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Impaired sex preference, but not social and social novelty preferences, following systemic blockade of oxytocin receptors in adult male mice

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ABSTRACT

The hypothalamic neuropeptide oxytocin (OT) is a powerful modulator of mammalian social behavior and its administration was shown to affect various types of social interactions. However, systematic examinations of the role of endogenous OT release in social behavior have heretofore been done only using genetically modified animal models in which the genes encoding either OT or the OT receptor (OTR) were mutated. While such genetic manipulations revealed various behavioral deficits, these deficits may involve developmental or long-term processes and do not prove the participation of acute OT release in the impaired behavior. Here we used a battery of social discrimination tasks to evaluate the effects of acute systemic OTR blockade, using a non-peptide, orally active OTR antagonist (L368,899), on social behavior of adult male C57BL/6 J mice. We found no effect of the pharmacological manipulation on the social preference and social novelty preference behaviors. However, the preference of a male mouse for investigating a female conspecific more than a male (sex preference behavior), was lost by administration of the OTR antagonist. Finally, we found that blocking OTR activity before social defeat prevented the consequent loss of social preference, suggesting a role for OT in the acquisition of aversive social memory. Overall, our results suggest that OT plays a role in modulating the salience of social stimuli and facilitating their memory, as predicted by the social salience theory, rather than in regulating the internal motivation of the subject for social interactions.

1. Introduction

Oxytocin (OT) is a neuropeptide synthesized in the hypothalamus, from where it is released either to the periphery *via* the posterior pituitary, or to various brain areas by direct hypothalamic innervation (Althammer et al., 2018; Lee et al., 2009). OT is known to be a powerful modulator of mammalian social behavior and its application was shown to affect various types of social interactions, including aggression, parental behavior, pair bonding and mating. Two main theories try to explain the effects of OT on mammalian social behavior. The approach/withdrawal hypothesis (Harari-Dahan and Bernstein, 2014) stipulates that oxytocin enhances approach behaviors and decreases withdrawal behaviors towards social stimuli. In contrast, the social salience hypothesis (Shamay-Tsoory and Abu-Akel, 2016) suggests that OT enhances the salience of emotional stimuli, regardless of their valence. While these theories are not necessarily mutually exclusive (Piva and Chang, 2018), one may assess their applicability for a given species and context by systematic examination of OT effects in various tasks performed by the same animals. Such systematic examination of the role of endogenous OT in social behavior has heretofore been done using genetically modified animal models, in which the genes encoding either OT (OT-KO) or the OT receptor (OTR-KO) were mutated (Crawley et al., 2007; Ferguson et al., 2000; Takayanagi et al., 2005). Although such genetic manipulations revealed various behavioral deficits, these deficits may involve developmental or long-term processes and do not prove the participation of acute OT release in the impaired behavior. Such participation may be directly examined by blocking OTR activity just before the behavioral test, thus preventing any influence of endogenous OT release on the examined behavior. However, a comprehensive analysis of the effects of acute blockade of OTR activity on murine social behavior was not reported yet.

Here we reasoned that by manipulating endogenous OT activity during multiple discrimination tasks with various stimuli, we will be able to tell which of the theories explains better the behavior of adult male mice. To that end, we used our recently published behavioral system (Netser et al., 2017), which enables a detailed analysis of the dynamics of murine investigation behavior, combined with a battery of social discrimination tasks which assess distinct aspects of social

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behavior, to evaluate the effect of acute systemic blockade of the OTR, using a non-peptide, orally active OTR antagonist (L368,899, henceforth termed OTRa), which is known to cross the blood brain barrier (Boccia et al., 2007). The first task is the social preference (SP, also termed sociability) test, assessing the preference of a subject for investigating a social stimulus as compared to an object. The second is the social novelty preference (SNP) test, assessing the ability of the subject to discriminate between novel and familiar social stimuli. The third is the sex preference (SxP) test, which evaluates the tendency of a subject to investigate a social stimulus of the opposite sex, as compared to a same sex stimulus. Finally, we addressed the involvement of OT in aversive social memory formation by checking the effect of OTRa administration on the reduction in social preference behavior, observed following a single experience of social defeat (see schematic description of the various tests in Supp. Fig. 1).

2. Methods

2.1. Animals

Subjects were naïve C57BL/6 J male mice (8–12 weeks old), commercially obtained (Envigo, Israel) and housed in groups of three to five animals per cage throughout the experiments. Stimuli were in-house grown C57BL/6 J, juvenile male mice (21–30 days old) for SP and SNP tests, and naïve adult male and female mice (8–12 weeks old) for the SxP. The aggressive stimuli for the defeat procedure were retired male breeders from the ICR (CD1) strain, commercially obtained (Envigo, Israel) and housed in isolation. All animals were kept on a 12-h light/12-h dark cycle, light on at 7p.m., with *ad libitum* access to food and water. Behavioral experiments took place during the dark phase under dim red light. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Haifa.

2.2. OTRa administration

L368,899 (Tocris), (10 mg) was dissolved in 10 mL saline and stored in small aliquots at −20 °C until the test day. Prior to the test, the dissolved L368,899 was diluted to 0.3 mg/mL in saline. Mice were administered with 3 mg/kg BW by intraperitoneal (I.P.) injection 30 min before the behavioral test, while control animals were injected with same volume of saline. The amount of OTRa applied here is higher than the one used in previous rodent studies (1 mg/kg BW) reporting behavioral effects of such administration (Blitzer et al., 2017; Olszewski et al., 2013). This amount was chosen as it yielded maximal effect in a previous study in rhesus monkeys (Boccia et al., 2007).

2.3. Behavioral assays

Habituation to injection took place one week before the behavioral assays and comprised three days of syringe stabbing with no injection and two days of 0.1 mL saline injection. All social discrimination tasks were conducted using our previously published automated experimental system (Netser et al., 2017). All animals underwent four tests sequentially: SP, SNP, SxP, and SP following social defeat. The SP and SNP tests were conducted on the same day, as previously described in detail (Netser et al., 2017). Briefly, the behavioral paradigm consisted of a 15 min open-field test, followed by a 15 min habituation to two empty chambers located in opposite corners of the arena. Thereafter, two chambers with social and object stimuli (one stimulus per chamber) were randomly located in opposite corners of the arena, and the SP test was performed for 5 min. Following the SP test, the chambers with the stimuli were removed from the arena, and the subject was left alone for 15 min. Then, the chambers were inserted again, this time to the other two corners of the arena, with one containing the same social stimulus used for the SP test (familiar stimulus) and the other containing a novel stimulus, and the SNP test took place for 5 min. At the end of the SNP test, the experimental subject was placed back in its home cage, while the stimuli mice were left in the chambers for the next experiment or placed back in their home cage at the end of the experimental session (Supp. Fig. 1A).

The SxP test was performed at least 2 days after the SP/SNP paradigm and consisted of 15 min habituation to the arena with empty chambers, followed by exposing the subject mouse for 5 min to both adult female and male stimuli (8–12 weeks old), which were confined to individual chambers randomly located at opposite corners of the arena (Supp. Fig. 1B).

The social defeat procedure, performed at least 2 days after the SxP test, included an unrestricted encounter with an aggressive retired breeder male mouse of the ICR strain for 5 min in the aggressor home cage. This was followed by an additional 25 min in the aggressor home cage while the subject was confined to a round perforated metal chamber (radius 45 mm × height 95 mm). The subject was then returned to its group-housed home cage and tested 24 h later with the SP task, as described above (Supp. Fig. 1C). In these experiments, we compared the behavior of three groups (Supp. Fig. 1C); Saline/saline animals were injected with saline before both the defeat and the SP test preformed a day later. OTRa/saline animals got OTRa injection before that defeat and saline injection before the SP test. Saline/OTRa animals got saline injection before the defeat and OTRa injection before the SP test.

2.4. Data analysis

Data analysis was performed by our custom-made TrackRodent software, as previously described (Netser et al., 2019).

2.5. Statistical analysis

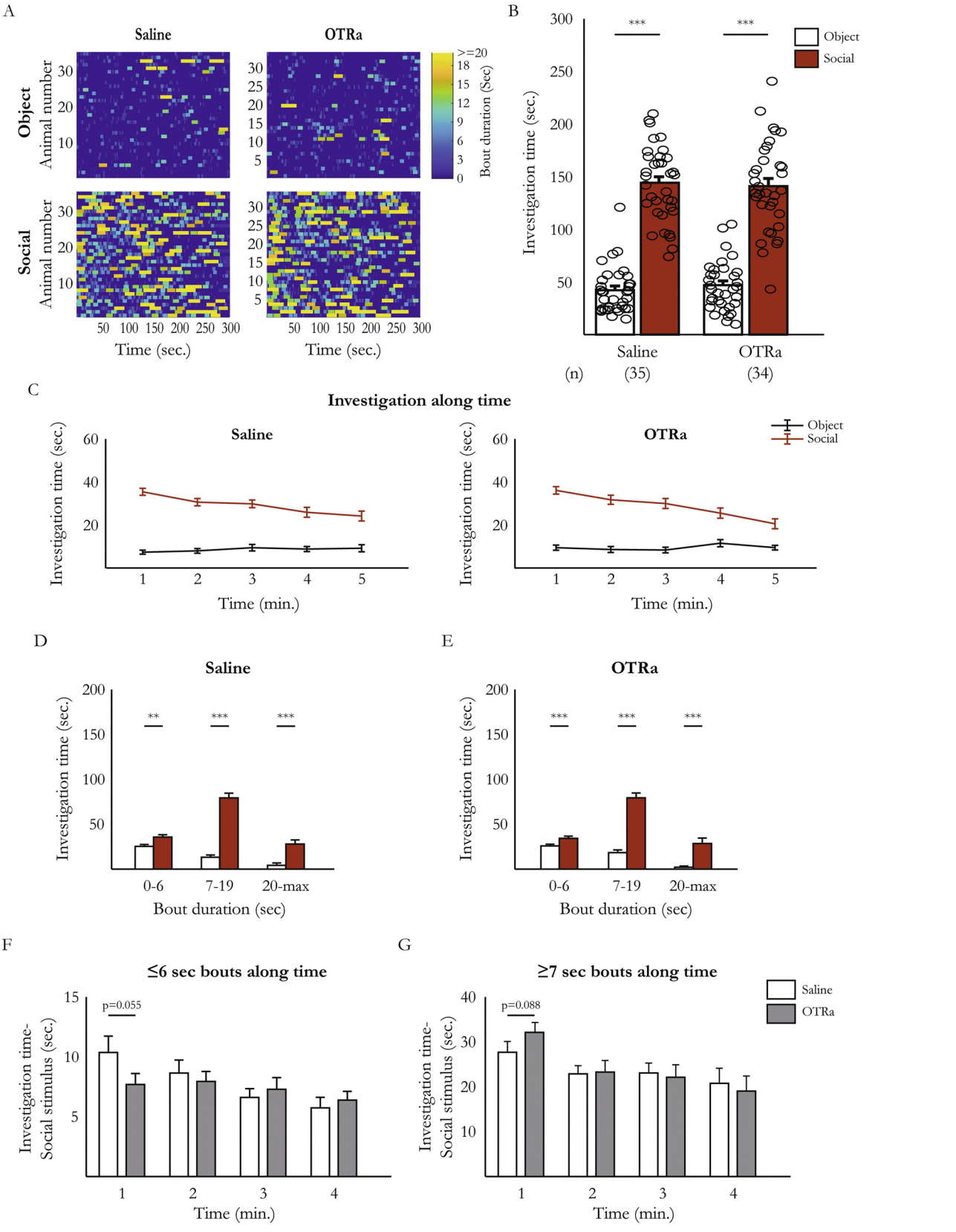
All statistical tests were performed using SPSS v21.0 (IBM). Kolmogorov–Smirnov was used for checking normal distribution of the dependent variables. A one-tailed paired t-test was used to compare between different conditions or stimuli for the same group, and a one-tailed independent t-test was used to compare a single parameter between distinct groups. For comparison between multiple groups and parameters a mixed analysis of variance (ANOVA) model was applied to the data. This model contains one random effect (ID), one within effect, one between effect, and the interaction between them. For comparison within a group using multiple parameters, a two-way repeated measures ANOVA model was applied to data. This model contains one random effect (ID), two within effects, one between effect and the interactions between them. All ANOVA tests were followed, if main effect or interaction were significant, by *post hoc* Student's t test. Significance was set at 0.05.

3. Results

3.1. Social preference

The SP test was preceded by 15 min of open-field test in the empty arena. We found no difference between the saline-injected (n = 35) and OTRa-injected (n = 34) adult male C57BL/6 J mice in either the ratio between time spent in the center and time spent in the periphery of the arena (Center/Surround, Supp. Fig. 2A; t-test, $t_{42} = 0.068$, $p = 0.473$), or in the distance traveled by the animals (Supp. Fig. 2B). Thus we found no change in the motor activity or anxiety level of the mice due to OTRa administration.

The heat maps of investigation behavior during the SP test performed by the OTRa and saline injected animals are shown in Fig. 1A. As apparent from these heat-maps, in which the duration of each social investigation bout is color coded, OTRa administration seems to affect only the dynamics of social investigation behavior. This is reflected by the relatively short duration of investigation bouts exhibited by saline-



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injected animals during the first 2 min of the test, in accordance with our previously published data (Netser et al., 2017). In contrast, OTRa injected animals displayed prolonged bouts of social investigation behavior already at the beginning of the test. Nevertheless, quantitative analysis of the investigation times showed that both groups of animals exhibited a similar preference for the social stimulus, with no significant difference between the two groups in the amount of time spent investigating each of the stimuli (Fig. 1B, within stimulus: F

Fig. 1. No change in social preference following OTRa administration.
(A) Heat-maps of investigation bouts towards the object (above) or social (below) stimuli along the 5-min session performed by adult male mice injected with either saline (left) or OTRa (right). Each line represent a single mouse. The heat-maps are color-coded according to the bout duration (see color code at the right side).
(B) Mean values of investigation time towards each of the stimuli, for the experiments shown in A. Each animal is represented by a circle. ***p < 0.001, *post hoc* t-test following main effect in ANOVA test (saline: $t_{34} = -12.083$, $p < 0.001$; OTRa: $t_{33} = -8.809$, $p < 0.001$, paired t-test).
(C) Mean values of investigation time towards each of the stimuli, for the saline (left) and OTRa (right) experiments shown in A, across the time course of the session (1-min bins).
(D) Mean values of investigation time for the three categories of investigation bouts (short, intermediate and long), for the saline experiments shown in A (short: $t_{34} = -2.949$, $p = 0.003$; intermediate: $t_{34} = -10.598$, $p < 0.001$; long: $t_{34} = -4.356$, $p < 0.001$, paired t-test). **p < 0.01, ***p < 0.001, *post hoc* t-test following main effect in ANOVA test.
(E) As in D, for the OTRa experiments (short: $t_{33} = -3.273$, $p = 0.001$; intermediate: $t_{33} = -8.681$, $p < 0.001$; long: $t_{33} = -4.104$, $p < 0.001$, paired t-test).
(F) Mean value of social investigation time for short bouts (≤6 s), across the session time (besides the last minute when a bias towards short bouts exist), compared between saline (white) and OTRa (grey) injections. *post hoc* t-test following main effect in ANOVA test.
(G) As in F, for the prolonged bouts (≥7 s).

(1,67) = 208.246, p < 0.001; between groups: F(1,67) = 0.029, p = 0.433; stimulus × group interaction: F(1,67) = 0.257, p = 0.307, mixed ANOVA. *post hoc*: saline group: $t_{34} = -12.083$, $p < 0.001$, OTRa group: $t_{33} = -8.809$, $p < 0.001$, paired t-test). Moreover, both groups showed a similar decline in their social preference behavior over time (Fig. 1C). When we categorized the investigation bouts into short (≤6 s), medium (> 6 s, ≤19 s) and long (> 19 s), we found that OTRa injection did not change the distribution of investigation time between the two stimuli (Fig. 1D-E, Saline: 0−6 s bouts: $t_{34} = -2.949$, $p = 0.003$, 7−19 s bouts: $t_{34} = -10.598$, $p < 0.001$, ≥20 s bouts: $t_{34} = -4.356$, $p < 0.001$; OTRa: 0−6 s bouts: $t_{33} = -3.273$, $p = 0.001$, 7−19 s bouts: $t_{33} = -8.681$, $p < 0.001$, ≥20 s bouts: $t_{33} = -4.104$, $p < 0.001$, paired t-test, Supp. Table 1). Nevertheless, when the distributions of short (≤6 s) and prolonged (> 6 s) bouts over time were analyzed (excluding the last minute when the session termination creates a bias towards short bouts), we found that, as reflected by the heat map, these parameters of the behavioral dynamics differed between the two groups. While saline-injected animals showed a higher level of short social investigation bouts during the first minute (Fig. 1F, Min 1: $t_{67} = 1.616$, $p = 0.055$, independent t-test), OTRa-injected animals displayed a higher level of long bouts during the first minute (Fig. 1G) and these differences were close to statistical significance (Fig. 1G, Min 1: $t_{67} = -1.366$, $p = 0.088$, independent t-test, Supp. Table 1). Thus, while OTRa administration did not change the social preference of the animals, it may change the dynamics of this behavior during the course of the test.

3.2. Social novelty preference

We then applied the same type of analysis to the SNP test, which took place 15 min following the SP test (Supp. Fig. 1). As for the SP test, both groups showed similar social novelty preference, reflected by higher investigation of the novel social stimulus as compared to the familiar one (Fig. 2A, within stimulus: F(1,66) = 26.698, p < 0.001; between groups: F(1,66) = 0.022, p = 0.442; stimulus x group interaction: F(1,66) = 0.138, p = 0.356, mixed ANOVA. *post hoc*: saline group: $t_{33} = 3.744$, $p < 0.001$, OTRa group: $t_{33} = 3.606$, $p = 0.001$, paired t-test) and this preference was reduced over time in both groups (Fig. 2B). The distribution of investigation bout length was also similar between the two groups (Fig. 2C–D, Saline: 0−6 s bouts: $t_{33} = 1.932$, $p = 0.031$, 7−19 s bouts: $t_{33} = 3.862$, $p < 0.001$, ≥20 s bouts: $t_{33} = 1.279$, $p = 0.105$; OTRa: 0−6 s bouts: $t_{33} = 1.141$, $p = 0.131$, 7−19 s bouts: $t_{33} = 3.326$, $p = 0.001$, ≥20 s bouts: $t_{33} = 1.507$, $p = 0.071$, paired t-test, Supp. Table 2). As for the SP test, the distribution of short (Fig. 2E) and prolonged (Fig. 2F) bouts towards the preferred (novel) stimulus over time did show some difference between the groups, with the saline injected animals performing more short bouts during the last stage of the test (Fig. 2E, Min 4: t66 = 1.564, p = 0.061, independent t-test), while the OTRa-injected animals performed more prolonged bouts towards the end of the test. (Fig. 2F, Min 4: t66 = −1.538, p = 0.064, independent t-test). These differences were close to statistical significance (the entire statistical results shown in Supp. Table 2). Overall, we did not observe any statistically significant change in the SP and SNP tests following OTRa administration.

3.3. Sex preference

In contrast to the previous tests, we did observe a very significant change in the SxP test following OTRa administration, as detailed in Fig. 3. Analysis of the investigation time showed that OTRa-injected animals lost their sex preference, unlike saline-injected animals, with a statistically significant interaction between stimulus and group (Fig. 3A-B, within stimulus: F(1,46) = 9.186, p = 0.002; between groups: F(1,46) = 1.513, p = 0.113; stimulus x group interaction: F(1,46) = 3.937, p = 0.027, mixed ANOVA. *post hoc*: Saline group: $t_{23} = 3.584$, $p = 0.001$; OTRa group: $t_{23} = 0.732$, $p = 0.236$, paired t-test, Supp. Table 3). The difference in sex preference behavior was also clearly observed in the analysis of the distinct bout durations (Fig. 3C–D, interactions: stimulus x group: F(1,46) = 3.677, p = 0.031; bout duration x group: F(2,92) = 0.2.824, p = 0.033, two-way repeated ANOVA, Supp. Table 3), and was mainly due to the reduction in prolonged investigation bouts towards the female (Fig. 3E–F, ≥7 s bouts: Min 1: $t_{46} = 2.473$, $p = 0.008$; Min 2: $t_{46} = 1.818$, $p = 0.038$; Min 3: $t_{46} = 0.734$, $p = 0.233$; Min 4: $t_{46} = 1.413$, $p = 0.082$, independent t-test, Sup. Table 3). It should be noted that the total investigation time was not different between the two groups (Mean ± SEM: saline: 181.38 ± 8.79, OTRa: 166.30 ± 8.55; independent t-test, $t_{46} = 1.230$, $p = 0.113$), suggesting that the OTRa-injected animals did not lose their general interest in social stimuli, as also suggested by the previous results of the SP test (Fig. 1). Thus, we conclude that OTRa administration blocks sex preference behavior in adult male mice.

3.4. Social defeat

Finally, in order to examine the effect of OTRa on social preference behavior following social defeat, we compared the behavior of three groups, as detailed in the Methods (Supp. Fig. 1C). When analyzing the distribution of social investigation time between the stimuli for the three groups (Fig. 4A), we found that control (saline/saline) and saline/OTRa animals lost their social preference following social defeat, in accordance with previous studies (Lukas and Neumann, 2014). In contrast, animals injected with OTRa before the social defeat experience (OTRa/saline animals) did show social preference following social defeat, suggesting the OTRa application before the defeat inhibited the formation of social fear memory. However, the differences between the groups were not statistically significant (within stimulus: F(1,42) = 8.309, p = 0.003; between groups: F(2,42) = 1.332, p = 0.138; stimulus x group interaction: F(2,42) = 0.200, p = 0.410. *post hoc*: saline/saline: $t_{14} = -1.432$, $p = 0.087$; OTRa/saline: $t_{13} = -2.137$, $p = 0.026$; saline/OTRa: $t_{15} = -1.510$, $p = 0.076$, paired t-test). Nevertheless, when the dynamics of social investigation

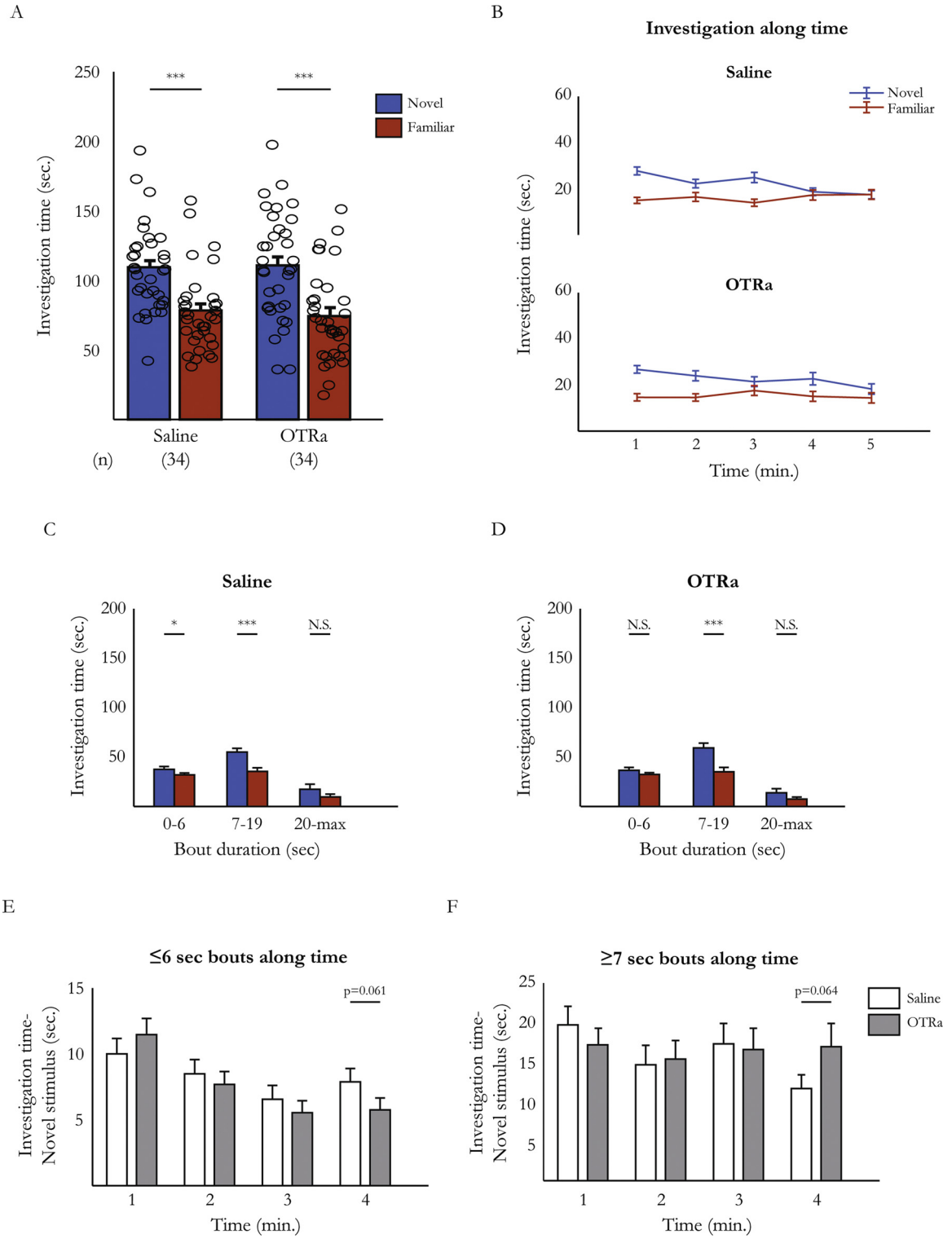


Fig. 2. No change in social novelty preference following OTRa administration.

(A) Mean values of investigation time towards each of the stimuli (Novel – blue, Familiar – red), for the social novelty experiments that followed the social preference experiments shown in Fig. 1, for the same animals. Each animal is represented by a circle. ***p < 0.001, *post hoc* t-test following main effect in ANOVA test (saline: $t_{33} = 3.744$, p < 0.001; OTRa: $t_{33} = 3.606$, p = 0.001, paired t-test).

(B) Mean values of investigation time towards each of the stimuli, for the saline (above) and OTRa (below) experiments shown in A, across the time course of the session (1-min bins).

(C) Mean values of investigation time for the three categories of investigation bouts (short, intermediate and long), for the saline experiments shown in A. *p < 0.05, ***p < 0.001, post hoc t-test following main effect in ANOVA test (short: t33 = 1.932, p = 0.031; intermediate: t33 = 3.862, p < 0.001; long: t33 = 1.279, p = 0.105, paired t-test).

(D) As in C, for the OTRa experiments (short: t33 = 1.141, p = 0.131; intermediate: t33 = 3.326, p = 0.001; long: t33 = 1.507, p = 0.071, paired t-test).

(E) Mean value of social investigation time for short bouts (≤6 s), across the session time (besides the last minute when a bias towards short bouts exist), compared between saline (white) and OTRa (grey) injections. *post hoc* t-test following main effect in ANOVA test.

(F) As in E, for the prolonged bouts (≥7 s).

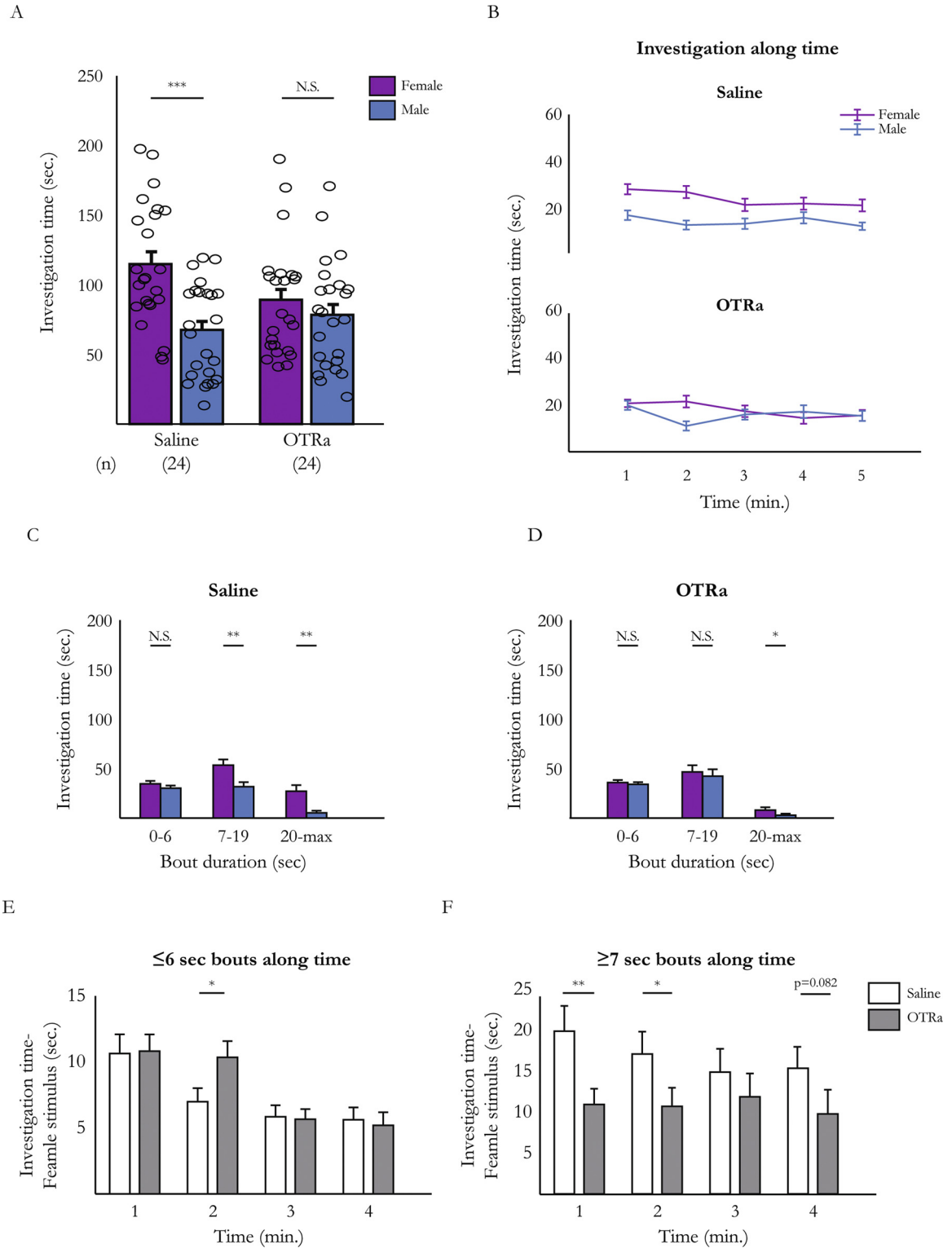


Fig. 3. Impaired sex preference following OTRa administration.

(A) Mean values of investigation time towards each of the stimuli (Female – purple, Male – blue), for the sex preference experiments. Each animal is represented by a circle. ***p < 0.001, *post hoc* t-test following main effect in ANOVA test (saline: $t_{23} = 3.584$, p = 0.001; OTRa: $t_{23} = 0.732$, p = 0.236, paired t-test).

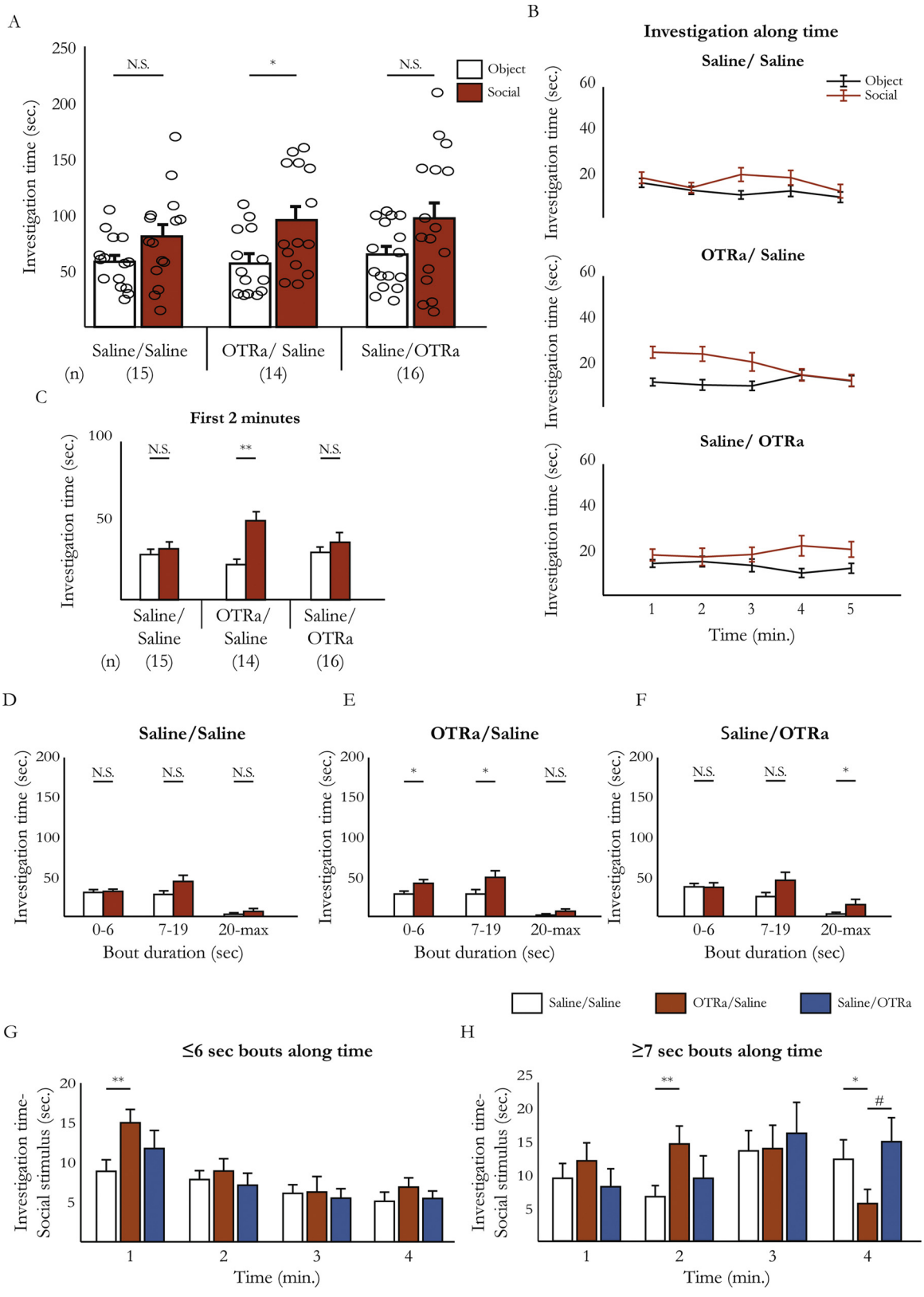
(B) Mean values of investigation time towards each of the stimuli, for the saline (above) and OTRa (below) experiments shown in A, across the time course of the session (1-min bins).

(C) Mean values of investigation time for the three categories of investigation bouts (short, intermediate and long), for the saline experiments shown in A. *p < 0.05, **p < 0.01, post hoc t-test following main effect in ANOVA test (short: t23 = 1.535, p = 0.069; intermediate: t23 = 2.784, p = 0.005; long: t23 = 3.170, p = 0.002, paired t-test).

(D) As in C, for the OTRa experiments (short: t23 = 0.465, p = 0.323; intermediate: t23 = 0.405, p = 0.345; long: t23 = 1.914, p = 0.034, paired t-test).

(E) Mean value of social investigation time for short bouts (≤6 s), across the session time (besides the last minute when a bias towards short bouts exist), compared between saline (white) and OTRa (grey) injections. *p < 0.05, **p < 0.01, post hoc t-test following main effect in ANOVA test.

(F) As in E, for the prolonged bouts (≥7 s).



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was examined (Fig. 4B) it was found that while OTRa/saline animals showed social preference already at the beginning of the test, which decreased with time, saline/OTRa animals showed the opposite dynamics, with social preference behavior developing towards the end of the test. Indeed, when the social and object investigation times for the first two minutes of the test were compared between the groups (Fig. 4C), the difference became much more profound and a statistically significant interaction between stimulus and group was observed (stimulus x group interaction: $F(2,42) = 2.759$, $p = 0.037$, mixed ANOVA. *post hoc*: saline/saline: $t_{14} = -0.485$, $p = 0.33317$; OTRa/saline:

Fig. 4. OTRa administration before social defeat blocks consequent loss of social preference.
(A) Mean values of investigation time towards each of the stimuli (Social – red, Object – white), for social preference experiments performed 24 h following a social defeat. Each animal is represented by a circle. *p < 0.05, post hoc t-test following main effect in ANOVA (saline/saline: t14 = −1.432, p = 0.087; OTRa/saline: t13 = −2.137, p = 0.026; saline/OTRa: t15 = −1.510, p = 0.076, paired t-test).
(B) Mean values of investigation time towards each of the stimuli, for the saline/saline (above), OTRa/saline (middle) and saline/OTRa (below) experimental groups shown in A, across the time course of the session (1-min bins).
(C) As in A, for the first two minutes of the social preference session. **p < 0.01, post hoc t-test following main effect in ANOVA (saline/saline: t14 = −0.485, p = 0.317; OTRa/saline: t13 = −3.435, p = 0.002; saline/OTRa: t15 = −0.730, p = 0.238, paired t-test).
(D) Mean values of investigation time for the three categories of investigation bouts (short, intermediate and long), for the Saline/Saline group shown in A. *p < 0.05, post hoc t-test following main effect in ANOVA test (short: t14 = −0.242, p = 0.406; intermediate: t14 = −1.554, p = 0.071; long: t14 = −0.792, p = 0.221, paired t-test).
(E) As in D, for the OTRa/Saline group (short: t13 = −1.785, p = 0.048; intermediate: t13 = −1.863, p = 0.043; long: t13 = −1.344, p = 0.101, paired t-test).
(F) As in D, for the Saline/OTRa group (short: t15 = 0.084, p = 0.467; intermediate: t15 = −1.546, p = 0.071; long: t15 = −1.906, p = 0.038, paired t-test).
(G) Mean value of social investigation time for short bouts (≤6 s), across the session time (besides the last minute when a bias towards short bouts exist), compared between the three experimental groups. *p < 0.05, **p < 0.01, post hoc t-test following main effect in ANOVA test.
(H) As in E, for the prolonged bouts (≥7 s).

$t_{13} = -3.435$, $p = 0.002$; saline/OTRa: $t_{15} = -0.730$, $p = 0.238$, paired t-test, Supp. Table 4). This suggested to us that while OTRa administration before the social defeat inhibited the effect of social fear memory, the other two groups displayed extinction of the fear memory as the test progressed.

Interestingly, when we analyzed the distinct durations of investigation bouts (Fig. 4D–H) we found that unlike the SP test under normal conditions (Fig. 1D–E), where most of the social preference is concentrated in prolonged bouts which reflects interaction with the social stimulus, in OTRa/saline animals the social preference was concentrated mainly in short bouts. Thus, despite the OTRa administration before the defeat, the animals still shortened their social investigation bouts (Fig. 4E, OTRa/saline: 0−6 s bouts: $t_{13} = -1.785$, $p = 0.048$, 7−19 s bouts: $t_{13} = -1.863$, $p = 0.043$, ≥20 s bouts: $t_{13} = -1.344$, $p = 0.101$, paired t-test, Sup. Table 4).

4. Discussion

The role of OT in regulating social behavior of mammals, including humans, has recently become the focus of a rapidly-growing body of studies. While the use of OT-KO and OTR-KO mouse lines has been highly informative, impaired behavior seen in these genetic models may be induced by long-term developmental processes affected by the lack of OT activity, rather than reflecting the role of acute OTR activation during behavior. On the other hand, various, sometimes contradicting effects of acute blockade of OTR activity were occasionally reported by several studies using various species and distinct developmental and behavioral contexts (see for example Hodges et al., 2019; Sakamoto et al., 2019).Thus, a systematic examination of the consequences of acute OTR blockade across multiple behavioral paradigms in adult mice, which may serve as a baseline for other studies, is much due. Here we systematically examined several types of social discrimination tasks to assess the effect of acute systemic blockade of OTR activity. In accordance with previous studies using OT-KO mice (Choleris et al., 2003; Crawley et al., 2007; Ferguson et al., 2000), but in contrast with studies using OTR-KO mice (Sala et al., 2013; Takayanagi et al., 2005), we found no significant effect of OTR blockade in the SP task. This result suggests that OT release does not regulate the motivation for social interaction with a novel social stimulus displayed by adult male mice. Notably, a similar result was previously reported using central administration of OTRa to female rats (Lukas and Neumann, 2014), while opposite results were obtained using central administration of OTRa to male rats and mice (Lukas et al., 2011). Differences in the reported effects of OTRa between peripheral and central administrations may be due to higher final concentration of OTRa using central administration. Another possibility is the involvement of peripheral mechanisms, which were blocked by the systemic administration of OTRa.

Similar to the SP test, we did not find any significant effect of OTRa injection in the SNP test, suggesting that OT is not involved in the regulation of social novelty preference. This result contradicts multiple studies showing a deficit in social novelty preference exhibited by OT-KO and OTR-KO mice (Macbeth et al., 2009; Sala et al., 2013; Takayanagi et al., 2005). It should be noted, however, that at least one comprehensive study reported no change in social novelty preference of two distinct lines of OT-KO mice (Crawley et al., 2007). Thus, there may be a split between OT-KO and OTR-KO models regarding the effect of the SP and SNP tasks. The discrepancy between our results, achieved using OTR antagonist administration and previous studies using OTR-KO mice in the SP and SNP tasks, may arise either due to insufficient dose of OTRa used by us or to other methodological differences such as the experimental systems used for the behavioral tests. However, it may also suggest that the deficits displayed by OTR-KO mice in the SP and SNP tests are consequences of pervasive developmental or other long-term processes and not caused by the prevention of OTR activity during the behavioral task.

In contrast to the SP and SNP tests, we did found a significant effect of OTR blockade in the SxP task. While saline-injected male mice showed the normal preference for a female over a male stimulus, OTRa-injected animals did not exhibit this preference. This result is in accordance with a previous study showing that sex discrimination in male mice depends on OT activity in the medial amygdala (Yao et al., 2017). As the medial amygdala is the primary target of chemosensory social information arriving *via* the vomeronasal system (Kang et al., 2011; Newman, 1999; Pro-Sistiaga et al., 2008; Takahashi, 2014), our data further supports a crucial role of OT in modulating social sensory information rather than in basic motivation or social interactions (Choe et al., 2015; Oettl et al., 2016).

Finally, as OT was shown to strongly affect the formation of social memory (Maroun and Wagner, 2016), we examined its effect on social fear memory following a single social defeat. First, we established that a single social defeat of adult male mice causes an abolishment of their social preference in a subsequent test (Toth and Neumann, 2013). We then found that OTRa injection before the defeat prevented the abolishment of social preference in the SP test conducted a day after the social defeat. This result supports a role for OT in the formation of social fear memory, in accordance with previous studies showing that blocking OTR activity in the lateral septum, either using OTRa or by genetic means, abolished the social fear memory which was induced in male mice by social defeat (Guzman et al., 2013, 2014). Moreover, OTRa injection before the SP test did not change the outcome of the social defeat, suggesting that OT affects social fear memory acquisition rather than recall. Similar conclusion was reached by a previous study using conditioned taste aversion (Olszewski et al., 2013). It should be noted, however, that in OTRa/saline animals the social preference was concentrated mainly in short bouts, rather than in long bouts as in undefeated animals. This suggests that despite the OTRa administration before the defeat, the animals still expressed some degree of social fear

and hesitated to interact with the social stimulus. These results are in line with the "social salience" hypothesis of OT function (Shamay-Tsoory and Abu-Akel, 2016), which suggests that OT is not "pro-social" but rather enhances the saliency of social stimuli, hence facilitating their memory regardless of their valence.

5. Summary

In summary, we employed a novel experimental system to examine the effect of pharmacological blockade of OTR on the dynamics of murine social behavior during various social discrimination tasks. Overall, our results suggest that OT plays a role in modulating the saliency of social stimuli and facilitating their memory, as predicted by the social salience theory, rather than in regulating the internal motivation of the subject for social interactions.

Competing interest statement

None of the authors has any competing interest in this manuscript.

CRediT authorship contribution statement

Shani Haskal de la Zerda: Conceptualization, Data curation, Formal analysis, Investigation, Methodology. **Shai Netser:** Project administration, Methodology, Formal analysis, Software. **Hen Magalnik:** Investigation, Data curation. **Shlomo Wagner:** Conceptualization, Funding acquisition, Supervision, Writing.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2020.104676.

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