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Early Emergence of Adult-Like Fear Renewal in the Developing Rat After Chronic Corticosterone Treatment of the Dam or the Pups

Bridget L. Callaghan and Rick Richardson
The University of New South Wales

Early adversity increases the risk of nearly all mental health disorders. Although tractable animal models exist to probe the psychobiology of early adversity, the empirical research has focused largely on adult outcomes. In the current studies we examined how early exposure to the stress hormone corticosterone (CORT) affects the maturation of fear extinction in *infant rats*. Whereas juvenile and adult rodents exhibit extinction learning that is relapse-prone (i.e., they show renewal and reinstatement of extinguished fear), under typical rearing conditions the extinction system in infant rats appears to be relapse-resistant (i.e., extinction leads to a permanent reduction in conditioned fear). In the current studies we tested the hypothesis that chronically exposing infant rats to CORT, either indirectly (through maternal drinking water) or directly (via insertion of an osmotic pump), leads to an early transition into the adult-like extinction system (i.e., evidence of the renewal effect). Although some differences were observed between the 2 methods of CORT exposure, the data showed that infants exposed to CORT via either method exhibited premature emergence of adult-like extinction learning (i.e., they exhibited the renewal effect). The theoretical implications of these findings for typical and abnormal development of emotion learning systems, as well as their practical applications in the context of prevention and treatment of mental health disorders, are discussed.

Keywords: corticosterone, stress, development, extinction, renewal

Early adversity is a strong predictor of mental health problems, increasing the risk of onset (and, in some cases, persistence) of nearly all mental health disorders (Kessler et al., 1997; Kessler et al., 2010; McLaughlin et al., 2010). Although there are tractable animal models available to probe the psychobiology of early adversity, the empirical research to date has focused largely on adult outcomes (but see Lupien et al., 2009). If we are to effectively prevent and treat mental health disorders, our understanding of the effects of adversity on the *developing* brain and on behavior early in life will be critical.

Primarily because of their potential for translation, the animal models of fear learning and fear inhibition (extinction) have received considerable attention from researchers interested in mental health (e.g., Graham & Milad, 2011; Milad & Quirk, 2012); exposure therapy (used in the treatment of numerous neuropsychiatric disorders) is modeled upon the process of extinction (Foa, 2000; Rothbaum & Davis, 2003). During a typical fear learning procedure a neutral conditioned stimulus ([CS]; such as a noise) is paired with an unconditioned stimulus (US; such as a footshock), eventually resulting in the expression of learned fear responses to the CS (e.g., freezing). In extinction training the animal is repeatedly exposed to the CS in the absence of the US, resulting in the learning of a new inhibitory association ("the CS no longer predicts the US") that competes with the original CS-US association (Bouton, 2002). Interestingly, we recently demonstrated that the processes mediating fear extinction differ across development and that the maturation of these processes is markedly affected by levels of stress in the rearing environment. Specifically, compared with standard-reared rats of the same age, infant (postnatal day [P] 17) rats exposed to adverse rearing (maternal-separation) exhibited greater fear relapse after extinction training, and precocious involvement of $GABA_A$ receptor activation in the expression of extinction (Callaghan & Richardson, 2011). The behaviors observed in infant maternally separated rats are typically observed in standard-reared rats only after they have transitioned from the infancy to juvenile period of development (i.e., approximately P23; Kim & Richardson, 2007a, 2007b; Yap & Richardson, 2007), suggesting that maternal-separation altered the natural trajectory of emotion learning system development.

One important question to emerge from the research just described relates to the mechanism via which maternal-separation leads to an accelerated emergence of adult-like fear extinction. One likely possibility is that exposure to stress hormones (i.e., corticosterone [CORT]), per se, is the critical factor mediating the early developmental transition seen in maternally separated pups. Indeed, CORT is ideally placed to act as an environmental signal for developing animals. CORT levels in the dam change in response to environmental stressors and are readily transmitted to infant offspring via lactation (Catalani et al., 2002; Catalani et al., 1993; Macrì et al., 2011). Hence, CORT might effectively inform

Bridget L. Callaghan and Rick Richardson, School of Psychology, The University of New South Wales.

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Correspondence concerning this article should be addressed to Bridget L. Callaghan, School of Psychology, The University of New South Wales, Sydney, Australia, 2052. E-mail: bcallaghan@unsw.edu.au

the infant about current and expected future environmental demands and program them to cope with such demands (Macrì et al., 2011). Consistent with its role as an environmental signal for stress, previous studies have demonstrated that exogenous manipulation of CORT levels can mimic the effects of environmental stressors. For example, in adult rats the same pattern of reorganization in the apical dendritic tree of CA3 neurons was achieved through either restraint stress or CORT administration (Magariños et al., 1998). In addition, male offspring of dams treated with high levels of CORT postpartum exhibited suppressed cell proliferation in the dentate gyrus (Brummelte et al., 2006), a finding that was similar to that observed in pups that were stressed through exposure to a predator odor (Tanapat, 1998). CORT has also been shown to be a critical factor in the regulation of developmental transitions. For example, infant rats treated with CORT exhibited an early transition in their behavioral response to an odor paired with shock (Moriceau et al., 2004; Moriceau et al., 2006; Stein et al., 2002), whereas adrenalectomy (that prevents the secretion of CORT) slowed down the developmental transition in behavioral responses toward predator odor (Takahashi, 1994). Because plasma levels of CORT are increased in suckling pups and their dams after maternal separation (Huot et al., 2002; Macrì et al., 2011), we assessed whether administering CORT to lactating dams or their infant pups (in the absence of rearing stress) was sufficient to mimic the effects of maternal separation on the developing fear extinction system.

General Method

Subjects

Experimentally naive Sprague-Dawley–derived rats, bred and housed at the School of Psychology, The University of New South Wales, were used. All rats were 17 days of age at the start of the experiments. The day of birth was designated as P0. Only males were used, and no more than one rat per litter was used per group. Rats were housed with their dam and littermates in plastic boxes (24.5 cm long × 37 cm wide × 27 cm high) covered by a wire lid, and food and water were available ad libitum. Rats were maintained on a 12 hr light/dark cycle (lights on at 7 a.m.). All animals were treated according to the principals of animal care and use outlined in the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (National Health and Medical Research Council, 2004), and all procedures were approved by the Animal Care and Ethics Committee at The University of New South Wales.

Maternal CORT Treatment

In Experiment 1, dams had their drinking water supplemented with either CORT (200 μg/ml; Sigma, St. Louis, MO) or Vehicle (VEH; 2.5% ethanol) across postnatal days (P) 2–14 inclusive (NB: pups do not independently consume water at these ages). The dose of CORT was chosen based on previous studies that had demonstrated an increase in basal CORT levels in both lactating dams and nursing pups after 200 μg/ml CORT supplementation in the dam's drinking water (Catalani et al., 1993). Dams consumed an average of 13.81 mg of CORT per day. The concentration of the vehicle had been used previously (Magariños et al., 1998).

Pup CORT Treatment

In Experiments 2 and 3, CORT was administered directly to pups via osmotic micropumps (Model 1007D, Alzet, Cupertino, CA). Micropumps were prefilled with either dH_2O or CORT dissolved in dH_2O under sterile conditions. CORT (~3 mg/kg/day) was administered at a rate of 0.5 μl/hr. Importantly, because the weight of the rats would increase across the 7 day infusion period, an approximate average weight of rats across that period was calculated and used to determine the drug concentration before pumps were loaded. Based on an average weight of 25 g across the treatment period (P7–14) a final dose of .075 mg/rat/day was calculated, meaning that rats would have received slightly more than 3 mg/kg/day when they were younger and slightly less toward the end of the infusion week when they were older. Because there were no previous studies that had used osmotic pumps to infuse CORT in infant rats (to our knowledge), we based the dose on past studies that had systemically injected the drug in infancy (Moriceau et al., 2004). The steady pumping rate of the device at 0.5 μl/hr would have been reached ~4–5 hr after implantation (Alzet, 2008). In Experiment 3 only, because we had already demonstrated that pups treated with VEH micropumps did not exhibit the renewal effect after extinction learning (i.e., in Experiment 2), and because of numerous previous demonstrations that renewal does not occur in standard-reared infant rats (e.g., Callaghan & Richardson, 2011; Cowan et al., 2013; Kim & Richardson, 2010), extinction behavior was examined only in pups that had been implanted with CORT pumps (i.e., do these animals exhibit renewal or not?).

Pup Surgery

The surgery procedure for implantation of osmotic pumps in infant rats was similar to that reported in previous studies (Thornton & Smith, 1997). On P7 rats were removed from the home cage, placed on a heating pad maintained at 30°C and anesthetized with isoflurane (Laser Animal Health, Sydney, New South Wales, Australia) mixed with oxygen. A small (1 cm) incision was made 1.5 cm from the base of the rat's tail on the dorsal surface. A subcutaneous pocket was created by insertion of a hemostat through the incision, and a sterile preloaded osmotic pump was inserted into the pocket with the delivery port facing 180 degrees from the incision site to prevent leakage from the incision. The wound was closed by two small (7 mm) wound clips. The wound clips remained in place for the 7-day period or fell out naturally when the wound had healed. After surgery the rats were injected with 0.01 ml of benacillin (300 mg/ml, intraperitoneally) and were left to recover for 15 min with their littermates before being returned to the dam. The entire surgery took less than 5 min and the rats recovered well. The dorsal placement of the micropump was chosen because it does not interfere with the feeding position of pups (Thornton & Smith, 1997).

One week after the micropump had been implanted, on the afternoon of P14, it was removed via a simple surgery that involved anesthetizing the rat (using the same procedure just described), reopening the original wound with scissors and removing the micropump. The incision was again closed with wound clips that remained in place or fell out naturally. The whole surgery lasted ~10 min (it took slightly longer for the anesthetic to take effect in the older pups) and rats were left to recover with their

littermates for 5 min before the dam was returned. All pups recovered well from surgery. Similar to past reports (e.g., Thornton & Smith, 1997), dams in the current studies tolerated the pups well after both surgeries and there were no instances of cannibalism or obvious abuse (e.g., bite marks).

Apparatus

Two experimental chambers served as different contexts in these experiments. In all experiments, training occurred in Context A, extinction occurred in Context B, and testing occurred in either A or B (i.e., the renewal effect was examined). Context A was a set of two identical chambers that were rectangular (13.5 cm long × 9 cm wide × > 9 cm high), with the front wall, rear wall, and ceiling constructed of clear Plexiglas. The floor and side walls consisted of stainless steel rods set 1 cm apart. Two high-frequency speakers were located 8 cm from either side of the chamber. A custom built constant-current shock generator could deliver shock to the floor of each chamber as required. Each chamber was housed within a separate wood cabinet so that external noise and visual stimulation were minimized. A red light-emitting diode (LED) located on the rear wall of the cabinet was the sole source of illumination in these chambers. A low, constant background noise (50 dB, measured by a TENMA sound level meter, Type 72–860) was produced by ventilation fans located within the cabinet. Context B was a set of two identical chambers that were rectangular (30 cm long × 30 cm wide × 23 cm high) and wholly constructed of Plexiglas, with the exception of the grid floor that was the same as in Context A. All the walls were transparent, except for the two side walls that consisted of vertical black and white stripes (each 5 cm wide). Two high frequency speakers were mounted on the ceiling of each of these chambers. The chambers were housed in separate wood cabinets so that external noise and visual stimulation was minimized. White and red LEDs located within the camera mount that was on the rear wall of the cabinet were the sole sources of illumination in these chambers. A low constant background noise (50 dB) was produced by the ventilation fans in the cabinet. Thus, the two sets of contexts differed primarily in their size and their visual features. The CS was a white noise; noise level in the chamber was increased by 8 dB when the CS was presented. A computer controlled all presentations of the CS and the footshock US. The software and hardware used were custom developed at The University of New South Wales.

Scoring, Exclusions, and Statistics

Freezing was scored using a time sampling procedure where the rat's behavior was observed every three seconds and scored as "freezing" or "not freezing." Freezing was defined as the absence of all movement except that required for respiration (Fanselow, 1980). These observations were then converted into a percentage score to indicate the proportion of total observations scored as freezing. A second scorer, unaware of the experimental condition of each rat, scored a random sample (30%) of all rats tested. The interrater reliability was very high across all experiments, ranging from .91 to .99, using intraclass correlation coefficients. Whenever a mixed-design analysis of variance (ANOVA) was used, if the assumption of sphericity was violated, the reported *p* values are for comparisons made with the Greenhouse-Geisser procedure; however, nominal *df* are reported in these cases.

Group sizes and levels of pre-CS freezing at test across all experiments are presented in Table 1. All data were analyzed with ANOVA and when significant differences in pre-CS freezing at test were detected (Experiment 2) the test data were analyzed with analysis of covariance (ANCOVA) using pre-CS freezing as a covariate. The same results were obtained, however, whether the data were analyzed with ANOVA or ANCOVA. *t* tests were used in post hoc analyses and in instances where only two groups were being compared (test data in Experiment 3). For all analyses, a *p* value of $<.05$ was considered statistically significant.

Three exclusions were made in Experiment 2; one rat was excluded for high baseline freezing (>50%) at test (Group VEH-different) and two rats were excluded for being statistical outliers (>3 *SD* from the group mean at test; one of these rats was from Group VEH-same and the other was from Group VEH-different).

CORT and Vehicle Consumption

In Experiment 1, CORT- and VEH-treated dams consumed an average of 69.05 ml and 67.35 ml of fluid per day, respectively, across the treatment period. Maternal water consumption did increase slightly as pups' age increased but this was similar for CORT- and VEH-treated dams. There was a main effect of day, $F(12, 216) = 6.618$, $p < .05$, but the interaction between day and group was not significant, $F > 1$ (see Figure 1). There were no differences in weight between P17 rats suckled by CORT- or VEH-treated dams, $t(40) = .79$, $p = .44$. Finally, implanted pups (in Experiments 2 and 3) gained weight normally across the treatment period and displayed all the normal gross indices of health. In Experiment 2, at the start of fear conditioning, on P17, there was no difference in weight between the pups infused with CORT or vehicle via osmotic micropumps (*M*s = 46.53 and 45.23*g*, respectively), $t(27) = .70$, $p = .49$.

Table 1
Mean (SEM) Levels of Pre-CS Freezing at Test for Pups That Were Nursed by Corticosterone (CORT)-Treated or Vehicle (VEH)-Treated Dams (Experiment 1) or That Were Implanted With an Osmotic Micro-Pump Loaded With CORT or VEH (Experiments 2 and 3)

Experiment	Group	N	% pre-CS freezing (*SEM*)
1	CORT-same	11	8.56 (4.30)
	CORT-different	11	9.55 (3.90)
	VEH-same	10	4.50 (2.66)
	VEH-different	10	17.5 (9.17)
2*	CORT-same	8	12.38 (5.87)
	CORT-different	8	11.25 (4.18)
	VEH-same	7	1.43 (1.00)
	VEH-different	6	3.33 (2.17)
3	CORT-same	10	5.75 (4.16)
	CORT-different	10	18.00 (6.21)

Note. Groups were tested in either the same (CORT-same/VEH-same) or in a different (CORT-different/VEH-different) context to extinction.
* Significant difference in pre-CS freezing at test between drug treatments ($<.05$).

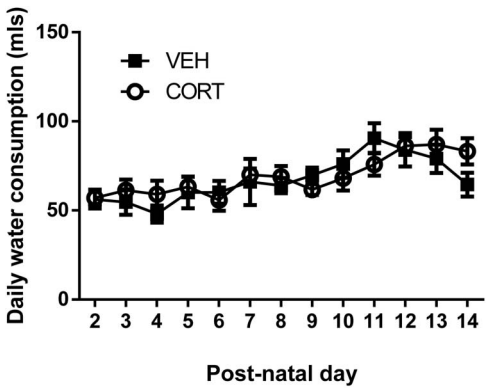

Figure 1. Daily water consumption in dams treated with vehicle (VEH) and those treated with corticosterone (CORT). There were no group differences in water consumption.

Experiment 1

Recently we reported that infant rats (i.e., P17) exposed to maternal-separation rearing exhibit an early transition into the adult-like extinction system (Callaghan & Richardson, 2011). That is, the infant maternally separated (MS) rats exhibited the renewal effect whereas the infant standard reared (SR) rats did not. In Experiment 1 we examined whether exposing lactating dams to CORT in the drinking water from P2–14, rather than putting them through the maternal-separation procedure, would be sufficient to cause the early transition into adult-like extinction in their infant pups.

Method

The design was a 2 × 2 factorial, with the first factor referring to maternal drug treatment (CORT or VEH) and the second referring to the context in which the animal was tested (same or different to the extinction training context). On Day 1, animals were placed in Context A, and after a 2-min adaptation period, a white noise CS (8 dB above background) was presented for 10 s. The shock US (0.6 mA, 1 s) was administered during the last second of the CS. The intertrial interval ([ITI]) ranged from 85 s to 135 s with a mean of 110 s. Six pairings of the CS and the US were given. Thirty to 60 s after the last pairing, rats were returned to their home cages. On Day 2, the animals were placed in Context B and after a 2 min adaptation period presented with 30 nonreinforced presentations of the 10 s CS with a 10 s ITI. The extinction session was 12 min in duration and 30–60 s after the last CS presentation, animals were returned to their home cages. The following day, at test, the animals were placed in either the same Context (B) or a different Context (A) to extinction training and their baseline level of freezing in the absence of the CS was recorded for 1 min. The CS was then presented and freezing was recorded for 2 min. All extinction and test sessions were recorded.

Results and Discussion

CS-elicited freezing during the 5 blocks of extinction training (each block is comprised of 6 CSs) for CORT- and VEH-nursed rats in Experiment 1 is presented in Figure 2a. As can be seen in that figure, freezing decreased across blocks, $F(4, 152) = 43.99$, $p < .001$. There was a main effect of maternal drug treatment, $F(1, 38) = 5.51$, $p < .05$, and of testing context, $F(1, 38) = 4.92$, $p < .05$, on within-session extinction that was driven by the lower levels of freezing exhibited by the "VEH-nursed same" group across extinction. None of the interactions were significant, largest $F = 1.69$, $p = .17$.

As can be seen in Table 1, levels of pre-CS freezing at test did not differ across groups, and this was supported by the statistics. There were no significant main effects or interactions on pre-CS freezing at test (largest $F = 1.81$, $p = .19$).

CS-elicited freezing at test is shown in Figure 2b. As can be seen in that figure, vehicle-nursed pups exhibited low levels of freezing at test regardless of the testing context (same or different to the extinction context). Freezing at test did, however, differ in the CORT-nursed pups. Specifically, CORT-nursed pups exhibited low levels of freezing when tested in the same context as extinction, but exhibited much higher levels of freezing when tested in a

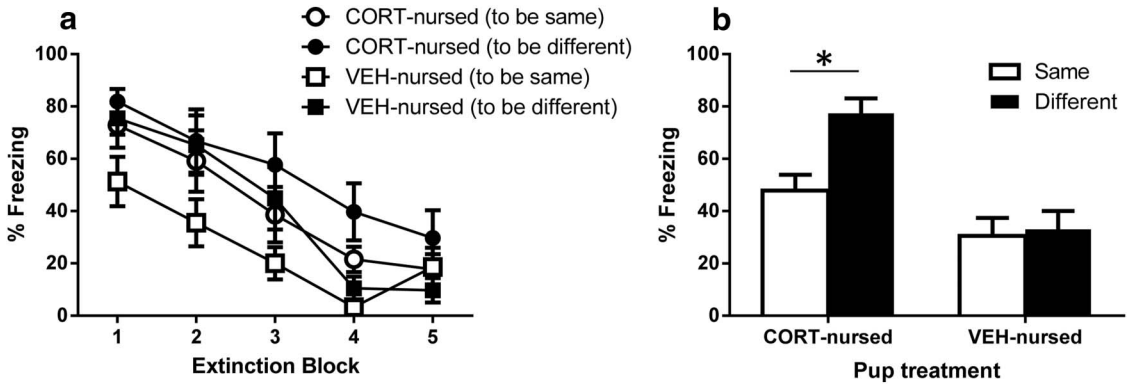

Figure 2. Mean (±*SEM*) levels of CS-elicited freezing during (a) extinction training, or (b) extinction retention test in Experiment 1. Rats were nursed by CORT- or VEH-treated dams (CORT-nursed and VEH-nursed, respectively) and were tested in the same or a different context to extinction training. Asterisk signifies a difference in freezing levels across test contexts in the CORT-nursed pups.

different context to extinction. In other words, the CORT-nursed rats exhibited the renewal effect at P17 whereas the VEH-nursed rats did not. The statistics confirmed this description of the data. There was a significant effect of maternal drug treatment, $F(1, 38) = 22.66$, $p < .001$, of test context, $F(1, 38) = 6.11$, $p < .05$, and a significant maternal drug treatment by test context interaction, $F(1, 38) = 4.20$, $p < .05$. Pairwise comparisons showed that the CORT-nursed group tested in a different context to extinction froze significantly more than the CORT-nursed group tested in the same context as extinction ($t(20) = -3.38$, $p < .01$) whereas the VEH-nursed groups showed low and similar levels of freezing regardless of test context ($t(18) = -.28$, $p = .78$).

In Experiment 1 we replicated past findings showing that SR P17 rats do not exhibit the renewal effect (Kim & Richardson, 2007b; Yap & Richardson, 2007). Further, and more importantly, we demonstrated that infants suckled by dams that had been treated with CORT in the drinking water did exhibit the renewal effect. This was not because of maternal CORT exposure impairing within-session extinction learning because all pups exhibited similar learning slopes across extinction training and extinguished fear to a similar level, regardless of maternal drug treatment. Importantly, although there were differences in freezing levels between groups across extinction training, the main effects detected during extinction could not possibly account for the effects observed at test. Specifically, the levels of CS-elicited freezing in the CORT-different and VEH-different groups were similar across extinction training yet differed dramatically at test (see Figure 2a and 2b). This experiment is the first to demonstrate that dam exposure to CORT affects the maturation of extinction behaviors in her pups.

Experiment 2

In Experiment 1, dam CORT treatment produced an adult-like extinction phenotype in the infant pups. The aim in Experiment 2 was to determine whether an early transition into adult-like extinction behavior could be elicited by alternative CORT treatment methods, or whether the effect was specific to dam CORT exposure. Specifically, in Experiment 2 we tested the hypothesis that direct manipulation of pup CORT levels, via implantation of an osmotic micropump, would also lead to an early transition between the infant and adult-like extinction systems.

Method

The design was a 2 × 2 factorial, with the first factor referring to pup drug treatment (CORT or VEH) and the second referring to the context in which the animal was tested (same or different to the extinction training context). Similar to Experiment 1, all pups were conditioned on P17 in Context A, extinguished the following day in Context B, and were then tested 24 hr later in either Context B (same as extinction) or Context A (different to extinction).

Results and Discussion

CS-elicited freezing during the five blocks of extinction training (each block is comprised of 6 CSs) for CORT- and VEH-treated rats is presented in Figure 3a. As can be seen in that figure, freezing decreased across blocks, $F(4, 100) = 92.74$, $p < .001$. The remaining effects and interactions were not significant (largest $F(4, 100) = 2.69$, $p = .08$).

As can be seen in Table 1, CORT-treated rats exhibited slightly higher (although still very low) freezing levels than VEH-treated rats during the pre-CS period at test, $F(1, 25) = 5.23$, $p < .05$. The remaining effects and interactions were not significant (all Fs < 1.0).

CS-elicited freezing at test is shown in Figure 3b. As can be seen in that figure, freezing differed at test between rats infused with CORT or VEH. Specifically, the CORT-treated pups exhibited high levels of freezing at test, regardless of context whereas the VEH-treated pups exhibited low levels of freezing regardless of context. The statistics confirmed this description of the data. There was a significant effect of pup drug treatment on CS-elicited freezing at test, $F(1, 24) = 8.55$, $p < .01$. However, there was no effect of test context and no test context by drug-treatment interaction (both Fs <1.0).

As in Experiment 1, and in previous work with standard-reared pups (Callaghan & Richardson, 2011; Kim & Richardson, 2007b),

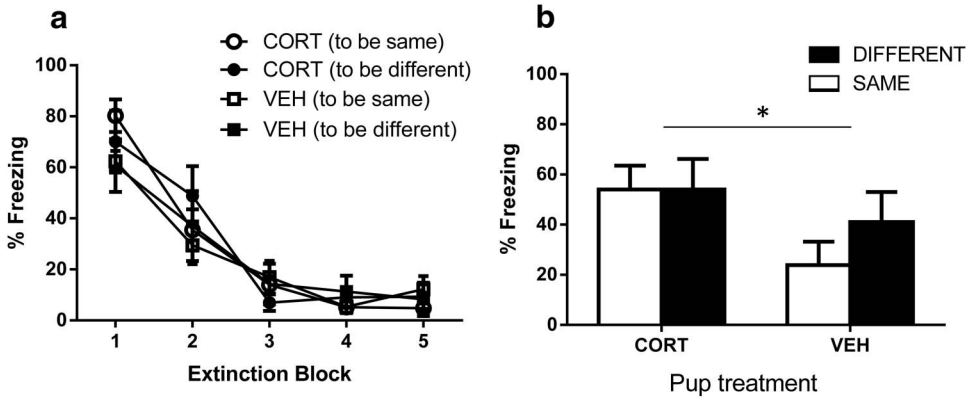

Figure 3. Mean (±*SEM*) levels of CS-elicited freezing during (a) extinction training, or (b) extinction test (in the same or different context to extinction training) in Experiment 2. Rats were implanted with an osmotic pump on P7 that released CORT or vehicle (CORT and VEH groups, respectively) until P14. Asterisk signifies a main effect of drug treatment on freezing levels at test.

renewal of extinguished fear was not observed in vehicle-treated pups in this experiment. Our hypothesis was that CORT-treated pups would, in contrast, exhibit renewal. That is, those animals tested in a context different from extinction would exhibit a return of fear, which was indeed observed. However, we were not able to conclude that the return of fear in these animals was because of a faster transition to the adult-like extinction system (that is characterized by renewal after extinction) because the CORT-treated pups tested in the extinction context also exhibited a return of fear (despite very low levels of freezing in this group at the end of extinction training). In other words, both groups of pups implanted with a micropump containing CORT exhibited poor retention of extinction, making any interpretations about the occurrence of renewal, or not, impossible. One approach to addressing this difficulty would be to increase the amount of extinction training such that those animals tested in the same context exhibit lower levels of freezing. This was done in Experiment 3.

Experiment 3

In Experiment 3, CORT-treated pups were given double the amount of extinction training as in Experiments 1 and 2 (60 trials instead of 30) and were then tested for context-mediated renewal after extinction training.

Method

Both groups of rats were directly exposed to CORT via an osmotic pump but were tested for their retention of extinguished fear in different contexts (i.e., either the extinction context or back in the conditioning context). Similar to Experiments 1 and 2, all pups were conditioned on P17 in Context A, extinguished the following day in Context B, and were then tested for renewal of extinguished fear in either Context A or B. However, unlike Experiments 1 and 2, extinction training occurred over 60 trials (instead of 30), increasing the length of that session from 12 to 22 min.

Results and Discussion

CS-elicited freezing during the 10 blocks of extinction training (each block is comprised of 6 CSs) for the two groups of CORT-treated rats is presented in Figure 4a. As can be seen, freezing decreased across blocks, $F(9, 162) = 15.98$, $p < .001$. There was no effect of test context and no test context by extinction block interaction (largest $F = 1.84$, $p = .19$).

Regardless of testing context, pre-CS freezing was low in both CORT-treated groups, $t(18) = -1.73$, $p = .10$ (see Table 1). CS-elicited freezing at test in CORT-treated rats is depicted in Figure 4b. As can be seen in that figure, freezing at test differed according to context. Specifically, CORT-treated rats tested in the same context to extinction exhibited significantly lower levels of freezing than CORT-treated rats tested in a different context to extinction ($t(18) = -2.67$, $p < .05$).

The results of Experiment 3 demonstrate that giving double the amount of extinction training to CORT-treated rats resulted in slightly better retention of extinction learning expressed in the same context as extinction (i.e., ~40% freezing in the same context after double extinction in this experiment vs. ~50% freezing in the same context after single extinction in Experiment 2). Although this decrease in freezing of CORT rats tested in the same context was small, it was sufficient to reveal evidence of the renewal effect in CORT-treated animals. Specifically, CORT-treated rats tested in the same context to extinction exhibited significantly lower levels of freezing than CORT-treated rats tested in a different context to extinction. In other words, infant rats treated with CORT via osmotic pump do appear to make an early transition into the adult-like extinction system; this effect is masked by poor extinction retention in the same context after 30 extinction trials but can be revealed after double extinction training. Importantly, however, even after double extinction training freezing in the same context remains relatively high in infant rats given CORT directly through an osmotic micropump.

General Discussion

The results of this study show that CORT treatment of dams or pups affects the development of extinction learning in the infants. Although vehicle-suckled/VEH-treated P17 pups exhibit the typical relapse-resistant extinction phenotype (i.e., no renewal effect), P17 pups suckled by a CORT-treated dam, or directly treated with CORT via osmotic micropump, were precocious in their exhibition

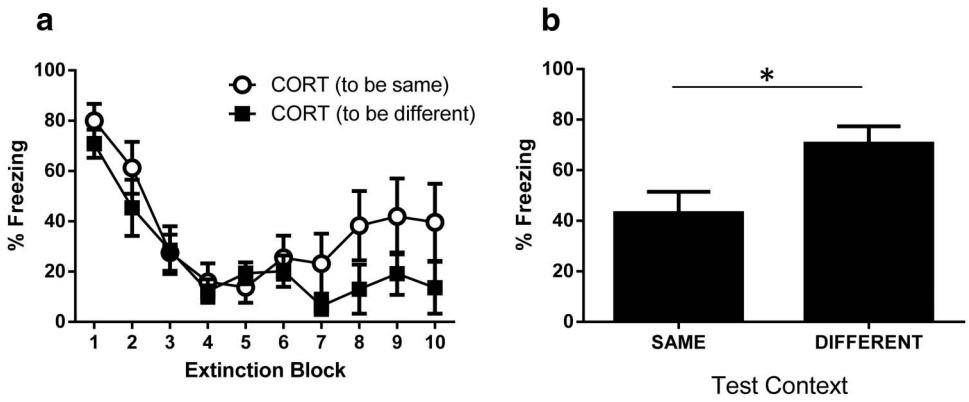

Figure 4. Mean (±*SEM*) levels of CS-elicited freezing during (a) extinction training, or (b) extinction test (in the same or different context to extinction training) in Experiment 3. Rats were implanted with an osmotic pump on P7 that released CORT until P14. Asterisk signifies a significant group difference in CS-elicited freezing at test.

of adult-like extinction behavior (i.e., they did show the renewal effect). These outcomes support the view that exposure to stress hormones in dams or pups is sufficient to cause an early transition into the adult-like extinction system and may be the mechanism via which maternal-separation stress led to a similar early extinction transition in our past work (Callaghan & Richardson, 2011).

The data in this article are consistent with previous studies demonstrating the importance of the stress hormone CORT in mediating a variety of developmental transitions. For instance, we have previously shown that pups suckled by CORT-treated dams exhibit an early transition into the adult-like fear retention system (Callaghan & Richardson, 2012). Specifically, memories formed early in development (e.g., at P17) are forgotten much faster than memories formed later in development (e.g., P24-adulthood). This phenomenon is termed infantile amnesia and occurs in most mammalian species (Callaghan et al., 2014). In our previous research we showed that rats exposed to maternal separation stress, or rats that were suckled by CORT-treated dams, exhibited an early developmental transition into adult-like fear retention. That is, although standard-reared animals forgot a fear association formed on P17 within a week, stress/CORT-exposed pups retained that fear association for much longer (up to 30 days), suggesting that these animals were displaying adult-like memory retention. Furthermore, as mentioned earlier, other studies have shown that CORT treatment accelerated the developmental transition in the behavioral response to an odor previously paired with shock (Moriceau et al., 2004; Moriceau et al., 2006). Specifically, P8 rats typically display a paradoxical approach response to an odor paired with a shock whereas rats trained at P10 or older display avoidance of shock-paired odors. If, however, rats are given an injection of CORT before conditioning on P8 then the more mature avoidance response will occur (Moriceau et al., 2004; Moriceau et al., 2006). The importance of CORT in triggering developmental transitions does not appear to only occur in rodents. Recent studies in young humans have shown that a developmental transition in amygdala-prefrontal cortex connectivity takes place between childhood and adolescence; amygdala and prefrontal areas are positively coupled during a functional task in childhood (passive viewing of fear faces) but are negatively coupled during this task in adolescence (Gee et al., 2013b). Critically, this developmental transition occurred earlier in children that were reared for a short time under adverse conditions (orphanage rearing), and post-MRI (magnetic resonance imaging) cortisol levels were found to mediate the relationship between adverse rearing and amygdala-prefrontal cortex connectivity (Gee et al., 2013a). Together with this other research, the current data suggest that CORT may act as a general environmental signal triggering the initiation of adult-like forms of learning and brain activity across numerous emotional (and possibly nonemotion related) systems.

One particularly interesting and unexpected outcome in this study was that infant rats directly exposed to CORT, via an implanted osmotic micropump, rather than indirectly exposed to CORT via their dams drinking it in their water, exhibited poor extinction retention when given 30 extinction trials and tested in the extinction context (i.e., compare test performance in CORT-treated rats tested in the same context in Experiments 1 and 2). The poor extinction retention in the directly exposed pups also stands in contrast to our past work, using the same parameters, with older rats (e.g., P24 and adult rats) and with maternally separated pups where good extinction retention is consistently observed (Callaghan & Richardson, 2011; Kim et al., 2011; Kim & Richardson, 2007a, 2007b). In fact, using the same parameters used in the present experiments, the only age group that we have found to exhibit poor extinction retention has been adolescents (Kim et al., 2011; McCallum et al., 2010).

There are several aspects of the CORT administration procedures used in the present experiments that might have led to the unexpected poor extinction retention in those pups directly exposed to CORT. One possibility is that the different doses of CORT used across the two procedures were responsible for the observed results. Indeed, the effects of CORT exposure on learning and HPA reactivity appear to differ according to level of exposure. For example, compared with rats that had been nursed by dams whose drinking water was supplemented with a moderate dose (100 μg/ml) of CORT, and to rats nursed by dams whose drinking water was not supplemented with CORT, pups nursed by dams treated with a low dose (33 μg/ml) of CORT made less errors on an intradimensional set shifting task as adults (Macrì et al., 2009). Unfortunately, plasma CORT levels were not measured in the present studies but it is likely that they differed between the two administration procedures; such variations in CORT levels could be responsible for the differences we observed in extinction retention. In future studies it would be useful to measure plasma CORT levels in pups on both of these procedures.

Another potential reason for the observed differences in extinction retention is that the two CORT administration procedures may have had different effects on maternal behavior. As mentioned earlier, previous studies have documented changes in maternal behavior after 200 μg/ml CORT supplementation in the dam's drinking water (Catalani et al., 1993). In that study, CORT-treatment was reported to increase the frequency with which dams engaged in "positive" mothering behaviors (e.g., licking and grooming and arched back nursing). Of interest to the authors, those behaviors exhibited by CORT-treated dams in past studies have been previously associated with reduced stress reactivity in adult offspring (Liu et al., 1997). However, studies have not yet examined the impact of increased positive maternal behaviors on the development of extinction learning in infant offspring. In addition, no studies have investigated whether changes in maternal behavior are observed after implantation of CORT micropumps in the pups. Similarly, as we did not monitor infant behavior (outside of extinction learning and retention) after either method of CORT administration it is unclear whether these procedures would have differentially affected infant behaviors (e.g., anxiety, proximity seeking to dam) that could potentially help to explain the differences in extinction retention. In an effort to determine whether maternal or pup behavior is critical for observed effects on extinction in developing pups, it will be important for future studies to monitor maternal and pup behavior during these two CORT administration procedures.

Yet another possible reason why the two CORT administration procedures differentially affected extinction retention is that rats were exposed to CORT at different times. When administered through the dam's drinking water pups were exposed to CORT for nearly 2 weeks (i.e., P2–14), but because of difficulties in implanting the osmotic micropumps in pups that were younger than P7, CORT was only given for a week in this procedure (i.e., P7–14). Stress is known to have different outcomes depending upon the

brain regions developing at the time of the exposure; that is, stress is believed to have a more potent and permanent effect on brain regions that are developing rapidly at the time of the insult (see Lupien et al., 2009, for a review). Hence, CORT administered through the dam's drinking water would have had a more potent effect on brain regions or neural functions that were developing across the first 2 weeks of life, whereas CORT given through osmotic pumps could be expected to have had a greater effect on brain regions or neural functions that were developing only in the second postnatal week. Neural structures involved in mature extinction behaviors (e.g., the prefrontal cortex) continue to develop across the entire postnatal period (Van Eden & Uylings, 1985). This suggests that CORT administered through either method (i.e., osmotic pump or maternal drinking water) could potentially have affected PFC development. However, given the rapid nature of the changes occurring in this structure across early life, which aspects of PFC development may have been affected by each method of CORT administration would likely differ. Such differences in timing of CORT treatments could, therefore, have contributed to the slightly dissimilar outcomes in extinction retention observed in the current experiments. Exactly which aspects of neural development might have been differentially affected by the CORT treatments that could potentially underlie differences in extinction retention is a question for future studies.

Regardless of the slight differences in outcome obtained with the two CORT treatment methods, the most important finding in the current study was that, regardless of the method of administration, CORT exposure in pups has a potent effect on the development of extinction. The same accelerated emergence of adult-like extinction behaviors were observed in infant pups whether CORT was administered for 1 week or 2, and whether it was given to the dam or directly to the pups. Importantly, these data demonstrate that the developmental trajectory of extinction is not fixed; rather, extinction development can be intricately controlled by both environmental and internal levels of CORT. The fact that trajectories of emotional development can be manipulated by CORT alludes to the possible advantages to be gained from such malleable maturation rates. For example, CORT may be a useful indicator of reduced parental investment or availability. Under such circumstances infants might be better equipped to extinguish fear in a nuanced, context-dependent manner (i.e., err on the side of caution). In conditions where parental investment or availability is high, however, infants may be advantaged by extinguishing fear permanently, leaving greater time to engage in other tasks (e.g., feeding).

One of the limitations in the current article is that our examination of how pup or maternal CORT-treatment affected extinction was restricted to male infants. Although this is characteristic of many studies that examine the effects of stress across early development, the absence of sex differences analyses limits the generalizability of these findings to females. Given that the rate of anxiety disorders is higher in women than in men (Breslau et al., 1998), and that sex hormones appear to play a significant role in extinction retention in female rodents and humans (Graham & Milad, 2013), highlights the need to characterize how stress/CORT-exposure may differentially affect the development of fear extinction in males and in females. Future studies examining these outcomes across sexes should ensure that adequate sample sizes of males and females are used to make meaningful comparisons across sex.

The findings in this article have numerous other theoretical and practical implications. For example, they may hint at the processes underpinning the normal developmental trajectory of fear extinction, the psychobiology of which has remained elusive. Specifically, it is possible that rising levels of circulating CORT at the termination of the stress hyporesponsive period (SHRP) act as a signal for developmental transitions in emotional learning. If this were the case, then situations that prematurely stimulate the adrenocortical response should lead to early transitions in systems that are regulated by CORT; the inverse of this should also be true if adrenocortical responses are maintained in a suppressed state beyond the typical end of the SHRP. On a more practical level, the findings presented here highlight the importance of the developmental approach when studying the effects of adversity on mental health functioning. We have shown that relapse after extinction training (a factor that impedes the effectiveness of exposure therapy in humans) is much more common in infant rats that have been exposed to CORT than in nonexposed pups. It is worth considering the possibility that higher relapse rates after therapies that rely on inhibitory learning may exist in early adversity-exposed populations, and that CORT levels might hold some promise as a treatment target to reduce relapse.

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