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Stephen M. Sivy  
*Gettysburg College*

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## **Abstract**

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## **Keywords**

juvenile rats, handling, play behavior, predatory threats

## **Disciplines**

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Effects of Neonatal Handling on Play Behavior and Fear Towards a Predator Odor in  
Juvenile Rats (*Rattus norvegicus*)

Stephen M. Sivy and Kelly A. Harrison

Department of Psychology  
Gettysburg College  
Gettysburg, PA 17325  
USA

Correspondence and proofs to:

S.M. Sivy  
Department of Psychology  
Gettysburg College  
Gettysburg, PA 17325  
USA

Email: [ssivy@gettysburg.edu](mailto:ssivy@gettysburg.edu)

### Abstract

The effects of brief daily separation, also known as “handling”, during the first 2 weeks of life on play behavior and fearfulness towards a predatory odor were assessed in juvenile rats. Handled rats were more playful than non-handled control rats and while handling had no effect on the direct response of these rats towards a predatory odor, handled rats did not exhibit a conditioned suppression of play when tested later in the same context where they had been exposed to the predatory odor. Handled rats were still wary of the environment in that they continued to show a heightened level of risk assessment behavior. These data suggest that early postnatal experiences may play a significant role in determining how an animal deals with predatory threats later in life.

Effects of neonatal handling on play behavior and fear towards a predator odor in  
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Early postnatal experiences can have a lasting impact on how mammals will respond to environmental challenges later in life. For example, it is well established that brief daily separations of rat pups from the dam during the first two weeks or so of life attenuates behavioral and hormonal responses to various stressors while longer periods of separation can have the opposite effect (Anisman, Zaharia, Meaney, & Merali, 1998; Meaney et al., 1991; Plotsky & Meaney, 1993; Sapolsky & Meaney, 1986). As brief maternal separation leads to increased licking and grooming of the pups by the mother, the stress-attenuating effects associated with brief periods of separation are thought to be largely dependent upon increased care directed by the mother towards the litter upon reunion (Caldji et al., 1998; Champagne, Weaver, Diorio, Sharma, & Meaney, 2003; Meaney, 2001; Zhang, Chretien, Meaney, & Gratton, 2005). This has been confirmed through subsequent studies showing that pups of dams that naturally engage in more licking and grooming tend to be less fearful (Menard, Champagne, & Meaney, 2004), are more likely to explore a novel environment (Caldji et al., 1998) and have an attenuated startle response (Zhang et al., 2005) when compared to rats raised by dams that engage in less licking and grooming.

Taken together, these data suggest that the quantity and quality of maternal care can have a lasting influence on a range of behavioral processes throughout life. Overall levels of fearfulness seem to be particularly susceptible to modulation by early experiences. Handled rats and rats of dams that engage in more licking and grooming are

more active when tested in a novel open field and are more likely to venture into the center of the field (Caldji et al., 1998; Madruga, Xavier, Achaval, Sanvitto, & Lucion, 2006; Menard et al., 2004), are more likely to approach a live predator when there is no opportunity for escape (Padoin, Cadore, Gomes, Barros, & Lucion, 2001), and are less likely to exhibit conditioned fear to cues that have been previously associated with foot-shock (Kosten, Lee, & Kim, 2006; Madruga et al., 2006). While these data would seem to suggest that handling decreases fearfulness in a variety of experimental paradigms, at least one study has reported enhanced fear conditioning in juvenile rats that were handled as newborns (Beane, Cole, Spencer, & Rudy, 2002), suggesting that handled rats may be susceptible to increased fearfulness. Since this latter study used pre-pubertal rats, while the other studies assessed behavior in adults, age of testing may be a critical factor in assessing the effects of handling on fear.

The young of many mammalian species regularly encounter a myriad of threats and stressors as they navigate into adulthood. For a small prey species, such as the rat, the time from weaning until adulthood can be particularly fraught with danger as young venture away from the relative protection of the nest. Safety can be further compromised during this developmental period by an increased compulsion to engage in risky behaviors (Galvan et al., 2006; Macri, Adriani, Chiarotti, & Laviola, 2002; Spear, 2000) and by the presence of regular bouts of rough-and-tumble play that may increase the risk of detection by predators (Bekoff & Byers, 1998; Burghardt, 2005; Panksepp, Siviy, & Normansell, 1984; Pellis & Pellis, 1998). Rats are particularly vulnerable to larger predators and have evolved a range of adaptive strategies to detect and avoid these predators. When confronted with a predatory threat, rats will cease most ongoing non-

defensive behaviors and engage in a variety of defensive behaviors that will depend on options available in the immediate environment (Blanchard & Blanchard, 1989, 2003). For example, providing an opportunity to hide will result in a rapid retreat to safety while freezing and/or attack are more likely to occur if there is no avenue of escape. These types of defensive behaviors can be readily obtained by the presence of an actual predator or simply by cues that predict the possible presence of a predator (Apfelbach, Blanchard, Blanchard, Hayes, & McGregor, 2005; Blanchard & Blanchard, 2003; Dielenberg & McGregor, 2001; Masini, Sauer, & Campeau, 2005; Takahashi, Nakashima, Hong, & Watanabe, 2005). As might be expected, fear of predation develops early in the rat, with both unconditioned and conditioned responses to cat odor being readily obtained at a fairly young age (Hubbard et al., 2004; Panksepp, 1998; Wiedenmayer & Barr, 2001).

Recent work from our lab (Siviy, Harrison, & McGregor, 2006) has shown that an acute exposure to the smell of a natural predator (cat) will virtually abolish play behavior in young rats. When tested later in the chamber where the cat odor was experienced, play continued to be suppressed up to 7 days after exposure, suggesting that strong contextual fear conditioning had been established. Providing rats with an opportunity to hide had no significant impact on how these rats responded to the predatory threat; play was reduced to a comparable extent in rats with or without an opportunity to hide. This highlights the relatively fragile nature of play when faced with a threat to survival and suggests that playfulness may be a sensitive barometer for fear and/or anxiety in the young animal. To better understand the extent to which early postnatal experiences can affect fearfulness using an innate stimulus in young rats, we assessed whether neonatal

handling would affect the unconditioned and conditioned consequences of exposing juvenile rats to a predatory odor.

## Methods

### Subjects

Rats were bred and born in the Gettysburg College animal facility from Sprague-Dawley rats originally obtained from Harlan Sprague-Dawley (Indianapolis, IN). Animals were housed in a colony room maintained at 22<sup>o</sup> C with a 12/12 hour light/dark cycle (lights on at 8 am) with food and water freely available. Rats were housed with the dam in solid bottom cages (48 x 27 x 20 cm) with corncob bedding and an ample supply of nesting material (Iso-Pads; Harlan Sprague-Dawley) until weaning at 21 days of age. Upon weaning, rats were re-housed with same-gender littermates in solid-bottom cages (48 x 27 x 20 cm) until testing began, at which point they were re-housed individually in smaller solid-bottom cages (27 x 21 x 14 cm).

### Apparatus

Play was assessed in a Plexiglas chamber (60 x 60 x 50 cm) that was painted black on all four sides. The floor of the chamber was covered with about 3 cm of pine shavings. A wooden hide box (20 x 26 x 50 cm) with a small (6 x 8 cm) opening was situated in one corner of the chamber. The room was darkened during testing and the outer chamber was illuminated by two 25W red light bulbs, whereas the hide box was not illuminated. Play bouts were videotaped with a camera that was directly above the outer chamber and an infrared-sensitive camera that was placed inside the hide box. Video input from both cameras was directed through a quad multiplexer and recorded for later

viewing. A time code was placed on the tape and behavior was scored using the Observer Video-Pro Video Analysis Program (Noldus Information Technology).

The collars used in this study were Petwear Adjustable Safety Collars (Rose America Corporation, Wichita, KS). Worn collars were obtained from a domestic cat (spayed female that spent most of the time indoors) that had been wearing the collar for approximately 2 months. The collar was cut into 2.5 cm pieces and only those pieces of the collar that came in direct contact with the fur of the cat were used. The collars were stored in airtight containers with a tuft of cat fur at  $-10^{\circ}\text{C}$  and warmed prior to testing by immersing the sealed container in hot ( $50^{\circ}\text{C}$ ) tap water for 10 minutes. Care was taken to insure that the collars never got wet and the collars were always handled with gloved hands.

### Procedure

Pregnant females were checked in the morning and late afternoon for births, with the day of birth designated as P0. Litters were culled on P1 to no more than eight pups and, when possible, culled to four males and four females. Eight litters were assigned to receive brief daily periods of maternal separation (“handling”) while eight litters were assigned to be non-handled controls. For those litters assigned to the handling condition, the handling procedure began on P2 and continued through P15. On each of these days, the mother was removed from the litter and placed in a holding cage. The entire litter was then transported in the home cage to an adjacent room. The pups were removed from the home cage and placed in a smaller container, also containing corncob bedding, which was kept warm with a heating pad. After 15 minutes, the pups were returned to the home cage, transported back to the colony room, and the mother returned to the litter.

This procedure was repeated for 14 consecutive days at approximately the same time (midway through the light phase of the light/dark cycle) each day. The non-handled control litters were left undisturbed, with the exception of weekly cage maintenance, until weaning.

Litters were weaned on P21 and re-housed in groups of same-gender littermates. Play testing and responsiveness to cat odor were tested over a 5-day period and rats were isolated 24 hours prior to the beginning of the 5-day testing regimen. Rats remained isolated throughout the testing period. In order to insure that at least one non-handled and one handled litter were always tested at the same time, thus assuring that the potency of the collar pieces would be comparable for both groups, the age of the rats on the first day of the testing regimen varied from 25 to 34 days. The mean ( $\pm$  SEM) age at the start of the testing regimen was  $28.3 \pm 1.0$  days for the non-handled controls and  $28.5 \pm 1.0$  days for the handled rats. Nine of the 16 litters were culled to an equal number of male and female pups, allowing for one pair of each gender to be tested with an unworn collar and one pair of each gender to be tested with a worn collar. For the remaining litters that were unbalanced in terms of gender or that had fewer than eight pups, all attempts were made to balance both gender and exposure within and across litters.

Rats were initially acclimated to the testing apparatus by allowing them to play with a same-treatment and same-gender littermate for 3 days. On each of these days, the rats were placed in the chamber for 5 minutes. Play was assessed on the first and third baseline days by recording the number of contacts directed by each rat to the nape of the other rat (nape contacts) and the number of times each rat was pinned by the other rat. A nape contact is scored if one rat brings its snout to within 1 cm of the nape of its partner,

whereas a pin is defined as occurring if a rat is on its back with at least three paws in the air (Panksepp et al., 1984; Siviy, 1998; Siviy & Panksepp, 1987; Vanderschuren, Niesink, & Van Ree, 1997). Risk assessment was also measured by quantifying the amount of time at least one of the rats was engaged in “head-out” behavior. Head-out behavior is a type of vigilant scanning of the environment from the relative safety of a confined space, such as a hide box, and has been suggested to be a sensitive measure of risk assessment (Blanchard & Blanchard, 1989; Dielenberg, Carrive, & McGregor, 2001; McGregor, Schrama, Ambermoon, & Dielenberg, 2002). An occurrence of head-out behavior was noted when at least one rat was inside the hide box, not moving, and had at least its entire head outside of the box and at least two hind paws within the box.

On the fourth test day, rats were exposed to either pieces of an unworn or worn cat collar. Two collar pieces were placed on the wall facing the opening of the hide box, and two pieces were placed on the one remaining wall without a hide box along it. The pieces of collar were kept in place by alligator clips that were situated approximately 5 cm from the base of the chamber and remained in the chamber for the entire 5 minute observation period. At the conclusion of this day of testing, the pine chips were removed from the chamber. Both the chamber and the hide box were wiped down with water, followed by alcohol. Fresh pine chips were then placed in the chamber. Rats were tested without any collars present on the next day.

### Statistical analyses

The data for each behavioral measure (nape contacts, pins, head out) over the two baseline days and over the two days assessing fear (exposure day and post-exposure day) were assessed by separate repeated measures Analyses of Variance (ANOVA). Gender

was included as a factor in all of the analyses. Any significant interactions were further analyzed using either independent samples or matched-samples t-tests, with a Bonferroni correction for multiple comparisons.

### Results

Play behavior on the two baseline days can be seen in Figures 1 and 2. Nape contacts (Figure 1) increased over the two days,  $F(1,58) = 103.21$ ,  $p < .001$ ,  $\eta^2 = .64$  and handled animals exhibited more nape contacts than control animals,  $F(1,58) = 10.11$ ,  $p < .005$ ,  $\eta^2 = .148$ . No other main effects or interactions were found to be statistically significant. Pinning (Figure 2) also increased over the two days of testing,  $F(1,58) = 107.18$ ,  $p < .001$ ,  $\eta^2 = .649$ , and handled animals pinned more than control animals,  $F(1,58) = 8.52$ ,  $p < .006$ ,  $\eta^2 = .128$ . As with nape contacts, no other main effects or interactions were significant. These data suggest that handled rats were more playful than non-handled rats.

Another way to quantify and characterize playfulness in the rat is to assess the likelihood of a rat being pinned in response to a nape contact. Pinning has been shown to be one type of defensive response made by young rats to contacts directed at the nape and has been suggested to reflect playfulness (Pellis & Pellis, 1990, 1991), so the relative playfulness of a rat can also be ascertained by determining the likelihood of a pin occurring in response to a nape contact. The play data from the two baseline days were converted to probabilities by dividing the number of pins by the number of contacts and analyzing these data as before. Data from 3 pairs of control animals could not be analyzed because they did not exhibit any nape contacts on first baseline day. When the data from the remaining animals was analyzed, there was a significant main effect of

group, with handled animals being more likely to respond to a nape contact with a pin than were control rats,  $F(1,55) = 4.803$ ,  $p < .05$ ,  $\eta^2 = .080$ . The mean ( $\pm$  95% CI) probability of being pinned in response to a nape contact over the two days was 0.241 ( $\pm$  .039) for handled rats and 0.183 ( $\pm$  .038) for control rats. With the exception of a main effect associated with day,  $F(1,55) = 120.12$ ,  $p < .001$ ,  $\eta^2 = .686$ , there were no other significant main effects or interactions. These data add support to our conclusion that handled rats tend to be more playful than non-handled rats.

Risk assessment, as measured by head out behavior, on the two baseline days can be seen in Figure 3. Handled animals exhibited less head out behavior,  $F(1,58) = 9.13$ ,  $p < .005$ ,  $\eta^2 = .136$ , and this was fairly constant over the two days as indicated by a non-significant main effect of day and non-significant interactions involving day. While the day x gender x group interaction approached significance,  $F(1,58) = 3.94$ ,  $p = .052$ ,  $\eta^2 = .064$ , this effect was not robust enough to stand up to subsequent analyses.

The effects of exposing control and handled rats to a worn cat collar on play can be seen in Figures 4 and 5. For nape contacts (Figure 4), there was a marginal main effect of group,  $F(1,54) = 4.02$ ,  $p = .05$ ,  $\eta^2 = .069$ , indicating that handled animals continued to be slightly more playful than controls on this measure. As expected, nape contacts were significantly reduced by exposure to a worn cat collar,  $F(1,54) = 11.62$ ,  $p < .005$ ,  $\eta^2 = .177$ , and this effect was especially pronounced on the exposure day, as indicated by a significant day X collar interaction,  $F(1,54) = 6.51$ ,  $p < .02$ ,  $\eta^2 = .108$ . Further analysis of this interaction indicated that both control and handled animals that were exposed to a worn cat collar had fewer nape contacts than those exposed to an unworn collar on the day of exposure, but neither group maintained this difference on the

post-exposure day. No other main effects or interactions were found to be significant. These data suggest that neither control nor handled rats exhibited a conditioned suppression of nape contacts when tested in the environment where they had experienced the smell of a predator.

Handled animals continued to pin more than control animals (Figure 5),  $F(1,54) = 8.85$ ,  $p < .005$ ,  $\eta^2 = .141$ , and exposure to a worn cat collar reduced this measure of play as well,  $F(1,54) = 5.72$ ,  $p < .05$ ,  $\eta^2 = .096$ . There was also a significant day x group x collar interaction,  $F(1,54) = 5.33$ ,  $p < .05$ ,  $\eta^2 = .090$ . Further analysis of this interaction indicated that control animals exposed to a worn cat collar pinned less than those exposed to an unworn collar on both the exposure day and the post-exposure day, indicating that non-handled control animals exhibited both an unconditioned and conditioned suppression of pinning. Handled animals exposed to a worn cat collar pinned less than those exposed to an unworn collar on the exposure day, indicating that these rats exhibited an unconditioned suppression of play towards the smell of a predator. However, handled animals did not exhibit a conditioned suppression of pinning on the subsequent day since pinning among those handled rats exposed to a worn collar on the previous day did not differ from those handled rats exposed to an unworn collar.

Risk assessment, as measured by head-out behavior on both the exposure and post-exposure days, can be seen in Figure 6. There was a significant effect of collar,  $F(1,54) = 14.13$ ,  $p < .001$ ,  $\eta^2 = .207$ , indicating that those rats exposed to a worn collar displayed more head out behavior than those exposed to an unworn collar. The lack of any interaction involving day and/or collar indicated that the effect of exposure to a worn

collar was comparable across days and comparable between the control and handled rats. No other main effects of interactions were found to be significant.

### Discussion

Since two landmark studies published some 50 years ago (Denenberg & Karas, 1959; Levine, Alpert, & Lewis, 1957), it has become well established that separating rat pups from the dam for brief daily periods during the first two weeks of life, an experimental protocol known as “handling”, can have a number of behavioral and hormonal consequences later in life. In particular, handled rats have been shown to be less fearful and are less reactive to a variety of stressors when tested later in life (for reviews see Boccia & Pedersen, 2001; Champagne & Curley, 2005; Meaney, 2001; Parent et al., 2005). In the present study, we separated pups from the mother for 15 minutes each day during the first two weeks of life and assessed subsequent play behavior and responsiveness to a predator odor when tested as juveniles. Our results indicate that these rats were more playful than non-handled control rats, responded predictably with less play and more risk assessment to the smell of a predator, but were less likely to exhibit a conditioned suppression of play when returned to a context where the predator odor had been previously experienced.

Prior to introducing any predatory threat, handled rats were found to be consistently more playful than non-handled controls. Previous work from our laboratory (Arnold & Siviy, 2002) found that handling did not affect the overall frequency of playful behaviors, although there were some subtle gender-dependent differences in how handled rats responded to playful overtures. While it isn't exactly clear what could be accounting for the difference between the results of these two studies, several possibilities can be

considered. First, the rats used in the present study were bred locally while our prior work used rats born to pregnant females that were obtained at 17 days gestation. Since prenatal stress has been shown to have effects opposite to that of handling (Vallee et al., 1997), it is possible that any handling-induced enhancement of play in our prior study may have been negated by the stress associated with shipping these rats while pregnant. Second, the environment in which the rats played differed between the two studies. The test chamber used in the present study was larger than that used in our earlier work and it also included additional complexity in the form of a hide-box. This may have led to opportunities for a richer exhibition of play behavior that may have increased the likelihood of detecting a difference between the groups.

With the exception of one marginal interaction involving day, gender, and group on risk assessment during the post-baseline testing, gender was not a significant source of variance on any of the measures. While there are a number of reports documenting gender effects on play in young rats (Meaney, Stewart, Poulin, & McEwen, 1983; Olioff & Stewart, 1978; Pellis, Pellis, & McKenna, 1994; Poole & Fish, 1976; Thor & Holloway, 1986), these are by no means universal (Almeida & De Araujo, 2001; Holloway & Suter, 2004; Panksepp et al., 1984) and can also be quite subtle (Arnold & Siviy, 2002; Pellis, Field, Smith, & Pellis, 1997; Pellis & Pellis, 1990). For example, Pellis and Pellis (1990) report minimal gender differences between male and female rats when tested in same-gender pairings at an age (31-35 days old) comparable to when rats were tested in the current study, where all testing was completed by 34 days of age. While it's possible that gender differences may have emerged had we continued testing

as the rats got older, it is clear from these data that gender differences are minimal, at best, under our testing conditions.

Why are handled rats more playful? One possibility is that these rats were more accustomed to experimenter contact, *per se*. While we cannot completely exclude this possibility, this explanation predicts that differences between these groups should have become less pronounced with time as the rats from the non-handled group became more accustomed to experimenter contact. While group differences in nape contacts did seem to decrease with continued testing, elevated levels of pinning in the handled rats stayed fairly constant throughout. Handled rats also tended to be consistently less wary of their environment during baseline testing, as indicated by less risk-assessment behavior from within the confines of a hide-box. This suggests that handled rats may consistently perceive the testing environment as less threatening than non-handled rats, perhaps making them more likely to engage in playful behaviors.

The smell of a predator can have a profound effect on the behavior of rats, with most non-defensive behaviors being suppressed while being replaced with various defensive behaviors (Blanchard & Blanchard, 2003; Blanchard, Yang, Li, Gervacio, & Blanchard, 2001; Dielenberg et al., 2001). Fear of predation develops early in the rat (Hubbard et al., 2004; Panksepp, 1998; Wiedenmayer & Barr, 2001) and recent work from our laboratory has shown that cat odor results in both unconditioned and conditioned suppression of play behavior in juvenile rats (Siviy et al., 2006). In the present study, the response of handled rats was comparable to that of non-handled rats when directly exposed to the smell of a predator; both handled rats and non-handled control rats played less and engaged in more risk assessment from the confines of the

hide-box. This suggests that handling does not affect the unconditioned response of a rat to the smell of a predator. We are aware of one other study that has looked at the effects of early handling on responsiveness to a predator (Padoin et al., 2001) and this study found that handled rats were more likely to approach a live cat and less likely to engage in vigilant scanning, a form of risk assessment. While rats in the present study were exposed to a partial predator cue only and had an opportunity to retreat into a hide box, Padoin and colleagues (2001) exposed rats to a live cat in an open field with no avenue of escape. Given that the response towards a predator depends upon the nature of the threat (actual predator compared to a predator cue) and the options available at the time of the confrontation (Blanchard & Blanchard, 2003), such methodological differences could readily account for these apparent differences in results. For example, if we assume that handled rats are less fearful overall (Caldji, Francis, Sharma, Plotsky, & Meaney, 2000; Caldji et al., 1998; Madruga et al., 2006; Menard et al., 2004), then they may be more likely to directly confront a potential threat when there is no option to escape but might still be expected to retreat to safety if that option is available.

Rats will continue to exhibit fear when returned to the same context in which a predator odor was experienced or presented with a cue previously associated with a predator odor (Blanchard et al., 2001; Dielenberg et al., 2001; Hubbard et al., 2004; Siviy et al., 2006). In the present study, we found evidence for contextual fear conditioning with pinning and risk assessment, but not for nape contacts. When compared to our previous work (Siviy et al., 2006), fear conditioning in the present study was not as robust. However, there are clear strain differences in the extent to which a predator odor can affect behavior (Apfelbach et al., 2005) and the most robust fear in our earlier work

was noted with Wistar rats, while Sprague-Dawley rats were used in the present study. In order to assess whether strain of rat may have been a factor in the magnitude of our effects and, more importantly, that the collars being used in the present study provided an effective source of odorant, a series of preliminary studies were done with Long-Evans rats, a strain that is known to be more fearful than Sprague-Dawley rats (Apfelbach et al., 2005). As expected, play was virtually abolished in Long-Evans rats with the same collars that produced a more modest reduction of play among Sprague-Dawley rats in the present study.

Despite these apparent strain differences in the magnitude of innate fear between studies, non-handled rats in the present study that were exposed to a predator odor still continued to pin less and show more risk assessment when returned to the chamber where they had previously encountered the predator odor than those not exposed to a predator odor. On the other hand, pinning among handled rats exposed to the predator odor did not differ on the post-exposure day from those not exposed to the odor. While this indicates a lack of contextual fear conditioning on this behavioral measure, these rats were still somewhat wary of the environment as they continued to exhibit significantly more risk-assessment behavior than those not exposed to a predatory odor.

Since handled rats were also more playful overall, the lack of conditioned fear on this measure could be reflecting higher levels of baseline play. In order to assess the likelihood of this possibility, the data for pins on the exposure and post-exposure days were re-analyzed with baseline levels of pinning as a covariate. The results of this analysis were unchanged (i.e., there was still a significant day x group x collar interaction) and indicate that the lack of conditioned fear for pinning is not an artifact

associated with higher levels of baseline play in these animals. Rather, these data suggest that early handling results in less fear conditioning when an innate fear stimulus is used. While these findings should be replicated with a strain that has a more robust response to predator odors, the data are consistent with handled rats being less fearful when tested as juveniles.

Previous studies using foot shock as an unconditioned stimulus to address fear conditioning in handled rats have not always yielded consistent results. Several studies have reported that contextual fear conditioning is attenuated in handled animals (Kosten et al., 2006; Madruga et al., 2006; Meerlo, Horvath, Nagy, Bohus, & Koolhaas, 1999) and our data would be consistent with these findings. However, one study has reported enhanced fear conditioning (Beane et al., 2002) and at least one has found conditioned fear to be unaffected by handling (Pryce, Bettschen, Nanz-Bahr, & Feldon, 2003). Due to the relatively small number of studies that have assessed conditioned fear in handled rats, there is no clear pattern that can easily explain these conflicting results. While Kosten and colleagues (2006) suggested that the enhanced fear conditioning observed by Beane et al. (2002) may reflect the younger age at which these rats were tested, the rats in the present study were also tested prior to puberty and we observed less fear conditioning. Since Beane and colleagues (2002) used Long-Evans rats, while the other cited papers used either Sprague-Dawley or Wistar rats, it is possible that strain may be an important variable in determining the effect of handling on contextual fear conditioning. The behavioral index used to assess fear conditioning may also be important in that we observed conditioned fear with risk assessment, but not with play. In any case, our results provide support for those studies showing that handling attenuates

contextual fear conditioning and extend these studies by demonstrating attenuated fear conditioning associated with a predatory threat. Yet, the discrepancies in results highlight the need for additional studies comparing different strains of rat.

Handling results in long-term changes to a number of neurobiological systems that have the potential for impacting both playfulness and fear conditioning in juvenile rats. The most prominent and well-established effects of handling are those associated with the hypothalamic-pituitary-adrenal (HPA) axis. In particular, stress-induced activation of the HPA axis is markedly attenuated in handled rats (Meaney, Aitken, Bodnoff, Iny, & Sapolsky, 1985; Meaney et al., 1991; Plotsky & Meaney, 1993) and this is believed to be due to enhanced negative feedback resulting from increased expression of glucocorticoid receptors in the hippocampus (Liu et al., 1997; Meaney et al., 1985). Restraint stress is a potent activator of the HPA axis and has been recently shown to decrease play in young rats (Romeo, Karatsoreos, & McEwen, 2006), suggesting that increases in corticosterone may dampen the playfulness of juvenile rats. Increased secretion of corticosterone has been reported following a bout of play (Gordon, Kollack-Walker, Akil, & Panksepp, 2002), leaving open the possibility that elevations in circulating glucocorticoids resulting from active rough-and-tumble play may act as a cue that contributes to terminating a play bout. Accordingly, attenuations in corticosterone may tend to make rats more playful.

High levels of corticosterone, resulting either from chronic administration of corticosterone (Kalynchuk, Gregus, Boudreau, & Perrot-Sinal, 2004; Pugh, Tremblay, Fleshner, & Rudy, 1997; Skorzewska et al., 2006) or from chronic restraint stress (Conrad, Magarinos, LeDoux, & McEwen, 1999) has been shown to enhance fear

conditioning. Conversely, adrenalectomized rats show less fear conditioning and this can be reversed with corticosterone treatment (Pugh et al., 1997). Finally, a positive correlation has been noted between behavioral inhibition and corticosterone levels in rats that are tested for contextual conditioning 24 hours after receiving foot shock, such that those rats showing the most conditioned fear are those that exhibit the highest corticosterone levels (Cordero, Merino, & Sandi, 1998). On the other hand, chronic corticosterone administration has been found to have no effect on the direct response of a rat to a predator odor (Kalynchuk et al., 2004). While still speculative, our behavioral data is fairly consistent with these findings in that handled rats exhibited less fear conditioning when tested 24 hours after exposure to a predator odor even though the direct response to the smell of a predator was unaffected.

Given the importance of detecting and avoiding predatory threats to a small prey species like the rat, one must wonder whether there is an adaptive advantage associated with less contextual fear conditioning when a predator cue is involved. It is perhaps significant that handling did not affect the direct response to cat odor, indicating that these animals would still respond in an adaptive way to a more immediate threat. Furthermore, rats were still wary of the environment on the post-exposure day even though play returned to baseline levels. Therefore, handled rats are still able to associate a particular context with a predatory cue, but are also able to maintain levels of play that are comparable to animals that have not experienced a predatory threat. These data also provide some insight into the extent to which play can occur in the face of threats to survival. It is often assumed that play will only occur once primary physiological needs have been met and when there is no immediate threat to survival. Showing that play in

handled animals can occur at baseline levels when an animal still senses a threat suggests that play behavior may not be as fragile as has often been assumed.

As mentioned earlier, the effects of handling are believed to be due to increases in maternal licking and grooming in response to brief daily separations (Caldji et al., 1998; Champagne, Francis, Mar, & Meaney, 2003; Meaney, 2001; Zhang et al., 2005) and it has been suggested that the quantity and quality of maternal care may provide an anticipatory cue as to the type of environment into which an animal has been born (Champagne & Curley, 2005; Meaney, 2001). Increased maternal licking and grooming, whether due to natural variations in maternal behavior or induced by brief daily separation, is thought to reflect or mimic the behavior of a mother that lives in an environment where resources are abundant and readily available. If so, then the present data suggest that playfulness and responsiveness to a predatory threat may also be sensitive to cues that predict availability of resources, with cues such as increased maternal care leading to a behavioral pattern of increased playfulness and less fearfulness.

Dealing effectively with predatory threats is an ongoing concern to a small prey species, such as the rat, and the price for not detecting these threats can be steep. However, remaining fearful when a threat is no longer imminent may be as detrimental to ultimate survival as being too bold in the face of a clear threat to safety (Dielenberg & McGregor, 1999). Therefore, an animal must use cues from the environment in order to take the best course of action as it navigates behaviorally through its world. When resources are abundant, less distance may need to be traveled in order to obtain food and water, thus decreasing the likelihood of being unnecessarily exposed to the risk of

predation. In such an ecological system, a rat may be able to afford to be not as vigilant as the rat that lives in an environment with more scarce resources. Obviously, a balance needs to be reached and many factors are likely to contribute towards determining the extent to which an animal will remain vigilant as a threat becomes less imminent. These data suggest that early postnatal experiences may be one important factor in helping determine the behavioral strategies that a rat will use to avoid predation after leaving the nest.

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## Figure Captions

Figure 1. Mean ( $\pm$  95% CI) number of nape contacts in control and handled rats during baseline testing.

Figure 2. Mean ( $\pm$  95% CI) number of pins in control and handled rats during baseline testing.

Figure 3. Mean ( $\pm$  95% CI) amount of time spent in risk assessment behavior during baseline testing, as determined by percentage of total time engaged in head out behavior.

Figure 4. Mean ( $\pm$  95% CI) number of nape contacts in control and handled rats for those that were either exposed to an unworn collar or a collar previously worn by a domestic cat. No collar was present on the post-exposure day. The horizontal dashed line is the mean number of nape contacts for each group averaged across the two baseline days.

Although gender was included as a factor in the analysis of these data, gender was not a significant factor and is not included for the sake of clarity. \*  $p < .05$ , compared to unworn collar

Figure 5. Mean ( $\pm$  95% CI) number of pins in control and handled rats for those that were either exposed to an unworn collar or a collar previously worn by a domestic cat. No collar was present on the post-exposure day. The horizontal dashed line is the mean number of pins for each group averaged across the two baseline days. Although gender was included as a factor in the analysis of these data, gender was not a significant factor and is not included for the sake of clarity. \*  $p < .05$ , compared to unworn collar

Figure 6. Mean ( $\pm$  95% CI) amount of time spent in risk assessment behavior in control and handled rats for those that were either exposed to an unworn collar or a collar previously worn by a domestic cat. No collar was present on the post-exposure day. The

horizontal dashed line is the mean amount of time spent in risk assessment for each group on the baseline day. Although gender was included as a factor in the analysis of these data, gender was not a significant factor and is not included for the sake of clarity.

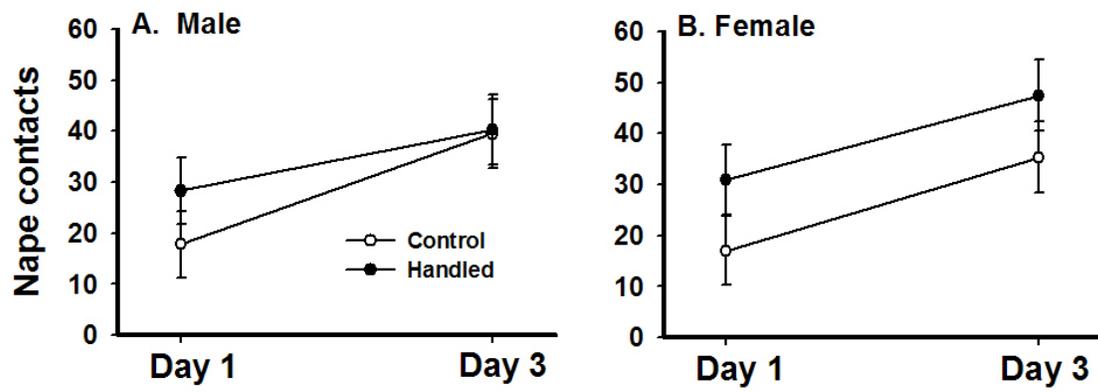


Figure 1

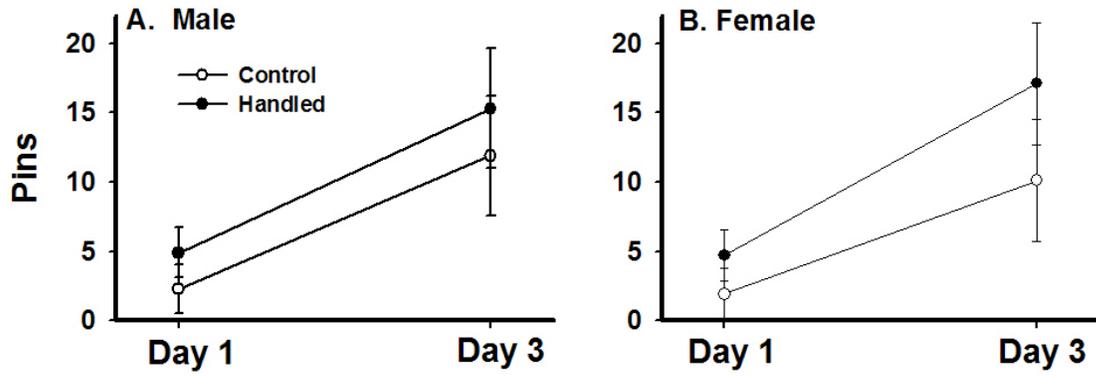


Figure 2

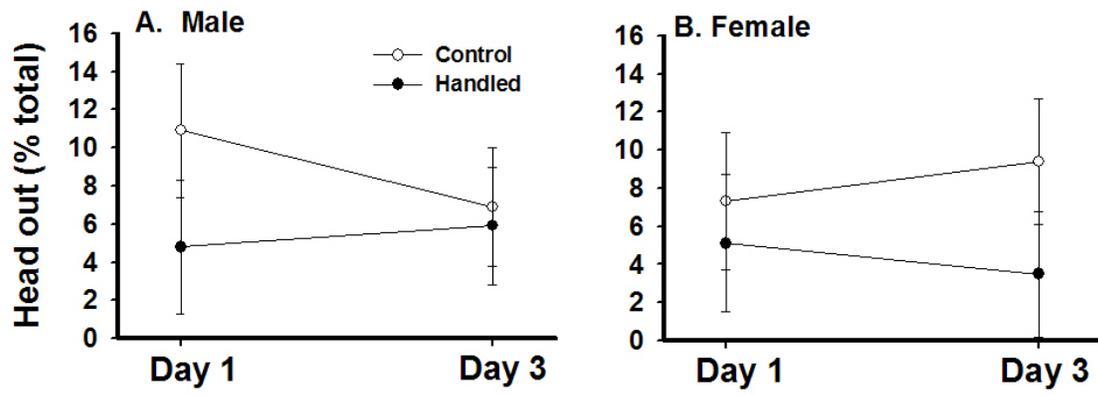


Figure 3

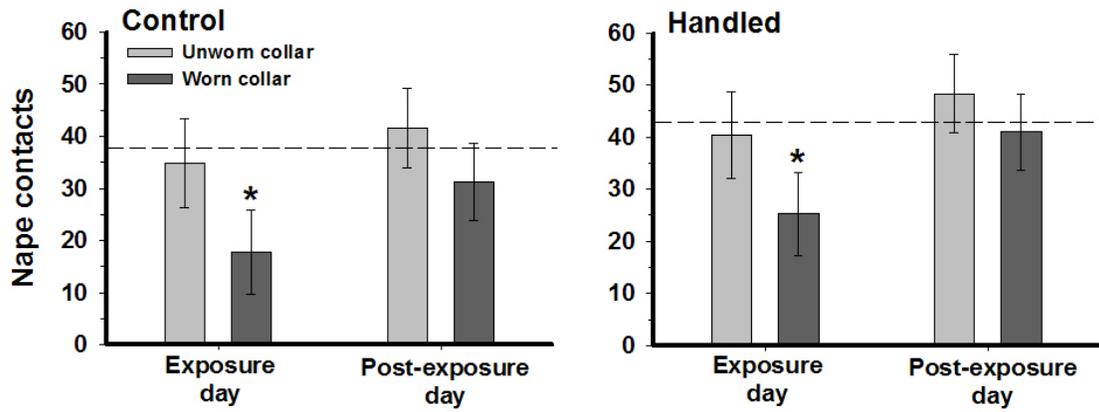


Figure 4

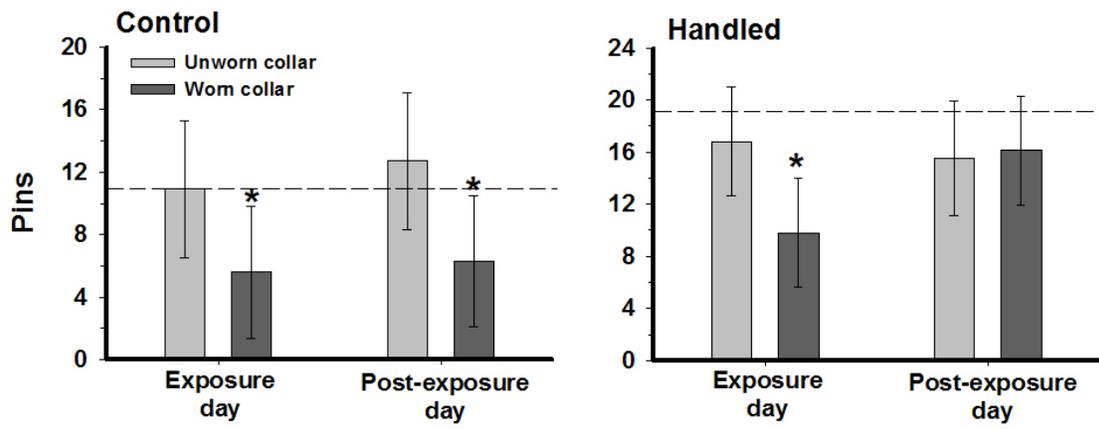


Figure 5

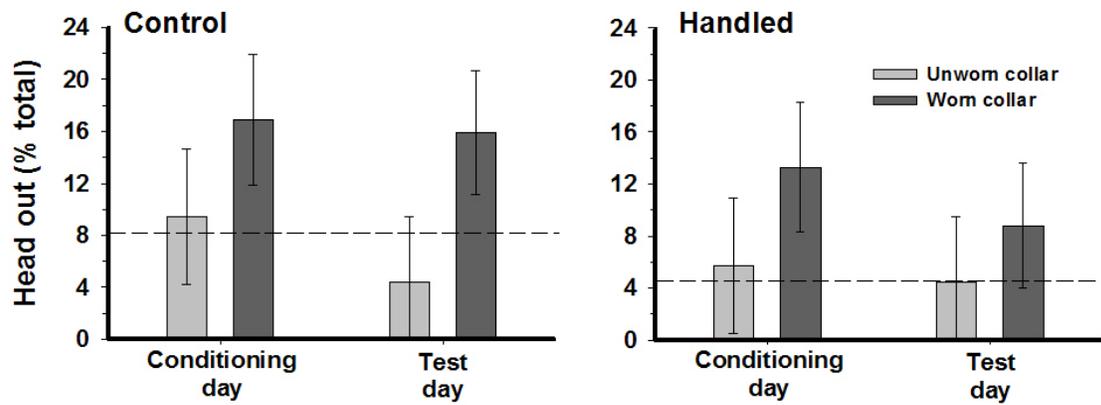


Figure 6

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