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Sex-Specific Effects of Stress on Oxytocin Neurons Correspond With Responses to Intranasal Oxytocin

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ABSTRACT

BACKGROUND: Oxytocin (OT) is considered to be a stress-buffering hormone, dampening the physiologic effects of stress. However, OT can also be anxiogenic. We examined acute and long-lasting effects of social defeat on OT neurons in male and female California mice.

METHODS: We used immunohistochemistry for OT and c-fos cells to examine OT neuron activity immediately after defeat (n = 6–9) and 2 weeks (n = 6–9) and 10 weeks (n = 4–5) later. We quantified *Oxt* messenger RNA with quantitative polymerase chain reaction (n = 5–9). Intranasal OT was administered to naïve and stressed mice tested in social interaction and resident-intruder tests (n = 8–14).

RESULTS: Acute exposure to a third episode of defeat increased OT/c-fos colocalizations in the paraventricular nucleus of both sexes. In the medioventral bed nucleus of the stria terminalis, defeat increased *Oxt* messenger RNA, total OT neurons, and OT/c-fos colocalizations in female mice but not male mice. Intranasal OT failed to reverse stress-induced social withdrawal in female mice and reduced social interaction behavior in female mice naïve to defeat. In contrast, intranasal OT increased social interaction in stressed male mice and reduced freezing in the resident-intruder test.

CONCLUSIONS: Social defeat induces long-lasting increases in OT production and OT/c-fos cells in the medioventral bed nucleus of the stria terminalis of female mice but not male mice. Intranasal OT largely reversed the effects of stress on behavior in male mice, but effects were mixed in female mice. These results suggest that changes in OT-sensitive networks contribute to sex differences in behavioral responses to stress.

Keywords: Anxiety, Bed nucleus of the stria terminalis, Mood disorders, Oxytocin, Paraventricular nucleus, *Peromyscus*, Posttraumatic stress disorder, Sex differences, Social behavior

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Oxytocin (OT) has diverse effects on social behaviors and behavioral responses to threat (1) by facilitating aspects of social bonding and cognition (2,3) and by exerting anxiolytic effects (4–9). Deficits in social processes and exaggerated responses to threat are common in stress-induced psychiatric disorders (10,11), so there has been strong interest in investigating OT as a potential therapeutic agent (12,13). Stress-induced psychiatric disorders are more common in women than men (14), so it is important to determine whether OT has similar effects in men and women. Several studies observed elevated OT levels in women with a diagnosis of posttraumatic stress disorder or depression (15–17). In most cases, increased OT levels were interpreted as an adaptive response to reduce the impact of a stressful environment. An alternative hypothesis is that increased OT activity could contribute to some aspects of psychopathology. Although some clinical studies (primarily in men) reported positive results using intranasal OT to treat mood disorders (18), others reported negative results. Intranasal OT was anxiogenic in patients undergoing a session of psychotherapy for depression (19) and increased the perception of social stress (20). In general, the effects of intranasal OT in women are understudied. In one study, intranasal OT enhanced blood oxygen level–dependent responses of the amygdala during an aversive social context in women but reduced this response in men (21). Studies in animal models also indicate that in social contexts, OT has different behavioral effects in females and males.

Social isolation stress increases OT/c-fos colocalizations in the paraventricular nucleus (PVN) during a social challenge, and this effect is stronger in female subjects compared with male subjects (22). Although exogenous OT blocked social defeat–induced social withdrawal in male rats and mice (23), OT did not reverse anxiogenic effects of social isolation in female prairie voles (24). Although effects of social defeat on endogenous OT systems were described in male rodents (25,26), to our knowledge, no data exist in female rodents. We studied the California mouse (*Peromyscus californicus*) to

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directly compare the effect of social defeat in males and females. Female California mice are aggressive (27), so male and female mice can be exposed to episodes of social defeat with similar intensity (28). Defeat induces more reactive coping strategies in female mice, such as social withdrawal and behavioral flexibility, whereas more proactive coping strategies are observed in male mice (29–31). First, we used OT/c-fos immunohistochemistry to examine acute responses to social defeat in the PVN and medioventral bed nucleus of the stria terminalis (BNSTmv). The BNSTmv and PVN contain populations of OT cell bodies and are important modulators social behavior and behavioral responses to stress (32). We tested whether these changes were specific to social contexts and used real-time polymerase chain reaction (PCR) to examine *Oxt* messenger RNA (mRNA). Finally, we examined the behavioral effects of intranasal OT in male and female mice exposed to defeat or control conditions. We tested these mice in the social interaction test because social interaction in an unfamiliar environment is reduced by defeat stress, and this effect is reversed with chronic but not acute antidepressant treatment (31,33). The resident-intruder test was used for ethological observation of fear and anxiety behaviors in a familiar environment, known as conditioned defeat (34). Our results support the hypothesis that defeat induces increases in the activity of OT neurons in females and that OT activity may contribute to some stress-induced changes in social behavior in females.

METHODS AND MATERIALS

Full details of experimental procedures are provided in the Supplement. See Supplemental Figure S1 for timelines. For brevity, California mice are referred to as mice.

Experiment 1: Acute Effects of Social Defeat on OT Neurons

Male and female mice were exposed to either one or three episodes of social defeat or handling control. A first cohort was euthanized 1 hour after a single episode of defeat or control (first day). A second cohort was euthanized 1 hour after a third episode of defeat or control (third day). Sections of hypothalamus and BNSTmv were double-labeled for OT and c-fos cells to estimate activation of OT immunoreactive neurons. Retro-orbital blood samples were taken immediately after the third day of defeat/control from a third cohort of mice. These samples were used for OT enzyme immunoassay.

Experiment 2: Long-lasting Effects of Social Defeat on OT Neurons

Male and female mice were randomly assigned to 3 days of defeat or control conditions and subsequently tested in a social interaction test 2 weeks later (28,31). Mice were euthanized 1 hour after behavior testing. We used OT/c-fos immunohistochemistry to assess the effects of defeat stress on the activity of OT neurons in the BNSTmv (Figure 1A–C) and PVN (Figure 1D–F).

Experiment 3: Effects of Social Context and Defeat on OT Neurons

Male and female mice were assigned to control or defeat conditions and subsequently tested in an open field test, a habituation-dishabituation test, and light/dark box 6–8 weeks later as previously described (35). Mice were then tested 10 weeks after the last episode of defeat in a modified social interaction test. Half of the mice were tested with an unfamiliar, same-sex target mouse during the interaction phase (target present). The other half were tested with an empty cage during the acclimation and interaction phases (target absent). Mice were euthanized 1 hour after testing.

Experiment 4: Long-lasting Effects of Social Defeat on *Oxt* Expression

Male and female mice were assigned to control or defeat stress and subsequently tested in the social interaction test 2 weeks later. Mice were then euthanized, and brains were flash frozen. The BNSTmv and PVN were microdissected from 500-μm sections cut on a cryostat. RNA extraction and real-time PCR were conducted as previously described (Supplemental Methods and Materials and Supplemental Figure S2) (29).

Experiment 5: Effects of Intranasal OT on Social Behavior

One set of male and female mice was exposed to defeat and subsequently tested in the social interaction tests 2 weeks later. Each mouse was treated 5 minutes before testing with 8.0 IU/kg OT, .8 IU/kg OT, or saline (36). The .8 IU/kg dose is roughly equivalent to a weight-adjusted dose used in human studies (8,9). The mice were individually housed for 3 days. Each mouse was treated again with the same dose of OT (or saline) 5 minutes before the resident-intruder test (same-sex intruder). A second set of mice was not exposed to defeat and tested in the same behavior tests. These mice were treated with either saline or .8 IU/kg OT, which had the most robust effects in stressed mice.

Estrous Cycle

In all experiments, we assessed estrous cycle stage post mortem because conducting vaginal lavage before testing disrupts behavior (37). There was no systematic bias in the distribution of estrous stage across treatment groups. In experiment 3, we also analyzed female cell count data using estradiol levels measured from postmortem trunk blood samples (Supplemental Methods and Materials) as a covariate. In previous studies, stress-induced social withdrawal in females persists across the estrous cycle (35) and is not blocked by ovariectomy (28).

Statistical Analysis

Normality of data and homogeneity of variance were checked with Q-Q plots and Levene's test. In most cases, two-way analysis of variance (ANOVA) was used to analyze effects of sex, social defeat stress, and the interaction. In experiment 3, three-way ANOVA was used to examine cell count data using sex, stress, and target status as fixed factors. We also conducted a preliminary analysis of covariance (ANCOVA) analysis on cell counts from female mice in experiment 3 using estradiol as a covariate and stress and target status as fixed factors. All colocalization data (expressed as a percentage of total OT cells) were square root arcsin transformed for

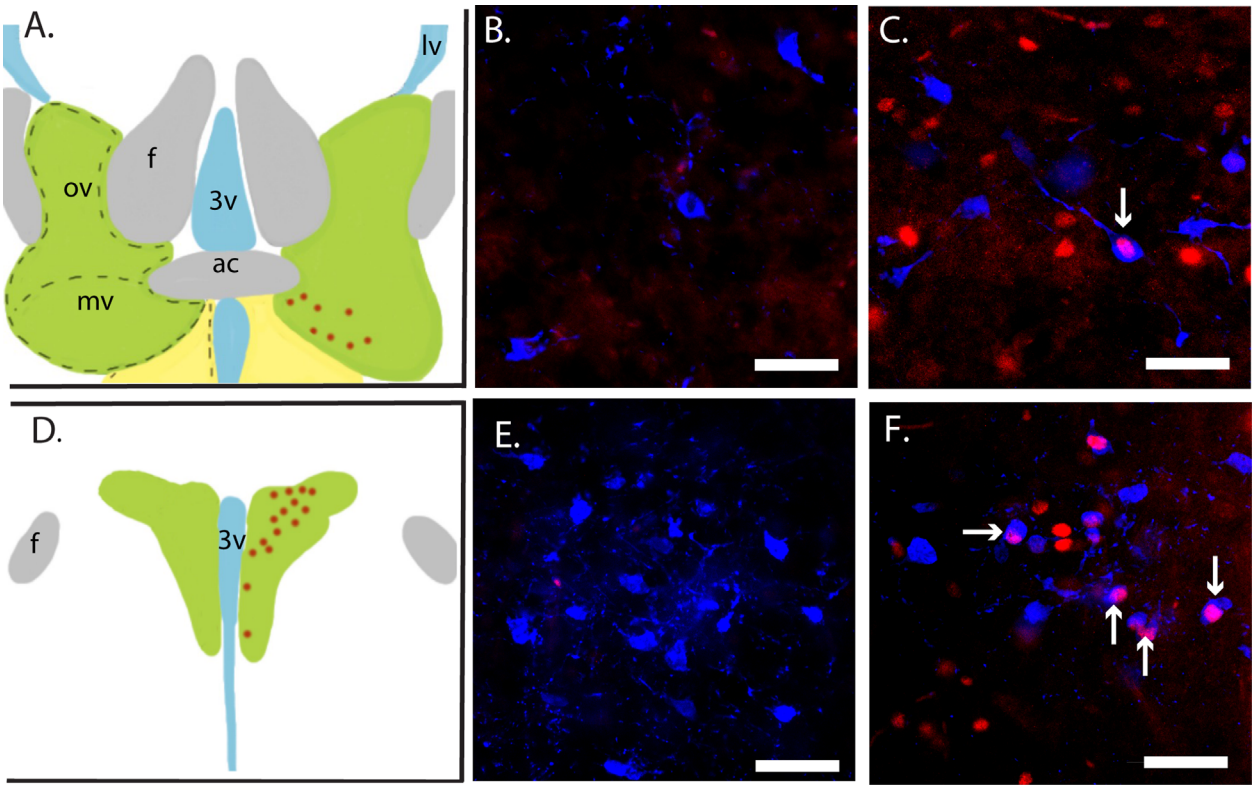

Figure 1. Populations of oxytocin (OT) neurons in the medioventral bed nucleus of the stria terminalis (BNSTmv) **(A–C)** and paraventricular nucleus **(D–F)**. In the BNSTmv, OT neurons (red dots) are clustered just lateral and ventral to the anterior commissure (ac). Representative photomicrographs demonstrating OT (blue) and c-fos (red) cells in the BNSTmv of control **(B)** and stressed **(C)** female mice; white arrow in **(C)** denotes colocalization. Scale bar = 50 μm. In the rostral paraventricular nucleus **(D)**, OT neurons (red dots) are adjacent to the third ventricle (3v). Representative photomicrographs demonstrating OT (blue) and c-fos (red) cells in the rostral paraventricular nucleus of control **(E)** and stressed **(F)** female mice; white arrows in **(F)** denote colocalizations. Scale bar = 50 μm. lv, lateral ventricle; f, fornix; ov, oval nucleus bed nucleus of the stria terminalis; mv, BNSTmv.

analysis. Planned comparisons were used to test the effect of defeat stress within sex. The social interaction ratio was calculated as time spent interacting with the target mouse divided by time spent interacting with an empty cage × 100 (38,39). Nonparametric tests were used for correlational analyses and gene expression data.

RESULTS

Experiment 1: Acute Effects of Social Defeat on OT Neurons

In the BNSTmv, which includes a population of excitatory neurons that induces aversion and reduces reward seeking (40), OT/c-fos cells were elevated immediately after a third day of defeat for both sexes (ANOVA, $p = .012$) (Figure 2A). Planned comparison showed that the effect of defeat on the third day was significant only in male mice. No differences were observed after one episode of defeat. In the PVN, we quantified rostral and caudal subregions separately based on previous work showing differences in glutamatergic input (41) and stress responsivity (29). Main effects of social defeat on OT/c-fos cells were observed in the rostral (ANOVA, $p = .019$) (Figure 2B) and caudal (ANOVA, $p = .01$) (Figure 2C) PVN after the first day of defeat as well as the third day of defeat (ANOVA, p values $< .01$) (Figure 2B, C). However, after the first day of defeat, planned comparisons showed colocalizations were increased in stressed male mice (p values $< .05$) but not female mice. After the third day of defeat, stressed male and female mice had increased OT/c-fos colocalizations. Effects of defeat in the supraoptic nucleus (Supplemental Figure S3A) matched the effects observed in the PVN. No differences in OT/c-fos cells were observed in other brain regions (Supplemental Figure S3), and there were no differences in plasma OT after the third day of defeat (Figure 2D).

Experiments 2–4: Long-lasting Effects of Social Defeat on OT Neurons

Defeat stress had long-lasting effects on OT neurons in the BNSTmv that were sex specific. In experiment 2, defeat stress increased the number of OT positive cells in the BNSTmv of female mice but not male mice (ANOVA, $p < .05$) (Figure 3A). Although stressed female mice had more OT positive cells than control mice, stressed female mice had a higher percentage of OT/c-fos positive cells after the social interaction test (ANOVA, $p < .05$) (Figure 3B). Analysis of the total number of OT/c-fos cells yielded identical results (Supplemental Table S1). No effects of stress were observed in male mice, and OT/c-fos colocalizations were not correlated with social

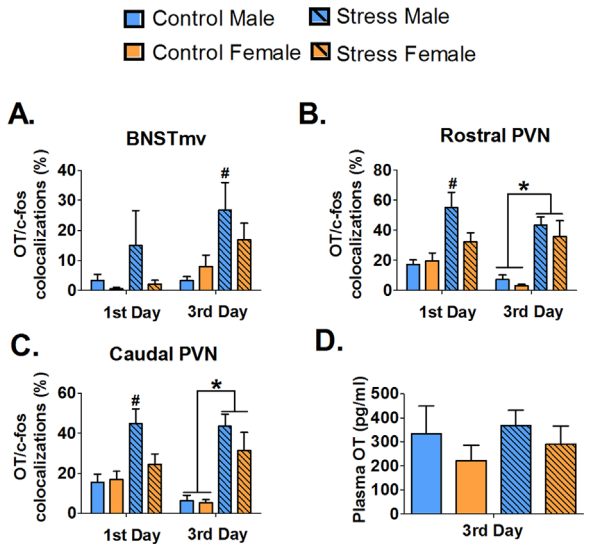

Figure 2. Acute effects of defeat on oxytocin (OT)/c-fos colocalizations in the medioventral bed nucleus of the stria terminalis (BNSTmv) **(A)** (n = 6–8 per group) and rostral and caudal paraventricular nucleus (PVN) **(B,C)** (n = 7–9 per group). A third day of defeat stress increased OT/c-fos colocalizations in the BNSTmv for male mice but not female mice **(A)**. In the rostral **(B)** and caudal **(C)** PVN, increased OT/c-fos cells were observed in male and female mice after a third day of defeat. Only male mice had increased OT/c-fos cells after the first day of defeat. No differences in plasma OT were observed after the third day of defeat **(D)**. Data are mean ± SE. $^{\#}p < .05$ compared with same-sex control mice; $^{*}p \leq .05$, main effect of stress.

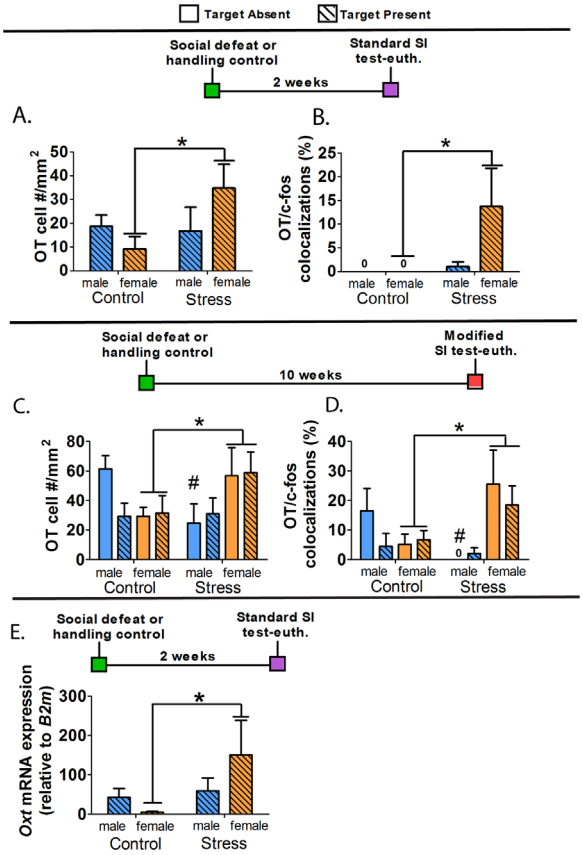

Figure 3. Long-lasting effects of defeat on oxytocin (OT) immunoreactive neurons in the medioventral bed nucleus of the stria terminalis. In experiment 2, all mice were tested in the social interaction test in the presence of a target mouse (hashed bars). Defeat increased the number of OT neurons **(A)** and OT/c-fos neurons **(B)** in female mice (n = 7 control mice, n = 8 stress mice) but not male mice (n = 8 control mice, n = 7 stress mice). In experiment 3, half of the mice were tested in the social interaction test in the absence of a target mouse (open bars). Defeat stress increased the number of OT neurons **(C)** and OT/c-fos neurons **(D)** in stressed female mice that were tested in the presence (n = 5 control mice, n = 5 stress mice) or absence (n = 5 control mice, n = 4 stress mice) of a novel target mouse. In experiment 4, defeat stress increased *Oxt* messenger RNA (mRNA) **(E)** in the medioventral bed nucleus of the stria terminalis in female mice (n = 5 control mice, n = 8 stress mice) but not male mice (n = 9 control mice, n = 8 stress mice). Graphs display mean ± SE. $^{*}p < .05$ effect of stress in female mice; $^{\#}p < .05$ effect of stress in male mice (target absent). SI test-euth, social interaction test followed by euthanasia.

interaction behavior. The increase in OT/c-fos colocalizations in stressed female mice could reflect a more or less continuous increase in the activity of BNSTmv OT neurons or increased activity specifically induced by interacting with a target mouse in the social interaction test. In experiment 3, half of the focal mice were euthanized without exposure to a target mouse (target absent). Stressed female mice had more OT cells ($p < .05$) (Figure 3C) and a greater percentage of OT/c-fos cells ($p < .05$) (Figure 3D) than control female mice in both the target present and target absent conditions. In a preliminary ANCOVA analysis, estradiol explained a significant proportion of variance for OT/c-fos cells (ANCOVA, $p = .03$) but not total OT cell counts (ANCOVA, $p = .66$). However, the overall effects of stress were essentially unchanged for OT and OT/c-fos (Supplemental Table S2). Stressed male mice had fewer OT cells and OT/c-fos colocalizations than control mice, but only in the target absent condition ($p < .05$) (Figure 3C, D). Finally, in experiment 4, real-time PCR analyses of mRNA in punch samples of the BNSTmv showed that stress increased *Oxt* mRNA in female mice but not male mice ($p < .01$) (Figure 3E). Gene expression changes were specific to *Oxt*, as there were no differences in vasopressin (*Avp*) mRNA (Supplemental Table S3). These data support the hypothesis that defeat stress increases OT production and the activity of OT neurons in the BNSTmv of female mice but not male mice.

Effects of defeat on OT neurons in the PVN were more subtle compared with the BNSTmv. Defeat stress affected OT immunoreactive neurons in rostral (Figure 4) but not caudal (Supplemental Figure S4) PVN. In experiment 2, defeat reduced the number of OT positive cells in rostral PVN of female mice (ANOVA, $p < .05$) (Figure 4A) but not male mice. There was a main effect of stress on the percentage of OT/c-fos positive cells (ANOVA, $p < .05$) (Figure 4B). Planned comparisons indicated that defeat increased OT/c-fos colocalizations in female mice but not male mice ($p < .05$). Furthermore, the percentage of OT/c-fos cells in the rostral PVN was negatively correlated with the social interaction ratio in female mice ($\rho = -.53$, $p < .05$) (Figure 5A) but not male mice ($\rho = .06$, $p = .87$) (Figure 5B). There was no change in the overall number of c-fos cells or OT/c-fos cells (Supplemental Table S1), suggesting that effects on colocalizations are driven primarily by decreases in

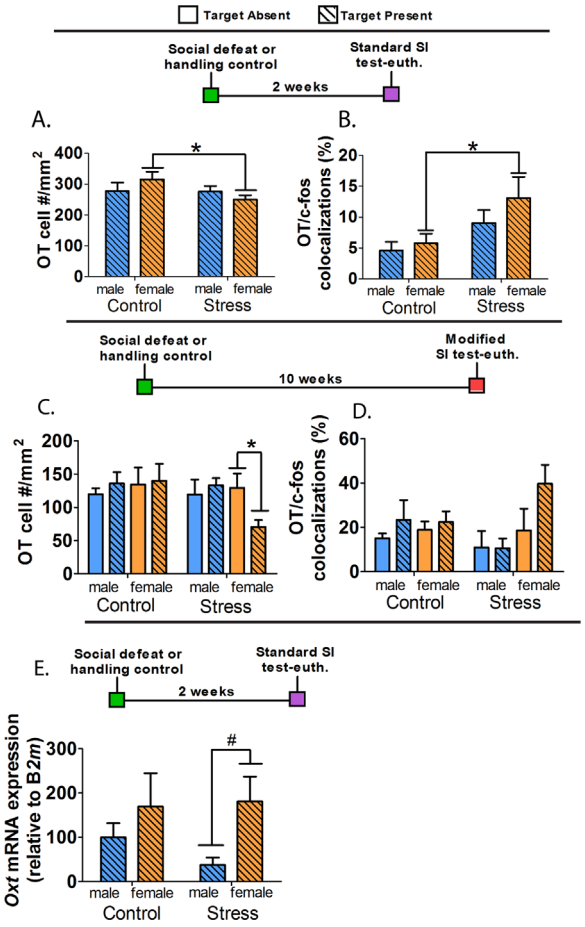

Figure 4. Long-lasting effects of defeat on oxytocin (OT) immunoreactive neurons in the rostral paraventricular nucleus. In experiment 2, all mice were tested in the social interaction test in the presence of a target mouse (hashed bars). Defeat reduced OT cell number **(A)** and increased OT/c-fos colocalizations **(B)** in female mice ($n = 7$ control mice, $n = 8$ stress mice) but not male mice ($n = 7$ control mice, $n = 8$ stress mice). In experiment 3, half of the mice were tested in the social interaction test in the absence of a target mouse (open bars). Stressed female mice had fewer OT neurons when tested in the target present condition ($n = 5$) compared with the target absent condition ($n = 4$) **(C)**. Control female mice did not differ between the target present ($n = 5$) or target absent ($n = 5$) condition. There was a nonsignificant trend for defeat to increase OT/c-fos neurons in the target present condition for female mice **(D)**. In experiment 4 **(E)**, there was no difference in *Oxt* messenger RNA (mRNA) between control female mice ($n = 7$) and stressed female mice ($n = 14$), but stressed female mice had higher *Oxt* than stressed male mice ($n = 5$). Graphs display mean ± SE. $^{*}p < .05$, effect of stress; $^{\#}p < .05$, sex difference. SI test-euth, social interaction test followed by euthanasia.

OT cell number in stressed female mice. In experiment 3, stressed female mice had fewer OT cells when tested in the target present condition (ANOVA, $p < .01$) (Figure 4C) compared with the target absent condition. There was also a nonsignificant trend for stress to increase percent OT/c-fos colocalizations (ANOVA, $p = .1$) (Figure 4D). Estradiol did not explain a significant proportion of variance in ANCOVA analysis for OT cell counts (ANCOVA, $p = .44$) or OT/c-fos colocalizations (ANCOVA, $p = .61$). The social interaction ratio was negatively correlated with percent OT/c-fos colocalizations in the rostral PVN of female mice ($\rho = -.86$, $p < .01$) (Figure 5C) and male mice ($\rho = -.73$, $p = .02$) (Figure 5D) that were tested in the target present condition but not in mice that were tested in the target absent condition (all p values $> .7$). If defeat stress affected the activity of OT neurons but not OT synthesis, defeat should have no effect on *Oxt* mRNA in PVN. Consistent with this hypothesis, real-time PCR analyses showed no effect of defeat on *Oxt* mRNA in male or female mice (Figure 4E). Stressed female mice had more *Oxt* mRNA than stressed male mice (Mann-Whitney, $p < .05$). There were no differences in *Crh* or *Avp* mRNA (Supplemental Table S3). Effects of stress on OT immunoreactive neurons in the PVN were observed in female mice tested in social contexts. Furthermore, in two experiments, we observed a negative relationship between OT/c-fos colocalizations in rostral PVN and social interaction ratio in female mice. No differences in OT cell counts or OT/c-fos colocalizations were observed in other hypothalamic subregions (Supplemental Figures S5 and S6).

Experiment 5: Effects of Intranasal OT on Behavior

The effects of intranasal OT on social interaction were sex dependent. In mice naïve to defeat, the low dose of OT reduced social interaction in female mice but not male mice (ANOVA, $p < .05$) (Figure 6A). Intranasal OT reduced mean social interaction time by 30 seconds, similar to the effect of social defeat stress (28,31). There was no effect of OT on behavior during the acclimation phase (Figure 6C). A similar sex × OT interaction was observed at the trend level in mice exposed to defeat (ANOVA, $p = .1$) (Figure 6B). There was a trend for the low dose to increase social interaction in male mice ($p = .06$) (Figure 6B). This effect may not be specific to social contexts because OT also increased time spent interacting with an empty cage during acclimation ($p < .05$) (Figure 6D). No effect of intranasal OT was observed in stressed female mice, but results from experiments 2–4 suggest that the activity of endogenous OT neurons was already elevated in these mice. During open field testing, intranasal OT increased time spent in the center of the open field in female mice but not male mice naïve to defeat (sex × drug ANOVA, $p = .03$) (Supplemental Figure S7A). There were no differences in stressed mice (Supplemental Figure S7B).

The low dose of OT reduced freezing behavior in stressed male and female mice during resident-intruder tests (Mann-Whitney, $p = .03$) (Figure 6F) but had no effect on naïve mice, which generally showed little freezing behavior (Mann-Whitney, $p > .4$) (Figure 6E). There were no main effects of OT, and there was no sex × drug interaction on number of bites or attack latency (all p values $> .3$) (Supplemental Figure S7). Intranasal OT did not affect escape behavior in naïve or stressed mice of either sex (all p values $> .1$) (Supplemental Figure S7).

DISCUSSION

Our results show that defeat induces enduring effects on the activity of OT neurons in female mice but not male mice. In the BNSTmv, defeat increased *Oxt* gene expression, the number of OT immunoreactive neurons, and OT/c-fos colocalizations

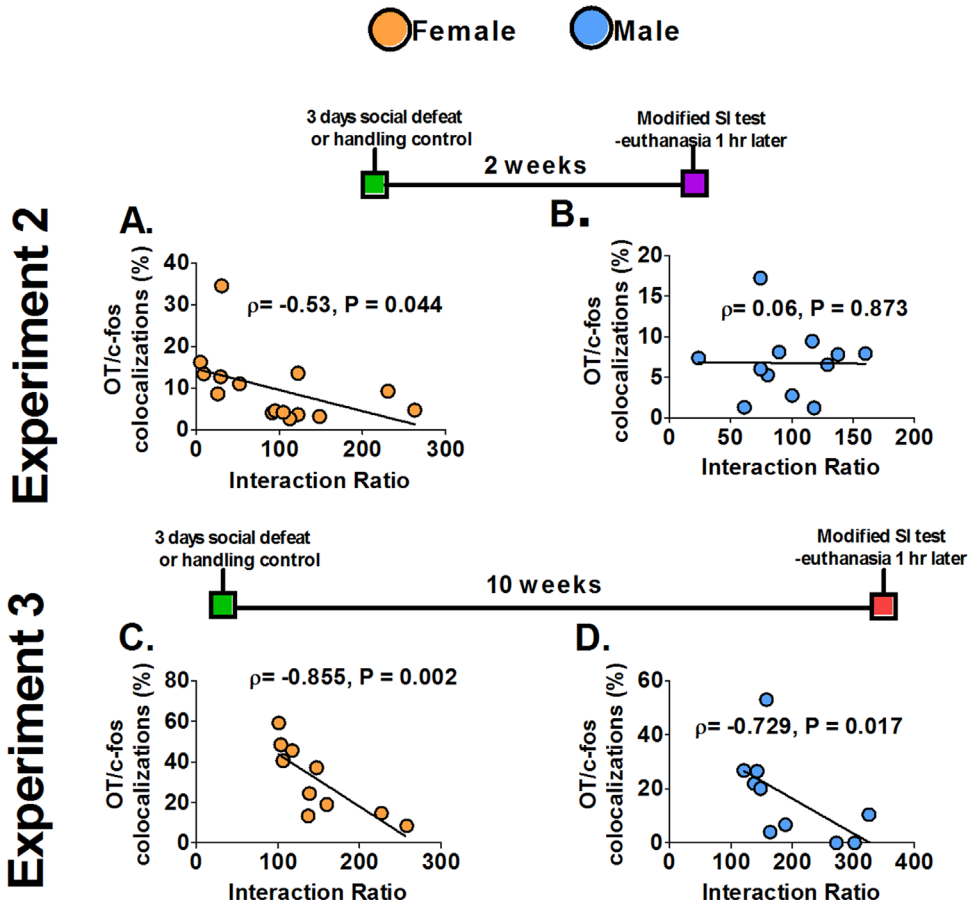

Figure 5. Correlations between percent oxytocin (OT)/c-fos colocalizations in the rostral paraventricular nucleus and social interaction ratio. Interaction ratio is defined as time spent in the cage zone during the interaction phase (target present) divided by the time spent in the cage zone during the acclimation phase and multiplied by 100. For female mice, OT/c-fos colocalizations in the rostral paraventricular nucleus were negatively correlated with the social interaction ratio in experiment 2 **(A)** and experiment 3 **(C)**. For male mice, there was no correlation in experiment 2 **(B)** and a negative correlation in experiment 3 **(D)**. SI, social interaction.

in female mice regardless of social context. In the PVN, stress-induced decreases in OT cell number and increases in OT/c-fos colocalizations were observed primarily in the presence of an unfamiliar mouse. Previous work showed that social isolation increased the reactivity of OT neurons in the PVN (22). Social isolation stress differs from defeat because isolation is continuously applied over several weeks. Our studies show that three brief episodes of social defeat can increase the activity of BNSTmv OT neurons for 10 weeks. We also observed different effects of intranasal OT in male and female mice. In stressed male mice, intranasal OT largely counteracted the effects of defeat on behavior. In stressed female mice, intranasal OT reduced freezing in the resident-intruder test but also reduced both social interaction in female mice naïve to defeat. The inhibitory effect of intranasal OT in female mice on social interaction mimics the effects of social defeat, which induces both social withdrawal and upregulation of OT in female mice. Together, these data suggest that increased OT activity could be an important mechanism contributing to sex differences in behavioral responses to stress.

The strongest effects of defeat stress on OT cells were observed in the BNSTmv. Stress-induced increases in OT immunoreactive cells and OT/c-fos colocalizations were observed in two sets of mice. The mice in experiment 3 had been tested in several behavior tests before the final social interaction test and euthanasia. Although it is possible that this experience could affect OT immunoreactive neurons, it is striking that effects of defeat on BNSTmv OT neurons in female mice were virtually identical in experiments 2 and 3. The increases in OT cells and OT/c-fos colocalizations were observed in the presence and the absence of a target mouse in the social interaction test. These findings, along with increased *Oxt* mRNA in stressed female mice, suggest that defeat stress induces a constitutive increase in the activity of BNSTmv OT neurons. The ventral bed nucleus of the stria terminalis has strong projections to the ventral tegmental area (42). Acute optical activation of excitatory ventral bed nucleus of the stria terminalis neurons projecting to the ventral tegmental area is anxiogenic and induces aversion (40). This suggests that sustained increases in the activity of OT neurons in the BNSTmv could have important effects on the mesolimbic dopamine system, which has been implicated as an important pathway mediating defeat-induced social withdrawal (35,38).

In the rostral PVN, the effects of defeat were also female biased, but they were more subtle than in the BNSTmv. In general, stressed female mice had a higher percentage of OT/c-fos colocalizations than control mice, but only when tested with a novel target mouse. We previously observed an increase in percent OT/c-fos colocalizations in rostral PVN of stressed female mice following a resident-intruder test (29).

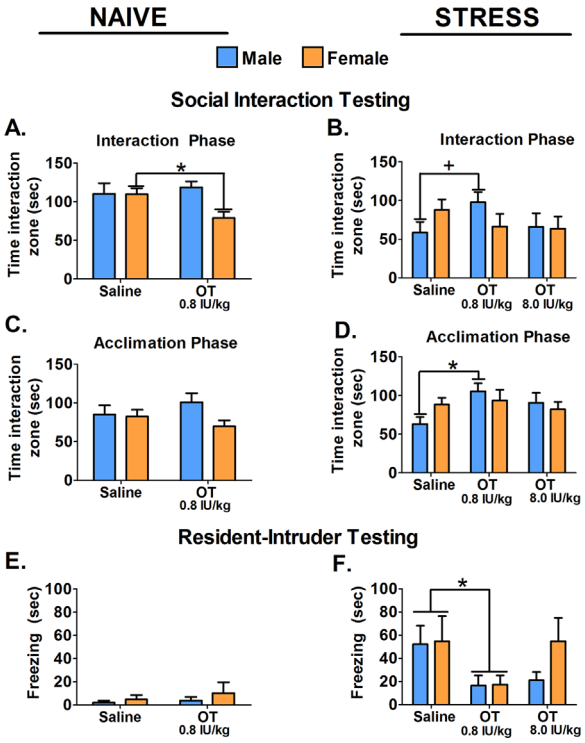

Figure 6. Effects of intranasal oxytocin (OT) on behavior in social interaction and resident-intruder tests in experiment 5. The .8 IU/kg dose reduced social interaction in female mice naïve to defeat **(A)** (saline, $n = 12$; .8 IU/kg, $n = 12$; 8.0 IU/kg, $n = 10$) but had no effect on stressed female mice **(B)** (saline, $n = 13$; .8 IU/kg, $n = 11$). There was a nonsignificant trend for .8 IU/kg OT to increase social interaction in stressed male mice **(B)** (saline, $n = 13$; .8 IU/kg, $n = 13$; 8.0 IU/kg, $n = 10$) and a significant increase in time spent with the empty cage **(D)**. Intranasal OT had no effect on behavior in male mice naïve to defeat **(A, C)** (saline, $n = 10$; .8 IU/kg, $n = 11$). Intranasal OT decreased freezing in stressed male and female mice **(F)** but had no effect on freezing in mice naïve to defeat **(E)**. Data presented as mean ± SE. $^{*}p < .05$, effect of OT; $^{+}p = .06$, effect of OT.

These effects appear to be driven by a rapid decrease in number of OT cells. Stressed female mice had fewer OT cells than control female mice, but only if tested with a novel target mouse. In experiment 3, there was no effect of defeat stress on OT neurons in female mice tested in the target absent condition. One possible explanation is that social encounters in stressed female mice increase release of OT, such that fewer OT neurons are detectable by immunohistochemistry. A similar reduction in vasopressin immunoreactivity was observed in male California mice. Male mice tested in the target present condition had about 30% less vasopressin immunoreactivity in the PVN than male mice tested in the target absent condition (29). An alternative hypothesis is that defeat stress reduces OT synthesis in the PVN. However, gene expression analyses did not support this hypothesis. Stressed female mice had levels of *Oxt* mRNA in the PVN similar to control female mice. Stressed female mice had more *Oxt* mRNA than stressed male mice. Further analysis, such as microdialysis, is needed to test whether there are changes in OT release or neuropeptide translation and degradation rates. Overall, these data suggest that in female mice, defeat increases the reactivity of rostral PVN OT neurons to social contexts.

The immunohistochemistry data showed very different long-lasting effects of defeat stress on OT activity in male and female mice. There was no evidence for enhanced OT activity in stressed male mice, and stressed male mice had less *Oxt* mRNA in the PVN than stressed female mice. Stressed male mice tested in the target absent condition also had fewer OT cells and OT/c-fos colocalizations in the BNSTmv compared with control mice. Although there was no effect of defeat on *Oxt* mRNA in male mice, these results suggest the possibility of a subtle decrease in OT activity in stressed male mice. The sex-dependent effects of defeat on OT provide insights into sex differences in the behavioral effects of intranasal OT.

Effects of Intranasal OT on Behavior

The effects of intranasal OT on behavior were context and sex dependent. Stressed male mice treated with the .8 IU/kg dose of intranasal OT showed a trend for increased social interaction behavior and showed significantly less freezing in the resident-intruder test. These results are consistent with previous reports that intracerebroventricular infusion of OT increases social interaction in male rats and mice *(Mus musculus)* exposed to defeat stress (23). Infusion of OT into the amygdala (43) or hippocampus (44) has also been reported to reduce freezing behavior. In female mice, intranasal OT had context-dependent anxiogenic and anxiolytic effects. In contrast to male mice, intranasal OT had no effect on social interaction behavior in stressed female mice. However, .8 IU/kg of OT decreased social interaction in female mice naïve to defeat, an effect that was similar in magnitude to social defeat. Female rats defeated by a lactating dam do not exhibit social withdrawal from an unfamiliar female rat, but social withdrawal was induced with intracerebroventricular infusion of OT in stressed female rats (45). It is unknown whether defeat stress increases the activity or release of endogenous OT neurons in female rats. All of the behavior tests described up to this point have used unfamiliar stimulus animals, and this may be an important factor because OT has more anxiogenic properties in ambiguous contexts (46). It has been proposed that OT modulates affective states by increasing the salience of social cues rather than determining the valence of those cues (18). Thus, OT might have fewer anxiogenic effects in the presence of familiar individuals, as previously observed in prairie voles (6). When stressed female mice were tested in their home cage during resident-intruder tests, intranasal OT exerted more anxiolytic effects by reducing freezing behavior. The context-dependent effects of OT in female mice could be due to context-dependent activation of different neural circuits. This hypothesis is supported by analyses of indirect markers of neural activity. Stressed male and female mice had more c-fos positive cells in the central nucleus of the amygdala but not the bed nucleus of the stria terminalis or PVN after a resident-intruder test than control mice (29). In contrast, stressed female mice (but not male mice) had more cells positive for phosphorylated cyclic adenosine monophosphate response element binding protein in the nucleus accumbens shell than control mice after a social interaction test, whereas there was

no difference in the central nucleus of the amygdala (35). Future studies need to integrate circuit level analyses with manipulations of the OT pathway in both males and females.

Acute Effects of Defeat

Acute effects of defeat on OT/c-fos colocalizations were more similar in male and female mice within the PVN, whereas in the BNSTmv, OT/c-fos responses were stronger in male mice. It is possible that the blunted acute responses of OT neurons to defeat in female mice contribute in part to the eventual emergence of the social withdrawal phenotype; if so, this would be consistent with previous work demonstrating a stress-buffering role for OT (6). The strong c-fos response of PVN OT neurons to social defeat we observed is likely associated with central release because peripheral OT levels were not elevated immediately after defeat.

Conclusions

Our results demonstrate that the effects of stress on OT systems are long lasting and sex dependent. We observed that defeat generally increased the activity of OT neurons in female mice. Elevated plasma OT was observed in women with posttraumatic stress disorder (15–17) and women in distressed interpersonal relationships (47). It was hypothesized that elevated OT activity in women functioned as a mechanism to facilitate social affiliation (48). An alternative hypothesis is that elevated OT function may contribute to some aspects of behavioral pathology. Our data suggest that familiarity may be a key factor modulating the effects of OT on behavior, with OT being more anxiogenic in unfamiliar contexts and more anxiolytic in familiar contexts. If this hypothesis is correct, OT receptor antagonists might have unanticipated therapeutic benefits in unfamiliar contexts. Further study of the long-lasting impact of psychosocial stressors on OT pathways should lead to a better understanding of the mechanisms underlying deficits in social behavior associated with psychiatric disorders.

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