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A Mother's Past Can Predict Her Offspring's Future: Previous Maternal Separation Leads to the Early Emergence of Adult-Like Fear Behavior in Subsequent Male Infant Rat Offspring

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Recent evidence has shown that pups exposed to maternal separation exhibit profound changes in their emotional development, for example, early emergence of adult-like fear retention and fear inhibition (Callaghan & Richardson, 2011; Callaghan & Richardson, 2012). Numerous studies have shown that maternal separation is also a significant stressor for the mother. However, no studies have examined how a mother's prior parenting experience affects emotion development of pups in her subsequent litters. In this study female rats were bred and were then separated from their pups (maternal separation, MS) or remained with their pups (standard rearing, SR). After those pups were weaned, females were bred again with all pups from the subsequent litters being standard reared. Hence, these subsequent litter pups had mothers that were either previously separated (MS_{SUB}) or not (SR_{SUB}) from their prior litter. Those pups underwent fear conditioning at postnatal Day 17 and tested for fear retention, or had their fear extinguished and then tested for the renewal effect. The results show that the MS_{SUB} infants respond similarly to infants that had been directly exposed to MS. That is, the MS_{SUB} infants exhibited better retention of fear and more relapse after extinction compared with SR_{SUB} infants. Further experiments demonstrated that MS_{SUB} rats were not more anxious than SR_{SUB} infants. Taken together, these experiments are the first to demonstrate that infant offspring exhibit atypical emotional development of fear conditioning (but not anxiety) as a consequence of their mother's prior exposure to stress.

Keywords: maternal-separation, stress, development, maternal transmission, fear

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It has been well established in humans as well as nonhuman animals that exposure to early life stress can significantly impact later functioning (Lupien, McEwen, Gunnar, & Heim, 2009; Sanchez, Ladd, & Plotsky, 2001), with one of the most potent stressors involving disruption of the mother-infant relationship. The disruption of this relationship has been modeled extensively using the maternal separation procedure. Adult animals that were maternally separated as infants exhibit a range of atypical phenotypes, including dysregulated stress responsiveness, depression-like behavior, anxiety-like behavior, and greater responsiveness to drugs of abuse compared with standard-reared animals (Aisa, Tordera, Lasheras, Del Río, & Ramírez, 2007; Daniels, Pietersen, Carstens, & Stein, 2004; Matthews, Robbins, Everitt, & Caine, 1999; Michaels & Holtzman, 2008; Moffett et al., 2006). These studies in adults demonstrate that maternal separation has a profound and long-lasting impact. Recently, the effects of maternal separation have been documented early in life. Specifically, maternally separated infant rats exhibit atypical fear regulation (Callaghan & Richardson, 2011; Callaghan & Richardson, 2012). Following Pavlovian fear conditioning, standard-reared rats trained in infancy tend to forget rapidly, and are less likely to exhibit relapse if this fear is extinguished, compared with rats trained at older ages (Kim, McNally, & Richardson, 2006; Kim & Richardson, 2007, 2010; Yap & Richardson, 2007). However, maternal separation was found to cause a shift in this normal pattern of fear regulation such that maternally separated infants retained fear memories for longer (Callaghan & Richardson, 2012), and were more likely to relapse after fear extinction (Callaghan & Richardson, 2011). These re-

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sults demonstrate that chronic disruption of the mother–infant relationship accelerates the developmental transition between the infant fear regulation system (infantile amnesia and relapse-resistant extinction) and the adult fear regulation system (enhanced retention and relapse-prone extinction). Taken together, this work shows that early life stress has a substantial impact on behaviors related to psychopathology in the directly exposed offspring, and that these effects emerge early in life and are long-lasting.

There is substantial evidence demonstrating that the impact of stress can persist beyond directly exposed individuals, affecting later generations. There are two ways the transmission of stress can be studied in rodents. The most frequently used model to date is a "vertical transmission" model, which involves exposing an animal to a stressor at one time point and studying the impact of that stress across multiple generations (i.e., the offspring, grandoffspring, and great-grandoffspring (Skinner, 2008; Skinner, Manikkam, & Guerrero-Bosagna, 2010). For example, it has been shown that exposing an adult female rat to toxic compounds during gestation results in increased physical disease risk and toxic stress responses in three subsequent generations (Crews et al., 2012). Multigenerational transmission of stress phenotypes has also been observed after exposure to maternal separation, with sex-specific depressive- and anxiety-like behavioral alterations observed across three generations (Franklin et al., 2010). Such research demonstrates that ancestral exposure to certain environmental factors can modify the phenotypes of multiple generations of descendants. In addition to this vertical transmission of stress across multiple generations, stress can also be transmitted within generations. This can be modeled by breeding rodent mothers multiple times after a period of stress to examine the persistent effects in caregivers that are passed on to subsequent offspring (so-called "horizontal transmission"). This mode of transmission has received less attention from nonhuman researchers, but may be an important pathway through which stress is transmitted between directly exposed and nonexposed individuals. This is evident from human generational studies, where offspring of parents who previously experienced trauma show atypical development. For example, the offspring of Holocaust survivors conceived after their parents had escaped the war, or after the war had ended, have inflated rates of various psychopathologies compared with Jewish individuals who did not have a parent who experienced the Holocaust (Yehuda, Bell, Bierer, & Schmeidler, 2008). Human generational research could be aided by the use of animal models, circumventing the ethical and logistical issues typically associated with studying humans. For example, an early study by Champagne and Meaney (2006) that examined horizontal transmission involved exposing pregnant rat dams to repeated prenatal restraint stress, and then breeding those dams a subsequent time. No further restraint stress occurred. The results revealed that adults exposed to gestational stress had heightened levels of anxiety-like behavior on the open-field test compared with standard-reared adults; importantly, the same heightened levels of anxiety-like behavior were observed in the adult offspring from the subsequent litter (i.e., a litter that was not directly exposed to the stress experience). Similar to findings in humans, this study demonstrates that atypical patterns of development can occur in offspring even when stress-exposure occurred only in the caregivers' past.

In the present study we aimed to further explore the transmission of stress within generations, and extend the results reported by Champagne and Meaney (2006). We aimed to extend their results in three ways. First, we examined whether the effects of prior maternal stress are also observed early in life (i.e., in infancy). Research on the effects of early life stress tends to focus on end-state function (i.e., studying adult animals), but given that many psychopathologies manifest in childhood (Jones, 2013), understanding what occurs in development can highlight early windows for intervention. Second, as most studies have previously examined *unlearned* fear responses, we examined whether offspring of stressed mothers demonstrate atypical *learned* fear behaviors. The ability to learn about fear and inhibit it (e.g., via Pavlovian conditioning and extinction) is highly clinically relevant, as anxiety disorders are said to emerge when these processes are dysregulated (Lissek et al., 2005; Milad et al., 2009). Further, studying learned fear has greater translational value in terms of therapeutic intervention, as the most widely used and empirically validated treatment for anxiety in humans is exposure therapy, which is modeled on the process of fear extinction (Milad, Rauch, Pitman, & Quirk, 2006). Finally, instead of prenatal stress, we examined whether atypical emotional development in subsequent offspring is evident after maternal exposure to a postnatal stressor (i.e., maternal separation). That is, we explored whether stress exposure during a different period of the mother's caregiving experience will lead to adverse outcomes for later offspring. Given the adverse effects found in mothers long after the termination of maternal separation (Boccia et al., 2007; Maniam & Morris, 2010; von Poser Toigo et al., 2012), we hypothesized that offspring subsequently born to previously stressed mothers will show atypical emotional development.

General Method

Subjects

Experimentally naive Sprague-Dawley derived rats, bred and housed at the School of Psychology, The University of New South Wales (UNSW), were used. Rats were housed with their mother in groups of eight in plastic boxes (24.5 cm long × 37 cm wide × 27 cm high) covered by a wire lid, with food and water available ad lib. The day of birth was designated as postnatal day (PND) 0, and experimental procedures began on PND17 or PND18. Only males were used, and no more than one rat per litter was used per group. Animals were maintained on a 12-hr light–dark cycle (lights on at 7:00 a.m.). Animals were treated according to the principles of animal care and use outlined in the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (7th ed., 2004). All procedures were approved by the Animal Care and Ethics Committee at UNSW.

Mother's Earlier Caregiving Experience: Maternal Separation or Standard Rearing

The breeding and rearing procedure is depicted in Figure 1. Multiparous, stress-naïve adult female rats were bred with stress-naïve adult males. After parturition, mothers and pups were exposed to maternal separation rearing (MS) or standard rearing (SR). Briefly, SR females remained with their pups in their home

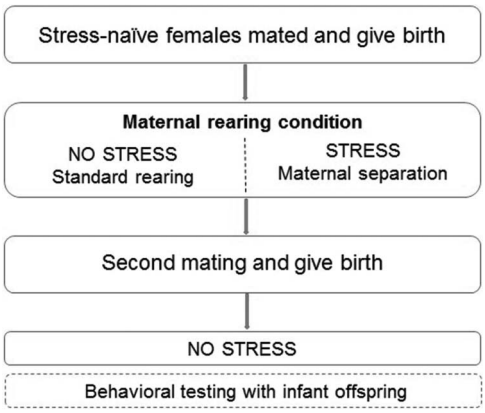

Figure 1. Breeding protocol. Female rats were bred and then either reared with relatively little disruption (i.e., standard rearing) or were separated from their pups for 3-hr daily when the pups were PND2-14 (maternal separation). After pups from both conditions were weaned, the mothers were mated again to produce the next litter. Note that no further stress occurred. Offspring of the second litter were the focus of the research reported in this study.

cages while MS females were separated from their pups for 3-hr a day on PND2-14 (see Callaghan & Richardson, 2011 for details). After pup weaning (approximately PND21), mothers were again bred with stress-naive males. No further MS occurred for any of these subsequent litters (i.e., all mothers stayed with the pups from the subsequent litters). The average interval between maternal separation and the birth of the subsequent litter (MS_{SUB}) was 55 days.[1]

Scoring and Analyses

In Experiments 1 and 2, freezing was used as the measure of learned fear, and was defined as the absence of all movement except that required for respiration (Fanselow, 1980). A time sampling procedure was used (i.e., each rat was scored as freezing or not every 3-s), and these observations were then converted into a percentage score to indicate the proportion of total observations scored as freezing.

Because conditional stimulus (CS) elicited freezing is difficult to detect when rats display high levels of baseline (pre-CS) freezing, any rat that exhibited baseline freezing >60% was excluded from the final analysis. Two out of 48 rats in Experiment 1 (from Group MS_{SUB} – 1 day) and three rats out of 48 in Experiment 2 (1 from Group MS_{SUB} – same, 1 from Group MS_{SUB} – different, and 1 from Group SR_{SUB} – different) were excluded for having high baseline freezing.

A second observer that was unaware of the experimental condition of each rat scored a random sample (30%) of rats tested in each experiment. The interrater reliability was high (rs = .92 to .98). SPSS (IBM SPSS Statistics 22) was used to analyze the data.

Experiment 1

Stimuli

The CS was a white noise that increased the noise level in the chambers by 8 dB. The US was a 0.6 mA, 1-s footshock administered by a custom built constant-current shock generator. The software and hardware used were custom developed at UNSW.

Method

The design was a 2 × 2 factorial, with the first factor referring to the mother's previous caregiving experience (maternal-separation or standard-rearing) and the second factor referring to the interval between conditioning and test (1 day or 10 days). At P17, animals were placed in the experimental chamber (see Supplemental Material) for a 2-min adaptation period which was immediately followed by a 10-s presentation of the white noise CS. The shock US was administered during the last second of the CS. Six pairings of the CS and the US were given. The intertrial interval (ITI) ranged from 85 to 135-s with a mean of 110-s. After the last pairing, rats were returned to their home cages. Test involved placing rats in the experimental chamber and, after a 1-min adaptation period, presenting the CS continuously for 2-min.

Results

A 2 (prior caregiving condition) × 2 (train-test interval) ANOVA on baseline freezing at test revealed a significant main effect of retention interval, $F(1, 38) = 10.71$, $p = .002$, with those tested after 1 day ($M = 15.05$, $SEM = 2.65$) exhibiting higher levels of baseline freezing than those tested after 10 days ($M = 4.31$, $SEM = 2.21$); no other effects were significant, largest $F(1, 38) = 1.42$, $p = .24$. Given the significant effect of retention interval on baseline freezing, difference scores for test were calculated (i.e., CS-elicited freezing minus baseline freezing). Figure 2 shows that MS_{SUB} and SR_{SUB} groups displayed similar levels of CS-elicited freezing when tested 1 day after conditioning. However, when tested 10 days after conditioning, MS_{SUB} rats had higher levels of CS-elicited freezing than did the SR_{SUB} rats. Statistical analyses yielded a significant effect of the mother's prior caregiving experience, $F(1, 38) = 5.76$, $p = .02$. No other effects were significant, including the interaction between the two conditions (largest $F = 3.37$, $p = .07$). Although the interaction was not significant, post hoc comparisons, using independent samples t tests, were done based on past findings in MS infants (discussed above). If Levene's test for equality of variances was violated, then the degrees of freedom were adjusted accordingly. These post hoc comparisons revealed that MS_{SUB} rats did not differ when tested 1 Day or 10 Days after conditioning, $t(17.61) = .53$, $p = .59$. In contrast, there was a significant difference between SR_{SUB} rats tested either 1 day or 10 days after conditioning, $t(18) = 2.51$, $p = .02$, 95% confidence interval (CI) [3.8–42.0], $d = .83$. A comparison of MS_{SUB} and SR_{SUB} rats at the 10 day interval revealed a significant difference, $t(16.25) = 2.33$, $p = .03$, 95% CI [2.44–53.6], $d = 0.69$. However, given that the Caregiving × Retention Interval interaction was not significant, these post hoc comparisons should be interpreted with caution.

The results of Experiment 1 provide partial support for the hypothesis that fear retention during infancy is affected by the mother's previous stressful experience of being separated from her

[1] Calculated from 17 out of 30 dams for which the dates were recorded.

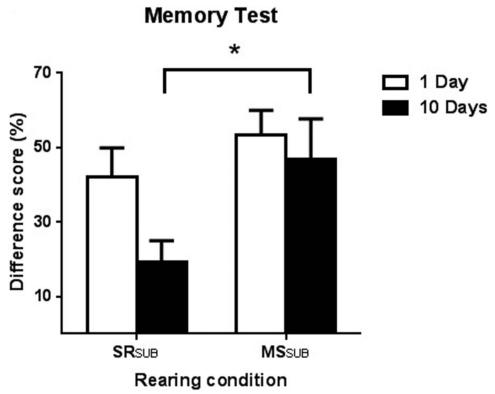

Figure 2. Mean (±*SEM*) difference scores (CS-elicited freezing minus baseline freezing) in rats born to previously stressed (MS_{SUB}) and standard-reared mothers (SR_{SUB}) when tested for fear retention 1 day (PND 18; white bars) or 10 days (PND27; black bars) after conditioning. At the 1 day interval, similar, and high, levels of fear was observed in the SR_{SUB} ($n = 10$) and MS_{SUB} ($n = 10$) rats. However, at the 10 day interval, MS_{SUB} ($n = 12$) rats exhibited higher levels of fear than SR_{SUB} ($n = 10$) rats. * Significant ($p < .05$) difference based on post hoc follow-up comparisons (independent samples *t* tests).

pups. As illustrated in Figure 2, and suggested by the exploratory post hoc comparisons, SR_{SUB} infants appeared to display typical infantile amnesia whereas MS_{SUB} infants did not. That is, the MS_{SUB} infants exhibited good fear retention that is characteristic of adult-like memory, just like what has been reported for infants directly exposed to the maternal separation procedure (Callaghan & Richardson, 2012).

Direct exposure to maternal separation has also been shown to lead to atypical behavior on another measure of emotional regulation—fear inhibition. After extinguishing a fear association in a particular context, older animals typically show relapse when re-exposed to the fear-eliciting stimulus in a different context. This failure to inhibit fear in a context different to where extinction training occurred is referred to as the renewal effect and is most commonly observed when animals are re-exposed to the original training context at test (termed ABA renewal). Infants, however, are typically relapse-resistant (see Revillo, Molina, Paglini, & Arias, 2013, for some boundary conditions for this effect), unless they had been directly exposed to maternal separation (Callaghan & Richardson, 2011). In the following experiment, we examined whether the renewal effect is also observed in infant offspring born to mothers who experienced maternal separation with her previous litter.

Experiment 2

Method

The design was a 2 × 2 factorial, with the first factor referring to the mother's previous caregiving experience (maternal-separation or standard-rearing) and the second factor referring to the test context in which the infant was tested after extinction (same or different to the extinction context). Across three consecutive days, PND17 rats underwent fear conditioning, extinction, and were then tested for relapse of extinguished fear (i.e., the renewal effect). The stimuli used (white noise and shock) were the same as in Experiment 1. The different chambers (see Supplemental Material) denoted as Context A or Context B were counterbalanced. During conditioning, rats were placed in Context A and underwent conditioning in the same way as in Experiment 1. The next day, rats in the "Same context" group received extinction training in either Context A or in Context B, and then the following day were tested in that same context (i.e., AAA or ABB condition). Rats in the "Different context" group received extinction training in Context B followed by test in Context A the next day (i.e., ABA condition).

Extinction involved a 2-min adaptation period, followed by five blocks of extinction, each consisting of six presentations of the 10-s CS with an ITI of 10-s. In total, the extinction session consisted of 30 nonreinforced presentations of the CS and was approximately 12-min in duration. Freezing was recorded during each presentation of the CS. Test involved the same procedure as in Experiment 1.

Results

Extinction. The effect of prior maternal caregiving experience on baseline freezing in pups during extinction training was not significant (*M*s = 38.95 & 23.05% for MS_{SUB} and SR_{SUB} groups, respectively; $t(43) = 1.77$, $p = .08$), but difference scores were analyzed for consistency with Experiment 1. The mean difference score on each block of extinction training is shown in Figure 3a, which illustrates that infants in both rearing conditions exhibited high levels of freezing during the first block, and lower levels of freezing on subsequent blocks. A mixed-design analysis of variance (ANOVA) revealed a significant effect of block, $F(4, 172) = 31.67$, $p < .001$. No other effects were significant (*F*s < 1). In other words, MS_{SUB} and SR_{SUB} rats extinguished fear at the same rate, and did not differ in overall levels of fear across extinction training.

Test. There were no significant effects on baseline freezing levels (average across all groups <15%; largest $F(1, 41) = 2.02$, $p = .16$). Levels of CS-elicited fear (difference scores) during test are shown in Figure 3b, which illustrates that when SR_{SUB} infants were tested in the same or different context to extinction training, they exhibited low levels of freezing. In contrast, infants that were born to a previously stressed mother (MS_{SUB}) exhibited high levels of fear when tested in a different context to extinction training, compared with MS_{SUB} rats tested in the same context. In other words, MS_{SUB} rats exhibited the renewal effect while SR_{SUB} rats did not. A 2 (prior caregiving condition) × 2 (test context) ANOVA confirmed this description of the data. The main effect of test context was significant, $F(1, 41) = 10.03$, $p = .003$, while that of prior maternal caregiving condition was not $F(1, 41) = 3.28$, $p = .07$; importantly, the interaction of these two factors was significant, $F(1, 41) = 3.99$, $p = .05$, $\eta^2_p = .089$.

Post hoc comparisons revealed there were no differences in levels of freezing between SR_{SUB} infants tested in the same or different context as extinction, $t(19) = .83$, $p = .41$. In contrast, MS_{SUB} infants tested in a different context exhibited significantly higher levels of fear compared with MS_{SUB} infants tested in the same context as extinction training, $t(22) = 3.64$, $p = .001$, 95% CI [14.87–54.24], $d = 1.48$.

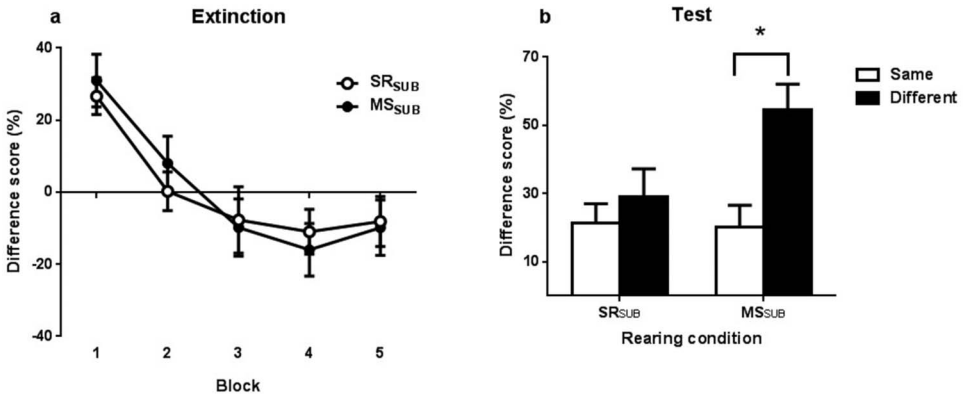

Figure 3. Mean (±*SEM*) difference scores (CS-elicited freezing minus baseline freezing) during (a) extinction training in PND18 SR_{SUB} ($n = 21$; open circles) and MS_{SUB} ($n = 24$; closed circles) rats. Each block represents the average freezing across 6 CS presentations; and (b) test in PND19 SR_{SUB} and MS_{SUB} rats tested in either the same (white bars; SR_{SUB}, $n = 11$; MS_{SUB}, $n = 12$) or different context (black bars; SR_{SUB}, $n = 10$; MS_{SUB}, $n = 12$) to extinction training. * Significant ($p < .01$) difference based on post hoc follow-up comparisons (independent samples t tests).

Experiment 3

The results of Experiments 1 and 2 show that the subsequent offspring of mothers exposed to postnatal stress with her prior litter acquire fear associations that persist for longer and that are more prone to relapse after extinction. These results were not because of MS_{SUB} infants being more sensitive to shock (see Supplemental Materials). As noted earlier, Champagne and Meaney (2006) reported that adult offspring of mothers that had been prenatally stressed with a previous litter were found to exhibit more anxiety-like behavior on the open field test; in the present experiment we explored whether this heightened anxiety is also evident in infancy.

Method

The two groups in this experiment differed only in terms of their mother's caregiving experience with her previous litter (MS or SR). At either P17 or P18, rats were tested for anxiety-like behavior on either the light-dark box or the elevated plus maze (different animals were used for each test). Tests were conducted in a dimly illuminated room (2.2 lux). A video camera was positioned above the apparatus to record the behavior of the animal. The light–dark box was a two compartment apparatus made of Perspex (each compartment measured 18 cm wide × 18.5 cm long × 20 cm high). One compartment had black opaque walls and black lid while the other had white opaque walls and a clear lid. The animal could move between the two compartments using a small opening (7.5 cm × 7.5 cm), which could be closed with a metal sliding door. During test, the animal was confined in the dark compartment for 1-min after which the door was opened, allowing access to the light compartment. The latency to enter the light compartment with all four paws was recorded, as was the number of entries into the light compartment and total time in the light compartment during the 5-min test. The floor of each compartment was cleaned with 70% ethanol between sessions.

The elevated plus-maze consisted of two open arms and two closed arms that met at the center to form a cross (arms measured 113 cm long × 10.5 cm wide; walls on closed arm measured 40 cm high). The apparatus was elevated 53 cm off the floor. At test, each animal was placed in the center of the maze facing an open arm. Test was 5-min long, and the number of entries into the open and closed arms, as well as the total time spent in each type of arm, was measured. All four paws of the animal had to cross into an arm to be considered an entry. At the end of the test, the maze was cleaned with 70% ethanol.

Results

Panels A and B in Figure 4 shows the mean response measures for the two groups on the light-dark box (data for total number of entries not shown). There were no differences in time to first entry, total number of entries, or total time in the light side of the compartment (largest $t = -.64$). Panel C in Figure 4 shows the mean response for the two groups on time in the open arm of the elevated-plus maze. There were no group differences in the number of open arm entries (data not shown) or time spent in the open arms (largest $t = .28$).

The results of Experiment 3 indicate that the mother's prior experience with postnatal stress does not heighten anxiety-like behavior in her future offspring when they are tested in infancy. Together with the results of Experiments 1 and 2, the results of this experiment suggest that prior maternal stress may only affect learned, but not unlearned, fear in her infant offspring.

General Discussion

The experiments in this study demonstrate that prior maternal stress affects the maturation of emotion regulation in her subsequent infant offspring, even when these offspring are not exposed directly to stress. Specifically, subsequent offspring of previously stressed mothers retained fear associations for longer and were more likely to relapse after extinction of fear during infancy than were infant offspring of standard mothers. This profile of fear responding is typically seen in older animals (Kim & Richardson,

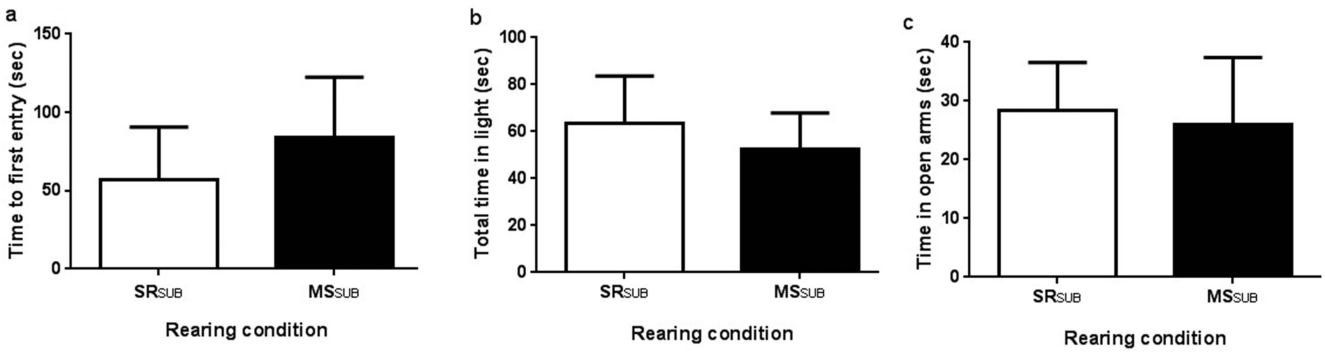

Figure 4. (a) Mean (±*SEM*) latency to enter the light side of the compartment and (b) Total time spent in light side of the light-dark box (SR_{SUB}, $n = 9$; MS_{SUB}, $n = 10$); (c) Mean (±*SEM*) time spent in the open arms of the elevated-plus maze (SR_{SUB}, $n = 10$; MS_{SUB}, $n = 9$).

2010), which suggests that infants of previously stressed mothers exhibit a precocious transition from the infant to the adult emotional regulation system. This same faster transition is also observed in infants directly exposed to the stress (Callaghan & Richardson, 2012, 2011). However, while those earlier studies showed that direct exposure to early life stress alters typical development of emotional regulation, what we show here is that the same pattern of accelerated emotional development can also occur indirectly via the mother's past exposure to stress.

Our findings that prior maternal stress alters emotional development in subsequent offspring is conceptually similar to the results reported in the study by Champagne and Meaney (2006) that was described earlier. In that study, gestational stress led to atypical emotional behavior (as measured by the open field test) in the offspring that the mother was carrying at the time of the stress as well as in her subsequent offspring. We have extended these results by showing that the effect of prior maternal stress on subsequent offspring emotional development (a) also occurs after a postnatal stressor (i.e., maternal separation), (b) leads to atypical expression and inhibition of learned fear, and (c) is also observed in infant offspring (i.e., Champagne & Meaney tested adults).

One major difference between our results and those reported by Champagne and Meaney (2006) is that we did not observe any effects on unlearned fear. There are a number of possible reasons for this difference. For example, we used different measures of unlearned fear (i.e., elevated plus maze and light-dark box) to Champagne and Meaney, who used the open field test. Another possibility is that this discrepancy may be because of methodological differences in the timing of the stress exposure (prenatal in Champagne and Meaney vs. postnatal in the current study). Finally, the different outcomes may be because of differences in the age of the animal at the time of testing, in that we tested infants while Champagne and Meaney tested adults. Indeed, studies have found age-dependent effects of prenatal stress on anxiety-like behavior (Dickerson, Lally, Gunnel, Birkle, & Salm, 2005; Estanislau & Morato, 2006), such that prenatal stress did not affect general fearfulness in postnatal Day 25–30 animals (juveniles) but did lead to heightened fear in postnatal Day 45 and older animals (late adolescence to adulthood). This suggests that the effects of stress on offspring anxiety-like behavior might only be detected later in life. It is possible that the measures of anxiety used in infancy are not sensitive to detect the effects of early life stress or that more time is required before these effects develop.

The mechanisms underlying the transmission of the effects of prior maternal stress on the emotional development of future offspring are not yet understood, but alterations in various hormonal levels after stress are likely to be involved. As one example, Maniam and Morris (2010) reported that dams exposed to the same maternal separation procedure used in this study have heightened basal plasma levels of the stress hormone corticosterone (CORT) compared with dams exposed to brief separations (15-min) on the same days. Further, these changes in CORT levels were observed 17 weeks after maternal separation had terminated, illustrating that this stressor causes a long-lasting alteration in the levels of CORT in the mothers. One way that elevated basal CORT levels in MS dams could be transmitted to subsequent offspring is in utero via placental transfer. The placenta acts as a barrier between the developing fetus and the maternal environment, and typically protects the fetus from circulating maternal stress hormones (for reviews, see Braun, Challis, Newnham, & Sloboda, 2013; O'Donnell, O'Connor, & Glover, 2009). However, chronic stress exposure diminishes the protective function of the placenta, thereby allowing the fetus to be exposed to atypical levels of glucocorticoids (Lucassen et al., 2009; Mairesse et al., 2007; Welberg, Thrivikraman, & Plotsky, 2005). It should be noted that the majority of these studies have been conducted using prenatal stress, and there is limited research on whether postnatal stress can exert enduring effects on placental function. This is a potential question for future research to investigate.

Another route by which elevated CORT levels in the mother could be transmitted to the infant is postnatally via the breastmilk (Macrì, Zoratto, & Laviola, 2011). Indeed, past studies have shown that exposure to CORT via the mother's breast milk leads to the early emergence of adult-like fear behavior. Specifically, Callaghan and Richardson (2012) reported that CORT mimics the effect of maternal separation in that chronic exposure to CORT via the mother's drinking water also led to the early emergence of adult-like fear retention in the infants. The role of CORT in permitting the expression of mature emotional regulation has also been shown in younger animals, such that PND8 infants given injections of CORT before odor-shock conditioning show odor avoidance instead of the paradoxical odor approach response typ-

ically observed in animals this age (Moriceau, Roth, & Sullivan, 2010; Moriceau, Wilson, Levine, & Sullivan, 2006). One way that CORT exposure could affect emotional development in the offspring is by altering activity in neural structures critical for mature emotional learning, such as the amygdala. In support of this, intraamygdala injections of CORT in PND8 animals led to activation of the amygdala in response to odor conditioning, an effect which is typically only seen in older animals (Moriceau et al., 2006). Together, these studies demonstrate that exposure to stress hormones can cause a precocious developmental shift in emotional regulation and suggests the same mechanism might also regulate the results reported in this study. It is possible there are other hormonal changes involved in the effects reported here. For example, oxytocin has been implicated in stress coping and regulates activity of the hypothalamic-pituitary-adrenal (HPA) axis (Amico, Mantella, Vollmer, & Li, 2004; Neumann, 2002; Neumann, Krömer, Toschi, & Ebner, 2000) and amygdala (Ebner, Bosch, Krömer, Singewald, & Neumann, 2005; Viviani et al., 2011). In addition, is has been demonstrated that the oxytocin system can become dysregulated in the mother after chronic stress (Champagne & Meaney, 2006).

In addition to long-term changes in neuroendocrine function, another physiological mechanism that could be responsible for the persistent effects of stress observed in the subsequent infant offspring are epigenetic changes to the mother after stress. While most epigenetic studies have focused on the effects of early life experiences on gene expression, there is mounting evidence that these changes can also occur in adults (Roth, 2012). For example, restraint stress in adulthood leads to patterns of methylation across several regions of the hippocampus that vary depending on the length of stress exposure (Hunter, McCarthy, Milne, Pfaff, & McEwen, 2009). In addition, chronic psychosocial stress consisting of exposure to cat odor and social instability leads to hypermethylation of brain-derived neutrophic factor (BDNF) in the hippocampus and posttraumatic stress disorder (PTSD) like behavioral changes (Roth, Zoladz, Sweatt, & Diamond, 2011). These data provide evidence that the social environment beyond infancy is capable of shifting patterns of gene expression with consequences for the future functioning of the individual (Champagne, 2010; Hunter, 2012). Thus, maternal separation (another type of chronic social stress) could potentially alter the dam's gene expression, contributing to alterations in her stress physiology and behavior; importantly, these effects could persist beyond a subsequent rearing experience to shape future offspring development.

While epigenetic changes (or biological inheritance) is proposed to be one route by which environmental experiences are passed onto later generations, another route involves social transmission (Dias, Maddox, Klengel, & Ressler, 2015; Klengel, Dias, & Ressler, 2015). This involves transfer of information to offspring via the rearing environment. For example, pioneering studies by Meaney and colleagues (see Meaney, 2001) documented naturally occurring variations in the frequency and quality of licking, grooming, and arched back nursing (LG-ABN) toward offspring. These maternal behaviors shape offspring development, such that adult offspring raised by low LG-ABN mothers tend to exhibit heightened anxiety-like behavior (i.e., increased startle responses, decreased open-field exploration, and dysregulated HPA activity (Caldji et al., 1998; Liu et al., 1997). Of importance for the current study is the finding that stressors can alter these grooming and nursing behaviors. Specifically, females previously characterized as high LG-ABN mothers became low LG-ABN mothers after chronic gestational stress (Champagne & Meaney, 2006). This alteration was long-lasting as these mothers continued to show low LG-ABN toward a subsequent litter, despite no additional exposure to the stressor. Changes in maternal behavior have also been observed after postnatal stress with dams exposed to an impoverished rearing environment (i.e., significantly reduced nesting material) showing an increase in the levels of abusive behaviors (e.g., stepping, throwing) directed toward pups (Raineki, Cortés, Belnoue, & Sullivan, 2012; Raineki, Moriceau, & Sullivan, 2010). The adolescent offspring of these abusive mothers exhibited depressive-like profiles as measured by the forced-swim test, as well as heightened amygdala activity in response to forced swim stress (Raineki et al., 2012). Overall, these studies indicate that parental behavior can shape the development of stress phenotypes in the offspring, and that alterations in maternal behavior is a promising mechanism for the heightened fear behavior observed on some measures in the current study.

In addition to direct maternal care, social transmission of stress effects can occur via parental modeling. In humans, displaying anxious behaviors in the child's presence has been shown to play a major role in the intergenerational transfer of fear and anxiety (Eley et al., 2015; Gerull & Rapee, 2002; Waters, West, & Mendes, 2014). Similar results have been found in rodent work, such that infant offspring can acquire fear to a conditioned stimulus vicariously through their mother (Chang & Debiec, 2016; Debiec & Sullivan, 2014). Furthermore, exposing mothers to a social aversive stimulus (i.e., adult male intruder) in the presence of their infant offspring leads to the precocious expression of fear to that stimulus in the offspring (Zuluaga, Agrati, Uriarte, & Ferreira, 2014). Together, these data provide further evidence that the mother can modulate her offspring's affective development. Given that offspring in the current study also display precocious fear behavior, it is possible that a similar parental display of heightened fear occurs in mothers after maternal separation. There have been several potential contributing mechanisms outlined above and it will be important for future studies to disentangle the effects of behavioral/social transmission and biological inheritance on offspring development (see Dias et al., 2015; Klengel et al., 2015).

The finding that prior caregiver stress impacts later offspring development has also been observed in humans. As noted previously, studies conducted on Holocaust survivors post-WWII indicate significant covariation between caregiver and child mental health (Betancourt, McBain, Newnham, & Brennan, 2015). Similarly, a study on survivors of the Khmer Rouge Regime in Cambodia found that maternal PTSD symptoms and level of trauma exposure significantly correlated with anxiety and depression in their teenage daughters (who had been conceived outside of the regime; Field, Muong, & Sochanvimean, 2013). In addition, the link between prior parental stress and offspring development has been demonstrated on controlled laboratory measures. Specifically, prior maternal abuse was related to heightened levels of dark-enhanced startle (a behavioral measure of anxiety) in their children, compared with peers of nonstressed mothers (Jovanovic et al., 2011). This relationship was not accounted for by the mother's psychopathology or the child's own trauma exposure; thus highlighting the unique link between past parental experi-

ences and outcomes in offspring. The results from the current study parallel these human findings by demonstrating that parental experiences exert persisting effects on offspring development. Such similarities highlight the translational value in using animals to study the effects of generational transmission of stress. Animal models such as the one described in this study will allow researchers to circumvent the various methodological and ethical constraints associated with studying stress in humans. We must now determine the mechanisms mediating these effects (e.g., persistent changes in levels of stress hormones and/or parenting behavior). Such information can then be used to develop treatments for caregivers that have experienced trauma, thereby limiting the generational impact of stress exposure on later offspring.

References

Aisa, B., Tordera, R., Lasheras, B., Del Río, J., & Ramírez, M. J. (2007). Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. *Psychoneuroendocrinology, 32,* 256–266. http://dx.doi.org/10.1016/j.psyneuen.2006.12.013

Amico, J. A., Mantella, R. C., Vollmer, R. R., & Li, X. (2004). Anxiety and stress responses in female oxytocin deficient mice. *Journal of Neuroendocrinology, 16,* 319–324. http://dx.doi.org/10.1111/j.0953-8194.2004.01161.x

Betancourt, T. S., McBain, R. K., Newnham, E. A., & Brennan, R. T. (2015). The intergenerational impact of war: Longitudinal relationships between caregiver and child mental health in postconflict Sierra Leone. *Journal of Child Psychology and Psychiatry, 56,* 1101–1107. http://dx.doi.org/10.1111/jcpp.12389

Boccia, M. L., Razzoli, M., Vadlamudi, S. P., Trumbull, W., Caleffie, C., & Pedersen, C. A. (2007). Repeated long separations from pups produce depression-like behavior in rat mothers. *Psychoneuroendocrinology, 32,* 65–71. http://dx.doi.org/10.1016/j.psyneuen.2006.10.004

Braun, T., Challis, J. R., Newnham, J. P., & Sloboda, D. M. (2013). Early-life glucocorticoid exposure: The hypothalamic-pituitary-adrenal axis, placental function, and long-term disease risk. *Endocrine Reviews, 34,* 885–916. http://dx.doi.org/10.1210/er.2013-1012

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P. M., & Meaney, M. J. (1998). Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proceedings of the National Academy of Sciences of the United States of America, 95,* 5335–5340. http://dx.doi.org/10.1073/pnas.95.9.5335

Callaghan, B. L., & Richardson, R. (2011). Maternal separation results in early emergence of adult-like fear and extinction learning in infant rats. *Behavioral Neuroscience, 125,* 20–28. http://dx.doi.org/10.1037/a0022008

Callaghan, B. L., & Richardson, R. (2012). The effect of adverse rearing environments on persistent memories in young rats: Removing the brakes on infant fear memories. *Translational Psychiatry, 2,* e138. http://dx.doi.org/10.1038/tp.2012.65

Champagne, F. A. (2010). Epigenetic influence of social experiences across the lifespan. *Developmental Psychobiology, 52,* 299–311.

Champagne, F. A., & Meaney, M. J. (2006). Stress during gestation alters postpartum maternal care and the development of the offspring in a rodent model. *Biological Psychiatry, 59,* 1227–1235. http://dx.doi.org/10.1016/j.biopsych.2005.10.016

Chang, D. J., & Debiec, J. (2016). Neural correlates of the mother-to-infant social transmission of fear. *Journal of Neuroscience Research, 94,* 526–534. http://dx.doi.org/10.1002/jnr.23739

Crews, D., Gillette, R., Scarpino, S. V., Manikkam, M., Savenkova, M. I., & Skinner, M. K. (2012). Epigenetic transgenerational inheritance of altered stress responses. *Proceedings of the National Academy of Sciences of the United States of America, 109,* 9143–9148. http://dx.doi.org/10.1073/pnas.1118514109

Daniels, W. M., Pietersen, C. Y., Carstens, M. E., & Stein, D. J. (2004). Maternal separation in rats leads to anxiety-like behavior and a blunted ACTH response and altered neurotransmitter levels in response to a subsequent stressor. *Metabolic Brain Disease, 19,* 3–14. http://dx.doi.org/10.1023/B:MEBR.0000027412.19664.b3

Debiec, J., & Sullivan, R. M. (2014). Intergenerational transmission of emotional trauma through amygdala-dependent mother-to-infant transfer of specific fear. *Proceedings of the National Academy of Sciences of the United States of America, 111,* 12222–12227. http://dx.doi.org/10.1073/pnas.1316740111

Dias, B. G., Maddox, S. A., Klengel, T., & Ressler, K. J. (2015). Epigenetic mechanisms underlying learning and the inheritance of learned behaviors. *Trends in Neurosciences, 38,* 96–107. http://dx.doi.org/10.1016/j.tins.2014.12.003

Dickerson, P. A., Lally, B. E., Gunnel, E., Birkle, D. L., & Salm, A. K. (2005). Early emergence of increased fearful behavior in prenatally stressed rats. *Physiology & Behavior, 86,* 586–593. http://dx.doi.org/10.1016/j.physbeh.2005.08.025

Ebner, K., Bosch, O. J., Krömer, S. A., Singewald, N., & Neumann, I. D. (2005). Release of oxytocin in the rat central amygdala modulates stress-coping behavior and the release of excitatory amino acids. *Neuropsychopharmacology, 30,* 223–230. http://dx.doi.org/10.1038/sj.npp.1300607

Eley, T. C., McAdams, T. A., Rijsdijk, F. V., Lichtenstein, P., Narusyte, J., Reiss, D., . . . Neiderhiser, J. M. (2015). The intergenerational transmission of anxiety: A children-of-twins study. *The American Journal of Psychiatry, 172,* 630–637. http://dx.doi.org/10.1176/appi.ajp.2015.14070818

Estanislau, C., & Morato, S. (2006). Behavior ontogeny in the elevated plus-maze: Prenatal stress effects. *International Journal of Developmental Neuroscience, 24,* 255–262. http://dx.doi.org/10.1016/j.ijdevneu.2006.03.001

Fanselow, M. S. (1980). Signaled shock-free periods and preference for signaled shock. *Journal of Experimental Psychology: Animal Behavior Processes, 6,* 65–80. http://dx.doi.org/10.1037/0097-7403.6.1.65

Field, N. P., Muong, S., & Sochanvimean, V. (2013). Parental styles in the intergenerational transmission of trauma stemming from the Khmer Rouge regime in Cambodia. *American Journal of Orthopsychiatry, 83,* 483–494. http://dx.doi.org/10.1111/ajop.12057

Franklin, T. B., Russig, H., Weiss, I. C., Gräff, J., Linder, N., Michalon, A., . . . Mansuy, I. M. (2010). Epigenetic transmission of the impact of early stress across generations. *Biological Psychiatry, 68,* 408–415. http://dx.doi.org/10.1016/j.biopsych.2010.05.036

Gerull, F. C., & Rapee, R. M. (2002). Mother knows best: Effects of maternal modelling on the acquisition of fear and avoidance behaviour in toddlers. *Behaviour Research and Therapy, 40,* 279–287. http://dx.doi.org/10.1016/S0005-7967(01)00013-4

Hunter, R. G. (2012). Epigenetic effects of stress and corticosteroids in the brain. *Frontiers in Cellular Neuroscience, 6,* 18. http://dx.doi.org/10.3389/fncel.2012.00018

Hunter, R. G., McCarthy, K. J., Milne, T. A., Pfaff, D. W., & McEwen, B. S. (2009). Regulation of hippocampal H3 histone methylation by acute and chronic stress. *Proceedings of the National Academy of Sciences of the United States of America, 106,* 20912–20917. http://dx.doi.org/10.1073/pnas.0911143106

Jones, P. B. (2013). Adult mental health disorders and their age at onset. *The British Journal of Psychiatry, 54,* s5–s10. http://dx.doi.org/10.1192/bjp.bp.112.119164

Jovanovic, T., Smith, A., Kamkwalala, A., Poole, J., Samples, T., Norholm, S. D., . . . Bradley, B. (2011). Physiological markers of anxiety are increased in children of abused mothers. *Journal of Child Psychol-*

ogy and Psychiatry, 52, 844–852. http://dx.doi.org/10.1111/j.1469-7610.2011.02410.x

Kim, J. H., McNally, G. P., & Richardson, R. (2006). Recovery of fear memories in rats: Role of gamma-amino butyric acid (GABA) in infantile amnesia. *Behavioral Neuroscience, 120,* 40–48. http://dx.doi.org/10.1037/0735-7044.120.1.40

Kim, J. H., & Richardson, R. (2007). A developmental dissociation of context and GABA effects on extinguished fear in rats. *Behavioral Neuroscience, 121,* 131–139. http://dx.doi.org/10.1037/0735-7044.121.1.131

Kim, J. H., & Richardson, R. (2010). New findings on extinction of conditioned fear early in development: Theoretical and clinical implications. *Biological Psychiatry, 67,* 297–303. http://dx.doi.org/10.1016/j.biopsych.2009.09.003

Klengel, T., Dias, B. G., & Ressler, K. J. (2015). Models of intergenerational and transgenerational transmission of risk for psychopathology in mice. *Neuropsychopharmacology, 41,* 219–231. http://dx.doi.org/10.1038/npp.2015.249

Lissek, S., Powers, A. S., McClure, E. B., Phelps, E. A., Woldehawariat, G., Grillon, C., & Pine, D. S. (2005). Classical fear conditioning in the anxiety disorders: A meta-analysis. *Behaviour Research and Therapy, 43,* 1391–1424. http://dx.doi.org/10.1016/j.brat.2004.10.007

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., . . . Meaney, M. J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science, 277,* 1659–1662. http://dx.doi.org/10.1126/science.277.5332.1659

Lucassen, P. J., Bosch, O. J., Jousma, E., Krömer, S. A., Andrew, R., Seckl, J. R., & Neumann, I. D. (2009). Prenatal stress reduces postnatal neurogenesis in rats selectively bred for high, but not low, anxiety: Possible key role of placental 11β-hydroxysteroid dehydrogenase type 2. *European Journal of Neuroscience, 29,* 97–103. http://dx.doi.org/10.1111/j.1460-9568.2008.06543.x

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience, 10,* 434–445. http://dx.doi.org/10.1038/nrn2639

Macrì, S., Zoratto, F., & Laviola, G. (2011). Early-stress regulates resilience, vulnerability and experimental validity in laboratory rodents through mother-offspring hormonal transfer. *Neuroscience and Biobehavioral Reviews, 35,* 1534–1543. http://dx.doi.org/10.1016/j.neubiorev.2010.12.014

Mairesse, J., Lesage, J., Breton, C., Bréant, B., Hahn, T., Darnaudéry, M., . . . Viltart, O. (2007). Maternal stress alters endocrine function of the feto-placental unit in rats. *American Journal of Physiology Endocrinology and Metabolism, 292,* E1526–E1533. http://dx.doi.org/10.1152/ajpendo.00574.2006

Maniam, J., & Morris, M. J. (2010). Long-term postpartum anxiety and depression-like behavior in mother rats subjected to maternal separation are ameliorated by palatable high fat diet. *Behavioural Brain Research, 208,* 72–79. http://dx.doi.org/10.1016/j.bbr.2009.11.005

Matthews, K., Robbins, T. W., Everitt, B. J., & Caine, S. B. (1999). Repeated neonatal maternal separation alters intravenous cocaine self-administration in adult rats. *Psychopharmacology, 141,* 123–134. http://dx.doi.org/10.1007/s002130050816

Meaney, M. J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual Review of Neuroscience, 24,* 1161–1192. http://dx.doi.org/10.1146/annurev.neuro.24.1.1161

Michaels, C. C., & Holtzman, S. G. (2008). Early postnatal stress alters place conditioning to both μ- and κ-opioid agonists. *The Journal of Pharmacology and Experimental Therapeutics, 325,* 313–318. http://dx.doi.org/10.1124/jpet.107.129908

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L., Shin, L. M., Lasko, N. B., . . . Rauch, S. L. (2009). Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biological Psychiatry, 66,* 1075–1082. http://dx.doi.org/10.1016/j.biopsych.2009.06.026

Milad, M. R., Rauch, S. L., Pitman, R. K., & Quirk, G. J. (2006). Fear extinction in rats: Implications for human brain imaging and anxiety disorders. *Biological Psychology, 73,* 61–71. http://dx.doi.org/10.1016/j.biopsycho.2006.01.008

Moffett, M. C., Harley, J., Francis, D., Sanghani, S. P., Davis, W. I., & Kuhar, M. J. (2006). Maternal separation and handling affects cocaine self-administration in both the treated pups as adults and the dams. *The Journal of Pharmacology and Experimental Therapeutics, 317,* 1210–1218. http://dx.doi.org/10.1124/jpet.106.101139

Moriceau, S., Roth, T. L., & Sullivan, R. M. (2010). Rodent model of infant attachment learning and stress. *Developmental Psychobiology, 52,* 651–660. http://dx.doi.org/10.1002/dev.20482

Moriceau, S., Wilson, D. A., Levine, S., & Sullivan, R. M. (2006). Dual circuitry for odor-shock conditioning during infancy: Corticosterone switches between fear and attraction via amygdala. *The Journal of Neuroscience, 26,* 6737–6748. http://dx.doi.org/10.1523/JNEUROSCI.0499-06.2006

Neumann, I. D. (2002). Involvement of the brain oxytocin system in stress coping: Interactions with the hypothalamo-pituitary-adrenal axis. *Progress in Brain Research, 139,* 147–162. http://dx.doi.org/10.1016/S0079-6123(02)39014-9

Neumann, I. D., Krömer, S. A., Toschi, N., & Ebner, K. (2000). Brain oxytocin inhibits the (re)activity of the hypothalamo-pituitary-adrenal axis in male rats: Involvement of hypothalamic and limbic brain regions. *Regulatory Peptides, 96,* 31–38. http://dx.doi.org/10.1016/S0167-0115(00)00197-X

O'Donnell, K., O'Connor, T. G., & Glover, V. (2009). Prenatal stress and neurodevelopment of the child: Focus on the HPA axis and role of the placenta. *Developmental Neuroscience, 31,* 285–292. http://dx.doi.org/10.1159/000216539

Raineki, C., Cortés, M. R., Belnoue, L., & Sullivan, R. M. (2012). Effects of early-life abuse differ across development: Infant social behavior deficits are followed by adolescent depressive-like behaviors mediated by the amygdala. *The Journal of Neuroscience, 32,* 7758–7765. http://dx.doi.org/10.1523/JNEUROSCI.5843-11.2012

Raineki, C., Moriceau, S., & Sullivan, R. M. (2010). Developing a neurobehavioral animal model of infant attachment to an abusive caregiver. *Biological Psychiatry, 67,* 1137–1145. http://dx.doi.org/10.1016/j.biopsych.2009.12.019

Revillo, D. A., Molina, J. C., Paglini, M. G., & Arias, C. (2013). A sensory-enhanced context allows renewal of an extinguished fear response in the infant rat. *Behavioural Brain Research, 253,* 173–177. http://dx.doi.org/10.1016/j.bbr.2013.07.027

Roth, T. L. (2012). Epigenetics of neurobiology and behavior during development and adulthood. *Developmental Psychobiology, 54,* 590–597. http://dx.doi.org/10.1002/dev.20550

Roth, T. L., Zoladz, P. R., Sweatt, J. D., & Diamond, D. M. (2011). Epigenetic modification of hippocampal BDNF DNA in adult rats in an animal model of post-traumatic stress disorder. *Journal of Psychiatric Research, 45,* 919–926. http://dx.doi.org/10.1016/j.jpsychires.2011.01.013

Sanchez, M., Ladd, C. O., & Plotsky, P. M. (2001). Early adverse experience as a developmental risk factor for later psychopathology: Evidence from rodent and primate models. *Development and Psychopathology, 13,* 419–449. http://dx.doi.org/10.1017/s0954579401003029

Skinner, M. K. (2008). What is an epigenetic transgenerational phenotype? F3 or F2. *Reproductive Toxicology, 25,* 2–6. http://dx.doi.org/10.1016/j.reprotox.2007.09.001

Skinner, M. K., Manikkam, M., & Guerrero-Bosagna, C. (2010). Epigenetic transgenerational actions of environmental factors in disease etiology. *Trends in Endocrinology and Metabolism, 21,* 214–222. http://dx.doi.org/10.1016/j.tem.2009.12.007

Viviani, D., Charlet, A., van den Burg, E., Robinet, C., Hurni, N., Abatis, M., . . . Stoop, R. (2011). Oxytocin selectively gates fear responses through distinct outputs from the central amygdala. *Science, 333,* 104–107. http://dx.doi.org/10.1126/science.1201043

von Poser Toigo, E., Diehl, L. A., Ferreira, A. G., Mackedanz, V., Krolow, R., Benitz, A. N., . . . Dalmaz, C. (2012). Maternal depression model: Long-lasting effects on the mother following separation from pups. *Neurochemical Research, 37,* 126–133. http://dx.doi.org/10.1007/s11064-011-0590-3

Waters, S. F., West, T. V., & Mendes, W. B. (2014). Stress contagion: Physiological covariation between mothers and infants. *Psychological Science, 25,* 934–942. http://dx.doi.org/10.1177/0956797613518352

Welberg, L. A., Thrivikraman, K. V., & Plotsky, P. M. (2005). Chronic maternal stress inhibits the capacity to up-regulate placental 11β-hydroxysteroid dehydrogenase type 2 activity. *The Journal of Endocrinology, 186,* R7–R12. http://dx.doi.org/10.1677/joe.1.06374

Yap, C. S., & Richardson, R. (2007). Extinction in the developing rat: An examination of renewal effects. *Developmental Psychobiology, 49,* 565–575. http://dx.doi.org/10.1002/dev.20244

Yehuda, R., Bell, A., Bierer, L. M., & Schmeidler, J. (2008). Maternal, not paternal, PTSD is related to increased risk for PTSD in offspring of Holocaust survivors. *Journal of Psychiatric Research, 42,* 1104–1111. http://dx.doi.org/10.1016/j.jpsychires.2008.01.002

Zuluaga, M. J., Agrati, D., Uriarte, N., & Ferreira, A. (2014). Social aversive stimuli presented to the mother produce the precocious expression of fear in rat pups. *Developmental Psychobiology, 56,* 1187–1198. http://dx.doi.org/10.1002/dev.21199

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