Priority Communication

Biological Psychiatry

Oxytocin Receptors in the Anteromedial Bed Nucleus of the Stria Terminalis Promote Stress-Induced Social Avoidance in Female California Mice

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ABSTRACT

BACKGROUND: The neuropeptide oxytocin (OT) is a key regulator of social and emotional behaviors. The effects of OT are context dependent, and it has been proposed that OT increases the salience of both positive and negative social cues. Here we tested whether the bed nucleus of the stria terminalis (BNST) mediates anxiogenic effects of OT.

METHODS: First, we studied the effects of systemic administration of an OT receptor (OTR) antagonist L-368,899 on social behavior in male and female California mice exposed to social defeat. We examined the effect of L-368,899 on G protein activation and used early growth response factor 1 immunohistochemistry to identify potential sites of OTR action. Finally, we examined the effects of L-368,899 infused in the BNST on behavior.

RESULTS: A single dose of systemic L-368,899 increased social approach in stressed female mice and decreased social approach in male mice naïve to defeat. L-368,899 prevented OT activation of G proteins and did not activate G proteins in the absence of OT. Intranasal OT, which reduces social approach in female mice but not male mice, increased early growth response factor 1 immunoreactivity in the nucleus accumbens core and anteromedial BNST in female mice but not in male mice. Stressed female mice that received an infusion of L-368,899 into the anteromedial BNST but not the nucleus accumbens core increased social approach and decreased social vigilance responses.

CONCLUSIONS: Our results suggest that OTR activation in anteromedial BNST induces a vigilance response in which individuals avoid, yet attend to, unfamiliar social contexts. Our results suggest that OTR antagonists may have unappreciated therapeutic potential for stress-induced psychiatric disorders.

Keywords: Bed nucleus of stria terminalis, Oxytocin, Sex differences, Social anxiety, Social defeat, Stress

https://doi.org/10.1016/j.biopsych.2017.08.024

Oxytocin (OT) is an evolutionary conserved neuropeptide that is a key regulator of social and emotional behaviors (1–4). Reports that OT has anxiolytic effects in humans (5–8) have sparked interest in the use of OT as a therapeutic. Nonetheless, it is clear in some cases that OT is anxiogenic (9). Intriguingly, postmortem analyses showed that humans with depression had more OT immunoreactive neurons in the hypothalamus (10). These results might reflect OT production as a homeostatic response to stress, but they could also indicate that OT contributes to behavioral pathology. When assessing these hypotheses, sex-specific effects of OT must be considered. Intranasal OT reduces amygdala reactivity in men (5,7) but increases it in women (11,12). Sex-specific effects of OT in humans are consistent with findings in animal research (1,13,14), indicating the importance including both males and females in studies of OT function.

We previously observed that social defeat increases the activity of OT neurons in the medioventral bed nucleus of the stria terminalis (BNSTmv) and paraventricular nucleus (PVN) in female but not male California mice (15). We also found that intranasal OT reduced social interaction (SI) in unstressed female mice, mirroring the effect of social defeat stress. We hypothesized that stress-induced increases in the activity of OT neurons contribute to social avoidance in female mice by activating OT receptors (OTRs). We studied the effects of systemic administration of the OTR antagonist (OTA) L-368,899 on social and nonsocial behavior in control and stressed male and female mice. To identify potential sites of action of OTRs, we used receptor autoradiography to examine effects of defeat on OTR binding. We then used immunohistochemistry to measure effects of intranasal OT or systemic administration of OTA on immediate early gene activation. Based on these results, we then performed site-specific injections of OTA in either the dorsolateral nucleus accumbens (NAcdl) core or anteromedial BNST (BNSTam) of stressed female mice. Our main finding is that a single dose of OTA, either systemically or

ISSN: 0006-3223

within the BNSTam, rapidly reverses stress-induced social avoidance in female mice, without affecting behavior in nonsocial contexts. Our results support the hypothesis that stress-induced increases in the activation OTR induce social withdrawal and imply that OTAs may have unappreciated therapeutic potential for stress-induced psychiatric disorders.

METHODS AND MATERIALS

Full details of experimental procedures are provided in the Supplement.

Animals

All studies on male and female California mice were approved by the institutional animal care and use committee and conformed to National Institutes of Health guidelines.

Social Defeat

Male and female mice were randomly assigned to control handling or social defeat for 3 consecutive days as described (16). Behavioral and receptor binding analyses were conducted 2 weeks after social defeat (17).

Effects of Systemic OTA Treatment

To inhibit OTRs, we used the high affinity nonpeptide OTA L-368,899 (L2540, Sigma-Aldrich, St. Louis, MO), which readily passes through the blood-brain barrier (18,19). Male and female mice exposed to social defeat or control conditions were given intraperitoneal injections of either saline (sterile phosphate-buffered saline) or one of two doses of L-368,899 (1 or 5 mg/kg). These doses were based on previous behavioral studies of L-368,899 (20–22). Injections were administered 30 minutes before testing based on pharmacokinetic data (23).

SI Test

SI testing was performed as previously described (16,24). The interaction-phase videos were later reanalyzed by an observer blinded to treatment assignment to manually score active investigation, time spent inactive in interaction zone, autogrooming, and time spent in sides (for details see Supplemental Table S1). We also manually recorded head orientation toward the target mouse when the focal mouse was outside of the interaction zone. This behavior is referred to as "risk assessment behavior" or vigilance [Supplemental Video 1, (25)].

Odor Preference Test

One day after SI, odor preference (OP) was assessed using the same arena used for SI. During OP phase, diluted urine of a known conspecific (cagemate odor) and an unknown conspecific (unfamiliar odor) were added to predefined interaction areas (Figure 1E). OP ratio was defined as time spent with the head in an unfamiliar odor zone divided by total time spent with the head in both cagemate and unfamiliar odor zones.

OTR Autoradiography

Male and female mice were exposed to control or defeat conditions and then euthanized 2 weeks later (26,27). Brains were snap frozen, sliced, and processed for OTR autoradiography as previously described (28).

Effects of Intranasal OT and Systemic OTA on Early Growth Response Factor 1 Immunoreactivity

Immunohistochemistry for early growth response factor 1 (EGR1) in mice treated intranasally with 0.8 IU of OT or saline (15) was used to identify possible sites of OT action. EGR1 has been shown to be useful as an indirect marker of neuronal activation in a variety of species and contexts (29–31). We used animals naïve to defeat because intranasal administration of OT did not affect social behavior in stressed female mice, while it reduced SI in naïve female mice, which mimics the effects that stress has on this behavior (full details are in the Supplemental Methods). Based on the results obtained in this study, we analyzed EGR1 expression in the NAcdl core and BNSTam of control and stressed animals receiving systemic injections of saline or OTA.

Site-Specific Injection of OTA

Seven days after social defeat, female mice were implanted with a bilateral guide cannula (Plastics One, Roanoke, VA) aimed at either the NAcdl core (anteroposterior: +0.51, mediolateral: ±1.5, dorsoventral: +6.0) or BNSTam (anteroposterior: +0.45, mediolateral: ±1.0, dorsoventral: +5.6) (24). After recovery, female mice were randomly assigned to receive bilateral 200-nL infusions of either artificial cerebrospinal fluid or L-368,899 (1 μg per side; Plastics One) into the NAcdl core or BNSTam that projected 1 mm past the guides. The dose used is within the range of previous studies using intracerebral injections (32–35). Thirty minutes later each mouse was run in the SI test. Histology was used to determine injection sites (see the Supplemental Methods).

Effects of OTA on OT Activation of G Proteins and β-Arrestins Recruitment

OT facilitates OTR coupling to several different G proteins and β-arrestins, allowing for multiple degrees of freedom for effects on neural activity (36,37). While L-368,899 is known to be a selective OTA, its effects on OTR signaling are unknown. This is an important question because some OTAs, such as atosiban, can activate selective inhibitory G protein pathways (36,38). We performed a complete bioluminescence resonance energy transfer (BRET) characterization of the coupling properties of L-368,899 (39) to test if this compound is a complete antagonist or shows biased agonism (see the Supplemental Methods).

Statistical Analysis

We used two-way analyses of variance (ANOVAs) to analyze receptor autoradiography (sex × stress), behavior from systemic OTA administration (stress × drug), behavior from site-specific injections (drug × injection site), OP ratio, and EGR1 immunoreactivity (sex × drug). For significant two-way ANOVAs, planned comparisons were used to detect differences between groups (package lsmeans in R [R Project for Statistical Computing, version 3.0.3, Vienna, Austria], Bonferroni, 0.95 confidence interval). Effect size is reported as Cohen's *d*. Spearman correlations were used to correlate autoradiography data with behavior. Finally, we performed a 3-way ANOVA to assess effects of estrous cycle (stress × drug × estrous) in the SI and OP tests. Estrous cycle was

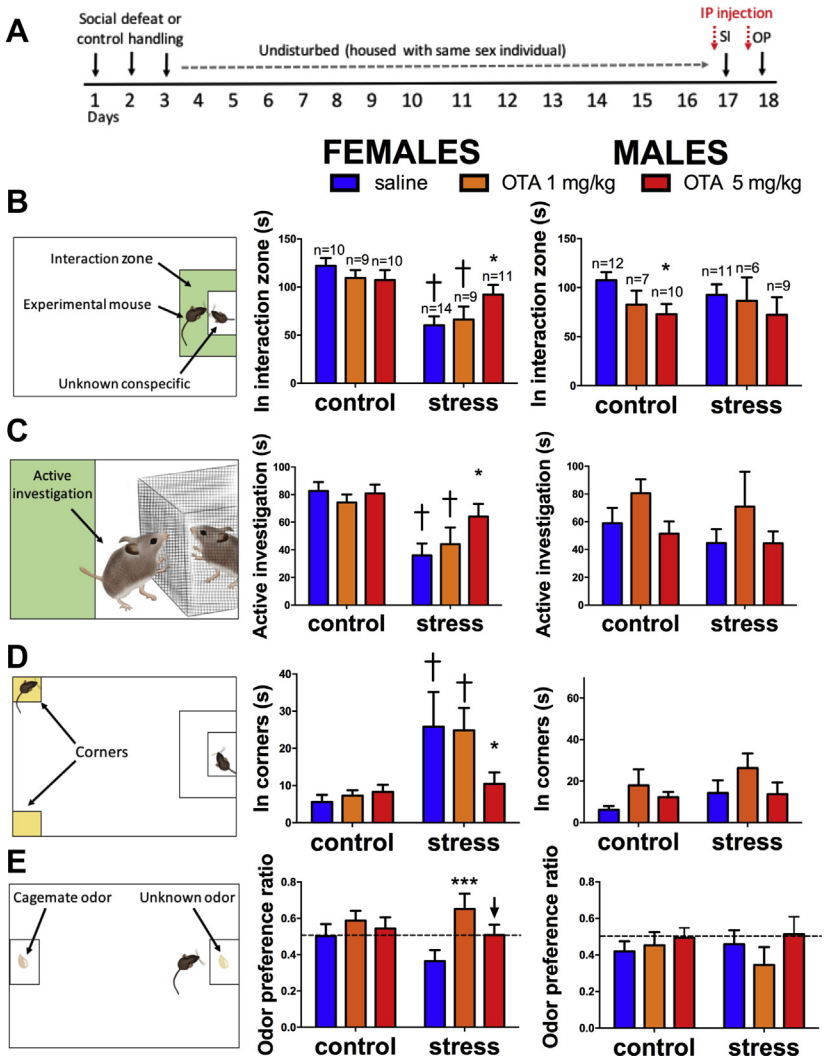

Figure 1. Effects of systemic administration of an oxytocin receptor antagonist (OTA) and social defeat stress on behavior. **(A)** Timeline of experiment. Mean and SEM time spent **(B)** in the interaction zone, **(C)** in active investigation, and **(D)** in corner zones during the social interaction (SI) test. **(E)** Mean and SEM of odor preference (OP) ratio during the OP test. The left column shows diagrams representing behavior measured, the middle column shows female data, and right column shows male data. *$p < .05$, effect of 5 mg/kg OTA vs. saline; †$p < .01$, effect of stress in animals treated with saline or OTA 1 mg/kg; ***$p < .01$, effect of 1 mg/kg OTA vs. saline in stressed animals; ↓$p = .08$, effect of 5 mg/kg OTA vs. saline in stressed animals. IP, intraperitoneal.

assessed postmortem to avoid disrupting behavior (40). There were no main effects of estrous cycle or interaction with stress or treatment (Supplemental Table S2).

RESULTS

Effects of Systemic Administration of OTA on SI Behavior

In female mice, there was evidence suggesting that the effects of systemic OTA treatment on SI behavior were different in control versus stressed female mice (Figure 1B; stress × drug interaction, $p = .06$). Stressed female mice spent less time in the interaction zone than control mice if they were treated with saline ($p < .001$, $d = 2.04$) or 1 mg/kg of OTA ($p < .01$, $d = 1.29$). In contrast, stressed female mice treated with 5 mg/kg of OTA spent significantly more time in the interaction zone than stressed female mice treated with saline ($p < .05$, $d = 1.03$) and were no different from control mice (all treatments, $d = 0.45$). Effects of OTA were stronger on the amount of time female mice actively interacted (by rearing or sniffing) with the cage containing the target mouse (Figure 1C; stress × drug interaction; $p < .001$). Stressed female mice showed reduced interaction with the target mouse if treated with saline ($p < .001$, $d = 1.6$) or 1 mg/kg dose of OTA ($p = .02$, $d = 1.1$), but not with 5 mg/kg dose of OTA ($d = 0.6$). In female mice, stress also significantly increased time spent in corners opposite the interaction zone (Figure 1D; main effect of stress; $p < .001$), but only in female mice receiving saline ($p = .01$, $d = 0.8$) or 1 mg/kg of OTA ($p = .047$, $d = 1.3$), and not 5 mg/kg of OTA ($d = 0.25$). There were no significant differences in any of these variables during the acclimation phase when the target mouse was absent (Supplemental Figure S1A, B) or other variables quantified during manual scoring (Supplemental Figure S2). During the open-field phase (when the empty cage was absent), there were no effects of treatment on time spent in center, but there was a main effect of stress-reducing time spent in center (Supplemental Figure S1C, $p < .001$).

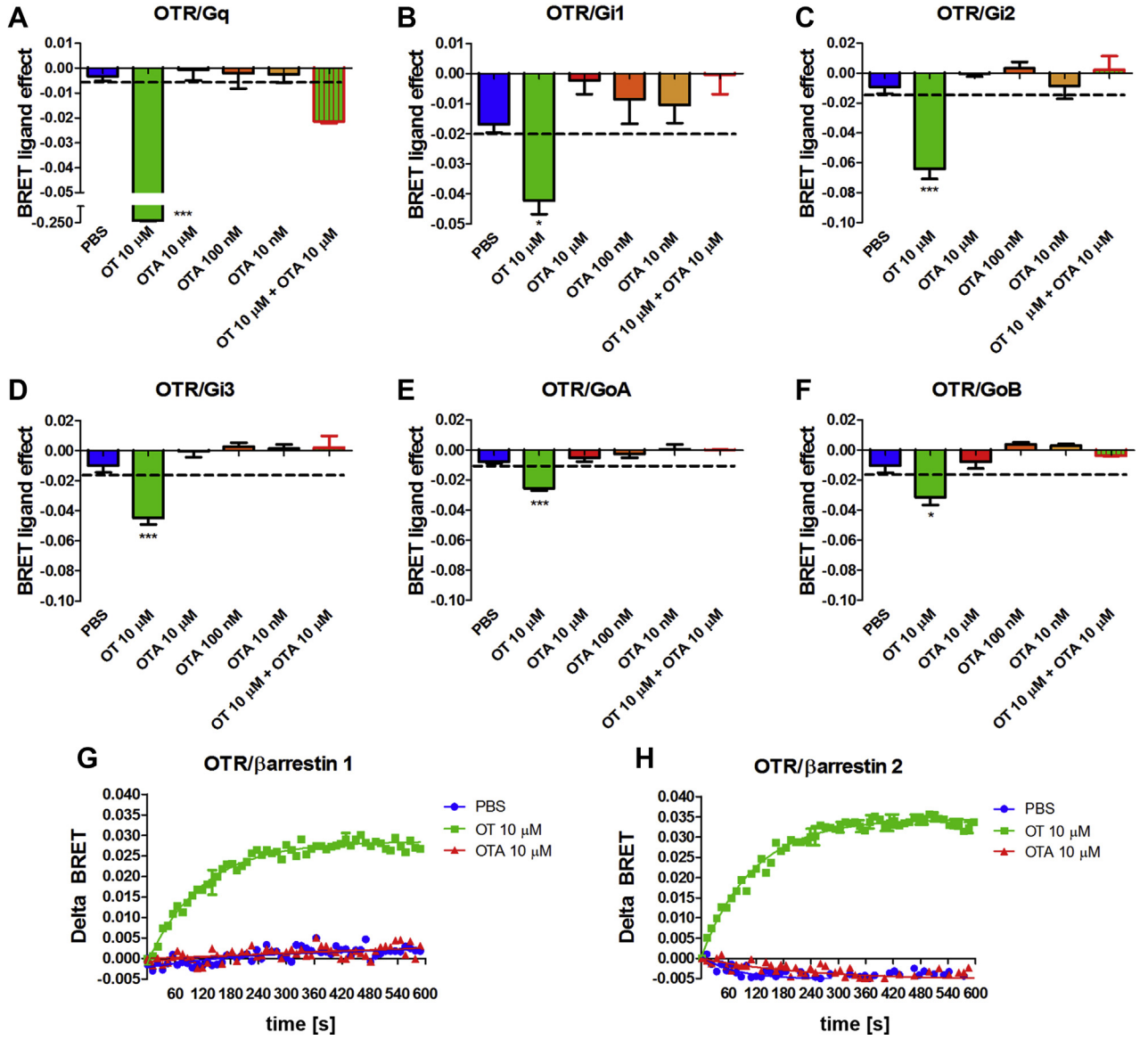

Figure 2. Effects of an oxytocin receptor (OTR) antagonist (OTA) on OTR activation of G proteins and β-arrestins recruitment. Mean and SEM of bioluminescence resonance energy transfer (BRET) ligand effect of OT, phosphate-buffered saline, OT + OTA, and three different concentrations of OTA for **(A)** Gq, **(B)** Gi1, **(C)** Gi2, and **(D)** Gi3; **(E)** GoA and **(F)** GoB activation; and **(G)** β-arrestin1 and **(H)** β-arrestin2 recruitment *$p < .05$, effect of drug treatment vs. phosphate-buffered saline (PBS); ***$p < .001$, effect of drug treatment vs. PBS.

In male mice, social defeat stress did not reduce SI behavior, consistent with previous studies (15,41,42). There was no significant effect of OTA on time spent in the interaction zone in the presence of a target mouse (Figure 1B; main effect of drug; $p = .1$). However, a planned comparison showed that 5 mg/kg of OTA reduced time spent in the interaction zone of control male mice ($p = .02$, $d = 1.25$). No significant differences were observed in stressed male mice. There were no significant differences in time spent in active investigation (Figure 1C) or in corner zones (Figure 1D). There were no effects of stress, treatment, or their interaction on time spent in interaction zone or corners during acclimation phase and time spent in the center of the open field during the open-field phase (Supplemental Figure S1).

Effects of Systemic Administration of OTA on OP Behavior

For female mice there was a main effect of treatment on OP during the interaction phase (Figure 1E; main effect of drug; $p < .001$). In stressed female mice, 1 mg/kg of OTA significantly increased preference for an unfamiliar odor versus cagemate odor compared with saline-treated animals ($p < .001$, $d = 0.94$). Intriguingly, the effect of 5 mg/kg of OTA was less pronounced ($p = .08$, $d = 0.78$). In control female mice,

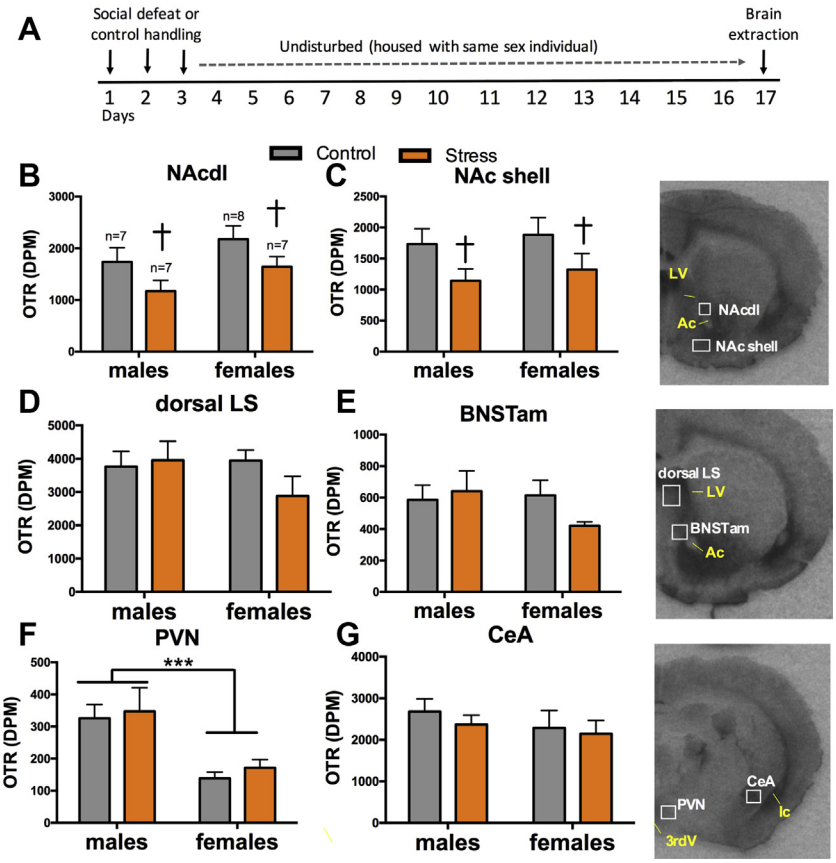

Figure 3. Oxytocin receptor (OTR) binding in naïve and stressed male and female mice. **(A)** Timeline of experiment. Mean and SEM of OTR binding in **(B)** the dorsolateral nucleus accumbens (NAcdl) core, **(C)** NAc shell, **(D)** dorsal lateral septum (LS), **(E)** anteromedial bed nucleus of the stria terminalis (BNSTam), **(F)** paraventricular nucleus (PVN), and **(G)** central nucleus of the amygdala (CeA). †$p < .05$, main effect of stress; ***$p < .01$, main effect of sex. Ac, anterior commissure; DPM, disintegrations per minute; Ic, internal capsule; LV, lateral ventricle, 3rdV, third ventricle.

OTA had no significant effect on preference ratios. Surprisingly, neither control nor stressed male mice showed a preference for any odor, and this was not affected by OTA treatment.

Effects of OTA on OT Activation of G Proteins and β-Arrestins Recruitment

Incubation with OT significantly reduced energy transfer (BRET) between all 6 G proteins analyzed (Figure 2; all $ps < .05$), consistent with previous observations that OT is capable of activating Gq and Gi/o pathways. None of the three concentrations of OTA alone led to activation of G proteins. However, OTA treatment fully prevented G protein activation by OT. Similarly, in cells coexpressing OTR-Renilla-luciferase and β-arrestin1-yellow fluorescent protein, OT increased the BRET ratio, indicating an agonist-induced association between the OTR and β-arrestin1. Similar results were observed using the β-arrestin2-yellow fluorescent protein construct. OTA treatment alone did not induce changes in BRET ratio. Thus, L-368,899 is a full antagonist at all known OTR-dependent second-messenger systems.

Effects of Stress on OTR Expression in Male and Female Mice

In the NAcdl core (Figure 3B; main effect of stress; $p = .03$) and NAc shell (Figure 3C; main effect of stress; $p = .03$), stressed male and female mice had significantly less OTR binding than control mice. In the PVN, female mice had significantly less OTR binding than male mice (Figure 3F; main effect of sex; $p < .001$) and there was no effect of stress. There were no effects of sex or stress in the dorsal lateral septum (Figure 3D), BNSTam (Figure 3E), or central nucleus of the amygdala (Figure 3G) or any other areas investigated (Supplemental Figure S3). In male mice, OTRs in the NAc shell and central nucleus of the amygdala were positively correlated with time spent in the interaction zone (Supplemental Table S3). Time spent in the interaction zone was not correlated with OTRs in female mice.

Effects of Intranasal Administration of OT and Systemic Administration of OTA on EGR1 Immunoreactivity

In the BNSTam there was evidence for sex-specific effects of intranasal OT on EGR1 immunoreactivity (Figure 4B; sex × drug interaction; $p = .08$). Planned comparisons showed that intranasal OT increased the number of EGR1 positive cells in female mice ($p = .03$, $d = 1.09$) but not in male mice ($d = 0.32$). A similar pattern was observed in the NAcdl core (Figure 4D; sex × drug interaction; $p = .06$). Planned comparisons showed that intranasal OT significantly increased EGR1-positive cells in female mice ($p = .03$, $d = 0.93$) but not in male mice ($p = .58$, $d = 0.31$). In the NAc shell, female mice had more EGR1

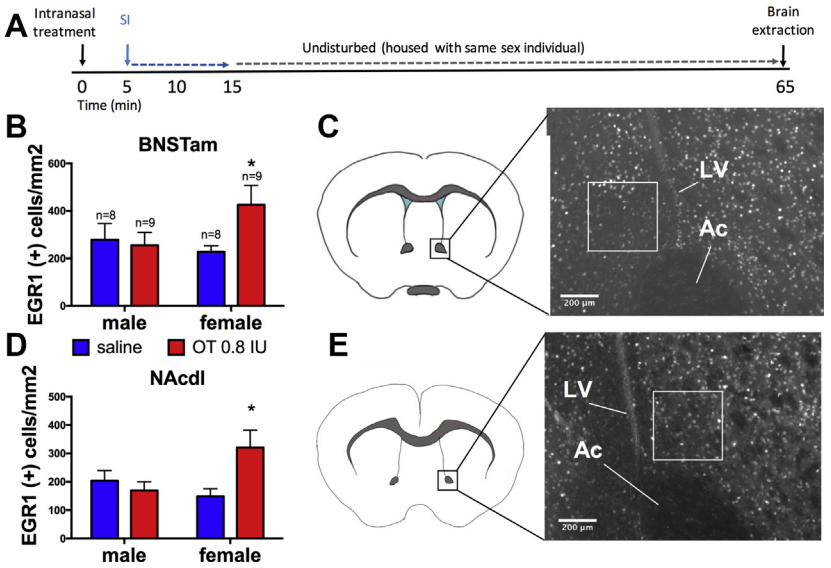

Figure 4. Effects of intranasal administration of oxytocin (OT) on early growth response factor 1 (EGR1) immunoreactivity in naïve male and female mice. **(A)** Timeline of experiment. Mean and SEM of EGR1-positive cells per mm^2 detected in the **(B)** anteromedial bed nucleus of the stria terminalis (BNSTam) and **(D)** dorsolateral nucleus accumbens (NAcdl) core. Diagrams of the **(C)** BNSTam and **(E)** NAc quantification regions with representative photomicrographs and box placement. *$p < .05$, effect of intranasal OT vs. saline. Ac, anterior commissure; LV, lateral ventricle; SI, social interaction.

positive cells than male mice (Supplemental Figure S4; main effect of sex; $p = .03$). There were no effects of stress, treatment, or their interaction on EGR1-positive cells detected in the ventromedial NAc core, lateral septum, or PVN (Supplemental Figure S4). There were no effects of stress or 5 mg/kg OTA treatment on EGR1 expression in the NAcdl core or BNSTam following the OP test (Supplemental Figure S5). However, female mice had more EGR1 positive cells in both the NAcdl core ($F_{1,59} = 47.58$, $p < .01$) and BNSTam ($F_{1,56} = 26.47$, $p < .01$) than male mice.

Effects of OTA Infusion Into BNSTam or NAcdl Core in Stressed Female Mice on SI

The effects of OTA on SI behavior were dependent on the site of injection (Figure 5B; injection site × drug; $p < .001$). Stressed female mice that received infusions of OTA into the BNSTam (Figure 5B, G, I) spent significantly more time in the interaction zone with the target mouse than female mice that received saline ($p < .001$, $d = 1.27$). In contrast, OTA injections into the NAcdl core (Figure 5B, F, H; $d = 0.07$) or misses (Figure 5B, Supplemental Table S4; $d = 0.12$) had no effect on time spent in the interaction zone with the target mouse. No effects of treatment or injection site were detected in time spent in corners during interaction (Figure 5C), time in the interaction zone during acclimation (Figure 5D), or distance traveled during open-field testing (Figure 5E).

Effects of OTA on Risk Assessment Behavior

While performing experiments we noticed that stressed female mice would orient toward the target mouse when outside of the interaction zone (Supplemental Video 1, Figure 6A). Head orientation was recorded by an observer blinded to treatment groups. In the systemic OTA experiment, the effect of OTA on time spent oriented toward the target mouse was different in control and stressed female mice (Figure 6B; stress × drug interaction, $p = .02$). Specifically, for saline-treated female mice, stressed mice spent more time oriented toward the target mouse than control female mice (Figure 6B; $p < .01$). Treatment with 5 mg/kg of OTA but not 1 mg/kg OTA eliminated the stress-induced increase in time spent oriented toward the target mouse (Figure 6B; $p < .01$). No differences in orientation responses were observed in control versus stressed male mice (Figure 6D) or during the acclimation phase when there was no target mouse present in control versus stressed female mice (Figure 6E). In site-specific studies on stressed female mice, only OTA infusion in the BNSTam reduced the amount of time the focal mouse was oriented toward the target (Figure 6C; $p < .05$). As in the systemic OTA experiment, OTA infusions in the BNSTam had no effect on orientation to an empty cage (Figure 6F). These results suggest that OTR activation in the BNST induces a response in which an individual avoids yet attends an unfamiliar social context.

DISCUSSION

Our results show that a single administration of systemic OTA is sufficient to reverse stress-induced deficits in SI behavior. To achieve similar effects with a selective serotonin reuptake inhibitor, 4 weeks of daily treatment was required (24). Local infusion of OTA into the BNSTam, but not into the NAcdl core, mimics the effects of systemic administration, indicating that the BNSTam is a critical site of action for OTR-dependent social withdrawal in female mice. Importantly, our data suggest that the effects of OTA are limited to social contexts. Results from the OP test suggest that defeat induces a preference for familiar social contexts and that OTRs facilitate this preference. Furthermore, analyses of orientation responses suggest that OTRs in the BNSTam promote a vigilance response to unfamiliar social contexts that inhibits social approach. The rapid action of OTA on social behavior suggests that further dissection of OTR-dependent behavioral phenotypes could lead to important insights for novel uses for OTR ligands.

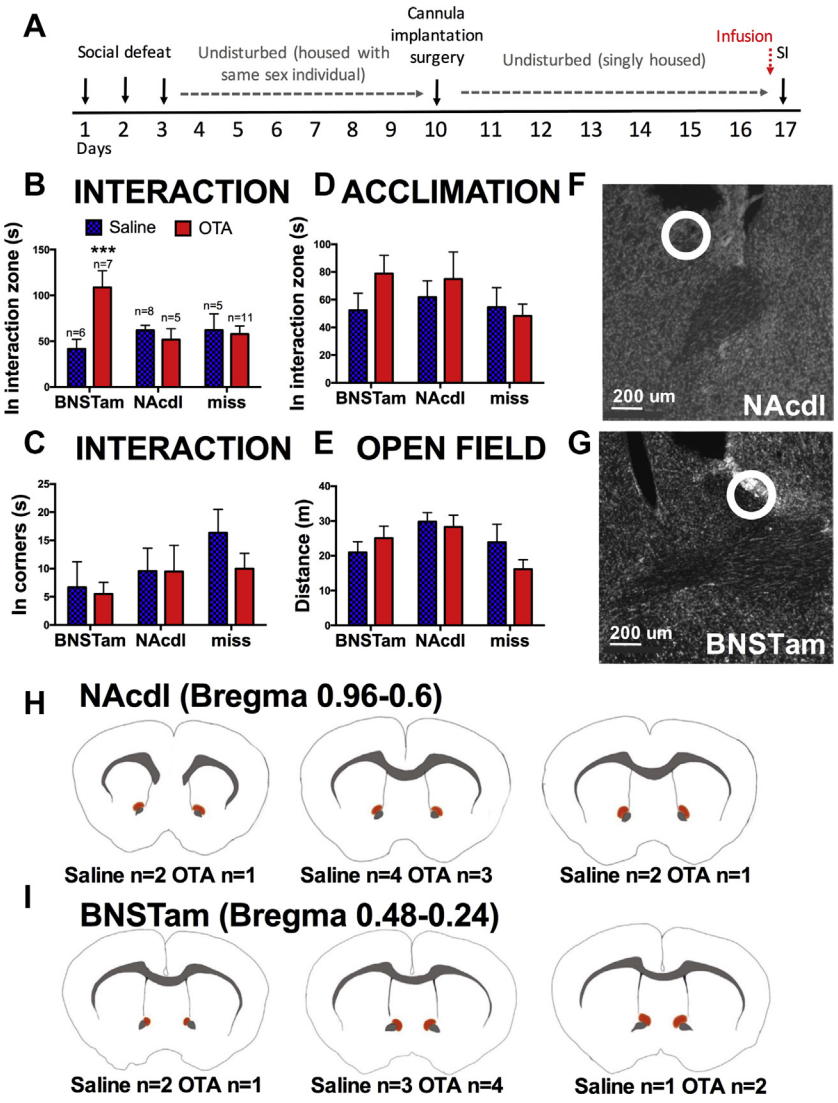

Figure 5. Effects of site-specific administration of an oxytocin receptor antagonist (OTA) on behavior in stressed female mice. **(A)** Timeline of experiment. Mean and SEM time spent **(B)** in the interaction zone and **(C)** in corners during social interaction (SI). There were no differences in time spent **(D)** in the interaction zone during acclimation or **(E)** in locomotor behavior during the open-field phase. Example of hits in Nissl-stained slices in the **(F)** dorsolateral nucleus accumbens (NAcdl) core and **(G)** anteromedial bed nucleus of the stria terminalis (BNSTam), with injection sites indicated by circles. Diagrams showing location of injections considered as hits for **(H)** NAcdl and **(I)** BNSTam. Under each diagram, the number of animals receiving injection in each site is indicated. ***$p < .01$, effect of OTA vs. saline in animals receiving injections in the BNSTam.

Sex-Specific Effects of Systemic Inhibition of OTRs

Our results show that OTR inhibition has opposing effects on social approach in male mice versus female mice in a novel environment. This is consistent with our previous data showing that intranasal OT induces social withdrawal in female mice but not in male mice, and that stress induces hyperactivation of OT neurons in female mice but not in male mice (15). Studies in rodents and humans have consistently shown robust sex-specific effects of OT on social behavior. For example, in rats, intracerebroventricular administration of OT increases social investigation of novel conspecifics in stressed male rats (32), but not in stressed female rats (43). Injections of OT into the lateral septum reduce social play in juvenile female mice but not in juvenile male mice (44). Similarly, in prairie voles, OT administration during development facilitates partner preference behavior in male voles (45) but not in female voles (46). In humans, intranasal OT increases anxiety in women but is anxiolytic in men following a social stress test (47). There are several possible explanations for sex-specific actions of OT.

An intuitive explanation for sex-specific effects of OT could involve sex differences in expression of OT or its cognate receptors including OTRs and vasopressin receptor 1A ($V_{1A}R$), as OT can activate multiple receptor types (48–50). Although sex differences in vasopressin immunoreactivity are well documented, there is little evidence for sex differences in OT immunoreactivity (51). In California mice, few sex differences in OT immunoreactivity are observed, and then only in mice exposed to defeat stress (15). For receptors, male rats have significantly more $V_{1A}R$ binding than female mice in 8 brain regions (52). However, other species report few or no sex differences in $V_{1A}R$ binding (53,54). In a previous study we considered the hypothesis that sex differences in $V_{1A}R$ expression or function contributed to stress-induced social withdrawal (27). There were few sex differences in $V_{1A}R$ binding in California mice and $V_{1A}R$ antagonist infusions into the

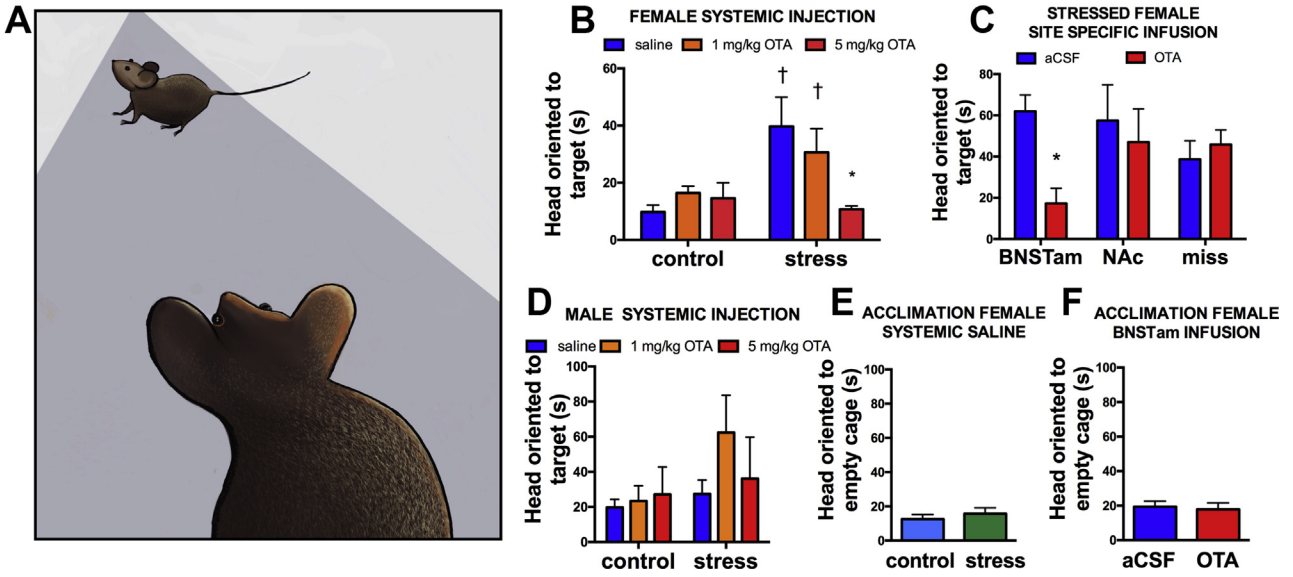

Figure 6. Effects of oxytocin receptor antagonist (OTA) on risk assessment behavior. **(A)** Drawing of focal mouse oriented toward target mouse. Mean and SEM time spent with head oriented toward the target mouse in **(B)** control and stressed female mice receiving systemic injections of saline, 1 mg/kg OTA, or 5 mg/kg OTA. **(C)** Stressed female mice receiving site-specific injections of artificial cerebrospinal fluid (aCSF) or OTA. **(D)** Control and stressed male mice receiving systemic injections of saline, 1 mg/kg OTA, or 5 mg/kg OTA. **(E)** There was no difference between control and stressed female mice during acclimation phase (when the cage was empty). **(F)** Similarly, OTA infusion into the anteromedial bed nucleus of the stria terminalis (BNSTam) had no effect during the acclimation phase. *$p < .05$, main effect of OTA vs. saline or aCSF, †$p = .01$, effect of stress vs. same-drug treatment (aCSF or 1 mg/kg OTA). NAc, nucleus accumbens.

BNST reduced SI in both male and female mice. Also, $V_{1A}R$ antagonist infusions into the NAc had no effects on SI in female mice. In the present study, we investigated whether sex differences or stress-induced changes in OTR expression could account for sex differences in behavioral responses to defeat. There were no sex differences in OTR expression, consistent with previous reports in other species (53,54). When effects of stress were observed, OTR expression was decreased, possibly as a negative feedback response to elevated OT release (55). On balance, it does not appear that sex differences in OT or OTR expression can account for the sex-specific effects of OTRs we report.

An alternative possibility is that there are sex differences in OTR activation of G proteins. OT facilitates OTR coupling to several different G proteins and β-arrestins, which provides multiple degrees of freedom for effects on neural activity (36,37). For example, OT activation of OTRs can inhibit inward rectifier currents of immortalized gonadotropin releasing hormone cells through activation of Gq_{11} whereas the same inward rectifier current can be activated by OT via OTR coupling with Gi/o protein (56). Interestingly, the OTR ligand atosiban, considered to be an OTA, actually activates Gi protein–mediated pathways in vitro (36,38) and in vivo (57). Atosiban mimicked the inhibitory effect of OT on sensory neurons of the spinal cord, providing clear evidence of its unique agonist activity on restricted OTR signaling pathways (57). Using BRET assays we showed that unlike atosiban, L-368,899 blocks OTR activation of Gq, Gi, and β-arrestin by OT. To our knowledge, no study has considered whether sex differences in G protein activation by a single receptor contributes to sex differences in behavioral effects. This would appear to be possible, as sex differences in the cellular trafficking of corticotropin-releasing factor receptors was linked to sex differences in the activity of norepinephrine neurons in the locus coeruleus (58). While biased agonists such as carbetocin (which selectively activates Gq) have been reported to have different behavioral effects than OT (59,60), no study has systematically compared these ligands in male and female mice. Our finding that a broad-spectrum OTA has opposite effects on social behavior in male and female mice suggest that this is a promising direction for further study.

Site-Specific Effects of OTRs on Social Avoidance

We used several approaches to identify a site of action of OTRs. The EGR1 immunoreactivity was effective at identifying nuclei that responded more strongly to intranasal OT in female mice compared with male mice. Intranasal OT reduced SI behavior in female but not in male California mice (15), so we hypothesized that the NAcdl core and/or BNSTam could be sites of OTR action on behavior. A single injection of OTA into the BNSTam, but not into the NAcdl core, increased social approach in stressed female mice. This is interesting because there has been increased focus on the BNST as a key locus contributing to stress-induced psychiatric disorders (61,62).

This sexually dimorphic forebrain structure is involved in the regulation of anxiety (63) and social behaviors including aggression (64,65) and attachment (66). The BNST is a heterogeneous structure containing both excitatory and inhibitory neurons, and it has strong connections with stress response circuits (67), motivational systems (68), and social behavior circuits (69). The BNST is thought to be a key center for integrating information from social and physical environments to

generate avoidance/approach responses (61). Anteromedial portions of the BNST receive inputs from the medial amygdala and posterior BNST and send direct projections to the PVN and central nucleus of the amygdala (70,71). Further study is needed to identify the downstream effects of OTR activation in the BNSTam. Interestingly, social defeat increases levels of brain-derived neurotrophic factor in BNSTam of female but not male California mice (24). Furthermore, infusion of a selective tropomysin receptor kinase B inhibitor into BNSTam had an identical effect as OTA, increasing social approach in stressed female mice. Infusion of tropomysin receptor kinase B inhibitor in unstressed female mice had no effect on behavior, suggesting that the role of the BNSTam in modulating social behavior is more important following a stressful experience. It has been reported that OT can increase brain-derived neurotrophic factor expression in hippocampus (72) and neuroblastoma (73) cells, but otherwise little is known about OT–brain-derived neurotrophic factor interactions.

Stress Induces Vigilance and Avoidance of Unfamiliar Social Contexts

An intriguing finding was that stressed female mice spent more time oriented toward the target mouse, but not an empty cage, when outside of the interaction zone. This response was inhibited by systemic or intra-BNST OTA treatment. Male rats confronted with threatening stimuli such as a predator or predator odor exhibit similar orienting responses (74), which have been described as risk assessment or vigilance behavior (25). It has been proposed that the BNST plays a critical role in "valence surveillance," in which an individual assesses social contexts and assigns a positive or negative valence (61). Similarly, the social salience hypothesis posits that OT enhances the salience of both positive and negative social experiences (75). Our results suggest that these hypotheses converge at the BNSTam, where OTR activation appears to inhibit social approach not by reducing social motivation, but rather by increasing vigilance toward unfamiliar and possibly dangerous social contexts. It has been previously reported that OTRs play a critical role in learning during aversive social contexts (76). Our results expand on this finding by showing that OTRs mediate behavioral responses that occur weeks after an aversive social experience.

Conclusions

The current findings combined with our previous work (15) suggest a model that social defeat induces hyperactivity of BNSTmv and PVN OT neurons, which in turn increase the activation of OTRs in the BNSTam to induce avoidance of unfamiliar social contexts in female mice. The empirical data support the hypothesis that elevated OT may contribute to social vigilance in novel contexts. Interestingly, intranasal OT has been reported to increase perceived social stress (9) and mistrust (77) in humans. Together, this evidence suggests that in unfamiliar contexts, OTAs may have unappreciated therapeutic potential for reducing social anxiety. Further study of OT and OTR-sensitive circuits on the behavioral effects of psychosocial stressors could greatly contribute to the understanding of mechanisms underlying social deficits associated with psychiatric disorders.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by Becas Chile Comisión Nacional de Investigación Científica y Tecnológica (to ND-W), National Institutes of Health (NIH) Grant No. F31 MH095253 (to MQS), a National Science Foundation Graduate Research Fellowships grant (to SAL), and NIH Grant No. R01 MH103322 (to BCT).

We thank D.A. Fox, J. Geng, A. Shackman, C.A. Marler, A.V. Williams, E.C. Wright, and M. Wilson for helpful discussions.

The authors report no biomedical financial interests or potential conflicts of interest.

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Received Jun 7, 2017; revised Aug 16, 2017; accepted Aug 29, 2017.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.biopsych.2017.08.024.

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