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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 physiological 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 to examine OT neuron activity immediately after defeat (n = 6-9) as well as two (n = 6-9) and ten weeks (n = 4-5) later. We quantified *Oxt* mRNA with qPCR (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 (PVN) of both sexes. In the medioventral bed nucleus of the stria terminalis (BNSTmv), defeat increased *Oxt* mRNA, total OT neurons, and OT/c-fos colocalizations in females but not males. Intranasal OT failed to reverse stress-induced social withdrawal in females, and reduced social interaction behavior in females naïve to defeat. In contrast, intranasal OT increased social interaction in stressed males 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 BNSTmv of females but not males. Intranasal OT largely reversed effects of stress on behavior in males but effects were mixed in females. These results suggest changes in OT sensitive networks contribute to sex differences in behavioral responses to stress.

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Keywords

Oxytocin; social behavior; *Peromyscus*; sex differences; mood disorders; bed nucleus of the stria terminalis; paraventricular nucleus; PTSD; anxiety

Introduction

Oxytocin (OT) has diverse effects on social behaviors and behavioral responses to threat (1) in part 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 (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 males and females. Intriguingly, several studies observed elevated OT levels in women diagnosed with post-traumatic stress disorder (PTSD) 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) have 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 also increased the perception of experiencing social stress (20). In general the effects of intranasal OT in women are understudied. In one study intranasal OT enhanced BOLD 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 females than males (22). Interestingly, while 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 have been described in male rodents (25, 26), to our knowledge no data exist in females. We studied the California mouse (*Peromyscus californicus*) to directly compare the effect of social defeat in males and females. Female California mice are aggressive (27), so males and females can be exposed to episodes of social defeat with similar intensity (28). Defeat induces more reactive coping strategies in females such as social withdrawal and behavioral flexibility whereas more proactive coping strategies are observed in males (29-31). First we used OT/c-fos immunohistochemistry to examine acute responses to social defeat in the paraventricular nucleus (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 PCR defeat to examine *Oxt* mRNA. Finally we examined the behavioral effects of intranasal OT in males and females exposed to defeat or control conditions. We used 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 stress-induced changes in social behavior in females.

Materials and methods

Full details of experimental procedures are provided in Supplementary methods. See Fig. S1 for timelines.

Experiment 1: Acute effects of social defeat on OT neurons

Males and females were exposed to either one or three episodes of social defeat or handling control. A first cohort was euthanized 1 hr following a single episode of defeat or control (first day). A second cohort was euthanized 1 hr after a third episode of defeat or control (third day). Sections of hypothalamus and BNSTmv were double-labeled for OT and c-fos to estimate activation of OT immunoreactive (-ir) 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

Males and females were randomly assigned to three days of defeat or control handling and then two weeks later tested in a social interaction test (28, 31). Mice were euthanized one hour after behavior testing. OT/c-fos immunohistochemistry was used to assess the effects of defeat stress on the activity of OT neurons in the BNSTmv (Fig. 1A-C) and PVN (Fig. 1D-F).

Experiment 3: Effects of social context and defeat on OT neurons

Males and females were assigned to control or defeat conditions and then tested in an open field test, a habituation-dishabituation test, and light dark box 6-8 weeks later as previously described (35). Ten weeks after the last episode of defeat, mice were tested 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 was exposed to an empty cage during both the acclimation and interaction phases (target absent). Mice were euthanized 1 hour after testing.

Experiment 4: Long lasting effects of social defeat on oxytocin (*Oxt*) gene expression

Males and females were assigned to control or defeat stress and then tested in the social interaction test two weeks later. Mice were then euthanized and brains flash frozen. The BNSTmv and PVN were microdissected from 500 μ m sections cut on a cryostat. RNA extraction and real-time PCR were conducted as previously described (see Supplementary methods, Fig S2.) (29).

Experiment 5: Effects of intranasal oxytocin on social behavior

One set of males and females was exposed to defeat and then tested in the social interaction tests two weeks later. Five minutes before testing each mouse was treated with either 8.0 IU/kg OT, 0.8 IU/kg OT, or saline (36). The 0.8 IU/kg dose is roughly equivalent to a weight-adjusted dose used in human studies (8, 9). Mice were then singly housed for 3 days. Five minutes before resident-intruder tests (same-sex intruder), each mouse was treated again with the same dose of OT (or saline). 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 0.8 IU/kg OT, which had the most robust effects in stressed mice.

Estrous cycle

In all studies 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 post-mortem trunk blood samples (see Supplementary methods) as a covariate. In previous studies we reported that stress-induced social withdrawal 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 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 ANCOVA analysis on cell counts from females 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 arc-sin transformed for 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). Non-parametric 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 (Fig. 2A, ANOVA, $p = 0.012$). Planned comparison showed the effect of defeat on the third day was significant only in males. 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 (Fig. 2B, ANOVA, $p = 0.019$) and caudal PVN (Fig. 2C, ANOVA, $p = 0.01$) after the first day of defeat as well as the third day of defeat (Fig. 2B&C, ANOVA p 's < 0.01). However

after the first day of defeat planned comparisons showed colocalizations were increased in stressed males (p 's < 0.05) but not females. After the third day of defeat both stressed males and females had increased OT/c-fos colocalizations. Effects of defeat in the supraoptic nucleus (SON, Fig. S3A) matched those observed in PVN. No differences in OT/c-fos cells were observed in other brain regions (Fig. S3) and there were no differences in plasma OT after the third day of defeat (Fig. 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 females but not males (Fig. 3A, ANOVA, $p < 0.05$). Even though stressed females had more OT positive cells than controls, stressed females had a higher percentage of OT/c-fos positive cells following the social interaction test (Fig. 3B, ANOVA, $p < 0.05$). Analysis of the total number of OT/c-fos cells yielded identical results (Table S1). No effects of stress were observed in males, and OT/c-fos colocalizations were not correlated with social interaction behavior. The increase in OT/c-fos colocalizations in stressed females 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 females had more OT cells (Fig. 3C, $p < 0.05$) and a greater percentage of OT/c-fos cells (Fig. 3D, $p < 0.05$) than control females 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 (ANCOVA, $p = 0.03$) but not total OT cell counts (ANCOVA, $p = 0.66$). However the overall effects of stress were essentially unchanged for both OT and OT/c-fos (Table S2). Stressed males had fewer OT cells and OT/c-fos colocalizations than controls, but only in the target absent condition (Fig. 3C-D, $p < 0.05$). Finally, in experiment 4 real-time PCR analyses of mRNA in punch samples of the BNSTmv showed that stress increased *Oxt* mRNA in females but not males (Fig. 3E, $p < 0.01$). Gene expression changes were specific to *Oxt*, as there were no differences in vasopressin mRNA (Table S3). In summary, these data support the hypothesis that defeat stress increases both OT production and the activity of OT neurons in the BNSTmv of females but not males.

Effects of defeat on OT neurons in the PVN were more subtle compared to the BNSTmv. Defeat stress affected OT-ir in rostral (Fig. 4) but not caudal PVN (Fig. S4). In experiment 2, defeat reduced the number of OT positive cells in rostral PVN of females (Fig. 4A, ANOVA, $p < 0.05$) but not males. There was a main effect of stress on the percentage of OT/c-fos positive cells (Fig. 4B, ANOVA $p < 0.05$). Planned comparisons indicated that defeat increased OT/c-fos colocalizations in females but not males (ANOVA, $p < 0.05$). Furthermore, the percentage of OT/c-fos cells in the rostral PVN was negatively correlated with the interaction ratio in females (Fig. 5A, $p = -0.53$, $p < 0.05$) but not males (Fig. 5B, $p = 0.06$, $p = 0.87$). There was no change in the overall number of c-fos cells or OT/c-fos cells (Table S1), suggesting that effects on colocalizations are driven primarily by decreases in OT cell number in stressed females. In experiment 3, stressed females had fewer OT cells when tested in the target present condition (Fig. 4C, ANOVA, $p < 0.01$) compared to the target

absent condition. There was also a non-significant trend for stress to increase percent OT/c-fos colocalizations (Fig. 4D, ANOVA, $p = 0.1$). Estradiol did not explain a significant proportion of variance in ANCOVA analysis for OT counts (ANCOVA, $p = 0.44$) or OT/c-fos (ANCOVA, $p = 0.61$). Interaction ratio was negatively correlated with percent OT/c-fos colocalizations in the rostral PVN of both females (Fig. 5C, $\rho = -0.86$, $P < 0.01$) and males (Fig. 5D, $\rho = -0.73$, $p = 0.02$) that were tested in the target present condition but not in mice that were tested in the target absent condition (all p 's > 0.7). If defeat stress impacted the activity of OT neurons but not OT synthesis, then 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 males or females (Fig. 4E). Interestingly, stressed females had more *Oxt* mRNA than stressed males (Mann-Whitney, $p < 0.05$). There were no differences in *Crh* or *Avp* mRNA (Table S3). In summary, effects of stress on OT-ir in the PVN were observed in females tested in social contexts. Furthermore, in two studies we observed a negative relationship between OT/c-fos colocalizations in rostral PVN and social interaction ratio in females. No differences in OT cell counts or OT/c-fos colocalizations were observed in other hypothalamic subregions (Figs. S5, S6).

Experiment 5: Effects of intranasal oxytocin 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 females but not males (Fig. 6A, ANOVA $p < 0.05$). Intranasal OT reduced mean social interaction time by 30 sec, similar to the effect of social defeat stress (28, 31). There was no effect of OT on behavior during the acclimation phase (Fig. 6C). A similar sex*OT interaction was observed at the trend level in mice exposed to defeat (Fig. 6B, ANOVA, $p = 0.1$). There was a trend for the low dose to increase social interaction in males (Fig. 6B, $p = 0.06$). This effect may not be specific to social contexts, as OT also increased time spent interacting with an empty cage during acclimation (Fig. 6D, $p < 0.05$). No effect of intranasal OT was observed in stressed females, 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 females but not males naïve to defeat (Fig. S7A, sex*drug ANOVA, $p = 0.03$). There were no differences in stressed mice (Fig. S7B).

The low dose of OT reduced freezing behavior in stressed males and females during resident-intruder tests (Fig. 6F, Mann-Whitney, $p = 0.03$) but had no effect on naïve mice, which generally showed little freezing behavior (Fig. 6E, Mann-Whitney, $p > 0.4$). There were no main effects of OT nor was there any sex*drug interaction on number of bites or attack latency (Fig. S7, all p 's > 0.3). Intranasal OT did not affect escape behavior in either naïve or defeated mice of either sex (Fig. S7, all p 's > 0.1).

Discussion

Our results show that defeat induces enduring effects on the activity of OT neurons in females but not males. In the BNSTmv, defeat increased *Oxt* gene expression, the number OT immunoreactive neurons, and OT/c-fos colocalizations in females 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 it is continuously applied over several weeks. Our studies show that three brief episodes of social defeat increase the activity of BNSTmv OT neurons for up to 10 weeks. We also observed different effects of intranasal OT in males and females. In stressed males, intranasal OT largely counteracted the effects of defeat on behavior. In stressed females, intranasal OT reduced freezing in the resident-intruder test but also reduced social interaction in females naïve to defeat. The inhibitory effect of intranasal OT in females on social interaction mimics the effects of social defeat, which induces social withdrawal and upregulation of OT in females. 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 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's possible this experience could impact OT-ir, it is striking that effects of defeat on BNSTmv OT neurons in females were virtually identical in experiments 2 and 3. The increases in OT cells and OT/c-fos colocalizations were observed in both the presence and absence of a target mouse in the social interaction test. These findings, along with increased *Oxt* mRNA in stressed females suggest that defeat stress induces a constitutive increase in the activity of BNSTmv OT neurons. Interestingly, the ventral BNST has strong projections to the ventral tegmental area (VTA)(42). Acute optical activation of excitatory ventral BNST neurons projecting to the VTA 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 more subtle than in the BNSTmv. In general stressed females had a higher percentage of OT/c-fos colocalizations than controls, but only when tested with a novel target mouse. Previously we observed an increase in percent OT/c-fos colocalizations in rostral PVN of stressed females following a resident-intruder test (29). Interestingly, these effects appear to be driven by a rapid decrease in OT cell number. Stressed females had fewer OT cells than controls, but only if tested with a novel target mouse. In experiment 3, there was no effect of defeat stress on OT neurons in females tested in the target absent condition. One possible explanation is that social encounters in stressed females may enhance release of OT, such that fewer OT neurons are detectable by immunohistochemistry. A similar reduction in vasopressin (AVP) immunoreactivity was observed in male California mice. Males tested in the target present condition had about 30% less AVP immunoreactivity in the PVN than males 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 females had similar levels of *Oxt* mRNA in the PVN as control females. In fact, stressed female had more *Oxt* mRNA than stressed males. Further analysis, such as microdialysis, is needed to test whether there are changes in OT release or neuropeptide

translation/degradation rates. Overall, these data suggest that in females 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 males and females. There was no evidence for enhanced OT activity in stressed males, and stressed males had less *Oxt* mRNA in the PVN than stressed females. Stressed males tested the target absent condition also had fewer OT cells and OT/c-fos colocalizations in the BNSTmv compared to controls. Although there was no effect of defeat on *Oxt* mRNA in males, these results suggest the possibility of a subtle decrease in OT activity in stressed males. 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. Interestingly stressed males treated with the 0.8 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 (icv) infusion of OT increases social interaction in male rats and mice exposed to defeat stress (23). Infusion of OT in to the amygdala (43) or hippocampus (44) has also been reported to reduce freezing behavior. In females intranasal OT had context-dependent anxiogenic and anxiolytic effects. In contrast to males, intranasal OT had no effect on social interaction behavior in stressed females. However 0.8 IU/kg of OT decreased social interaction in females naïve to defeat, an effect that was similar in magnitude to social defeat. In rats, females defeated by a lactating dam do not exhibit social withdrawal from an unfamiliar female, but social withdrawal was induced when stressed females were also infused with OT icv (45). It is unknown whether defeat stress increases the activity or release of endogenous OT neurons in female rats. An important point is that all of the behavior tests described up to this point have used unfamiliar stimulus animals. This may be an important factor, as 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 (47). Thus OT might have less anxiogenic effects in the presence of familiar individuals, as previously observed in prairie voles (6). Interestingly when stressed females 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 females could be due to context-dependent activation of different neural circuits. This hypothesis is supported by analyses of indirect markers of neural activity. Stressed females and females had more c-fos positive cells in central nucleus of the amygdala (CEA) but not BNST or PVN following a resident-intruder test than controls (29). In contrast stressed females (but not males) had more phosphorylated CREB positive cells in the nucleus accumbens shell than controls following a social interaction test while there was no difference in the CEA (35). Future studies will need to integrate circuit level analyses with manipulations of the OT pathway.

Acute effects of defeat

Acute effects of defeat on OT/c-fos colocalizations were more similar in males and females within the PVN while in the BNSTmv OT/c-fos responses were stronger in males. It is possible that the blunted acute responses of OT neurons to defeat in females contribute in part to eventual emergence of the 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 as peripheral OT levels were not elevated immediately after defeat.

Conclusion

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 females. It is interesting to consider that elevated plasma OT has been observed in women with PTSD (15-17) as well as women in distressed interpersonal relationships (48). It has been hypothesized that elevated OT activity in women functioned as a mechanism to facilitate social affiliation (49). An alternate 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 correct, oxytocin receptor antagonists might have unanticipated therapeutic benefits in novel 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Populations of oxytocin neurons in the BNSTmv (A-C) and PVN (D-F). In the BNSTmv, OT neurons (red dots) are clustered just lateral and ventral from to the anterior commissure (ac). lv, lateral ventricle; f, fornix; ov, oval nucleus BNST; mv, BNSTmv. Representative photomicrographs demonstrating OT (blue)/c-fos (red) cells in the BNSTmv of control (C) and stressed (D) females, white arrow denotes colocalization. Scale bar = 50 μ m. In the rostral PVN (D) oxytocin neurons (red dots) are adjacent to the third ventricle (3v). Representative photomicrographs demonstrating OT (blue)/c-fos (red) cells in the rostral PVN of control (E) and stressed (F) females, white arrows denote colocalizations. Scale bar = 50 μ m.

Figure 2.

Acute effects of defeat on OT/c-fos colocalizations in the BNSTmv (A; n = 6-8 per group) and rostral and caudal paraventricular nucleus (PVN; n = 7-9 per group). A third day of defeat stress increased OT/c-fos colocalizations in the BNSTmv for males but not females (A). In the rostral (B) and caudal (C) PVN increased OT/c-fos cells were observed in both males and females after a third day of defeat. Only males 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 $\pm$ SE. #p < 0.05 compared with same sex control, *p = 0.05, main effect of stress.

Figure 3.

Long lasting effects of defeat on OT-ir in the medioventral bed nucleus (BNSTmv). 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 OT neurons (A) and OT/c-fos neurons (B) in females (control $n = 7$, stress $n = 8$) but not males (control $n = 8$, stress $n = 7$). In experiment 3, half of the mice were tested in the social interaction test in the absence of target mouse (open bars). Defeat stress increased the number of OT neurons (C) and OT/c-fos neurons (D) in stressed females that were tested in the presence (control $n = 5$, stress $n = 5$) or absence (control $n = 5$, stress $n = 4$) of a novel target mouse. In experiment 4 defeat

stress increased *Oxt* mRNA (E) in the BNSTmv in females (control n=5, stress n=8) but not males (control n = 9, stress n = 8). Graphs display mean $\pm$ SE. *p < 0.05 effect of stress within females. #p < 0.05 effect of stress within males (target absent).

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Figure 4.

Long lasting effects of defeat on OT-ir in the rostral paraventricular nucleus. In experiment 2, all mice were tested in the social interaction test in the presence of a target mouse (hatched bars). Defeat reduced OT cell number (A) and increased OT/c-fos colocalizations (B) in females (control $n = 7$, stress = 8) but not males (control $n = 7$, stress = 8). In experiment 3, half of the mice were tested in the social interaction test in the absence of target mouse (open bars). Stressed females had fewer OT neurons when tested in the target present condition ($n = 5$) compared to the target absent condition ($n = 4$). Control females did not differ between the target present ($n = 5$) or target absent ($n = 5$) condition. There was a non-significant trend for defeat to increase OT/c-fos neurons in the target present condition (D).

In experiment 4 (E), there was no difference in *Oxt* mRNA between control (n = 7) and stressed (n = 14) females but stressed females had higher *Oxt* than stressed males (n = 5). Graphs display mean $\pm$ SE *p < 0.05, effect of stress; #p < 0.05, sex difference.

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Figure 5. Correlations between percent OT/c-fos colocalizations in the rostral paraventricular nucleus (PVN) 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 acclimation phase and then multiplied by 100. For females, OT/c-fos in the rostral PVN was negatively correlated with interaction ratio in experiment 2 (A) and experiment 3 (C). For males, there was no correlation in experiment 2 (B) and a negative correlation in experiment 3 (D).

Figure 6.

Effects of intranasal OT on behavior in social interaction and resident intruder tests in experiment 5. The 0.8 IU/kg dose reduced social interaction in females naïve to defeat (A, saline $n = 12$, 0.8 IU/kg $n = 12$, 8.0 IU/kg $n = 10$) but had no effect on stressed females (B, saline $n = 13$, 0.8 IU/kg $n = 11$). There was a nonsignificant trend for 0.8 IU/kg OT to increase social interaction in stressed males (B, saline $n = 13$, 0.8 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 males naïve to defeat (A&C, saline $n=10$, 0.8 IU/kg $n = 11$). Intranasal OT decreased freezing in stressed males and females (F) but had no effect on freezing in

mice naïve to defeat (E). Data presented as mean $\pm$ SE. * $p < 0.05$ effect of OT, + $p = 0.06$ effect of OT.

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