that improved social fear extinction is due to altered social motivation, as mating with or without ejaculation did not alter social preference or social novelty preference (S-Fig. 2). We rather suggest that ejaculation-induced reduction in stress levels are involved in the facilitation of social fear extinction, although this possibility needs to be examined.

4.4. *Mating differentially activates hypothalamic OXT neurons*

Sexual activity and mating are robust stimuli for the OXT system, as indicated by increased activity of OXT neurons of both PVN and SON shown in male rats (Witt and Insel, 1994). Activation of hypothalamic OXT neurons may particularly reflect elevated OXT secretion into the blood from magnocellular neurons projecting to the neurohypophysis. Indeed, OXT concentrations in human plasma and saliva sampled after sexual activity (independent of the occurrence of orgasm) was found to significantly increase (Carmichael et al., 1987; Jong et al., 2015). Moreover, the elevated neuronal activity within the hypothalamic PVN in response to mating may also reflect the rise in OXT release within the PVN (Waldherr and Neumann, 2007), and within the LS from OXT neurons projecting to this region (Menon et al., 2018).

However, we did not aim to study the effects of mating on activity

patterns of OXT neurons *per se*, but rather compared the differential effects of mating either resulting in ejaculation or not. Interestingly, neuronal activity of PVN-OXT neurons of Ej^+ mice was not found to be particularly higher compared to Ej^- mice. However, we only found a positive correlation between the percentage of cFos-positive OXT neurons (reflecting ejaculation-induced activity) and mating duration and number of intromissions in the PVN (Figs. 2F, 2G). In contrast, unexpectedly, a lower number of cFos-positive OXT neurons was found in the SON of Ej^+ compared with Ej^- mice (Fig. 2D). Although brain OXT is essentially involved in the regulation of various aspects of sexual behaviors (Argiolas and Melis, 2004; Argiolas et al., 1989; Caquineau et al., 2006; Pattij et al., 2005), the differential recruitment of OXT neurons of the SON and PVN by mating and the occurrence of ejaculation, and their functional involvement in mating-induced behavioral alterations in mice remains to be shown.

4.5. Mating induces OXT release within the LS

Indeed, the LS receives OXTergic projections from both PVN and SON (Menon et al., 2018) and abundantly expresses the OXTR described in rats (Oliveira et al., 2021) and mice (Menon et al., 2022). OXTR signaling within the LS facilitates social fear extinction in male and female mice (Menon et al., 2018; Zoicas et al., 2014), enhances aggression in virgin female rats (Oliveira et al., 2021), mediates social memory in male rats (Lukas et al., 2013), and reinforces social stress-induced contextual fear in male mice (Guzman et al., 2013) (for review see (Menon et al., 2022)). Thus, our finding of stimulated release of OXT within the septum during mating (Fig. 4A) is in agreement with the local effects of OXT on various aspects of social behaviour. However, to which extent locally released OXT is essential for the regulation of male mating behaviour is unknown. Also, monitoring of septal OXT release using microdialysis did not allow to specifically differentiate between the various phases of mating, such as approach, mounting and ejaculation; temporal fluctuations in the local concentration of OXT in the extracellular fluid, e.g., during the short event of ejaculation, are rather integrated during the 30-min sample period. In men it was found that plasma OXT increases during self-stimulation with a maximum level during orgasm and ejaculation (Carmichael et al., 1987; Kruger et al., 2003; Murphy et al., 1987; Ogawa et al., 1980). Using more sensitive approaches such as OXT sensors (Qian et al., 2023) will enables us to monitor, whether the septal release of OXT follows similar patterns, i.e., steadily rises during mating and peaks during ejaculation. The mating-induced release of OXT was specific for LS; within the amygdala OXT release was found to rise both during contact with a non-receptive female (Contact group) and during mating (Fig. 4D). Thus, contrary to the LS, the presence of the female was sufficient to stimulate OXT release within the amygdala. A similar effect has also been observed in both male and female rats, wherein interactions with a conspecific was shown to lead to OXT release within the central amygdala (Dumais et al., 2016). This suggest a cross species mechanism involving OXT release within the amygdala during social interactions in male mice and rats.

4.6. Mating with ejaculation activates the vLS

To obtain more evidence for the possible role of the LS in ejaculation-induced facilitation of social fear extinction, we analyzed the expression of the immediate early genes cFos and of pErk, reflecting the neuronal activation during mating (i.e. after 90 min) and social fear extinction (i.e. after 10 min), respectively. Surprisingly, we identified a reduced number of pErk-positive cells in the vLS in the Ej^+ group compared to the Ej^- during social fear extinction (Fig. 3B), whereas no difference was found in the dLS (Fig. 3C). In this context, however, we also have to consider that the LS mainly consists of GABAergic neurons (Sheehan et al., 2004). Thus, it is possible that the elevated activity of OXT neurons and increased release and availability of OXT within the LS during mating (with ejaculation) results in activation of local GABAergic signaling, which subsequently suppresses overall vLS activity in response to social exposures during extinction. Indeed, muscimol-induced activation of GABAergic signaling in the LS was shown to facilitate social fear extinction in male mice (Bludau et al., 2023). This hypothesis is corroborated by the positive correlation between cFos and pErk within the LS (Figs. 3E, 3F) suggesting a priming effect of mating on social fear extinction via the OXTR-expressing cells of the LS.

4.7. Septal OXT mediates ejaculation-induced facilitation of social fear extinction

We were further able to establish a causal relationship between mating-induced release of OXT and facilitation of social fear extinction by blocking OXTR-mediated signaling within the LS prior to mating. Although the experimental design was risky, as brain OXT is involved in mating behavior (Argiolas and Melis, 2004), locally restricted OXTR interference did not interfere with sexual behaviour (Figs. 5B, 5C). Thus, OXTR signaling within the LS does not seem to be a major player in the regulation of mating behavior. In fact, OXT was rather described to be functional in the PVN, medial preoptic area, sacral parasympathetic nucleus or the spinal cord (Argiolas and Melis, 2013) in this context.

Importantly, blockade of OXTR within the LS moderately impaired social fear extinction, in both Ej^- and Ej^+ mice (Fig. 5E), providing evidence that OXT release within the LS during mating is contributing to the mating-induced facilitation of social fear extinction. The contribution of central OXT to the effects of mating on social fear extinction was confirmed by icv infusion of the OXTR-A. However, in these pharmacological experiments, the difference in extinction behavior between the SFC^+/Ej^+ and SFC^+/Ej^- Veh groups was abolished, and the facilitated extinction specifically found in the SFC^+/Ej^+ mice compared with SFC^+/NM/Veh mice was absent (Fig. 5E). As the effect of ejaculation on social fear extinction was robust in otherwise untreated mice (Fig. 1J) and could be repeatedly found in several experiment, surgical intervention together with pharmacological manipulation within the LS are possibly interfering with such an obviously sensitive effect.

In summary, we can show that pre-extinction mating of male SFC^+ mice, especially when culminating in ejaculation, leads to the facilitation of social fear extinction. This is likely due to mating-induced release of OXT within the LS, as blockade of local OXTR-mediated signaling partly prevents this effect. This also suggests OXT actions in other brain regions, and/or the involvement of other local systems including dopamine, serotonin and opioids (Argiolas and Melis, 2004; Pfaus, 1999) in this social fear-reducing effect of mating.

We can, so far, only speculate about the evolutionary benefits of such fear-reducing effects of mating, especially after ejaculation. For instance, mating in their home cage could enhance aggression in male mice, which would consequently drive interaction with the stimulus presented thereafter during extinction as it could be considered as an intruder. Indeed copulation with a receptive female is known to enhance offensive attacks in male rats (Flannelly et al., 1982). Additionally, during mating, male and female animals have to overcome all aspects of social avoidance and social fear. Low social fear is more relevant in females as in most mammals, males are substantially larger compared to females. Thus, it would be of particular interest, whether the described OXT-dependent reduction in social fear expression can also be found in virgin female mice. Interestingly, an anxiolytic effect of mating could only be revealed after paced-mating, but not after forced mating, in virgin female rats (Nyuyki et al., 2011).

Our results extend previous findings in lactating mice and rats demonstrating that activation of the endogenous OXT system exerts a positive effect not only on anxiety levels and stress responsiveness (Slattery and Neumann, 2008), but also on extinction of social fear induced by a traumatic social event (Menon et al., 2018). In general, the OXT system is highly responsive to various social stimuli (Menon and Neumann, 2023), and has been repeatedly shown to enhance social

salience independent of valence and, thus, mediates the bidirectional consequences of social interactions on fear and anxiety (Ferreira et al., 2019; Gao et al., 2020; Guzman et al., 2013; Menon and Neumann, 2023; Mikami et al., 2020; Nowak et al., 2013; Yuan et al., 2018). Also in humans, positive relationships or social company before or during cognitive behavioral therapy potentiates the therapeutic effect (Hori et al., 2018; Rapee et al., 2015; Stone et al., 2019; Strauss et al., 2017). Understanding the mechanisms underlying the effect of specific physiological states such as mating on fear and anxiety could improve the support of patients suffering from anxiety disorders.

CRediT authorship contribution statement

Ilayda Birben Fahlıoğulları: Data curation, Formal analysis. **Christopher Sommer:** Data curation, Formal analysis. **Cindy Grossmann:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Rohit Menon:** Conceptualization, Data curation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Inga D Neumann:** Funding acquisition, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rohit Menon reports was provided by German Research Foundation. Inga D. Neumann reports financial support was provided by German Research Foundation. Cindy Grossmann reports financial support was provided by German Research Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2024.107083.

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