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Acute Early-Life Stress Results in Premature Emergence of Adult-Like Fear Retention and Extinction Relapse in Infant Rats

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Running Head: Acute Stress and Emotional Learning in Infant Rats

Abstract

Recent studies have shown that chronic early-life stress results in precocious expression of the adult-like phenotype of fear retention and inhibition. However, it is unknown whether the experience of acute early trauma has the same effects as exposure to chronic early stress. In the present study, a 24 h period of maternal deprivation on postnatal day (P) 9 was used as an acute early-life stressor. In infancy (P16-17), maternally-deprived and standard-reared rats were conditioned to fear a noise paired with shock. In Experiments 1 and 2, fear to the noise was then extinguished before rats were tested for context-mediated fear renewal or stress-induced fear reinstatement. In Experiments 3a and 3b, conditioned rats were tested for fear retention 1, 7, or 14 days after training. Whereas standard-reared infants exhibited relapse-resistant extinction and infantile amnesia (i.e., behaviors typical of their age), maternally-deprived infants exhibited the renewal and reinstatement effects (i.e., relapse-prone extinction) and showed good retention of fear over the 7- and 14-day intervals (i.e., infantile amnesia was reduced). In other words, similar to rats exposed to chronic early-life stress, rats exposed to acute early stress expressed an adult-like profile of fear retention and inhibition during infancy. These findings suggest that similar mechanisms might be involved in the effects of acute and chronic stress on emotional development, and may have implications for our understanding and treatment of emotional disorders associated with early adversity.

Key Words: maternal deprivation, development, fear conditioning, extinction, infantile amnesia

The detrimental effects of early-life adversity on mental health, in particular the increased risk for mood and anxiety disorders, are well-documented (Kessler, Davis, & Kendler, 1997). However, a limitation of the existing research is the focus on chronic sources of early stress, such as childhood abuse, neglect, or institutionalization (e.g., Cicchetti & Toth, 2005; Heim & Nemeroff, 1999; Nelson III et al., 2007). It has been suggested that single-event and repetitive traumas are categorically different (Terr, 1991). Yet, relative to what is known about the effects of chronic stress on emotional functioning, we know far less about the effects of discrete traumas on emotional functioning, especially early in life. Because early-life appears to be a 'critical-period' of vulnerability to mental health problems (Kessler et al., 1997), understanding the effects of acute trauma on emotional functioning early in life might aid in the development of more effective treatments for mental health problems across the lifespan.

We have recently demonstrated that chronic early-life stress affects emotional learning in infant rats, resulting in behaviors which might put them at greater risk for anxiety (Callaghan & Richardson, 2011, 2012). Specifically, using a rodent model of infant neglect (maternal separation, 3 h per day from post-natal days [P] 2-14), we examined the effect of an adverse rearing environment on two forms of emotional learning known to be disrupted in individuals with anxiety disorders, fear retention and fear extinction (Graham & Milad, 2011; Shin & Liberzon, 2010). Under normal rearing conditions, infant rats exhibit rapid forgetting of learned associations, a ubiquitous, cross-species phenomenon referred to as infantile amnesia (for review, see Campbell & Spear, 1972; Josselyn & Frankland, 2012). In addition, infant rodents typically do not demonstrate fear relapse following extinction training (for review, see Kim & Richardson, 2010). In other words, the infant emotional learning phenotype is characterized by rapid forgetting and relapse-resistant extinction of fear memories. Initially, these findings appear somewhat contradictory to epidemiological

evidence that early life is a period of vulnerability to anxiety. However, we have shown that expression of the infant emotional learning phenotype is modulated by early experiences. Specifically, we (Callaghan & Richardson, 2011, 2012) demonstrated that infant rats exposed to chronic early-life stress exhibit longer retention of fear memories and greater relapse following extinction training, behaviors typical of fear learning and extinction in adults. Considering the ecological niche inhabited by infants (e.g., infants are generally protected from external threats and their needs provided for by a caregiver), precocious transition to the adult emotional learning phenotype might result in inappropriate expression of fear, which could help to explain the increased incidence of anxiety amongst those exposed to early trauma. In the present study we examined whether adult-like emotional learning is also exhibited by infants exposed to an acute early-life stressor.

In order to examine the impact of *acute* early-life stress, an alternate form of the maternal separation procedure, maternal deprivation (MD), has been used in some studies. In contrast to the chronic procedure, MD involves a single, 24 h separation of pups from dams. Deprivation typically takes place during the *stress hyporesponsive period* (SHRP; a period of attenuated hypothalamic pituitary adrenal [HPA] axis responses to stress occurring across P4-14) and has both short- and long-term consequences for deprived animals. In the short-term, MD has been shown to disrupt the SHRP; rat pups exhibit significant increases in corticosterone (CORT; the main glucocorticoid in rats) during deprivation, as well as augmented HPA axis responses to stress during deprivation and after reunion with the mother (Levine, Huchton, Wiener, & Rosenfeld, 1991; Rosenfeld, Wetmore, & Levine, 1992; Suchecki, Nelson, Van Oers, & Levine, 1995). In the long-term, this procedure has been shown to increase animals' psychological vulnerability. For example, adult maternally-deprived rats exhibit schizophrenia-like symptoms and heightened anxiety (Barbosa Neto et al., 2012; Ellenbroek & Riva, 2003; Faturi et al., 2010). However, to our knowledge there

have been no studies examining the behavioral impact of MD during infancy. This line of investigation may provide insight into the mechanism underlying the impact of acute early stress on psychological vulnerability and enable the identification of similarities or differences between animals exposed to chronic and acute early-life stress. In the current series of experiments we examined whether MD results in an early transition between the infant and adult-like emotional learning systems.

General Method

Subjects

Subjects were experimentally naïve male Sprague-Dawley derived rats, bred and housed in the School of Psychology at The University of New South Wales. Rats were housed with their dam and littermates (culled to a maximum of 8 pups per litter) and maintained on a 12-hour light/dark cycle (lights on at 0700) with food and water available *ad libitum*. No more than one rat from each litter was allocated to any given experimental group. All animals were treated in accordance with *The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 7th Edition* (2004), and all procedures were approved by the Animal Care and Ethics Committee at The University of New South Wales.

Maternal Deprivation

All animals from a given litter were assigned to either the maternal deprivation (MD) or standard-rearing (SR) condition. On postnatal day (P) 9, MD pups were separated from their dams. All pups in each MD litter were removed from the home cage, weighed, and placed together in an incubator maintained at approximately 27 °C by a heat pad. Three centimetres of bedding was provided so pups could behaviorally thermoregulate as needed. Neither food nor water was provided to pups during the deprivation period, as they do not independently ingest solids or water at this age. At the end of the deprivation period (24 hours later), pups were weighed and returned to their dam. SR litters were not separated from

their dam for any extended period of time. In Experiments 1, 2, and 3a, SR litters were exposed to the same handling cues as MD rats (i.e., they were weighed on P9 and 10).

Apparatus

Two types of chambers were used to produce distinct contexts that differed in terms of both size and visual characteristics. One type, Context A, was a set of two identical rectangular chambers (13.5 cm long x 9 cm wide x 9 cm high). The front wall, rear wall, and ceiling were constructed of clear Plexiglas, while the floor and side walls consisted of stainless steel rods spaced 1 cm apart. A shock could be delivered through the floor by a custom-built constant-current shock generator. Two high-frequency speakers were fitted on either side of the chamber. The second type, Context B, was a set of two identical rectangular chambers (30 cm long x 30 cm wide x 23 cm high). The ceiling and walls were clear Plexiglas, while the floor was made of stainless steel rods spaced 1 cm apart. The two side walls were covered by a pattern of 5 cm wide vertical black and white stripes. Two high-frequency speakers were positioned on the ceiling of the chamber.

Experimental chambers were individually housed in separate wood cabinets to minimize external noise and visual stimulation. Each cabinet was fitted with a white light-emitting diode (LED), a red LED, and an infrared camera to allow sessions to be recorded. However, in Context A, the white LED was switched off, so the red LED provided the sole source of illumination in those chambers. Both white and red LEDs were used in Context B. Cabinets were also equipped with ventilation fans that produced a constant low-level (50 dB) background noise. Chambers were wiped clean with tap water after each experimental session.

Scoring, Exclusions, & Statistics

Freezing was scored using a time sampling procedure whereby each rat was scored every 3 s as freezing or not freezing. Freezing was defined as the absence of all movement

except that required for respiration (Fanselow, 1980). Percentage freezing scores were calculated for each subject to indicate the proportion of total observations scored as freezing. A random sample (30%) of the test data (or difference score data, as appropriate) was cross-scored by a second observer unaware of the experimental condition of each rat. Inter-rater reliability was very high across all experiments, r_{50} = .93.

As high levels of pre-CS freezing make it difficult to detect CS-elicited freezing, any rat that exhibited > 60% pre-CS freezing was excluded from the final analysis. Additionally, any rats that were statistical outliers (i.e., > 3 *SD* from the group mean) at test were excluded from the final analysis. This resulted in the following exclusions: in Experiment 1, 1 rat from Group SR-same, 1 rat from Group MD-same, and 2 rats from Group SR-different; in Experiment 2, 1 rat from Group MD-US exposure; in Experiment 3b, 1 rat from Group SR-14 days.

For all analyses, values of $p < .05$ were considered statistically significant. Where the assumption of sphericity was violated, the Greenhouse-Geisser procedure was used and the reported *p* values modified accordingly (but the nominal *df* are reported in these cases).

Results

Pup Weight

In Experiments 1, 2, and 3a, pups were weighed on P9 (prior to MD), P10 (after MD), and P17. Collapsed across these experiments, maternal deprivation resulted in significant changes to pups' weight (shown in Table 1), which is consistent with previous studies (e.g., Rentesi et al., 2010). There were no differences between groups prior to MD on P9. However, immediately after the deprivation period (i.e., P10), MD pups were lighter than SR pups and this difference was maintained at P17. This description was confirmed by statistical analysis; there were significant main effects of day, rearing condition, and a significant day x rearing condition interaction, smallest $F(1, 45) = 19.89$, $p < .001$. Pairwise comparisons revealed

significant differences on P10, $t(45) = 4.01$, $p < .001$, and on P17, $t(45) = 8.72$, $p < .001$; there was no difference between rearing conditions on P9, $t(45) = -1.49$, $p = .14$.

Experiment 1

Recent work from our laboratory has shown that, unlike SR infants, rats that have experienced chronic stress early in life exhibit adult-like, relapse-prone extinction as infants (Callaghan & Richardson, 2011). In Experiment 1, we examined whether an acute form of early-life stress would have a similar impact on extinction retention. Specifically, we tested whether a single, 24 h episode of maternal deprivation on P9 would cause infant rats to precociously express context-mediated renewal of fear following extinction training.

Method

A 2 x 2 between-subjects design was employed, with the factors referring to rearing condition (MD or SR) and test context (same or different to the extinction context). On Day 1, P17 rats were conditioned in Context A. After a 2 min adaptation period, six pairings of a white noise CS (8 db above background, 10 s in duration) and a shock US (0.6 mA, 1 s) were presented. The US was administered in the final second of the CS, and the intertrial interval (ITI) ranged from 85 – 135 s with a mean of 110 s. Thirty to 60 s after the final CS-US pairing, rats were returned to their home cages. On Day 2, rats were placed in Context B and after a 2 min adaptation period received 30 non-reinforced presentations of the 10 s CS with a 10 s ITI. Thirty to 60 s after the last CS presentation, rats were returned to their home cages. On Day 3, animals were tested in either the same context (B) or a different context (A) to extinction training. Levels of freezing were recorded throughout a 1 min adaptation period (baseline) and a 2 min presentation of the CS.

Results and Discussion

A significant difference was observed between rearing conditions in pre-CS freezing at extinction, $M_{SR} = 6.83$, $M_{MD} = 26.46$; $t(45) = 3.64$, $p = .001$. Due to this difference,

baseline freezing was used as a covariate in the subsequent ANCOVA of CS-elicited freezing during extinction. However, the same results were obtained if the data were analyzed without the covariate. Freezing to the CS decreased across the 5 blocks of extinction training, $F(4, 176) = 5.01$, $p = .002$, as shown in Figure 1a. The main effect of rearing condition and the rearing condition x extinction block interaction were not significant, largest $F(4, 176) = 1.11$, $p = .35$. In other words, rearing condition did not affect levels of conditioned fear or the rate of extinction.

Baseline freezing at test is presented in Table 2. The effect of context and the rearing condition x context interaction were non-significant, largest $F(1, 43) = 2.39$, $p = .13$. There was a trend toward a significant effect of rearing condition, $F(1, 43) = 4.01$, $p = .052$. Given this trend, test data were analyzed using ANOVA with no covariate, ANCOVA with baseline freezing as a covariate, and ANOVA using difference scores (freezing during the CS minus baseline freezing). The same results were obtained regardless of the method of analysis used, so results are presented using raw test scores with no covariate. To further address this concern, a supplementary analysis was conducted in which 2 rats in the MD-different group that exhibited the highest levels of baseline freezing were excluded. This led to a reduction in mean baseline freezing for this group (adjusted $M_{baseline} = 6.00$) but no change in the level of freezing at test (adjusted $M_{test} = 43.62$). Analysis of these data demonstrated that the effect of rearing condition on baseline freezing was not significant, $F(1, 41) = 1.75$, $p = .19$, but did not change the results of the analysis of the test data.

All rats exhibited low levels of CS-elicited freezing when tested in the same context as extinction training (see Figure 1b). However, in the different context, levels of freezing differed between rearing conditions. Whereas SR rats exhibited the same low levels of freezing in both contexts, MD rats exhibited higher levels of freezing in the different context. In other words, while P17 SR rats did not exhibit context-mediated renewal of fear, MD

infant rats did. The statistical analysis confirmed this description of the data; there were significant main effects of rearing condition, $F(1, 43) = 11.69$, $p = .001$, test context, $F(1, 43) = 16.53$, $p < .001$, and a significant rearing condition x test context interaction, $F(1, 43) = 5.65$, $p = .02$. Follow-up comparisons revealed that SR rats showed similar low levels of freezing in both contexts, $t(22) = -1.79$, $p = .09$, whereas MD animals showed significantly higher levels of freezing in the different context compared to the same context, $t(21) = -3.69$, $p = .002$.

In Experiment 1 we replicated previous research showing that standard-reared infant rats do not exhibit the renewal effect (Callaghan & Richardson, 2011; Kim & Richardson, 2007; Yap & Richardson, 2007). Of greater interest is the finding that an acute episode of stress, a 24-hour period of maternal deprivation, during the SHRP results in precocious expression of context-mediated renewal. Given that rearing condition did not affect CS-elicited freezing during extinction, this result was not due to maternal deprivation increasing levels of conditioned fear or impairing within-session extinction. This experiment demonstrates that an acute stressor early in life increases the vulnerability of infant rats to exhibit relapse of extinguished fear, similar to the effect of chronic early-life stress (Callaghan & Richardson, 2011).

Experiment 2

In Experiment 1, we showed that acute maternal deprivation resulted in the early emergence of context-mediated renewal. The aim of Experiment 2 was to determine whether this finding reflected a general transition from the infant, relapse-resistant extinction phenotype to the adult-like, relapse-prone extinction phenotype. To do that, we tested the hypothesis that MD rats, but not SR rats, would exhibit reinstatement of extinguished fear following a post-extinction reminder treatment (exposure to a shock US).

Method

A 2 x 2 between-subjects design was employed, with the factors referring to rearing condition (MD or SR) and reminder treatment (context- or US-exposure). In order to equate age at test with Experiment 1, all rats were 16 days old at the start of the experiment. Subjects were conditioned in Context A on Day 1 and received extinction training in Context B on Day 2, as per the procedures in Experiment 1. However, in this experiment all rats were placed back into Context B on Day 3 for a period of 2 min 30 s where they were either exposed to a 0.4 mA reinstating footshock after 2 mins (group US-exposure) or not (group context-exposure). All rats were tested in Context B on Day 4 using the same procedure described in Experiment 1.

Results and Discussion

The effect of rearing condition on pre-CS freezing at extinction was not significant, M_{SR} = 19.20, M_{MD} = 15.90; $t(31) = .57$, $p = .58$. As in Experiment 1, rearing condition had no effect on levels of conditioned fear expressed at the start of extinction training or on the rate of extinction. CS-elicited freezing decreased across extinction blocks, $F(4, 124) = 20.58$, $p < .001$, as shown in Figure 2a. The effect of rearing condition and the rearing condition x extinction block interaction were not significant, largest $F(4, 124) = 0.73$, $p = .54$.

Levels of pre-CS freezing at test are presented in Table 2. The effect of rearing condition and the rearing condition x reminder treatment interaction were non-significant, largest $F(1, 29) = 3.57$, $p = .07$. However, there was a significant effect of reminder treatment such that animals in the US-exposure groups exhibited higher levels of pre-CS freezing compared to animals in the context-exposure groups, $F(1, 29) = 14.49$, $p = .001$. Because of the differences in pre-CS freezing, test data are presented as difference scores (per cent freezing during the CS minus per cent freezing during baseline). The same results were obtained if the data were analyzed by ANCOVA.

CS-elicited freezing at test is presented in Figure 2b. Regardless of reminder treatment, SR rats exhibited low levels of freezing at test. In contrast, MD rats exhibited much higher levels of freezing following a post-extinction reminder shock (US-exposure group) than after mere exposure to the context. In other words, infant MD rats exhibited clear reinstatement of extinguished fear whereas the SR infants did not. The statistical analysis confirmed this description of the data; there were significant main effects of rearing condition, $F(1, 29) = 7.72$, $p = .009$, reinstatement, $F(1, 29) = 6.09$, $p = .02$, and a significant rearing condition x reinstatement interaction, $F(1, 29) = 5.34$, $p = .028$. Follow-up comparisons revealed that SR rats showed similar, low levels of freezing following context- or US-exposure, $t(14) = -.11$, $p = .91$, whereas MD US-exposure rats exhibited significantly higher levels of freezing than MD context-exposure rats, $t(15) = -3.31$, $p = .005$.

Thus, in Experiment 2 we replicated past research showing that SR infant rats do not exhibit reinstatement of extinguished fear (Callaghan & Richardson, 2011; Kim & Richardson, 2007). More importantly, we demonstrated that MD infants exhibit reinstatement of extinguished fear following a post-extinction reminder shock. This result is consistent with the finding from Experiment 1 that MD infants exhibit the renewal effect and supports the conclusion that depriving rats of maternal care for a single 24 h period on P9 increases rates of fear relapse during infancy. In other words, acute early-life stress results in precocious expression of the adult-like, relapse-prone extinction phenotype in infant rats, similar to the effects of chronic maternal separation (Callaghan & Richardson, 2011).

Experiment 3a

In Experiments 1 and 2 we demonstrated that an acute, 24 h episode of maternal deprivation results in an early transition to the adult-like, relapse-prone extinction phenotype, similar to past findings using chronic stress. As mentioned earlier, chronic stress also reduces expression of infantile amnesia in rats (i.e., stress increases retention over extended intervals;

Callaghan & Richardson, 2012). For this reason, in Experiment 3a we examined whether MD rats would also exhibit an early transition to the adult-like memory system, resulting in extended retention of fear memories over a 7 day interval.

Method

A 2 x 2 between-subjects design was employed, with the factors referring to rearing condition (MD or SR) and retention interval (1 day or 7 days). P17 rats were conditioned in Context A on Day 1, as per Experiment 1. Rats were then tested at one of two intervals, either 1 day or 7 days later. Testing took place in context A using the same procedure described in Experiment 1.

Results and Discussion

Significant differences in pre-CS freezing were detected at test (see Table 2 for pre-CS means). Specifically, there was a significant effect of retention interval such that animals exhibited higher levels of pre-CS freezing at the 1 day test than at the 7 day test, $F(1, 50) = 7.44$, $p = .009$. The effect of rearing condition and the rearing condition x retention interval interaction were non-significant, largest $F(1, 50) = 2.13$, $p = .15$. As in Experiment 2, test data are presented as difference scores due to the differences in pre-CS freezing. However, the same results were obtained if the data were analyzed by ANCOVA.

CS-elicited freezing at test is presented in Figure 3. Observation of the figure suggests that all rats exhibited high levels of CS-elicited freezing, or good retention, 1 day after conditioning. In contrast, at the 7 day interval freezing in the MD rats remained high, whereas the SR rats showed low levels of freezing, suggesting that only the SR rats had forgotten. The statistical analysis was supportive of this description; the effect of retention interval was not significant, $F(1, 50) = 0.43$, $p = .52$, but there was a significant main effect of rearing condition, $F(1, 50) = 4.27$, $p = .04$, and a significant rearing condition x retention interval interaction, $F(1, 50) = 4.79$, $p = .03$. Follow-up comparisons revealed that MD animals

showed similar, high levels of freezing at the 1 day and 7 day tests, $t(26) = -1.07$, $p = .29$. In the SR groups, there was a trend towards animals exhibiting lower levels of freezing at the 7 day test compared to the 1 day test, $t(24) = 2.00$, $p = .059$. Hence, although the data were in the hypothesised direction, no firm conclusions regarding the occurrence of forgetting in one group over the other could be drawn. In order to see whether MD infant rats could retain a fear memory that SR infants had forgotten a further experiment was conducted testing retention performance in MD and SR infants at a longer interval.

Experiment 3b

Experiment 3b was designed to test whether MD infant rats would continue to exhibit good fear retention after a longer interval (14 days) in which SR infants would likely exhibit more pronounced forgetting.

Method

The methods used were exactly the same as in Experiment 3a except that animals were tested either 1 day or 14 days after training.

Results and Discussion

Similar to Experiment 3a, animals exhibited significantly higher levels of pre-CS freezing at the 1 day test than at the 14 day test, $F(1, 26) = 9.90$, $p = .004$ (see Table 2 for pre-CS means). The effect of rearing condition and the rearing condition x retention interval interaction for pre-CS freezing were non-significant, largest $F(1, 26) = 0.49$, $p = .49$.

Test data are presented as difference scores due to differences in pre-CS freezing, consistent with Experiment 3a, though the same results were obtained if the data were analyzed by ANCOVA. SR infants exhibited high levels of CS-elicited freezing (good retention) 1 day after training, but low levels of CS-elicited freezing (poor retention) 14 days after training (see Figure 4). In contrast, MD infants exhibited high levels of CS-elicited freezing, or good retention, at both intervals. This description of the data was confirmed by

the statistical analysis. The effect of rearing condition was non-significant, $F(1, 26) = 0.95$, $p = .34$, but there was a significant effect of retention interval, $F(1, 26) = 6.25$, $p = .019$, and a significant rearing condition x retention interval interaction, $F(1, 26) = 6.23$, $p = .019$. Follow-up comparisons showed that MD rats exhibited similar, high levels of freezing at the 1 day and 14 day tests, $t(13) = 0.002$, $p = .998$. On the other hand, freezing at the 14 day interval was significantly lower than at the one day interval in the SR rats, $t(13) = 3.95$, $p = .002$.

In Experiments 3a and 3b we replicated prior research demonstrating that SR rats exhibit rapid forgetting of a fear association when trained on P17 (e.g., Callaghan & Richardson, 2012; Campbell & Campbell, 1962; Kim, McNally, & Richardson, 2006). Secondly, and more importantly, we showed that rats subjected to a 24 h period of maternal deprivation on P9 do not exhibit infantile amnesia over extended retention intervals of up to 14 days. Rather, MD rats trained on P17 exhibit excellent retention of fear memories, similar to the findings reported in infant rats exposed to chronic forms of early-life stress (Callaghan & Richardson, 2012).

General Discussion

The results of this set of experiments demonstrate that an acute episode of early-life stress, maternal deprivation for 24 h on P9, causes a precocious transition from the infant to adult-like phenotype of fear memory retention and extinction. In a replication of previous findings in infant rodents, the standard-reared (SR) infants in the current experiments exhibited infantile amnesia and resistance to fear relapse following extinction (i.e., did not show the renewal and reinstatement effects). In contrast to SR infants, however, the maternally-deprived (MD) rats exhibited good fear retention and relapse-prone extinction (i.e., renewal and reinstatement of extinguished fear). These results cannot be attributed to differences in rates of learning or extinction, as SR and MD infants expressed similar levels

of freezing at the start and end of extinction training (Experiments 1 and 2), as well as in the 1 day retention test (Experiments 3a and 3b).

The present findings fit with recent research (Callaghan & Richardson, 2011, 2012) showing that chronic early stress, in the form of daily maternal separation, accelerates the transition to adult-like emotional learning. In those experiments, rats were separated from their mothers for 3 h a day from P2-14 and trained on P17 to fear a white noise CS. It was shown that maternally-separated rats exhibited good retention of fear memories for up to 30 days after training (Callaghan & Richardson, 2012). Further, maternally-separated rats displayed fear relapse when tested in a context that differed from the extinction context (i.e., renewal of fear) and when given a post-extinction reminder treatment (i.e., reinstatement of fear; Callaghan & Richardson, 2011). In other words, chronic maternal separation resulted in relapse-prone extinction and better fear retention during infancy, much like what was observed in the MD rats in the present studies.

It is unclear what the consequences of a precocious transition to the adult-like phenotype of fear retention and extinction might be. One could speculate that the accelerated development of these processes may be adaptive in the context of stressful rearing conditions; the ability to remember aversive events for longer and exhibit relapse in new contexts or after secondary aversive events might be advantageous for animals reared in an environment with low or unreliable parental presence. However, such changes, even if they are adaptive in the short-term, are likely to come at a cost. Specifically, early maturation of some learning tasks might interfere with later learning or create competition for neural systems which are developing at the same time (Bjorklund, 1997). The importance of developmental timing has been elegantly demonstrated in studies of perceptual systems. For example, unusual early (prenatal) stimulation of the visual system was shown to accelerate visual development in quail chicks but also interfered with auditory preferences indicative of species-typical

attachment (Lickliter, 1990). Similarly, reductions in responding to olfactory cues have been demonstrated in rats following surgical opening of pups' eyelids to allow premature visual stimulation (Kenny & Turkewitz, 1986). While it is unclear which specific brain regions or behaviors might be compromised as a result of precocious emotional learning maturation, the ultimate consequence of this increased neural competition during development may be reflected in the heightened incidence of schizophrenic- and anxiety-like symptoms in MD adults (Barbosa Neto et al., 2012; Ellenbroek & Riva, 2003; Faturi et al., 2010; Rentesi et al., 2010).

In our earlier work (Callaghan & Richardson, 2011), we suggested that the effects of chronic early-life stress on fear retention and extinction might be the result of accelerated development of the brain regions involved in expression of these behaviors (i.e., the amygdala, prefrontal cortex, and hippocampus). While in the present experiments it is unlikely that these emotion circuits would mature over a single 24 h period of stress, both chronic and acute forms of maternal separation are known to disrupt the *stress hyporesponsive period* (SHRP), resulting in increased basal and stress-induced levels of CORT secretion (Gareau, Jury, Yang, MacQueen, & Perdue, 2006; Levine et al., 1991; Suchecki et al., 1995). It is possible that these stress-induced increases in glucocorticoid release might expose young animals to a level of CORT which exceeds a threshold necessary to initiate a cascade of changes culminating in accelerated neural maturation and an early transition to the adult-like emotional learning systems. Indeed, we have previously demonstrated (Callaghan & Richardson, 2012) that elevation of CORT levels during the SHRP is sufficient to cause the early transition to adult-like emotional learning. In that study, we administered CORT to nursing mothers through the drinking water when pups were P2-14 and found that CORT treatment mimicked the effects of maternal separation. Specifically, the offspring of CORT-treated mothers exhibited good fear retention over a 10 day interval when

conditioned on P17. Unpublished data from our lab also shows that this CORT treatment leads to precocious expression of fear relapse following extinction training (Callaghan & Richardson, in prep). Furthermore, past work by Sullivan and colleagues suggests that early exposure to CORT causes premature maturation of a different form of emotional learning, odor avoidance. Normally, rat pups exhibit an approach response to odors paired with aversive stimuli on P8, a paradoxical response thought to encourage attachment to the primary caregiver (Camp & Rudy, 1988; Moriceau, Wilson, Levine, & Sullivan, 2006). This period of enhanced approach learning is short-lived; by P12 rat pups typically exhibit the adult-like avoidance response to odors previously paired with an aversive stimulus. These distinct behavioral responses are associated with distinct patterns of brain activation (Moriceau et al., 2006). However, early exposure to a stressful rearing environment (or exogenous CORT) causes rats to precociously express the mature odor avoidance response and engage the mature neural circuitry at P8 (Moriceau, Shionoya, Jakubs, & Sullivan, 2009; Moriceau et al., 2006). Further, reducing CORT exposure through injections of a CORT antagonist or adrenalectomy delays expression of odor avoidance in P12 rats and prevents the premature transition to mature responding in P8 rats subjected to stress or exogenous CORT (Moriceau et al., 2009; Moriceau et al., 2006), indicating that exposure to glucocorticoids is both necessary and sufficient to terminate the “sensitive period” of paradoxical attachment seeking.

Considering that a single infusion of CORT was sufficient to cause the precocious expression of avoidance responses to an odor paired with shock, Moriceau et al. (2006) suggested that the transition between the two odor-learning circuits is not dependent on neuroanatomical maturation but is better described as a developmental “switch” regulated by CORT exposure. In other words, animals are equipped to respond with the mature system but require an environmental or endogenously generated cue to do so. A similar explanation may

account for the present results. Specifically, infant and adult-like phenotypes of fear retention and extinction also rely on distinct neural circuits. In adults, expression of learned fear and inhibition of fear following extinction both require activation of the medial prefrontal cortex (mPFC; Sotres-Bayon & Quirk, 2010). In contrast, it appears that infants do not engage the mPFC during expression of learned fear (Li, Kim, & Richardson, 2012) nor during extinction retrieval (Kim, Hamlin, & Richardson, 2009), despite evidence showing that the PFC is functionally active at this stage of development (Nair, Berndt, Barrett, & Gonzalez-Lima, 2001). Hence, it may be the case that the developmental switch signaling the transition between the fear retention and extinction systems is also mediated by exposure to stress or stress hormones.

Although we used maternal deprivation as a model of acute stress in the current studies, it is necessary to consider the possibility that maternal deprivation may act like a chronic stressor through long-term disruptions to normal mother-pup interactions. For example, the extended period of separation may permanently alter maternal hormone levels or behavior, including disruptions to lactation or feeding. While we are not aware of any studies that directly examine changes to maternal behavior or CORT levels in response to deprivation, this possibility is supported by our finding that MD pups do not recover weight lost during deprivation (see Table 1). However, there is some evidence to suggest that loss of maternal care *during* the deprivation period is important for changes to HPA axis function. Specifically, mimicry of certain maternal behaviors (i.e., licking and nursing) via manual stroking and feeding of pups during maternal deprivation has been shown to prevent deprivation-induced rises in ACTH and CORT (Suchecki, Rosenfeld, & Levine, 1993). While these results do not exclude the possibility that mother-pup interactions are disrupted upon reunion, they do suggest that the acute loss of maternal cues is an important factor in

HPA axis dysfunction, which we have suggested may be critical for the behavioral changes observed in MD animals.

To further challenge the suggestion that maternal deprivation is a chronic stressor "in disguise", it has been shown that MD rats exhibit neuroendocrine changes that are both distinct from the changes seen in chronically separated rats and similar to those seen in humans that have experienced acute stress. While both acute deprivation and chronic separation disrupt the SHRP and result in hyper-activation of the HPA axis in the short-term (Gareau et al., 2006; Levine et al., 1991; Rosenfeld et al., 1992), rats exposed to these two procedures exhibit divergent profiles of HPA axis responding when tested later in life. Maternally-separated rats continue to exhibit exaggerated HPA axis responses to stress into adulthood, similar to the profile seen in depressed individuals and those with a history of early-life abuse (Heim, Newport, Bonsall, Miller, & Nemeroff, 2001; Holsboer, 2001; Ladd et al., 2000). However, the HPA axis profile of MD rats appears to change, and even reverse, with age. Specifically, it has been shown that, as early as P20, rats exposed to maternal deprivation during the SHRP exhibit *hyporeactivity* of HPA axis responses to stress, with some hyporeactivity exhibited until at least P60 (Suchecki & Tufik, 1997; Van Oers, De Kloet, & Levine, 1998; Van Oers, De Ronald Kloet, & Levine, 1997). This pattern of HPA axis suppression bears similarities to the neuroendocrine profile of post-traumatic stress disorder (PTSD), which is characterized by low basal cortisol levels (Yehuda, 2001).

Although the chronic maternal separation and acute maternal deprivation procedures appear to result in distinct HPA response profiles, and may therefore model different disorders, the present results suggest that both procedures lead to the same behavioral outcomes in infancy (i.e., accelerated maturation of fear retention and extinction). Thus, it is possible that accelerated development of emotional learning during infancy could be a general risk factor for a range of mental health problems. This fits with the suggestion that

particular experiences increase vulnerability to a range of problems rather than increasing risk for specific outcomes (e.g., Kessler et al., 1997; McLaughlin et al., 2010). For example, Kessler et al. (1997) examined a range of acute and chronic childhood adversities and found consistent associations between adversity and the onset of various psychological disorders. However, there was little evidence to suggest unique associations between specific childhood adversities and specific disorders. At the molecular level, a recent genome-wide analysis identified a number of shared risk loci for five major psychological disorders (autism spectrum disorder, attention deficit-hyperactivity disorder, bipolar disorder, major depressive disorder, and schizophrenia; Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013), which suggests that other risk factors, including genetic factors, may not be unique to particular disorders but increase psychological vulnerability in general.

In summary, we have shown that an acute episode of stress during the SHRP results in precocious expression of an adult-like phenotype of fear retention and inhibition. This implies that one of the factors that may be associated with increased risk of mental health problems following acute early stress in humans is that such stress may affect the development of emotional responding systems. This question should be the focus of future translational work. Such developmental alterations are of clinical interest because they suggest that treatments such as exposure-based therapy might be less effective in young people following traumatic events due to increased rates of relapse. The present findings also indicate that there are similarities between the effects of acute and chronic forms of early-life stress, specifically in their impact on infants' emotional development. However, further research is required to disentangle differences in long-term responses to these distinct types of stressor.

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Table 1

Mean (± SEM) Pup Weight (g) for Standard-Reared (SR) and Maternally-Deprived (MD) Litters on Post-Natal Days (P) 9, 10, and 17, Collapsed Across Experiments 1, 2, and 3a.

Rearing Condition	*n*	P9	P10*	P17*
SR	27	24.05 (± 0.47)	26.92 (± 0.51)	44.57 (± 0.71)
MD	20	25.16 (± 0.59)	23.95 (± 0.52)	36.08 (± 0.59)

* Indicates a significant difference between groups, $p < .05$.

Table 2

Group Ns and Mean (± SEM) Levels of Pre-CS Freezing at Test for Maternally-Deprived (MD) and Standard-Reared (SR) Groups in Experiments 1 (Renewal of Extinguished Fear), 2 (Reinstatement of Extinguished Fear), and 3a and b (Retention of Conditioned Fear).

Experiment	Group	*n*	Pre-CS freezing %
1	SR-same	12	1.25 (± 0.68)
	MD-same	11	7.72 (± 3.35)
	SR-different	12	6.19 (± 2.32)
	MD-different	12	13.12 (± 5.61)
2*	SR-context exposure	8	9.31 (± 5.17)
	MD-context exposure	9	6.24 (± 3.45)
	SR-US exposure	8	31.43 (± 5.91)
	MD-US exposure	8	17.79 (± 4.26)
3a*	SR-1 day	14	24.29 (± 4.89)
	MD-1 day	14	15.00 (± 2.88)
	SR-7 days	12	10.00 (± 5.02)
	MD-7 days	14	7.79 (± 3.39)
3b*	SR-1 day	7	16.12 (± 5.43)
	MD-1 day	8	21.22 (± 4.94)
	SR-14 days	8	6.25 (± 2.99)
	MD-14 days	7	5.68 (± 3.62)

* Indicates a significant difference ($p < .05$) between groups in pre-CS levels of freezing. In Experiment 2, there was a significant effect of reinstatement exposure (context vs. US) on baseline freezing. In Experiments 3a and 3b, there was a significant effect of retention interval (1 day vs. 7 days and 1 day vs. 14 days, respectively) on baseline freezing.

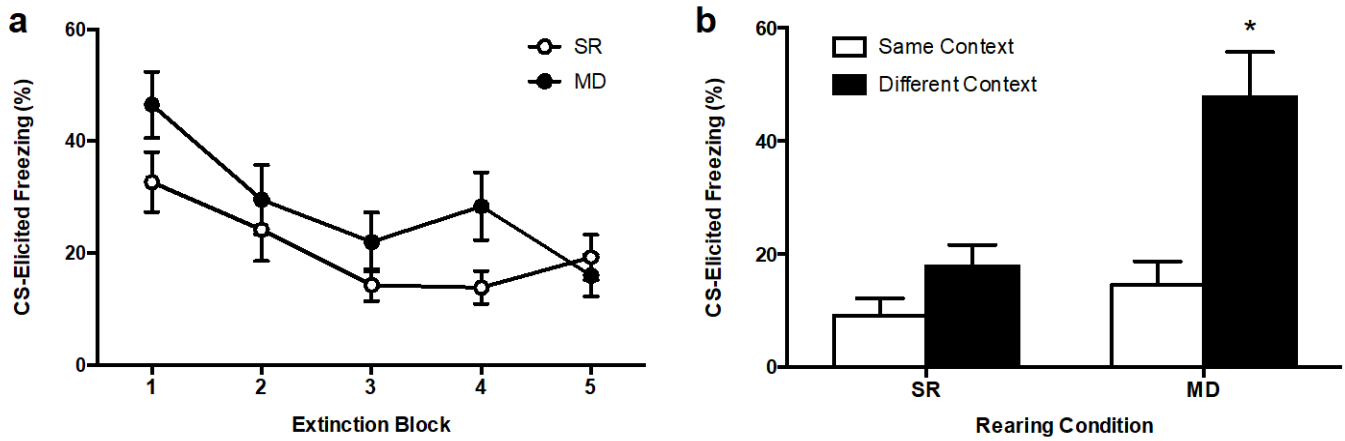

Figure 1. (a) Mean (± *SEM*) levels of CS-elicited freezing across 5 blocks (6 CS presentations per block) of extinction training for standard-reared (SR; open circles) and maternally-deprived (MD; closed circles) rats. Regardless of rearing condition, rats exhibited high levels of fear at the start of extinction that decreased across blocks. (b) Mean (± *SEM*) levels of CS-elicited freezing for SR and MD rats tested in the same context (white bars) as extinction training or in a different context (black bars). Only MD rats demonstrated high levels of fear when tested in a different context to extinction training. * $p < .05$ from MD-same group.

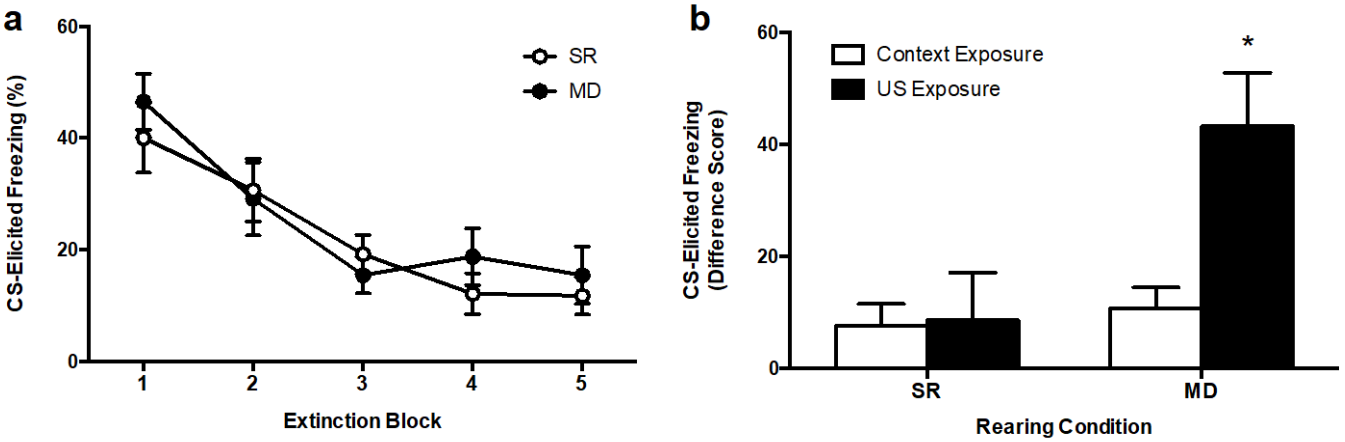

Figure 2. (a) Mean (± *SEM*) levels of CS-elicited freezing across 5 blocks (6 CS presentations per block) of extinction training for standard-reared (SR; open circles) and maternally-deprived (MD; closed circles) rats. Regardless of rearing condition, rats had high levels of freezing at the start of extinction that decreased across blocks. (b) Mean (± *SEM*) levels of CS-elicited freezing (freezing during the CS minus pre-CS freezing) for SR and MD rats given no reminder (context exposure; white bars) or a reminder treatment (US exposure; black bars). Only MD rats demonstrated high levels of fear when tested 24 h after a reminder footshock. * $p < .05$ from MD-context exposure group.

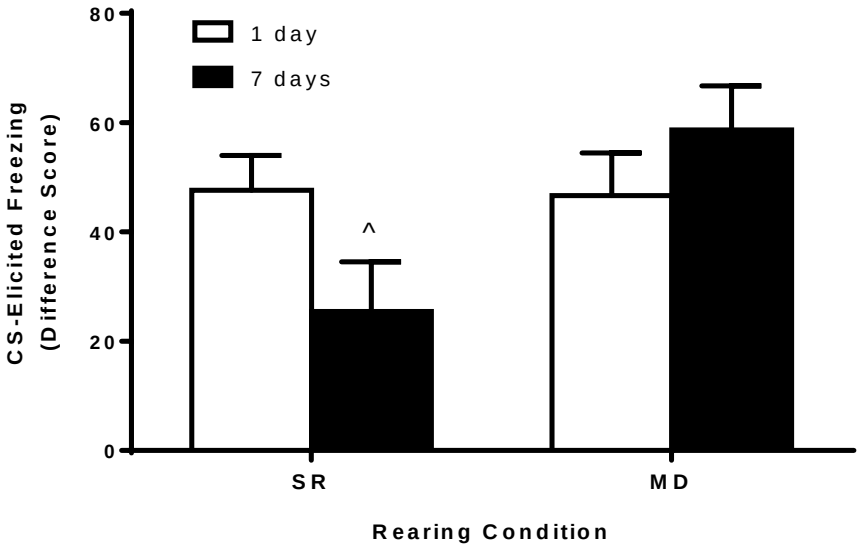

Figure 3. Mean (± *SEM*) levels of CS-elicited freezing (percentage freezing during the CS minus pre-CS freezing) for standard-reared (SR) and maternally-deprived (MD) rats tested 1 day (white bars) or 7 days (black bars) after conditioning. MD rats demonstrated high levels of fear when tested 7 days after initial fear conditioning. ^ $p = .059$ from SR-1 day group.

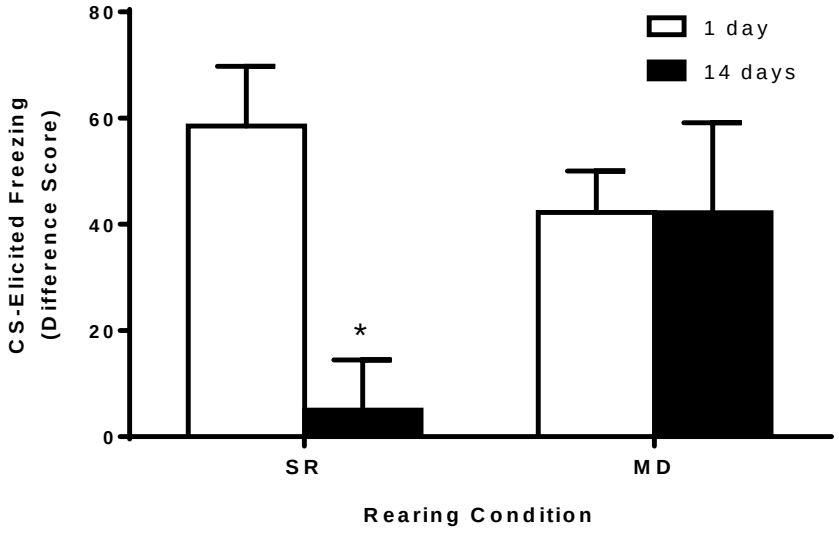

Figure 4. Mean (± *SEM*) levels of CS-elicited freezing (percentage freezing during the CS minus pre-CS freezing) for standard-reared (SR) and maternally-deprived (MD) rats tested 1 day (white bars) or 14 days (black bars) after conditioning. MD rats demonstrated similar levels of fear regardless of test interval. However, SR rats exhibited significantly lower levels of fear after 14 days. * $p < .05$ from SR-1 day group.

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