

Neonatal Handling Enhances Contextual Fear Conditioning and Alters Corticosterone Stress Responses in Young Rats

Melinda L. Beane, Michael A. Cole, Robert L. Spencer,
and Jerry W. Rudy

Department of Psychology, University of Colorado, Boulder, Colorado 80309

Received December 29, 2000; revised June 4, 2001; accepted July 10, 2001

Previous studies have indicated that neonatal handling influences development of hypothalamic–pituitary–adrenal (HPA) control of corticosterone. In addition, corticosterone influences memory consolidation processes in contextual fear conditioning. Therefore, neonatal handling may affect hippocampal-dependent memory processes present in contextual fear conditioning by influencing the development of HPA control of corticosterone. To investigate the effects of neonatal handling on early learning, rat pups were either handled (15-min removal from home cage) on the first 15 days after birth or left undisturbed in their home cage. Handled rats and nonhandled rats were fear conditioned at 18, 21, or 30 days of age and then tested at two time points—24 h following conditioning and at postnatal day 45. Subsequently, at approximately postnatal day 60, rats were exposed to restraint stress and corticosterone levels were assessed during restraint and recovery. Handled and nonhandled rats did not differ significantly in their freezing response immediately following footshock on the conditioning day. However, when tested for contextual fear conditioning at 24 h following conditioning and at postnatal day 45, handled rats showed more freezing behavior than nonhandled rats. When exposed to restraint stress, handled rats had a more rapid return of corticosterone to basal levels than nonhandled rats. These results indicate that neonatal handling enhances developmentally early memory processes involved in contextual fear conditioning and confirms previously reported effects of neonatal handling on HPA control of corticosterone. © 2002 Elsevier Science (USA)

Key Words: stress; corticosterone; handling; hippocampus; contextual fear conditioning.

Neonatal handling of rats has been used to investigate the impact of early developmental experiences on neurobehavioral plasticity. In previous studies, early

handling has been reported to enhance two-way active avoidance learning (Escorihuela, Tobena, and Fernandez-Tereul, 1994), to protect against age-related declines on hippocampal-dependent learning (Meaney, Aitken, van Berkel, Bhatnagar, and Sapolsky, 1988), to reduce emotional reactivity (Levine and Mullin, 1966; Vallee, Mayo, Dellu, Le Moal, Simon, and Maccari, 1997), and also to decrease the magnitude and/or duration of hypothalamic–pituitary–adrenal (HPA) responses to stress (Liu, Diorio, Tannenbaum, Caldji, Francis, Freedman, Sharma, Pearson, Plotsky, and Meaney, 1997; Meaney *et al.*, 1988; Meaney, Aitken, Sharma, Viau, and Sarrieau, 1989; Vallee *et al.*, 1997; Viau, Sharma, Plotsky, and Meaney, 1993). Furthermore, early handling has been correlated with a lifelong increase in glucocorticoid receptor mRNA and protein expression in the hippocampus (Meaney, Aitken, Bodnoff, Iny, Tatarewicz, and Sapolsky, 1985; O'Donnell, Laroque, Seckl, and Meaney, 1994; Meaney, Diorio, Francis, Widdowson, La Plante, Caldji, Sharma, Seckl, and Plotsky, 1996) and increased negative feedback sensitivity to glucocorticoids (Meaney *et al.*, 1989).

By influencing the activity of the HPA axis, neonatal handling may substantially impact learning and memory, as varying levels of plasma corticosterone have been shown to affect hippocampal-dependent learning and memory (de Kloet, Oitzl, and Joels, 1999). Removal of corticosterone, blockade of glucocorticoid receptors, or high levels of glucocorticoids have been associated with impaired hippocampal-dependent learning and memory (Conrad, Lupien, and McEwen, 1999; Oitzl and de Kloet, 1992; Pugh, Tremblay, Fleshner, and Rudy, 1997b; McLay, Freeman, and Zadina, 1998; de Kloet, de Kock, Schild, and Veldhuis, 1988; Luine, Spencer, and McEwen, 1993; Yau, Olsson, Mor-

ris, Meaney, and Seckl, 1995), whereas low to moderate levels of corticosterone normalize adrenalectomy-induced deficits in hippocampal-dependent learning and memory (Pugh *et al.*, 1997b; Oitzl and de Kloet, 1992). For example, removal of circulating corticosterone, through adrenalectomy, impairs spatial learning, as measured in the Morris water maze (Oitzl and de Kloet, 1992) or Y maze (Conrad, Lupien, Thanasoulis, and McEwen, 1997) and adrenalectomy or pharmacological blockade of glucocorticoid receptors impairs memory consolidation involved in contextual fear conditioning of adult rats (Pugh *et al.*, 1997b; Pugh, Fleshner, and Rudy, 1997a) and passive avoidance learning of day old chicks (Sandi and Rose, 1994). Likewise, acute or chronic high concentrations of glucocorticoids have been shown to impair spatial learning (Conrad *et al.*, 1999; Mclay *et al.*, 1998; Luine *et al.*, 1993). Interestingly, acute elevations of glucocorticoids also impair *in vivo* hippocampal long-term potentiation and primed-burst potentiation (Diamond, Bennett, Fleshner, and Rose, 1992; Pavlides, Watanabe, Magarinos, and McEwen, 1995).

In addition to increasing the density of glucocorticoid receptors in the hippocampus, neonatal handling may increase the rate at which the hippocampus matures neuroanatomically, thereby impacting early hippocampal-dependent learning and memory. Normally, for rats, during the first 3 weeks after birth, glucocorticoid receptor numbers in the hippocampus steadily increase, approaching adult values at the end of the third week (Meaney and Aitken, 1985; Rosenfield, Sutanto, Levine, and de Kloet, 1988; Sarrieau, Sharma, and Meaney, 1988). Differences in early hippocampal-dependent learning and memory have also been observed toward the end of the third week postnatally. Specifically, using the Morris water maze to assess learning and memory, Rudy, Stadler-Morris, and Albert (1987) found that rats 21 days old were able to solve the hidden platform task, whereas 19-day-old rats were unable to solve the same task. Likewise, using fear conditioning to assess learning and memory, Rudy (1993) found that 23-day-old rats showed more contextual fear conditioning than 18-day-old rats. It was hypothesized that the long-term memory system supporting contextual fear conditioning was not available to 18-day-old rats, although a more recent study challenges the notion that 18-day-old rats perform qualitatively differently than older rats (Pugh and Rudy, 1996). If postnatal handling accelerates the rate of neuroanatomical maturation in the hippocampus, then handled rats may develop HPA

control of corticosterone sooner than nonhandled rats, facilitating memory processes for hippocampal-dependent learning.

The experiments that follow were designed to investigate the impact of postnatal handling on contextual fear conditioning, an aspect of fear conditioning that has been shown to be dependent on normal hippocampal function (Kim, Rison, and Fanselow, 1993; Phillips and LeDoux, 1992). For this task rats are placed in a novel environment and briefly shocked via an electrified floor grid. Upon subsequent reexposure to the shock environment rats will display a conditioned fear response, most evident from their freezing posture. Rats will also display a conditioned freezing response to a noncontextual cue, such as an auditory tone, that had been paired with footshock. A number of studies have determined that the contextual conditioned fear response is hippocampal dependent, while the conditioned cue response is not (Kim and Fanselow, 1992; Kim, Rison, and Fanselow, 1993; Phillips and LeDoux, 1992, 1994; Selden, Everitt, Jarrard, and Robbins, 1991; Young, Bohenek, and Fanselow, 1994).

For this study we also wanted to determine if the neonatal handling had long-lasting effects on HPA control of corticosterone secretion, as has been seen in other studies (Meaney *et al.*, 1989; Vallee *et al.*, 1997). We hypothesized that neonatal handling (administered postnatal days 1–15) would enhance early hippocampal-dependent learning and memory processes involved in contextual fear conditioning, through decreased HPA stress reactivity that would be evident during later exposure to acute stress. Early learning and memory associated with contextual fear conditioning were assessed by measuring the amount of freezing that was elicited by the context in which the unconditioned stimulus (footshock) occurred. For this experiment, 24-h retention of contextual fear was examined in 18-, 21-, and 30-day-old rats. In addition to the immediate effects of neonatal handling possible on contextual fear conditioning in young rats, previous studies indicate that the effects of neonatal handling persist and are evident during exposure to stress in adulthood. Therefore, the long-term retention of contextual freezing behavior was examined in all rats at a later time point (day 45). In addition, HPA stress reactivity was assessed by measuring the plasma corticosterone levels during and following challenge with acute stress (1-h restraint) in a subset of animals around 60 days of age.

MATERIALS AND METHODS

Subjects

Subjects were male and female Long–Evans-derived, hooded rat pups bred at the University of Colorado. Litters were culled to nine pups (five male and four female) 1 day after birth. Pups were weaned on day 22 and housed in same-sex groups of four. Rats were maintained on a 12:12 h light–dark cycle and had free access to food and water. The day of birth was designated day 0.

Handling

Litters were randomly assigned to either the handled or the nonhandled condition. Handling was performed on days 1 through 15, once a day, between 0700 and 0800 h. For the handling procedure, the dam of each handled litter was removed temporarily from the home cage and placed singly into a clean maternity cage. Then, each litter of pups was removed and placed together in a clean polycarbonate cage (29 × 18 × 13 cm), lined with paper towels. After 15 min, the pups were returned to their home cage. The dam was then returned to the home cage. For days 1 through 15, cage maintenance was not performed. During this time, other than handling, all litters were left undisturbed.

Apparatus

Fear conditioning occurred in two clear plastic conditioning chambers (26 × 21 × 24 cm) with window screen on top, each placed inside an Igloo ice chest (54 × 27 cm) with a clear plastic window (30 × 18 cm) in the door for observing the rats. Each Igloo chest had a white interior, with a speaker, a light bulb and a ventilation fan (providing 68-dB background noise) mounted inside. Beneath each conditioning chamber was a removable floor made of stainless steel rods, spaced either 1.2 or 0.5 cm center to center. The rod floors were wired to a shock generator and scrambler (Lafayette Instruments Model 8240415-SS) to deliver a 2-s, 0.4-mA shock. The rods and floor of each chamber were rinsed with water before training or testing each rat. A 3000-Hz tone at 75 dB was the auditory stimulus. The tester scoring the rats sat approximately 2 ft away from the rats.

Fear Conditioning Procedure

Contextual fear conditioning was assessed in separate groups of rats at three ages: 18, 21, and 30 days.

On day 16, rats were tail-marked for identification and were moved to an animal room in close proximity to the conditioning test room. Conditioning occurred between 0800 and 1000 h. Two minutes after being placed into the conditioning chambers, rats received a 2-s shock. Three minutes later, 18- and 21-day-old rats received a second 2-s shock. During the 3-min inter-shock interval freezing behavior was recorded as a measure of short-term memory and general unconditioned freezing behavior. Rats conditioned at 30 days of age received only one shock since our previous studies have demonstrated that by this age one shock is sufficient for producing a robust conditioned fear response (Rudy, 1993). Each shock was preceded by a 20-s presentation of the auditory stimulus that terminated with the onset of the shock. Rats were returned to their home cage 30 s after termination of the second shock.

Approximately 24 h after the conditioning session, fear conditioning was assessed. To assess contextual fear conditioning, each rat was placed in the conditioning chamber for 5 min. Freezing behavior was measured using a time-sampling procedure. Every 10 s, each subject was judged as either active or freezing at the instant the sample was taken. Freezing was defined as the absence of visible movement except for respiration movements.

Rats were subsequently tested on the same day for fear conditioning to the auditory cue. However, due to improper tone frequency presentation on the test day there was an unusually low level of freezing on some test days. Consequently, we cannot make a definitive statement about these results.

Subject Assignment

Subjects were randomly assigned to each treatment condition. Seven litters contributed to each neonatal handling treatment condition. Only two littermates, one male and one female, were assigned to a particular age of conditioning treatment group. To determine if the effects of neonatal handling on contextual fear conditioning persisted, rats previously conditioned at day 18, 21, or 30 were tested at a second time point, at postnatal day 45. As before, each subject was placed in the conditioning chamber for 5 min and freezing behavior was assessed every 10 s.

Corticosterone Response to Novel Stress

Sixteen rats, approximately 60 days old, from four of the litters described above were used as subjects.

These rats had been previously conditioned (counter-balanced for age of conditioning), tested at 24 h following conditioning, and retested at day 45 to context. Eight rats (4 male and 4 female) had been handled and 8 rats (4 male and 4 female) were nonhandled.

For the 60-min restraint stress, rats weighing under 250 g (females) were restrained in small size Plexiglas restraint tubes (5 cm in diameter \times 17 cm in length), whereas rats weighing over 250 g (males) were restrained in larger size restraint tubes (6.5 cm in diameter \times 19 cm in length). Blood samples (approximately 100 μ l) were taken from the tail vein. The first blood sample was taken within 3 min after placing the rat in the restrainer in order to determine basal corticosterone levels. Subsequently, samples were taken every 30 min, with the last sample taken 120 min after the initial basal sample was taken. After 60 min of restraint, each rat was returned to the home cage. Thereafter, each rat was briefly returned to the restrainer at 30 and 60 min after restraint to obtain recovery blood samples. Plasma was stored at -20°C until subsequent corticosterone measurement.

Corticosterone Measure

Plasma corticosterone was measured by radioimmunoassay. Plasma samples were diluted in 0.01 M phosphate-buffered saline and heated for 1 h at 60°C to inactivate corticosteroid binding globulin. Corticosterone standards (10–2000 pg) and samples were incubated overnight with antiserum (B3-163 Endocrine Sciences, Tarzana, CA) and [^3H]corticosterone (20,000 cpm/tube). Dextran-coated activated charcoal was used to separate antibody-bound steroid from free steroid. The intra-assay coefficients of variation for this assay were 13.3 and 11.4%, for 20- μ l control samples containing approximately 5 and 20 $\mu\text{g}/100$ ml of corticosterone, respectively.

Data Analysis

Due to concerns about the non-independence that most likely is associated with behavior of rats born and raised in the same litter (Abbey and Howard, 1973), we analyzed the data both on an individual basis and on a "unit of analysis" basis. For the unit of analysis consideration we used the average score of the male and female littermates that were tested on the same occasion. Although this approach reduces the overall sample size by a factor of 2, it also may reduce the sample standard deviation. We found that the use of individual scores or unit scores both yielded the

same statistically significant effects and have chosen to present the analysis based on the individual scores. Multiway mixed model analysis of variances (ANOVAs) were performed for both the freezing behavior and plasma corticosterone levels. Due to the repeated measures factors present within each analysis, standard post-hoc tests were not performed (Myers, 1979). Data represented on graphs are the means \pm SEMs.

RESULTS

Effect of Neonatal Handling on Immediate Freezing Response (Short-Term Memory) to Fear Conditioning

Freezing behavior of rats conditioned at 18 or 21 days of age was scored during the 3-min interval between the first and second shocks. There was not a significant difference between nonhandled ($n = 16$) and handled ($n = 12$) rats in the percentage of time spent freezing (mean \pm SEM): 37.2 ± 5.2 for nonhandled rats and 45.8 ± 7.7 for handled rats.

Effect of Neonatal Handling on Fear Conditioning (Long-Term Memory)

An initial $2 \times 3 \times 2 \times 2$ mixed design ANOVA (handling condition \times age of conditioning \times sex \times retention interval) indicated that there was no significant differences in levels of freezing between rats conditioned at postnatal days 18, 21, or 30, nor a difference in freezing between male and female rats. Moreover, the age of conditioning or sex did not significantly interact with handling condition. Consequently, age at the time of conditioning and sex were collapsed into single groups, and an ANOVA with a handled group and a nonhandled group and one repeated measure (time of testing—24 h postconditioning and postnatal day 45) was performed on freezing response data (Fig. 1). This analysis indicated a significant main effect of handling, $F(1,40) = 13.4$, $P < 0.01$, and of time of testing, $F(1,40) = 36.0$, $P < 0.01$. There was not a significant handling by time of testing interaction. Thus, neonatally handled rats showed significantly higher levels of freezing than nonhandled rats when tested for contextual fear conditioning at 24 h following conditioning. Although, as expected, the overall levels of freezing behavior were less when rats were retested on postnatal day 45, there was still

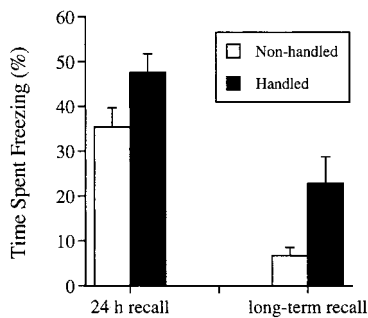


FIG. 1. Neonatal handled rats displayed increased contextual fear conditioning (time spent freezing) when tested both 24 h after training and several weeks later. Rats subjected to fear conditioning on postnatal day 18, 21, or 30 were tested for freezing behavior in the conditioning context 24 h after conditioning and on postnatal day 45. Data from rats across different ages of conditioning have been pooled. Rats were handled on postnatal days 1–15 ($n = 18$) or left undisturbed during this time in their home cage (nonhandled, $n = 24$).

significantly greater freezing behavior in handled rats than in nonhandled rats.

Effect of Neonatal Handling on Corticosterone

As shown in Fig. 2, handled rats showed a more extensive shutoff of stress-induced corticosterone than nonhandled rats within the time frame examined. ANOVA revealed a significant handling \times time interaction, $F(4,56) = 3.36$, $P = 0.02$. If the 0 time point is omitted from the overall ANOVA, there still remains a significant handling \times time interaction, $F(3,42) = 3.13$, $P = 0.04$. There was no overall effect of gender on plasma corticosterone levels, nor an interaction with handling condition.

DISCUSSION

Previously, studies have found that glucocorticoids selectively influence hippocampal-dependent memory consolidation processes in contextual fear conditioning (Pugh *et al.*, 1997a,b) and that neonatal handling reduces HPA reactivity to stress (Meaney *et al.*, 1989; Vallee *et al.*, 1997). This suggests that by influencing development of HPA control over corticosterone secretion, neonatal handling may enhance hippocampal-dependent memory processes present in contextual fear conditioning. This study provides some indirect support for this hypothesis. We found that neonatal handling significantly enhanced contextual fear conditioning. Moreover, handled rats from

the same study displayed a greater shutoff of corticosterone levels after challenge with novel stress than did unhandled rats.

These results indicate that in young rats, long-term memory processes needed for contextual fear conditioning can be enhanced by postnatal handling. In addition, the effects of neonatal handling persist, later influencing stress reactivity and behavioral responses to contextual elements similar to those present in the original conditioning context. Thus, postnatal handling significantly increased freezing behavior seen upon return to the conditioning chamber at postnatal day 45 and facilitated the termination of an HPA response to acute stress challenge.

One consideration of this study was that neonatal handling may accelerate maturation of the hippocampus such that hippocampal-dependent memory processes needed to form a memory representation of context become available sooner (Rudy and Morledge, 1994). The results of this study did not provide support for this assertion, as age at the time of conditioning did not significantly affect contextual fear conditioning. These results suggest that memory processes needed for contextual learning were available to rats as young as 18 days old. Previously, Jacobs and Nadel (1985) suggested that early learning was context independent. Similarly, Rudy and Morledge (1994) proposed that rats conditioned at day 18 were unable to form and/or consolidate a memory representation of

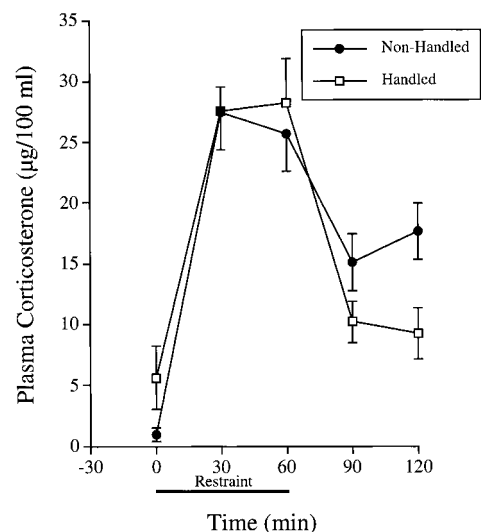


FIG. 2. Enhanced shutoff of stress-induced plasma corticosterone in neonatal handled rats when stress challenged at approximately postnatal day 60. Rats were pseudo-randomly selected from non-handled or neonatally handled litters ($n = 8$; 4 male/4 female) and challenged with 1 h of restraint stress.

context and thus showed less freezing to context than rats conditioned at day 23. However, in contrast to earlier studies, Pugh and Rudy (1996) found that rats by day 18 were able to acquire independent representation of the visual and tactile features of the context. The present study also found no differences in contextual fear conditioning between 18-day-old rats and older rats. One possible explanation for the lack of an age-related effect on contextual fear conditioning in this study may be due to the use of two shocks during training of 18- and 21-day-old rats compared to the use of one shock in previous studies that did see an age-related difference in performance.

The finding that postnatal handling increases contextual fear conditioning is consistent with the hypothesis that neonatal handling enhances hippocampal-dependent memory processes involved in contextual fear conditioning. Other researchers have argued that neonatal handling protects against age-related declines in hippocampal-dependent memory processes and tasks (Escorihuela, Tobena, and Fernandez-Teruel, 1995; Meaney *et al.*, 1988). Furthermore, it has also been shown that corticosterone selectively influences hippocampal-dependent memory processes involved in contextual fear conditioning (Pugh *et al.*, 1997a,b). Therefore, neonatal handling influences on the development of HPA control of corticosterone may consequently influence hippocampal-dependent memory processes involved in contextual fear conditioning.

Several different factors related to the experience of neonatal handling (i.e., short-term daily maternal separation) may be responsible for the long-term behavioral and physiological consequences produced by this manipulation. These factors may include activation of stress responses within the pup, transient hypothermia of the pup, or even changes induced in the pup by increased maternal attention (e.g., licking) after reunion of the pup with a mother (Denenberg, Brumaghim, Haltmeyer, and Zarrow, 1967; Liu *et al.*, 1997). Regardless of the precipitating event, one of the key underlying mechanisms of neonatal handling may be an increased concentration of glucocorticoid receptors in the hippocampus (Meaney *et al.*, 1985, 1988; Liu *et al.*, 1997). Researchers have hypothesized that neonatal handling may influence HPA control of corticosterone by increasing negative feedback sensitivity to corticosterone in the hippocampus (Meaney *et al.*, 1989; Viau *et al.*, 1993). Thus, these increased receptors may account for an enhanced negative feedback sensitivity to corticosterone and facilitated corticosterone shutoff following exposure to stress. Our results are

consistent with earlier findings (Meaney *et al.*, 1988; Vallee *et al.*, 1997) that handled rats suppress corticosterone levels faster than nonhandled rats following exposure to acute stress. We note, however, that the extent of the altered corticosterone response to restraint that we report in this study is not as great as that reported by Meaney *et al.* (1989), in which handled rats had diminished corticosterone responses during stress as well as throughout a 2-h period after stress. One intriguing possible explanation for our dampened response is that the litter culling procedure that we used partially attenuated subsequent effects of handling vs nonhandling. Although the brief disruption of the litters necessary to remove excess pups may seem trivial, Barbazanges, Vallee, Mayo, Day, Simon, Le Moal, and Maccari (1996) have demonstrated that the minor manipulation of momentary removal (<1 min) of the mother from the home cage on the first day of birth was sufficient to alter plasma corticosterone responses after restraint in the adult rat.

The extent to which neonatal handling affected the shutoff of the plasma corticosterone response to stress did not vary with gender. Moreover, we did not see an overall gender effect on plasma corticosterone responses to stress. This is contrary to other reports that generally find higher stress-induced plasma corticosterone levels in female rats than male rats (Burgess and Handa, 1992). This gender difference appears to depend on activational effects of estrogen. The gender difference is not evident prepubertally, and the magnitude of corticosterone response in female rats is positively correlated with fluctuations in estrogen either across the estrous cycle or via experimental manipulations (Burgess and Handa, 1992; Critchlow, Liebelt, Bar-Sela, Mountcastle, and Lipscomb, 1963). It is possible that the rats in this present study, although undoubtedly past puberty by 60 days of age, had not yet fully developed this sexually dimorphic state.

Future studies should determine whether the difference between handled and nonhandled rats in the shutoff of corticosterone responses to restraint generalizes to the fear conditioning session. Glucocorticoid hypersecretion during and after conditioning may interfere with consolidation of memory for the contextual features present during shock. Thus, neonatal handling may facilitate contextual fear conditioning by moderating the level of corticosterone secretion during postconditioning memory consolidation.

Additional studies are also necessary to establish the degree to which neonatal handling selectively affects the hippocampal-dependent component of fear conditioning. Neonatal handling has been found to

alter a variety of behaviors in the adult animal, including two-way active avoidance learning (Escorihuela *et al.*, 1994), open-field behavior, plus maze behavior (Vallee *et al.*, 1997), and Morris water maze performance of aged rats (Meaney *et al.*, 1988). Whether these effects of neonatal handling are hippocampal dependent has not been tested. As noted under Materials and Methods, due to improper tone presentation on some of the test days in the present study, we are not able to draw conclusions about the effect of neonatal handling on auditory cue fear conditioning (a non-hippocampal-dependent process). However, we observed a similar degree of freezing between non-handled and handled rats on the day of conditioning during the interval between the first and second shocks. This immediate response to shock has been demonstrated to represent short-term memory for shock associated stimuli and this short-term memory does not appear to be hippocampal dependent (Kim *et al.*, 1993). Thus, our short-term memory results suggest that neonatal handling did not produce an overall nonspecific increase in freezing behavior.

REFERENCES

- Abbey, H., and Howard, E. (1973). Statistical procedure in developmental studies on species with multiple offspring. *Dev. Psychobiol.* **6**, 329–335.
- Barbazanges, A., Vallee, M., Mayo, W., Day, J., Simon, H., Le Moal, M., and Maccari, S. (1996). Early and later adoptions have different long-term effects on male rat offspring. *J. Neurosci.* **16**, 7783–7790.
- Burgess, L. H., and Handa, R. J. (1992). Chronic estrogen-induced alterations in adrenocorticotropin and corticosterone secretion, and glucocorticoid receptor-mediated functions in female rats. *Endocrinology* **131**, 1261–1269.
- Conrad, C. D., Lupien, S. J., and McEwen, B. S. (1999). Support for a bimodal role for type II adrenal steroid receptors in spatial memory. *Neurobiol. Learning Memory* **72**, 39–46, doi:nlme/1988.3898.
- Conrad, C. D., Lupien, S. J., Thanasoulis, L. C., and McEwen, B. S. (1997). The effects of type I and type II corticosteroid receptor agonists on exploratory behavior and spatial memory in the Y-maze. *Brain Res.* **759**, 76–83.
- Critchlow, V., Liebelt, R. A., Bar-Sela, M., Mountcastle, W., and Lipscomb, H. S. (1963). Sex difference in resting pituitary-adrenal function in the rat. *Am. J. Physiol.* **205**, 807–815.
- De Kloet, E. R., De Kock, S., Schild, V., and Veldhuis, H. D. (1988). Antiglucocorticoid RU 38486 attenuates retention of a behavior and disinhibits the hypothalamic-pituitary adrenal axis at different brain sites. *Neuroendocrinology* **47**, 109–115.
- De Kloet, E. R., Oitzl, M. S., and Joels, M. (1999). Stress and cognition: are corticosteroids good or bad guys? *Trends Neurosci.* **22**, 422–426.
- Denenberg, V. H., Brumaghim, J. T., Haltmeyer, G. C., and Zarrow, M. X. (1967). Increased adrenocortical activity in the neonatal rat following handling. *Endocrinology* **81**, 1047–1052.
- Diamond, D. M., Bennett, M. C., Fleshner, M., and Rose, G. M. (1992). Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation. *Hippocampus* **2**, 421–430.
- Escorihuela, R. M., Tobena, A., and Fernandez-Teruel, A. (1994). Environmental enrichment reverses the detrimental action of early inconsistent stimulation and increases the beneficial effects of postnatal handling on shuttlebox learning in adult rats. *Behav. Brain Res.* **61**, 169–173.
- Escorihuela, R. M., Tobena, A., and Fernandez-Teruel, A. (1995). Environmental enrichment and postnatal handling prevent spatial learning deficits in aged hypoemotional (roman high-avoidance) and hyperemotional (roman low-avoidance) rats. *Learning Memory* **2**, 40–48.
- Jacobs, W. J., and Nadel, L. (1985). Stress-induced recovery of fears and phobias. *Psychol. Rev.* **92**, 512–531.
- Kim, J. J., and Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science* **256**, 675–677.
- Kim, J. J., Rison, R. A., and Fanselow, M. S. (1993). Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. *Behav. Neurosci.* **107**, 1093–1098.
- Levine, S., and Mullin, R. F. (1966). Hormonal influences on brain organization in infant rats. *Science* **152**, 1592–1966.
- Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P. M., and Meaney, M. J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* **277**, 1659–1662.
- Luine, V. N., Spencer, R. L., and McEwen, B. S. (1993). Effects of chronic corticosterone ingestion on spatial memory performance and hippocampal serotonergic function. *Brain Res.* **616**, 65–70.
- Mclay, R. N., Freeman, S. M., and Zadina, J. E. (1998). Chronic corticosterone impairs memory performance in the Barnes maze. *Physiol. Behav.* **63**, 933–937.
- Meaney, M. J., and Aitken, D. H. (1985). The effects of early, postnatal handling on development of hippocampal glucocorticoid receptors: temporal parameters. *Dev. Brain Res.* **22**, 301–304.
- Meaney, M. J., Aitken, D. H., Bodnoff, S. R., Iny, L. J., Tatarewicz, J. E., and Sapolsky, R. M. (1985). Early, postnatal handling alters glucocorticoid receptor concentrations in selected brain regions. *Behav. Neurosci.* **99**, 765–770.
- Meaney, M. J., Aitken, D. H., Sharma, S., Viau, V., and Sarrieau, A. (1989). Neonatal handling alters adrenocortical negative feedback sensitivity and hippocampal type II glucocorticoid receptor binding in the rat. *Neuroendocrinology* **50**, 597–604.
- Meaney, M. J., Aitken, D. H., van Berkel, C., Bhatnagar, S., and Sapolsky, R. M. (1988). Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science* **239**, 766–768.
- Meaney, M. J., Diorio, J., Francis, D., Widdowson, J., LaPlante, P., Caldji, C., Sharma, S., Seckl, J. R., and Plotsky, P. M. (1996). Early environmental regulation of forebrain glucocorticoid receptor gene expression: Implications for adrenocortical responses to stress. *Dev. Neurosci.* **18**, 49–72.
- Myers, J. L. (1979). *Fundamentals of Experimental Design*, 3rd ed., pp. 302–303. Allyn & Bacon, Boston.
- O'Donnell, D., Larocque, S., Seckl, J. R., and Meaney, M. J. (1994). Postnatal handling alters glucocorticoid, but not mineralocorti-

- coid messenger RNA expression in the hippocampus of adult rats. *Mol. Brain Res.* **26**, 242–248.
- Oitzl, M. S., and de Kloet, R. E. (1992). Selective corticosterone antagonists modulate specific aspects of spatial orientation learning. *Behav. Neurosci.* **106**, 62–71.
- Pavlidis, C. Watanabe, Y., Magarinos, A. M., and McEwen, B. S. (1995). Opposing roles of type I and type II adrenal steroid receptors in hippocampal long-term potentiation. *Neuroscience* **68**, 387–394.
- Phillips, R. G., and LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav. Neurosci.* **106**, 274–285.
- Phillips, R. G., and LeDoux, J. E. (1994). Lesions of the dorsal hippocampal formation interfere with background but not foreground contextual fear conditioning. *Learning Memory* **1**, 34–45.
- Pugh, C. R., Fleshner, M., and Rudy, J. W. (1997a). Type II glucocorticoid receptor antagonists impair contextual but not auditory-cue fear conditioning in juvenile rats. *Neurobiol. Learning Memory* **67**, 75–79.
- Pugh, C. R., and Rudy, J. W. (1996). A developmental analysis of contextual fear conditioning. *Dev. Psychobiol.* **29**(2), 87–100.
- Pugh, R. C., Tremblay, D., Fleshner, M., and Rudy, J. W. (1997b). A selective role for corticosterone in contextual-fear conditioning. *Behav. Neurosci.* **111**, 503–511.
- Rosenfield, P., Sutanto, W., Levine, S., and de Kloet, E. R. (1988). Ontogeny of type I and type II corticosteroid receptors in the rat hippocampus. *Dev. Brain Res.* **42**, 113–118.
- Rudy, J. W. (1993). Contextual conditioning and auditory cue conditioning dissociate during development. *Behav. Neurosci.* **107**, 887–891.
- Rudy, J. W., and Morledge, P. (1994). Ontogeny of contextual fear conditioning in rats: Implications for consolidation, infantile amnesia, and hippocampal system function. *Behav. Neurosci.* **108**, 227–234.
- Rudy, J. W., Stadler-Morris, S., and Albert, P. (1987). Ontogeny of spatial navigation behaviors in the rat: Dissociation of 'proximal'—and 'distal'—cue-based behaviors. *Behav. Neurosci.* **101**, 62–73.
- Sandi, C., and Rose, S. P. R. (1994). Corticosteroid receptor antagonists are amnesic for passive avoidance learning in day-old chicks. *Eur. J. Neurosci.* **6**, 1292–1297.
- Sarrieau, A., Sharma, S., and Meaney, M. J. (1988). Postnatal development and environmental regulation of hippocampal glucocorticoid and mineralocorticoid receptors in the rat. *Dev. Brain Res.* **43**, 158–162.
- Selden, N. R. W., Everitt, B. J., Jarrard, L. E., and Robbins, T. W. (1991). Complimentary roles for the amygdala and hippocampus in aversive learning to explicit and contextual cues. *Neuroscience* **42**, 335–350.
- Vallee, M., Mayo, W., Dellu, F., Le Moal, M., Simon, H., and Maccari, S. (1997). Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: Correlation with stress-induced corticosterone secretion. *J. Neurosci.* **17**, 2626–2636.
- Viau, V., Sharma, S., Plotsky, P. M., and Meaney, M. J. (1993). Increased plasma ACTH responses to stress in nonhandled compared with handled rats require basal levels of corticosterone and are associated with increased levels of ACTH secretagogues in the median eminence. *J. Neurosci.* **13**, 1097–1105.
- Yau, J. L. W., Olsson, T., Morris, R. G. M., Meaney, M. J., and Seckl, J. R. (1995). Glucocorticoids, hippocampal corticosteroid receptor gene expression and antidepressant treatment: Relationship with spatial learning in young and aged rats. *Neuroscience* **66**, 571–581.
- Young, S. L., Bohenek, D. L., and Fanselow, M. S. (1994). NMDA processes mediate anterograde amnesia of contextual fear conditioning induced by hippocampal damage: Immunization against amnesia by context preexposure. *Behav. Neurosci.* **108**, 19–29.