Neuropsychopharmacology (2016) 41, 2160–2170

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Moderate Stress-Induced Social Bonding and Oxytocin Signaling are Disrupted by Predator Odor in Male Rats

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In times of stress, social support can serve as a potent buffering mechanism that enhances resilience. In humans, stress can promote protective affiliative interactions and prosocial behavior. Yet, stress also precipitates psychopathologies characterized by social withdrawal such as post-traumatic stress disorder (PTSD) and depression. The factors that drive adaptive *vs* maladaptive social responses to stress are not yet clear. Rodent studies have focused on pair-bonded, opposite-sex mates and suggest that a variety of stressors can induce social support-like behaviors. However, between same-sex conspecifics—particularly males—stress effects on social bonding are less understood and often associated with aggression and social unrest. We thus sought to investigate if a moderate stressor—3 h of acute immobilization—impacts social-support behaviors differently when experienced in a neutral *vs* more innately threatening context (ie, paired with predator odor). We found that moderate stress increased social support-seeking behavior in rat cagemates and facilitated long-term sharing of a limited water resource, decreased aggression, and strongly defined dominance ranks (an indicator of home cage stability). In contrast, experiencing the same stressor in the presence of predator odor eliminated the positive behavioral effects of moderate stress. Importantly, hypothalamic oxytocin (OT) signaling increased coincident with stress in a neutral—but not a predator odor—context. Our results define a novel rodent model of divergent stress effects on social affiliation and OT signaling dependent on odor context with particularly strong relevance to stress-related disorders such as PTSD, which are characterized by a disrupted ability to seek and maintain social bonds.
Neuropsychopharmacology (2016) **41,** 2160–2170; doi:10.1038/npp.2016.16; published online 16 March 2016

INTRODUCTION

Social support can serve as a powerful protective mechanism that promotes stress resilience and positively influences health and life expectancy (House *et al*, 1988; Taylor, 2011). In humans and other highly social species, the presence of a conspecific can dampen the stress response, a phenomenon termed social buffering (Kikusui *et al*, 2006). However, stress is also often associated with asocial patterns of behavior (Sandi and Haller, 2015) and increasing vulnerability to psychopathologies such as post-traumatic stress disorder (PTSD) (American Psychiatric Association, 2013a) and depression (American Psychiatric Association, 2013b) which are characterized by refusal of social support and social withdrawal. Thus, whether a stressor will promote adaptive affiliative behaviors or increased anti-social tendencies remains difficult to predict.

Rodent models have provided often-conflicting views on how stress affects social behavior. In monogamous rodents such as prairie voles (*Microtus ochrogaster*), a variety of stressors drive increased affiliative behaviors with a pair-bonded opposite-sex mate, resulting in dampened stress responses (reviewed in Beery and Kaufer, 2015). Yet in non-pair-bonded or non-monogamous social relationships, stress is often construed as an initiator of social unrest or aggression, such as in resident-intruder (Koolhaas *et al*, 2013) or social defeat paradigms (Blanchard *et al*, 2001). The link between stress and aggression is particularly prominent among male rodents.

Among humans, while men have been shown to exhibit a tend-and-befriend (Taylor *et al*, 2000) response to stress with opposite-sex romantic partners (Ditzen *et al*, 2008; Kirschbaum *et al*, 1995), their stress response among other (non-romantic partner) males has historically been characterized as more aggressive or flight-or-flight. However, recent studies in humans suggest that stress can increase beneficial affiliative interactions between men outside of monogamous relationship pairs (Dawans *et al*, 2012; Takahashi *et al*, 2007; Zucker *et al*, 1968). Understanding how stress affects the seeking and use of social support outside of the narrow scope of monogamous, opposite-sex

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Received 28 August 2015; revised 27 January 2016; accepted 28 January 2016; accepted article preview online 2 February 2016

pairs has great importance to treating stress-associated diseases, which typically occur in much more varied and heterogeneous social contexts.

Rodent models have been used extensively to study stress and its neurobiological underpinnings, often with great relevance to human psychopathology. However, to our knowledge, no rodent model examining how stressor context (a moderate *vs* a more innately threatening stressor) differentially impacts social support behaviors or social dynamics among males exists. We therefore sought to determine whether male rodents of a non-monogamous but social species, rats, show a divergent response in their social affiliative behavior and long-term social bonds after exposure to moderate stress in a neutral odor *vs* predator odor context. Our results define a novel rodent model of divergent stress effects on social function and oxytocin (OT) signaling with strong relevance to stress-related psychiatric disorders like PTSD, which is characterized by a disrupted ability to seek and maintain social bonds.

MATERIALS AND METHODS

For additional details, please see Supplementary Materials and Methods.

Experimental Subjects

Adult 3-month-old male Sprague Dawley rats were pair-housed upon arrival at our facility and maintained on a 12 : 12-h light–dark cycle (lights on at 0700 hours) with *ad libitum* access to food and water unless otherwise noted. All animal care and procedures were approved by the University of California-Berkeley Animal Care and Use Committee.

Acute Immobilization Stress

Rats were restrained in Decapicone bags (Braintree Scientific, Braintree, MA) and cagemates were placed together in a cage inside a fume hood (0900–1200 hours). For the immobilization+fox urine and immobilization+peppermint groups, a cotton ball infused with 1 ml of fox urine or peppermint extract was placed in the cage. Fox urine was used in a separate hood in a separate room from peppermint. After immobilization, cagemates were both returned to their home cage at the same time.

Dark Cycle Home Cage Observations

Huddling, fighting/allogrooming, solitary resting/self-grooming, home cage exploration, and eating/drinking were recorded with a video camera under red light at 1930, 2300 and 0600 hours. Behavior was quantified with a frequency approach similar to that used for maternal care observations of post-parturitent dams (Champagne *et al*, 2003). Examples of some of these behaviors are in Supplementary Movie S1.

Water Access Task

Water bottles were removed at the start of the dark cycle (1900 hours). At the onset of the light cycle rats were marked with washable, nontoxic marker and water was returned. Up to 2 min of water access behavior was recorded by video camera and scored by a blind observer. Examples of water access behavior are in Supplementary Movies S2 and S3.

Tube Dominance Test

The tube test consists of placing two animals at opposite ends of a narrow, plexiglass tube, and allowing the dominant animal to drive its opponent backwards and out of the tube (Supplementary Movie S4). Tube dominance testing was adapted for rats from the original mouse protocol (Lindzey *et al*, 1961).

Elevated Plus Maze

Rats were allowed to explore an elevated plus maze (EPM) for 5 min. Videos were scored by a blind observer for entries into closed and open arms and time in open and closed arms.

Serum Corticosterone Sampling

Rats were lightly anesthetized with isoflurane and rapidly decapitated immediately after the end of immobilization. Trunk blood was collected and centrifuged at 2000 *g* for 15 min. Serum was extracted and stored at − 80 °C until assayed using a Corticosterone EIA kit (Enzo Life Sciences, Farmingdale, NY).

Real-Time Quantitative PCR (qPCR)

After rapid decapitation (immediately following immobilization) brains were collected and frozen at − 80 °C. Hypothalami were dissected from fresh frozen tissue and quantitative PCR (qPCR) was run on Trizol-extracted, DNase-treated (DNA-free, Ambion, Carlsbad, CA) RNA. Primers were designed using NCBI Primer BLAST software. Two-step qPCR was run following the manufacturer's instructions for iScript Reverse Transcription Supermix and Sso Advanced Universal SYBR Green Supermix (BioRad, Hercules, CA) on a BioRad CFX96 real-time PCR machine. Specificity of primer pairs was confirmed using melt curve analysis. Ct values were determined using PCR Miner (Zhao and Fernald, 2005) and normalized to the ribosomal protein L16P (RPLP) reference gene. Fold change in mRNA expression is relative to control (non-stressed) rats. Primer sequences are listed in Supplementary Table S1.

Oxytocin (OT) Protein Quantification

Protein was extracted from fresh frozen hypothalami (collected immediately or 19 h after immobilization) by homogenizing in RIPA buffer plus protease (1 : 100; Calbiochem, Billerica, MA) and phosphatase inhibitors (1 : 10; Roche, Basel, Switzerland). Total protein concentrations were determined using a BCA Kit (Pierce, Waltham, MA). OT protein concentrations were quantified (in duplicate) using an OT ELISA Kit (Enzo Life Sciences).

Statistical Analysis

Home cage behaviors and water access variables during repeated deprivation were analyzed using repeated measures ANOVA with *post hoc* Sidak's or Tukey's multiple

comparisons to compare treatment groups within behavior or timepoint, as appropriate/noted. Two-way ANOVA was used to analyze timed water access behaviors, EPM behaviors, body weight, and cagemate difference in body weight in single-tested rats. The fraction of pairs showing water monopoly *vs* distribution was compared using a χ^2-test. Tube dominance data was analyzed using survival curve analysis (log-rank (Mantel-Cox) test) or by χ^2 testing of resolved *vs* unresolved pairs at the control median cutoff. In both tube and water tests, when three groups were used, an overall χ^2-test was followed by planned pairwise χ^2-tests. Correlation was performed using Pearson correlation coefficient. $P<0.05$ was considered significant in all experiments.

RESULTS

Acute Moderate Stress Increased Huddling in the Home Cage

To investigate affiliative social behavior after stress, we assessed home cage social interactions between adult male rat cagemates after they were both exposed to an acute, moderate stressor—3 h of immobilization. This model was chosen as a moderate stressor because we previously showed that this stressor stimulates hippocampal growth factor expression, adult neural stem cell proliferation and enhanced memory function (Kirby *et al*, 2013), all of which are impaired by more prolonged stressors (Conrad *et al*, 1999; McEwen, 2001; Smith *et al*, 1995). During the dark cycle after stress (~6 h later; Figure 1a), acute immobilization increased cagemate social behaviors, an effect primarily driven by greater huddling (eg, resting or self-grooming while in physical contact with cagemate) with no difference in active social behavior (eg, fighting or allogrooming) (Figure 1b–d and Supplementary Movie S1). Control pairs, in contrast, displayed more resting or grooming alone early in the dark cycle (Figure 1e and f) and more non-social exploration later in the dark cycle (Figure 1g). The frequency of eating and drinking did not differ between groups (Supplementary Figure 1).

Acute Moderate Stress Increased Long-Term Resource Sharing

In primates, enhanced social affiliation is often associated with greater cooperation and resource sharing (De Waal, 1986), behaviors that can significantly impact individual and group fitness (West *et al*, 2007). We therefore adapted a resource competition task—the water access task—to assess resource sharing between cagemates 2, 7 or 14 days after immobilization (Figure 2a and Supplementary Movie S2). Following a 12 h water deprivation, immobilized cagemates spent more time sharing (simultaneously drinking from) a waterspout (Figure 2b), had a decreased latency to the second rat's initial access to the waterspout (Figure 2c), and showed less time with no rat accessing the spout compared with controls (Figure 2d and Supplementary Figure 2a). The difference in water distribution between cagemates was particularly marked when the number of pairs with one rat monopolizing water access was quantified: a monopoly of water access (defined as only one rat of the pair having sole access to the spout) was not observed in any of the immobilized pairs (0/24, 0%), while 6/16 (38%) of control pairs had a monopolizing rat (Figure 2e). No effect of immobilization on body weight (Supplementary Figure 2b) nor pair difference in body weight was found (Supplementary Figure 2c). Immobilization also did not significantly alter anxiety-like behavior or activity in an EPM either 1 or 13 days after stress (Supplementary Figure 3a–c). These findings highlight an overall more efficient strategy for obtaining and sharing water in immobilized pairs of rats compared with control pairs that arose within 2 days of acute stress and lasted up to 2 weeks.

Acute Moderate Stress Reduced Aggression Between Cagemates

We next sought to track dynamic changes in resource sharing strategies within the same cage over time. One week after pair housing, baseline resource sharing behavior was determined using the water access task. The task was then repeated in the same pairs of rats 2, 7 and 14 days after immobilization (Figure 3a). Repeated water deprivation led to increased equality and efficiency in gaining access to water in both groups (Supplementary Figure 4a–e). Consistent with the relatively docile nature of Sprague Dawley rats (Ulrich and Azrin, 1962), aggressive/agonistic behaviors (ie, pushing or shoving cagemate away from the waterspout) were rare in the first water test. With repeated resource deprivation, however, agonistic behaviors nearly tripled in control pairs while remaining low in immobilized pairs (Figure 3b and Supplementary Movie S3). Therefore, while the efficiency in acquiring a limited resource increased regardless of stress exposure, the manner in which cagemates negotiated water access was qualitatively different in control *vs* immobilized pairs.

Acute Moderate Stress Improved the Resolution of the Home Cage Dominance Hierarchy

Aggression and agonistic behavior are common in social species when dominance hierarchies are unsettled (van Kreveld, 1970) and can lead to social disruption and instability which are associated with chronic stress and increased incidence of mental illness in humans (Taylor *et al*, 1997). To assess the saliency of the dominance hierarchy within a cage (ie, sharp distinction in social rank positions between cagemates), we determined the time to dominance resolution over four trials in the tube dominance test 2 weeks after immobilization (Supplementary Movie S4). We found that across all four trials in the tube test, immobilized pairs resolved the task faster than control pairs (Figure 3c and Supplementary Movie S5), and within the first trial alone, all immobilized pairs (6/6) had resolved dominance when only half of the control pairs had (3/6) (Figure 3d). Body weight and cagemate body weight difference did not differ between groups (Supplementary Figure 4f and g). The shorter latency to resolution is not likely due to general changes in activity patterns or anxiety caused by immobilization, as EPM testing revealed no significant difference in anxiety-like behavior (Figure 3e and f) or in general activity levels (Figure 3g) between groups. These findings imply strong and well-defined social rank positions in rats exposed to an acute

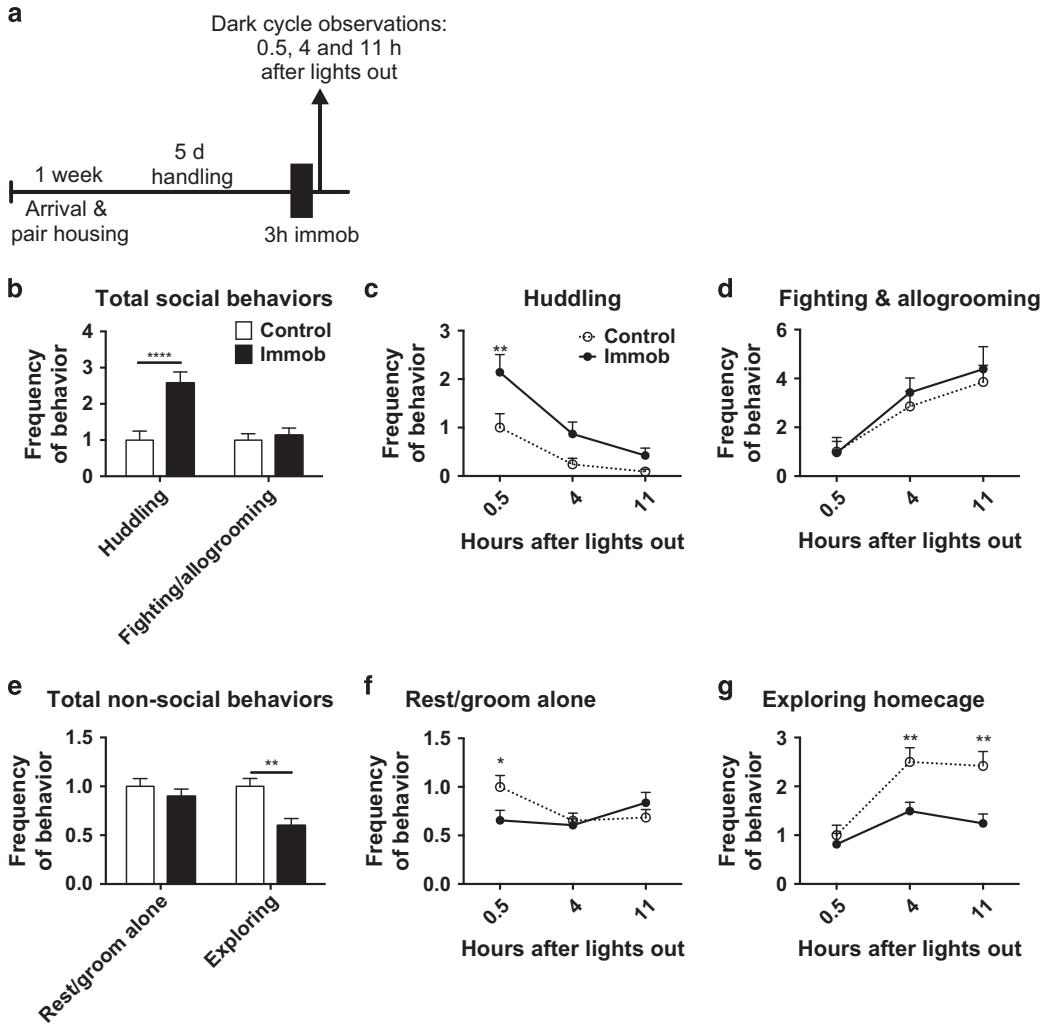

Figure 1 Acute moderate stress increased cagemate social interaction. (a) Timeline. (b) Acute immobilization (black bars) led to an increase in dark cycle cagemate social behaviors (stress effect: $F_{1,33} = 12.70$, $p = 0.0011$), driven primarily by increased huddling (interaction stress × group: $F_{1,33} = 7.219$, $p = 0.0112$) compared with control (open bars). (c) Huddling decreased over time but was consistently more prevalent in immob cagemates (black circles) *vs* controls (open circles) (time effect: $F_{2,66} = 13.30$, $p < 0.0001$; stress effect: $F_{1,33} = 13.66$, $p = 0.0008$). (d) Fighting/allogrooming increased over time in both groups similarly (time effect: $F_{2,66} = 14.88$, $p < 0.0001$; stress effect: not significant, ns). (e) Non-social behaviors were significantly lower in immob pairs (stress effect: $F_{1,33} = 14.61$, $p = 0.0006$). (f) Early in the dark cycle, immob pairs showed significantly less solitary resting/grooming than controls (interaction stress × time: $F_{2,66} = 3.625$, $p = 0.0321$). (g) Immob pairs showed less non-social home cage exploration than controls, particularly later in the dark cycle (time effect: $F_{2,66} = 15.26$, $p < 0.0001$; stress effect: $F_{1,33} = 13.38$, $p = 0.0009$; interaction: $F_{2,66} = 3.115$, $p = 0.0510$). All data are mean ± SEM relative to control or 0.5 h control, as appropriate ($n = 14$ pairs control, $n = 21$ pairs immob). Two-way RM ANOVA with *post hoc* Sidak's comparisons con v. immob: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$. ANOVA, analysis of variance; RM, repeated measures. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

moderate stressor 2 weeks earlier and are consistent with the beneficial nature of dominance hierarchies for reducing aggression (Berdoy *et al*, 1995) and increasing socio-environmental stability (van Kreveld, 1970).

Predator Odor Stress Eliminates the Prosocial Effects of Acute Moderate Stress

To determine whether experiencing the same physical stressor but in a more threatening context would differentially impact cagemate social behavior, we added a predator's odor (fox urine) to acute immobilization (Figure 4a). Exposure to fox urine increases anxiety (Hebb *et al*, 2004) and disrupts memory function (Morrow *et al*, 2000) in rodents and therefore represents a likely model for a more threatening stressor than acute immobilization alone. It is also commonly used to model PTSD-like symptoms in rodents (Janitzky *et al*, 2015) and may therefore represent a good stimulus for rejection of social support.

Similar to immobilization-alone, acute immobilization with a neutral odor (peppermint) increased affiliative social huddling behaviors. Immobilization with fox odor, however, partially negated the beneficial effects of acute moderate stress, dampening huddling compared with rats exposed to immobilization+peppermint (Figure 4b and c and Supplementary Figure 5a–d). The frequency of eating and drinking did not differ between groups (Supplementary Figure 5e).

We next investigated the long-term consequences of experiencing immobilization stress when combined with fox odor exposure. Two weeks after stress, rats were tested in

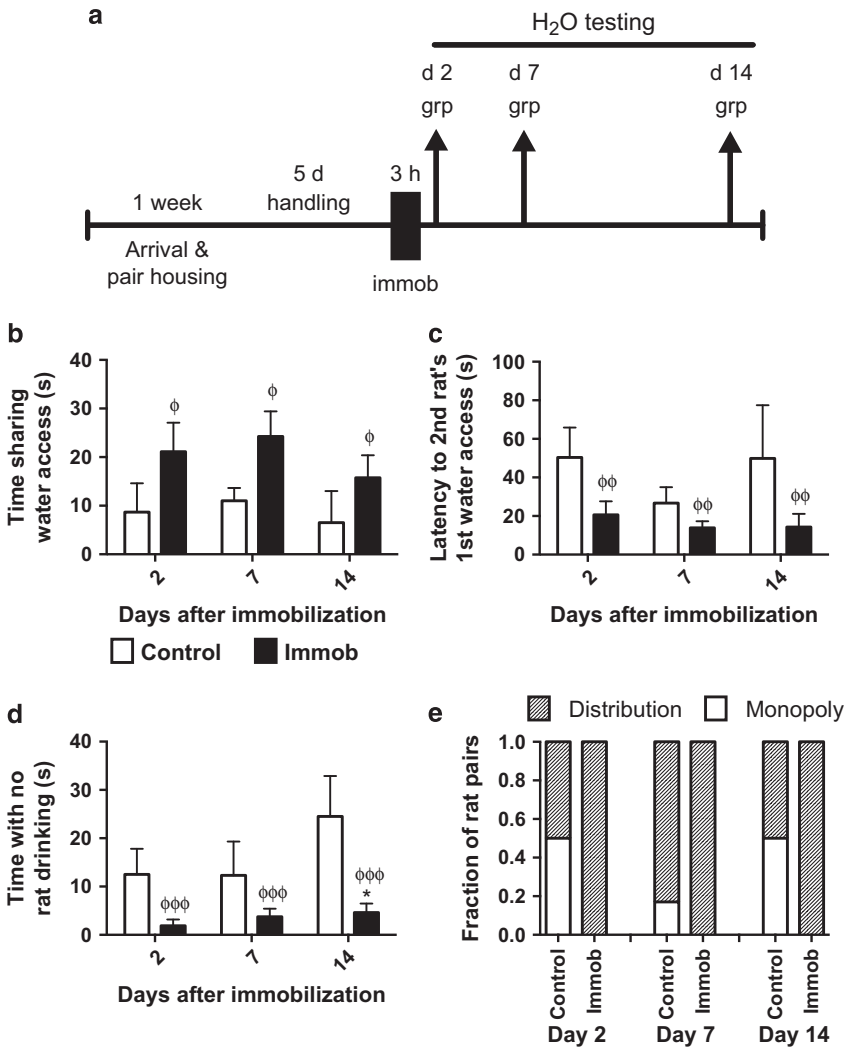

Figure 2 Acute moderate stress led to lasting facilitation of resource sharing. (a) Timeline. Behavior was monitored in the first minute of water access following 12 h of water deprivation. Immob pairs (black bars) spent more time sharing water access (b), had shorter latencies for the second cagemate to access the water (c) and had less time with no rat accessing the water (d) (stress effect: $F_{1,34} = 6.718$, $^{\Phi}p = 0.014$; $F_{1,34} = 8.844$, $^{\Phi\Phi}p = 0.0054$; $F_{1,34} = 15.03$, $^{\Phi\Phi\Phi}p = 0.0005$, respectively) than control pairs (open bars). Data are mean ± SEM Two-way ANOVA. (e) Some rat pairs had only one rat accessing the water (a monopoly, solid color) while others had both rats accessing the water (a distribution, shaded) in the first minute. Significantly more control pairs had a monopolizing rat compared with immob pairs ($\chi^2 = 10.59$, d.f. = 1, $p = 0.0011$). Con: $n = 6$, 6, and 4 pairs, d 2, d 7, and d 14, respectively. Immob: $n = 8$ pairs/d for all reported measures. ANOVA, analysis of variance. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

the water access task to assess resource sharing. Similar to our previous results, immobilized pairs exposed to peppermint odor had no rats (0/6, 0%) monopolizing the waterspout. In contrast, 3/6 pairs (50%) previously exposed to immobilization plus fox odor had a monopolizing rat, similar to controls (4/6 pairs, 67%) (Figure 4d and Supplementary Figure 6a–d). Importantly, waterspout sharing was positively correlated with the amount of huddling exhibited 2 weeks earlier (Figure 4e), suggesting a potentially strong relationship between this affiliative behavior and long-term sharing of resources.

In the tube test, peppermint-immobilized cagemates readily resolved dominance (6/6 pairs resolved, 100%), while pairs exposed to fox urine during immobilization showed resolution dynamics similar to controls (3/6 pairs resolved, 50% for both groups) (Figure 4f and Supplementary Figure 6e). Overall, these findings imply that the addition of fox urine to the stressor prevents the development of affiliative social behavior between cagemates after stress and negates the enhanced sharing of a limited resource and resolution of the dominance hierarchy driven by moderate acute immobilization.

Immobilization-Induced Increase in Hypothalamic Oxytocin Signaling is Prevented by Predator Odor

We next asked what the neurobiological correlates of social affiliation after stress might be. We found similar elevations in serum corticosterone (CORT), the primary rat stress hormone, after immobilization in both the neutral and predator odor exposure conditions (326.0 and 383.0 ng/ml, $p = 0.3614$; Figure 5a and b) suggesting that CORT levels may not drive the differential social responses to these two stressors.

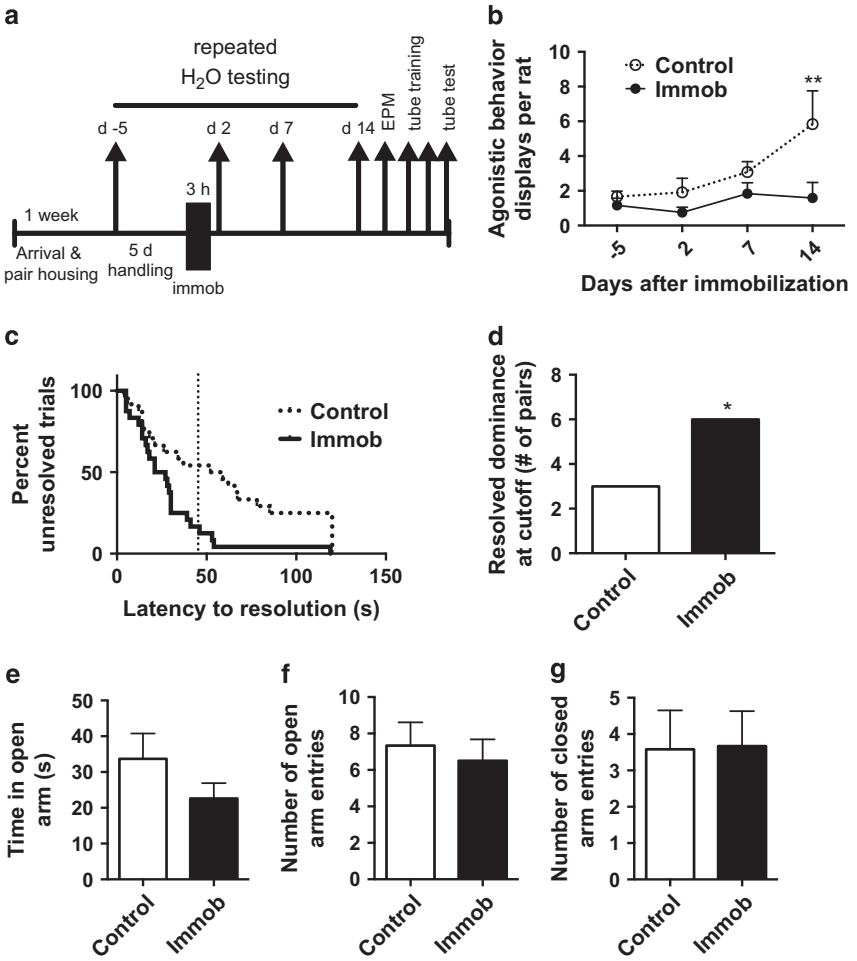

Figure 3 Acute moderate stress reduced aggression and increased cagemate differences in social rank. (a) Timeline. (b) Acute immobilization (black circles) reduced aggression/agonistic behaviors compared with controls (open circles) over repeated water deprivation (stress effect: $F_{1,22} = 5.083$, $p = 0.0344$; time effect: $F_{3,66} = 3.996$, $p = 0.0112$). Data are mean ± SEM Two-way RM ANOVA with *post hoc* Sidak's comparisons within day: $^{**}p < 0.01$). (c) The latency to resolve each trial of the tube test was significantly reduced in immob *vs* con pairs ($p = 0.0021$). Dashed line represents median time to task resolution in control pairs. Log-rank (Mantel-Cox) test. (d) Pairs were categorized as having a median time to resolution (over the four trials) either above or below the control median. All immobilized pairs had resolved dominance at cutoff ($\chi^2 = 4.000$, d.f. = 1, $^{*}p = 0.0455$). Rats were tested on an EPM several hours after the last (d 14) water access test. There was no significant effect of immobilization stress on time in open arms (e) and open arm entries (f). (g) Movement between closed arms (entries) was also not significantly changed by immobilization stress. Data are mean ± SEM Student's *t*-test. $n = 6$ pairs or 12 rats/group, as appropriate. EPM, elevated pulse maze. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

The neuropeptides OT and arginine vasopressin (AVP) are both important mediators of social behaviors in rodents and primates, including humans (Choleris *et al*, 2013). We therefore measured expression of OT and AVP in the hypothalamus, their primary area of expression in the brain. Hypothalamic OT protein levels significantly increased in peppermint-immobilized but not fox urine-immobilized rats immediately after stress (1.875 ng/mg immob+pepp rats compared with 0.6538 ng/mg immob+fox and 0.3905 ng/mg control animals; stress effect: $F_{2,28} = 3.892$, $p = 0.0323$), interaction stress × group: $F_{2,28} = 10.24$, $p = 0.0005$). By 19 h after the end of immobilization, OT levels had returned to baseline (Figure 5c). In addition, OT receptor (OTR) mRNA expression in hypothalamus significantly increased in peppermint-immobilized animals compared with controls (Figure 5d), while OT mRNA expression significantly decreased in fox urine-immobilized rats (Figure 5e). There was no change in AVP or vasopressin 1a receptor (V1aR) expression across any of the groups (Figure 5f and g). All together, these findings imply heightened OT signaling in response to moderate stressor exposure which is diminished by adding a threatening predator odor stimulus. These opposing OT responses in the two different stressor conditions therefore provide an interesting neurobiological correlate of the social consequences of stress and further emphasize the potentially divergent consequences of stress for prosocial behavior and its neural mechanisms.

DISCUSSION

Our results define a novel rodent model of increased social affiliation and OT signaling coupled with long-term enhancement of putative prosocial behavior that can be altered by the context in which the stressor is experienced. We found that moderate acute immobilization increased

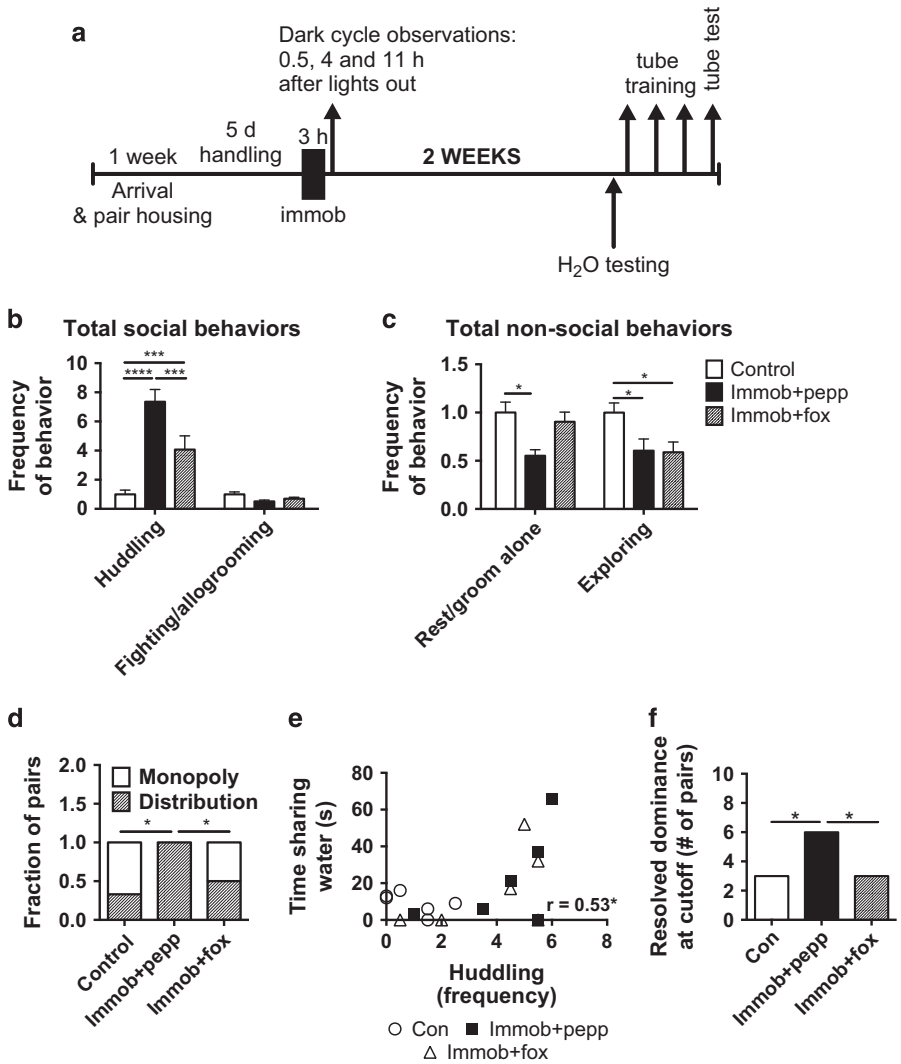

Figure 4 Predator odor stress eliminated the prosocial effects of acute moderate stress. (a) Timeline. (b) Immobilization with peppermint (immob+pepp, black bars) increased huddling compared to controls (open bars), while immobilization with fox urine odor (immob+fox, shaded bars) significantly reduced this increase (group effect: $F_{2,15} = 14.61$, $p = 0.0003$; interaction group x behavior: $F_{2,15} = 21.33$, $p < 0.0001$). (c) Solitary resting/self-grooming was significantly lower in immob+pepp pairs compared with control animals. Both immob+pepp and immob+fox odor pairs had significantly lower non-social exploring compared with controls (group effect: $F_{2,15} = 7.526$, $p = 0.0055$). Data are mean ± SEM relative to control. Two-way RM ANOVA with *post hoc* Sidak's comparisons within behavior between groups: $^{*}p < 0.05$, $^{***}p < 0.001$, $^{****}p < 0.0001$. (d) In the first minute of water access, group significantly altered distribution of waterspout access (overall $\chi^2 = 6.078$, d.f. = 2, $p = 0.0479$). Immob+pepp pairs showed significantly fewer monopolizing rats than both control and immob+fox pairs (*post hoc* planned χ^2 comparisons: immob+pepp v. con: $\chi^2 = 6.000$, d.f. = 1, $p = 0.0143$; immob+pepp v. immob+fox: $\chi^2 = 4.000$, d.f. = 1, $p = 0.0455$; $^{*}p < 0.05$). Immob+fox did not differ from control (immob+fox v. con: $\chi^2 = 0.3429$, d.f. = 1, $p = 0.5582$). Solid color = distribution; shaded = monopoly. (e) Time spent sharing the waterspout (both rats drinking simultaneously) during the first 2 min of water access correlated significantly with huddling frequency in the first half hour of the dark cycle after stress ($r = 0.53$, $^{*}p = 0.0226$). Pearson correlation. (f) Significantly more immob+pepp pairs had resolved dominance at the median control cutoff time than control or immob+fox pairs ($\chi^2 = 4.500$, d.f. = 2, $p = 0.1054$, ns; *post hoc* planned χ^2 comparisons: immob+pepp v. con/immob+fox: $\chi^2 = 4.500$, d.f. = 1, $p = 0.0339$, $^{*}p < 0.05$). $n = 6$ pairs/group for all reported measures. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

social support-seeking behaviors, specifically huddling, between rat cagemates the night after stress. Huddling—also termed adjacent lying or passive social contact—is remarkably similar to contact behavior shown by monogamous voles with their pair-bonded mate and is a well-established social bonding behavior (Carter *et al*, 1986). Rats also increase huddling behaviors after administration of the oxytocinergic stimulant (±)-3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy')—which in humans increases feelings of closeness to others and a desire to interact socially (Dumont and Verkes, 2006; Ramos *et al*, 2013). Such affiliative social contact in rats has been linked to several health benefits, such as increased cancer resistance and reduced early all-cause mortality (Yee *et al*, 2008). Similarly in humans, affiliative physical contact and social support powerfully influence health and life expectancy (House *et al*, 1988; Taylor, 2011). However, there are other potential explanations for huddling behavior aside from social bonding. Notably, a few previous studies have shown a connection between stress and increased fear-stimulated physical contact in rats (Bowen *et al*, 2013; Latané, 1969). However, these studies largely describe defensive aggregation

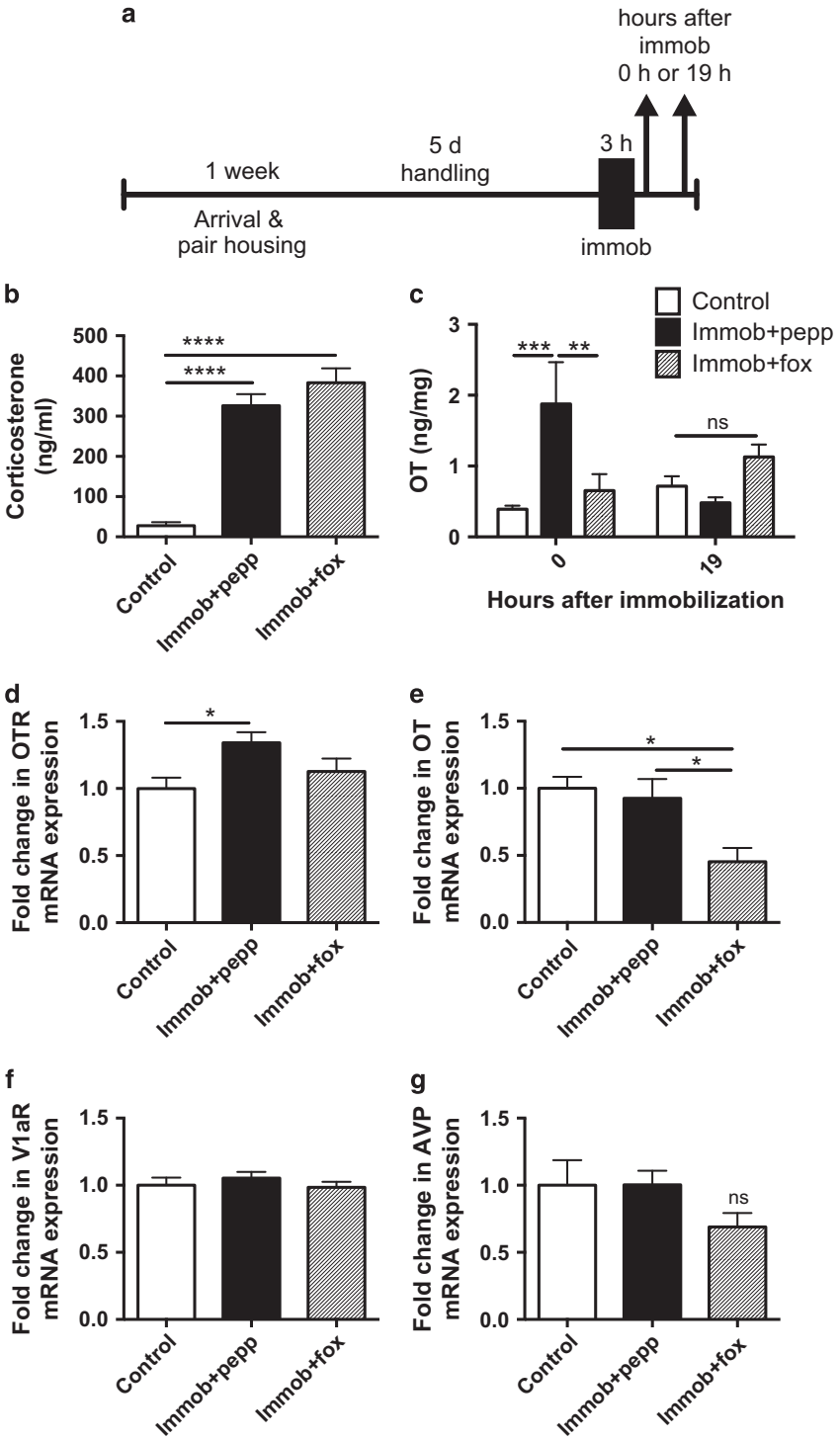

Figure 5 Hypothalamic OT signaling is increased after moderate—but not predator odor—stress. (a) Timeline. (b) Serum CORT increased significantly in both immob groups compared with controls (open bars $F_{2,13} = 54.27$, $p < 0.0001$). Data are mean ± SEM ($n = 4$ immob+pepp, $n = 6$ immob+fox, $n = 6$ control). One-way ANOVA with *post hoc* Tukey's comparisons between groups: ****$p < 0.0001$. (c) OT protein levels in the hypothalamus were significantly greater in immob+pepp rats (black bars) compared with immob+fox (shaded bars) and control animals immediately after the end of stress (stress effect: $F_{2,28} = 3.892$, $p = 0.0323$, interaction stress × group: $F_{2,28} = 10.24$, $p = 0.0005$). OT levels had returned to baseline 19 h after the end of immobilization. Data are mean ± SEM ($n = 4$ immob+pepp, $n = 6$ immob+fox, $n = 6$ control). Two-way ANOVA with *post hoc* Tukey's comparisons within timepoint between groups: **$p < 0.01$, ***$p < 0.001$. (d) OTR mRNA levels increased significantly in immob+pepp animals compared with controls after the end of stress ($F_{2,15} = 3.999$, $p = 0.0406$). (e) OT mRNA levels were significantly decreased in immob+fox animals compared with both immob+pepp and controls ($F_{2,15} = 6.853$, $p = 0.0077$). (f, g) There was no change in vasopressin (AVP) or vasopressin receptor 1a (V1aR) mRNA levels in any of the groups. Data are mean ± SEM relative to control ($n = 6$ for all groups). ANOVA, analysis of variance; AVP, arginine vasopressin; CORT, corticosterone; OT, oxytocin; OTR, OT receptor. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

to a present threat, a behavior that is qualitatively different from the more passive huddling behavior we observed hours after the end of stress. It is also possible that huddling behavior reflects an attempt at thermoregulation or some lingering defensive instincts after stress. Huddling engaged in for seemingly non-social reasons could still stimulate social bonding, however, and to better clarify the nature of cause and effect between social bonding and huddling will require further research.

We found that stressed cagemates exhibited greater long-term cooperative behavior and reduced aggression, as well as increased salience of the home cage dominance hierarchy. Although the findings of increased putatively prosocial behavior coupled with increased dominance salience might seem counterintuitive, they are consistent with the beneficial nature of settled dominance hierarchies in multiple species. Well-defined dominance hierarchies are a sign of social stability which reduces aggression and improves social integration (van Kreveld, 1970). In primates, well-settled dominance hierarchies are characterized by low overt aggression, greater resource sharing and more contact behavior (De Waal, 1986). Stress—resulting from inter-group conflict, for example—has even been reported to serve as an antecedent to greater resource sharing in primates (Carpenter, 1942), including humans (Gavrilets and Fortunato, 2014). In the present studies, we found early benefits in prosocial behavior after stress (huddling the night after stress) on a background of very low levels of aggressive behavior in all groups, suggesting that enhanced prosocial behavior may drive social behavior changes after stress. However, we cannot infer whether an undetected stress-induced decrease in aggression led to increased social behavior and the cause and effect between aggression and prosocial behavior remains an avenue for further investigation. In addition, use of a more aggressive species than the docile Sprague Dawley used in the present studies might reveal different behavioral consequences of stress.

Most previous rodent studies of stress effects on social behavior have used pair-bonded, monogamous rodents to demonstrate a phenomenon called stress buffering, where pair-bonded opposite-sex mates reduce their response to stress via prosocial, affiliative behaviors such as huddling (Smith and Wang, 2014) (reviewed in (Beery and Kaufer, 2015)). Outside of monogamously bonded adult rodents, developmental studies of human and nonhuman primates have provided evidence for both impaired social functioning and enhanced resilience after exposure to early life stress (Dettmer and Suomi, 2014; Gunnar, 2000; Parker *et al*, 2006). How the social behaviors of same-sex adults change after stress is less well understood.

One prominent model of non-monogamous adult social stress response, known as tend-and-befriend (Taylor *et al*, 2000), proposes that females seek social support and strengthen social ties to recover from a stressor. This model is intended to contrast with the inherently more aggressive and anti-social (ie, fight-or-flight) response of males. However, relatively few studies of the effects of different stressors on social dynamics among adult male rodents exist and of those that do, most center on short-term effects following prolonged isolation and interactions with unfamiliar conspecifics (Nosjean *et al*, 2014). Our results indicate that familiar males can show increased huddling behavior with each other after stress, a potential sign of greater affiliation and social bonding, accompanied by a long-term reduction in aggression when competing for limited resources. How the social strategy after stress might differ if rats were unfamiliar with each other before stress or if only one rat in a dyad were stressed remain questions for future study.

Our findings suggest a different view of stress compared with stress buffering models. Buffering models construe stress as a negative experience to be 'forgotten' via use of social support, a kind of sink for negative experiences or emotions. Our data, in contrast, suggest that changes in social behavior after a stressor could result in long-term changes in the social environment. We propose that in the case of a moderate stressor, stress may lead to behaviors suggestive of stress bonding. Within the frame of this 'stress bonding' model, our findings provide a possible explanation for how a brief stressor might induce a long-term benefit to cognitive health and increase stress resilience, ie, by changing the day-to-day life of conspecifics.

We found that the social effects of moderate stress were eliminated when the stressor was experienced in a more innately threatening context (ie, in the presence of fox odor). Fox odor is a natural predator odor that induces a strong stress response in rodents and is frequently used as a model of PTSD (Janitzky *et al*, 2015). Most notably, fox odor exposure induces memory impairments weeks after the stress (Morrow *et al*, 2000), in contrast to our moderate stressor, which we have shown to enhance contextual memory in a similar timescale (Kirby *et al*, 2013). It has been recently hypothesized that disruption of contextual memory is the key feature of PTSD pathology (Desmedt *et al*, 2015) and that an urgent need exists for distinguishing between adaptive and maladaptive stress responses. Although reduced social behavior in the context of an animal being preyed upon in the wild may be an adaptive response, in domesticated species like humans, an anti-social response after trauma is likely maladaptive because the individual would not benefit from social support. A breakdown of stress-induced enhancement of social support could be strongly relevant to the maladaptive stress response in neuropsychiatric disorders like PTSD, where social withdrawal and a lack of social support are defining risk factors (Brewin *et al*, 2000).

Despite divergent social responses to stress in the moderate and more threatening stressor contexts, we found no difference in stress hormone (CORT) responses to the two stressors. Our results are in agreement with studies showing that similar CORT elevations can drive either positive and negative outcomes (eg, in cognitive function and performance) based on the psychological context in which the stress is experienced (reviewed in (Kim *et al*, 2015)).

As a potential neurobiological correlate of stress-context driven behavioral differences, we found strong parallels between the social and oxytocinergic system response to the different stressors. Overall, OT and OTR levels reflected enhanced oxytocingeric tone after moderate immobilization stress and this effect was negated by adding predator odor to the context. Oxytocinergic signaling strongly drives social affiliation and attachment/pair bonding in rodents (prairie voles) (Aragona and Wang, 2004), and interpersonal trust, empathy, and prosocial behavior in humans (Choleris *et al*,

2013). It therefore seems likely that the observed increase in OT may modulate the moderate stress-induced increase in affiliation between cagemates, lending support to our 'stress bonding' model. The location and causative nature of hypothalamic OT action in this model remains undetermined, however. Hypothalamic OTergic fibers project to many distinct brain regions (medial prefrontal cortex, piriform cortex, basolateral and basomedial amygdala, and hippocampus (Choleris *et al*, 2013; Štefánik *et al*, 2015)) involved in the regulation of social behavior. Given the low central availability of peripherally administered OTR antagonists (Viero *et al*, 2010), future experiments requiring local OT infusion into these diverse brain regions will address this question.

Accounts of bonding and increased displays of prosocial behavior following stressful experiences such as combat or natural disasters abound in human societies. It is tempting to consider that built into the stress response is a concomitant activation of a mechanism to seek out and give social support and increase stress resilience. We show here a potent modulation of social dynamics after acute moderate stress in male rat pairs that implies such a reciprocal social support arrangement. Our results further define a novel rodent model of how a moderate, cognition-enhancing stressor contrasts with a PTSD-relevant stressor to drive differential social behavior responses and neurohormonal mediators of social bonding. Future work will address whether the rise in OT drives the behavioral changes, as well as, investigate mechanisms underpinning the differential OT response and its modulation of the observed divergent social responses to stress.

FUNDING AND DISCLOSURE

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

SEM and EDK designed and performed experiments, analyzed data, and wrote the manuscript. KLPL performed experiments. DK designed experiments and wrote the manuscript. We thank IB Bartal, K Taravosh-Lahn, and AK Beery for helpful comments on the manuscript and DK Hernandez, MC Goff and JM Castellano for technical assistance. This work was supported by the NIMH BRAINS innovator award to DK.

REFERENCES

American Psychiatric Association. Depressive Disorders. In: Diagnostic and statistical manual of mental disorders. 5th edn, 2013a, http://dx.doi.org/10.1176/appi.books.9780890425596.dsm04.

American Psychiatric Association. Trauma- and Stressor-Related Disorders. In: Diagnostic and statistical manual of mental disorders. 5th edn, 2013b, http://dx.doi.org/10.1176/appi.books.9780890425596.dsm07.

Aragona BJ, Wang Z (2004). The prairie vole (*Microtus ochrogaster*): an animal model for behavioral neuroendocrine research on pair bonding. *ILAR J* **45**: 35–45.

Beery AK, Kaufer D (2015). Stress, social behavior, and resilience: Insights from rodents. *Neurobiol Stress* **1**: 116–127.

Berdoy M, Smith P, Macdonald DW (1995). Stability of social status in wild rats: age and the role of settled dominance. *Behaviour* **132**: 193–212.

Blanchard RJ, McKittrick CR, Blanchard DC (2001). Animal models of social stress: effects on behavior and brain neurochemical systems. *Physiol Behav* **73**: 261–271.

Bowen MT, Kevin RC, May M, Staples LG, Hunt GE, McGregor IS (2013). Defensive aggregation (huddling) in *Rattus norvegicus* toward predator odor: individual differences, social buffering effects and neural correlates. *PLoS One* **8**: e68483.

Brewin CR, Andrews B, Valentine JD (2000). Meta-analysis of risk factors for posttraumatic stress disorder in trauma-exposed adults. *J Consult Clin Psychol* **68**: 748–766.

Carpenter CR (1942). Societies of monkeys and apes. *Biol Symp* **8**: 177–204.

Carter CS, Getz LL, Cohen-Parsons M (1986). Relationships between social organization and behavioral endocrinology in a monogamous mammal. *Adv Study Behav* **16**: 109–145.

Champagne FA, Francis DD, Mar A, Meaney MJ (2003). Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiol Behav* **79**: 359–371.

Choleris E, Pfaff PW, Kavaliers M (2013). *Oxytocin, Vasopressin and Related Peptides in the Regulation of Behavior*. 1st edn. Cambridge University Press: Cambridge, UK.

Conrad CD, Lupien SJ, McEwen BS (1999). Support for a bimodal role for type II adrenal steroid receptors in spatial memory. *Neurobiol Learn Mem* **72**: 39–46.

Dawans von B, Fischbacher U, Kirschbaum C, Fehr E, Heinrichs M (2012). The social dimension of stress reactivity: acute stress increases prosocial behavior in humans. *Psychol Sci* **23**: 651–660.

De Waal FB (1986). The integration of dominance and social bonding in primates. *Q Rev Biol* **61**: 459–479.

Desmedt A, Marighetto A, Piazza P-V (2015). Abnormal fear memory as a model for PTSD. *Biol Psychiatry* **78**: 290–297.

Dettmer AM, Suomi SJ (2014). Nonhuman primate models of neuropsychiatric disorders: influences of early rearing, genetics, and epigenetics. *ILAR J* **55**: 361–370.

Ditzen B, Schmidt S, Strauss B, Nater UM, Ehlert U, Heinrichs M (2008). Adult attachment and social support interact to reduce psychological but not cortisol responses to stress. *J Psychosom Res* **64**: 479–486.

Dumont GJH, Verkes RJ (2006). A review of acute effects of 3,4-methylenedioxymethamphetamine in healthy volunteers. *J Psychopharmacol* **20**: 176–187.

Gavrilets S, Fortunato L (2014). A solution to the collective action problem in between-group conflict with within-group inequality. *Nat Commun* **5**: 3526.

Gunnar MR. Early adversity and the development of stress reactivity and regulation. In: Nelson CA (ed.). *The Effects of Early Adversity on Neurobehavioral Development Minnesota Symposia on Child Psychology (Series) v. 31*. Mahwah: New Jersey, 2000, pp 163–200.

Hebb ALO, Zacharko RM, Gauthier M, Trudel F, Laforest S, Drolet G (2004). Brief exposure to predator odor and resultant anxiety enhances mesocorticolimbic activity and enkephalin expression in CD-1 mice. *Eur J Neurosci* **20**: 2415–2429.

House J, Landis K, Umberson D (1988). Social relationships and health. *Science* **241**: 540–545.

Janitzky K, D'Hanis W, Kröber A, Schwegler H (2015). TMT predator odor activated neural circuit in C57BL/6J mice indicates TMT-stress as a suitable model for uncontrollable intense stress. *Brain Res* **1599**: 1–8.

Kikusui T, Winslow JT, Mori Y (2006). Social buffering: relief from stress and anxiety. *Philos Trans R Soc Lond B Biol Sci* **361**: 2215–2228.

Kim EJ, Pellman B, Kim JJ (2015). Stress effects on the hippocampus: a critical review. *Learn Mem* **22**: 411–416.

Kirby ED, Muroy SE, Sun WG, Covarrubias D, Leong MJ, Barchas LA *et al* (2013). Acute stress enhances adult rat hippocampal neurogenesis and activation of newborn neurons via secreted astrocytic FGF2. *Elife* **2**: e00362.

Kirschbaum C, Klauer T, Filipp S-H, Hellhammer DH (1995). Sex-specific effects of social support on cortisol and subjective responses to acute psychological stress. *Psychosom Med* **57**: 23.

Koolhaas JM, Coppens CM, de Boer SF, Buwalda B, Meerlo P, Timmermans PJA (2013). The resident-intruder paradigm: a standardized test for aggression, violence and social stress. *J Vis Exp* e4367.

Latané B (1969). Gregariousness and fear in laboratory rats. *J Exp Social Psychol* **5**: 61–69.

Lindzey G, Winston H, Manosevitz M (1961). Social dominance in inbred mouse strains. *Nature* **191**: 474–476.

McEwen BS (2001). Plasticity of the hippocampus: adaptation to chronic stress and allostatic load. *Ann N Y Acad Sci* **933**: 265–277.

Morrow BA, Roth RH, Elsworth JD (2000). TMT, a predator odor, elevates mesoprefrontal dopamine metabolic activity and disrupts short-term working memory in the rat. *Brain Res Bull* **52**: 519–523.

Nosjean A, Cressant A, de Chaumont F, Olivo-Marin J-C, Chauveau F, Granon S (2014). Acute stress in adulthood impoverishes social choices and triggers aggressiveness in preclinical models. *Front Behav Neurosci* **8**: 447.

Parker KJ, Buckmaster CL, Sundlass K, Schatzberg AF, Lyons DM (2006). Maternal mediation, stress inoculation, and the development of neuroendocrine stress resistance in primates. *Proc Natl Acad Sci USA* **103**: 3000–3005.

Ramos L, Hicks C, Kevin R, Caminer A, Narlawar R, Kassiou M *et al* (2013). Acute prosocial effects of oxytocin and vasopressin when given alone or in combination with 3, 4-methylenedioxymethamphetamine in rats: involvement of the V1A receptor. *Neuropsychopharmacology* **38**: 2249–2259.

Sandi C, Haller J (2015). Stress and the social brain: behavioural effects and neurobiological mechanisms. *Nat Rev Neurosci* **16**: 290–304.

Smith AS, Wang Z (2014). Hypothalamic oxytocin mediates social buffering of the stress response. *Biol Psychiatry* **76**: 281–288.

Smith MA, Makino S, Kvetnansky R, Post RM (1995). Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J Neurosci* **15**: 1768–1777.

Štefánik P, Olexová L, Kršková L (2015). Increased sociability and gene expression of oxytocin and its receptor in the brains of rats affected prenatally by valproic acid. *Pharmacol Biochem Behav* **131**: 42–50.

Takahashi T, Ikeda K, Hasegawa T (2007). Social evaluation-induced amylase elevation and economic decision-making in the dictator game in humans. *Neuro Endocrinol Lett* **28**: 662–665.

Taylor SE (2011). Social support: a review. *The Oxford Handbook of Health Psychology* 189–214.

Taylor SE, Klein LC, Lewis BP, Gruenewald TL, Gurung RA, Updegraff JA (2000). Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychol Rev* **107**: 411–429.

Taylor SE, Repetti RL, Seeman T (1997). Health psychology: what is an unhealthy environment and how does it get under the skin? *Annu Rev Psychol* **48**: 411–447.

Ulrich RE, Azrin NH (1962). Reflexive fighting In response to aversive stimulation1. *J Exp Anal Behav* **5**: 511–520.

van Kreveld D (1970). A selective review of dominance-subordination relations in animals. *Genet Psychol Monogr* **81**: 143–173.

Viero C, Shibuya I, Kitamura N, Verkhratsky A, Fujihara H, Katoh A *et al* (2010). Oxytocin: crossing the bridge between basic science and pharmacotherapy. *CNS Neurosci Ther* **16**: e138–e156.

West SA, Griffin AS, Gardner A (2007). Evolutionary explanations for cooperation. *Curr Biol* **17**: R661–R672.

Yee JR, Cavigelli SA, Delgado B, McClintock MK (2008). Reciprocal affiliation among adolescent rats during a mild group stressor predicts mammary tumors and lifespan. *Psychosom Med* **70**: 1050–1059.

Zhao S, Fernald RD (2005). Comprehensive Algorithm for Quantitative Real-Time Polymerase Chain Reaction. Available at http://dx.doi.org/10.1089/cmb.2005.12.1047 12: 1047–1064.

Zucker RA, Manosevitz M, Lanyon RI (1968). Birth order, anxiety, and affiliation during a crisis. *J Pers Soc Psychol* **8**: 354–359.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)