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Keywords
juvenile rats, social buffering, fear, play

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Effects of Prepubertal Social Experiences on the Responsiveness of Juvenile Rats to Predator Odors

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Abstract

The extent to which social variables may modulate the fear associated with a predator cue was assessed in juvenile rats. Cat odor reduced play to a comparable extent in both socially housed and isolate housed rats, although socially housed rats exhibited more risk assessment during extinction. Rats that had played previously in the context used for assessing fear hid slightly less when exposed to cat odor than those rats that had not played previously in the testing context. However, no other differences were found between these two groups suggesting that prior social experience with the testing context has minimal effects on fear. In a direct test of a 'social buffering' hypothesis, rats that were tested for contextual fear conditioning in the presence of an unfamiliar partner were less fearful than those rats tested alone. These data are consistent with a social buffering hypothesis and suggest that positive social cues may help animals cope with the threat of predation.

Key words: fear anxiety play social juvenile adolescent social buffer conditioning

1. Introduction

Anxiety is one of the most common psychiatric disorders among children, with approximately 9% of all children being diagnosed with some type of anxiety disorder. Anxiety among children and adolescents also appears to have increased in prevalence in recent decades. For example, one recent report (Twenge, 2000) noted a significant increase in the incidence of anxiety among both young adults (college students) and younger school children from 1952 to 1993. In fact, the average anxiety score measured in the 1980s for school-age children was higher than that seen among psychiatric patients in the 1950s. Anxiety in children and adolescents can lead to additional problems, such as difficulty in school, alcohol and drug abuse, and other psychiatric conditions such as depression (Williams and Miller, 2003). Childhood and adolescence is also a vulnerable developmental period during which the foundation for developing an anxiety disorder during adulthood could be established (Heim and Nemeroff, 2001). Until fairly recently, however, little attention has been directed to understanding the phenomenology and neurobiological substrates of fear and anxiety as exhibited by children and adolescents (Heim and Nemeroff, 2001, Kagan, 2001).

The ability to detect situations that should elicit fear and/or anxiety and to exhibit appropriate responses in such situations appears fairly early in development. Using factor analysis, Doremus and colleagues (Doremus et al., 2006) determined that adolescent and adult rats exhibit the same constellation of behaviors in the elevated plus maze, suggesting that the underlying processes that lead to anxiety in this model are comparable before and after puberty. While the mechanisms that can lead to anxiety may be present in adolescents, there do seem to be differences in what can elicit anxiety

between adolescents and adults. For example, adolescent rats exhibit more anxiety in a light-dark box test (Slawecki, 2005) but fail to exhibit an anxiogenic response in the elevated plus maze following withdrawal from acute alcohol exposure (Doremus et al., 2003). Gender may also be a factor as adolescent male rats will exhibit an anxiolytic response to chronic nicotine administration, while adolescent females have an anxiogenic response to chronic nicotine, as do both adult males and females (Elliott et al., 2004). Stimuli that can produce fear in the young animal also seem to vary somewhat within the age period prior to puberty as well. For example, the presence of an unrelated adult male rat can induce fear, as measured by immobility and analgesia, in rats younger than 14 days of age but not in 26 day old rats. On the other hand, cat odor is ineffective as a fear stimulus at 14 days of age but produces fear in the 26 day old rat (Wiedenmayer and Barr, 2001). A subsequent study found that 18 day old rats exhibit fear towards cat odor that is comparable to that of adult rats (Hubbard et al., 2004). All of this suggests that the neural machinery necessary to elicit fear is fully operational at a fairly early age, although there do seem to be age-related differences with regard to the types of stimuli that can elicit fear and anxiety in rats. These data highlight the importance of using caution when generalizing to the adolescent condition from studies conducted in the adult rat.

While fear circuits appear to be fully engaged by the time before weaning, pre-pubertal rats still engage in a number of behaviors that can easily place them in jeopardy (Laviola et al., 1999, Spear, 2000). As maternal care begins to wane, the young of most mammals also begin to engage in varied forms of play behavior. In the young rat, play generally takes the form of rough-and-tumble wrestling among littermates and same-age conspecifics (Panksepp et al., 1984, Vanderschuren et al., 1997, Pellis and Pellis, 1998,

Siviy, 1998, Burghardt, 2005) which begins around the time of weaning, peaks at about 35 days of age, and then slowly wanes around puberty. Rough-and-tumble play is neither subtle nor quiet and, as such, might be expected to increase the likelihood of being detected by predators. As with any behavior, play must occur against a backdrop of everyday threats to survival and is fairly sensitive to disruption by a number of threats such as hunger (Baldwin and Baldwin, 1976, Siviy and Panksepp, 1985), sudden changes in lighting (Siviy and Baliko, 2000), and non-specific stressors such as restraint (Romeo et al., 2006). Fear of predation might also be expected to have an impact on play and, indeed, cat odor has been shown to be a potent inhibitor of play (Panksepp, 1998, Siviy et al., 2006). For example, we found that play was virtually abolished when rats were exposed to the smell of a cat in a familiar testing chamber and remained suppressed for up to 7 days after exposure when returned to the same chamber where the cat odor had been experienced (Siviy et al., 2006). Providing an opportunity to hide had no effect on either the unconditioned or conditioned suppression of play; 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 putative threat to survival and suggests that changes in playfulness may be a sensitive barometer for fear and/or anxiety in the young animal.

How an organism responds to threats in the environment may depend to some extent on the social context in which the animal lives and in which that threat is experienced. The social life of young mammals can be fairly rich and social experiences that occur prior to puberty may have a significant impact on how the adult phenotype unfolds (Champagne and Curley, 2005). Before independent locomotion is attained, the

primary social contact for the newborn is with the mother and active interactions directed by the mother towards the infant can have a lasting influence on the later development of that infant (Suomi, 1997, Meaney, 2001, Parent et al., 2005). For rats, the quality of maternal care during the first couple of weeks of life seems to be particularly important for laying down a foundation of emotional tone that pups from a particular litter will carry with them as they mature. Rats that are raised by dams that naturally engage in more licking and grooming tend to be less fearful (Menard et al., 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 those raised by dams that engage in less licking and grooming. All in all, these data have shown that increased maternal care during the first couple of weeks of life results in a rat that responds to anxiety-provoking stimuli in a way that suggests lower levels of fearfulness and anxiety (Anisman et al., 1998, Meaney, 2001, Parent et al., 2005).

The effects on offspring that are associated with naturally occurring higher levels of licking and grooming by the mother can be experimentally mimicked through brief periods (e.g., 15 minutes) of daily separation during the first two weeks of life. Since two landmark studies published roughly 50 years ago (Levine et al., 1957, Denenberg and Karas, 1959), a number of studies have characterized the behavioral and hormonal consequences of brief daily separation (Meaney et al., 1991, Boccia and Pedersen, 2001, Brunson et al., 2001). The effects of this manipulation, commonly known as “handling”, seem to be due to increased maternal care (i.e., more licking and grooming) induced by the repeated separation and reunion (Caldji et al., 1998, Meaney, 2001, Champagne et al., 2003b, Zhang et al., 2005). In order to determine whether early maternal experiences

could have an impact on how young rats deal with a predator threat, we recently assessed the extent to which handling could affect predator odor-induced reductions in play (Siviy & Harrison, 2008). Using a standard protocol for handling (Meaney et al., 1985, Meaney et al., 1991, Meerlo et al., 1999) we separated pups from the dam for 15 minutes each day from post-natal day 1 through 15. Pups were weaned at around 21 days of age, housed individually, and tested over a five day period. Play was tested in an open field that also provided an opportunity to hide in a small enclosed box. Prior to any predator threat, handled rats were consistently more playful than non-handled controls and seemed less fearful in that handled rats exhibited less risk assessment behavior from within the confines of the hide box. When exposed to a worn cat collar, both handled and non-handled rats played less and showed more risk assessment with no differences between the two groups. However, handled rats did not show a conditioned suppression of play when tested on the following day while non-handled rats that were previously exposed to a worn collar continued to play less than those exposed to an unworn collar. Interestingly, handled rats still had an elevated level of risk assessment on the day after exposure suggesting that they were still somewhat wary of the testing environment. These data show that a manipulation thought to promote increased maternal care during the first two weeks of life can have a significant impact on how an animal will deal with a predator threat during the juvenile period.

As can be seen from these studies, play is fairly sensitive to disruption by fear-provoking stimuli such as cues associated with predators and this disruption may be modulated by affectively positive social influences. When looking at the effects of predator odors on play, however, it is important to appreciate that our assessment of fear

is deeply intertwined with an active social behavior where both rats are exposed to the fearful stimulus in pairs at the same time and in a chamber that has become associated with play. In these paradigms, rats are also housed in isolation except for brief daily opportunities to play so the stress associated with chronic isolation housing (Angulo et al., 1991, Hol et al., 1994, Frisone et al., 2002, Von Frijtag et al., 2002, Arakawa, 2003) might be expected to influence how an animal responds when an additional stressor is superimposed onto a chronic level of isolation stress. In an attempt to disentangle how some of these social variables may be affecting fear in a predator odor model, we have begun to systematically assess the extent to which social factors may modulate fear to a predator odor in young rats. In addition to the parametric utility of these types of studies, this line of research may also provide useful insight into potential non-pharmacological approaches to treating anxiety disorders in young children.

2. Experiment 1: Effects of social housing

2. 1. Introduction

Few studies have systematically assessed the time course of extinction when cat odor is used to induce fear and we were initially struck by the sustained reluctance to play long after the predatory cue had been removed. Adamec and colleagues (Adamec and Shallow, 1993, Adamec et al., 2004) found that rats and mice will display signs of anxiety for up to 3 weeks after a brief unprotected exposure to a live cat. Yet one might expect the response to an actual predator under these types of conditions to be more potent than that to cues indicating the possible presence of a predator. Indeed, mice exposed to a live predator have a more protracted response than mice exposed to the room which is permeated by cat odors (Adamec et al., 2006). In any event, we were

struck by the strength of the conditioned suppression of play that was observed in our earlier work (Siviy et al., 2006).

In order to magnify the amount of play that is exhibited during the brief (5 minute) test period that we routinely use in our lab, rats are chronically isolated over the course of the experiment and this was the procedure used in all of our initial studies looking at the effects of cat odor on play behavior. Social isolation, especially during the pre-pubertal period, can enhance the responsiveness of rats to stressors when tested later in life (Hol et al., 1994, Van den Berg et al., 1999a, Van den Berg et al., 1999b, Weiss et al., 2004) so it is possible that the sustained expression of conditioned fear in our play model may be reflecting the housing conditions being used in these studies. In order to assess this possibility, the present study compared the unconditioned and conditioned fear towards a predator odor in chronically isolated rats to rats that were housed together and only isolated for 4 hours before each 5 minute test.

2.2 Materials and Methods

2.2.1 Subjects

Thirty-two male Long-Evans rats were obtained from Harlan Sprague-Dawley at approximately 25 days of age. Animals were housed either individually (n = 8 pairs) in solid bottom cages (27 X 21 X 14 cm) or in groups of four (n = 8 pairs) in solid bottom cages (48 X 27 X 20 cm). Food and water were freely available. The colony room was maintained at 22^{O} C with a 12/12 hr reversed light/dark cycle (lights off at 08:00). All housing and testing was done in compliance with the NIH Guide for Care and Use of Laboratory Animals using a protocol approved by the Institutional Animal Care and Use Committee at Gettysburg College.

2.2.2 Apparatus

Play was assessed in a Plexiglas chamber (60 x 60 x 50 cm) that was painted black on all four sides (Figure 1). 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 recorded with a camera that was directly above the outer chamber and an infrared-sensitive camera that was placed inside the hide box. Video ouput from both cameras was directed through a quad multiplexer, encoded into an mpeg file, and scored later using behavioral observation software (Observer XT: 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 at -10^{O} C and warmed prior to testing by immersing the sealed container in hot (50 ^{O} 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.

2.2.3 Procedure

Rats from the socially housed group were placed in individual housing for four hours prior to testing so that a quantifiable amount of play would occur during the 5 minute test session. Previous studies from our lab and others have shown that this level

of isolation is adequate for producing a sub-maximal amount of play behavior (Niesink and Van Ree, 1989, Siviy et al., 1997, Varlinskaya et al., 1999). All rats were initially acclimated to the testing apparatus by allowing them to play with a rat from the same housing condition for 3 days. On each of these days, the rats were placed in the chamber for 5 minutes. Rats played with the same partner throughout the experiment.

Play was assessed on the third baseline day 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 and Panksepp, 1987, Vanderschuren et al., 1997, Siviy, 1998). The location of the rats was also recorded and the relative amount of time spent in the hide box by one rat, both rats, or neither rat was used as an index of hiding. A measure of risk assessment was also measured by quantifying the amount of time at least one of the rats was engaged in a "head-out" posture on the baseline day, conditioning day, and on extinction days 1, 3, 5, and 7. 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 and Blanchard, 1989b, Dielenberg et al., 2001, McGregor et al., 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 a piece of a cat collar that had been previously worn by a domestic cat. The collar piece was placed on the wall facing the

opening of the hide box and kept in place by an alligator clip that was situated approximately 5 cm from the base of the chamber. 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 alcohol. Fresh pine chips were then placed in the chamber. Rats were tested without any collars present over the next seven days.

2.2.4. Results and discussion

The results for play behavior can be seen in Figure 2. As expected, rats that were housed in isolation exhibited more nape contacts, $t(14) = 2.764$, $p < .02$, and more pins, $t(14) = 4.794$, $p < .001$, on the baseline day than those housed socially. When exposed to a worn cat collar, both measures of play were virtually abolished. In order to determine whether exposure to a worn cat collar had a differential effect on play behavior in socially housed and isolated rats over the course of extinction, the data from the exposure day and the 7 extinction days were submitted to a 2 X 8 Analysis of Variance (ANOVA). For nape contacts, there was a significant effect of day, $F(7,98) = 10.11$, $p < .001$, with this measure of play increasing over days, indicative of extinction. There was also a marginal effect of group, $F(1,14) = 3.23$, $p = .094$, although this was no longer apparent when baseline levels of nape contacts were included as a covariate in the analysis. For pinning, the only significant effect was that of day, $F(7,98) = 4.813$, $p < .001$, again indicating that extinction was occurring. Including baseline levels of pinning as a covariate in this analysis yielded the same results. The lack of any significant day x group interaction on either measure of play suggests that extinction was comparable between the two housing conditions.

When exposed to a worn collar, both rats tended to be together in the hide box for most of the test period. This continued into extinction and was comparable between the two groups (data not shown). While there were no differences between the two housing groups in baseline levels of risk assessment (Figure 3), socially housed rats exhibited more risk assessment overall than isolate-housed rats as indicated by a significant main effect of group, $F(1,14) = 4.65$, $p < .05$.

These data show that housing conditions can have mixed effects on how a juvenile rat will respond to a predatory threat. Both measures of play were virtually abolished in the presence of a worn collar, remained suppressed throughout seven days of extinction, and were not sensitive to modulation by housing conditions. The amount of time spent in the hide box was also not sensitive to modulation by housing condition. This suggests that the sustained conditioned suppression of play previously observed with this model (Siviy et al., 2006) cannot be easily accounted for by housing condition. However, socially housed rats maintained an elevated level of risk assessment, compared to isolated rats. The measure for risk assessment used in the present study is comparable to that used by others (Blanchard and Blanchard, 1989b, Dielenberg et al., 2001, McGregor et al., 2002) and involves scanning of the main testing chamber from the relative safety of a smaller hide box. This could be considered a fairly adaptive response in that the rat can monitor the outer chamber (where the odor was previously experienced) but easily retreat into a highly defensible position while engaged in this posture. Without a concomitant change in play and/or hiding, however, it is difficult to interpret the differences observed in risk assessment between the two groups.

When confronted with a predator threat, the defensive response to that threat unfolds over time (Blanchard and Blanchard, 1989a, Blanchard et al., 1990b, Blanchard et al., 1993) so while higher levels of risk assessment in the socially housed rats did not correspond to an earlier return of playfulness in these rats it is perhaps noteworthy that the rats were only tested for a brief 5 minute daily session. Longer observation periods may be needed in order to more fully assess whether heightened risk assessment among socially housed rats can lead to an earlier recovery of play in this paradigm. Nevertheless, these data show that housing condition can have an impact on at least one measure of fear in the juvenile rat while not affecting other measures.

3. Experiment 2: Effects of prior social experience with test chamber

3.1 Introduction

The preceding experiment suggests that housing conditions could not readily account for the magnitude of fear conditioning indexed by levels of play in our earlier work although the amount of risk assessment observed during extinction was sensitive to housing condition. Another factor that could be contributing to the magnitude of fear being observed in our paradigm is the prior social experience associated with the testing chamber. In this model, animals have played repeatedly in the testing chamber prior to being exposed to the worn cat collar. Since rats will associate a specific context with opportunities for play (Knutson et al., 1998, Siviy, 1998), it is possible that neural substrates being sensitized as a result of prior play experience may also result in an altered response to a predator odor. In order to assess whether this possibility could account for our data, the following experiment compared fear in rats that had experienced

play in the testing chamber to those of rats that had never experienced playful interactions in the testing chamber.

3.2. Materials and Methods

3.2.1 Subjects and apparatus

Thirty two male Long-Evans rats were obtained from Harlan Sprague-Dawley at approximately 25 days of age. Housing conditions were the same as in the previous experiment except that all rats were housed in the larger solid-bottom cages (48 X 27 X 20 cm), with half of the animals housed individually and the other half housed in groups of four. An additional 16 rats were used to provide a comparably active play target for those animals that were to experience play in the testing chamber. The apparatus was the same as that used in the preceding experiment.

3.2.2 Procedure

One half of the rats from each housing group were placed in the testing chamber with a playful unfamiliar partner for 5 minutes each day while the other half was placed in the testing chamber individually for 5 minutes each day. Those rats not allowed to play in the testing chamber were given an opportunity to play with an unfamiliar playful partner in their home cage later in the day on each of these days. This protocol continued for seven days, at which time all rats were re-housed socially. On the following day, all of the rats were placed individually in the testing chamber and recorded for 5 minutes to obtain baseline measures. On the next day, all of the rats were again placed individually in the testing chamber and recorded for 5 minutes, except that a piece of a worn cat collar was placed along one wall as in the preceding experiment. This was the conditioning day. At the end of this day of testing, the chamber was cleaned as before and fresh pine

shavings were placed in the chamber. On the third day of testing, the rats were placed individually in the testing chamber for 5 minutes without any collar. Fear was quantified by the amount of time spent in the hide-box (hiding) and the amount of time spent engaged in head-out behavior (risk assessment).

3.3 Results and Discussion

Neither housing nor prior test chamber experience had an effect on time in the hide box or risk assessment when assessed on the baseline day. The results from the conditioning day and test day can be seen in Figure 4. Housing condition was not a significant source of variance for either measure on either day, so the figure shows the results collapsed across housing condition for the sake of clarity. For hiding, there was a significant effect of context on the conditioning day, $F(1,27) = 6.12$, $p < .05$, with those animals having prior play experience in the test chamber hiding slightly less than those rats without prior play experience in the test chamber. There was no difference between the two context groups for hiding on the test day, nor were there any differences on either day for risk assessment.

These data show that when rats were exposed to a worn cat collar in a context that had been previously associated with play, they were found to spend slightly less time in the hide box (86.1% of total time compared to 93.5% for those that did not previously play in the chamber). This was a subtle effect and did not carry over to the subsequent day when testing for conditioned fear. There were also no significant differences between the two groups in risk assessment. These data suggest that prior social experience with the testing context has minimal effects on how an animal will respond to a predator odor when experienced in that context.

In light of the previous experiment, it is curious that there was not a significant effect of housing in this experiment. However, housing conditions in the preceding experiment were constant throughout testing such that rats returned to their housing condition (social or isolate) immediately after testing while all of the rats in the present experiment were re-housed socially prior to testing in this experiment. This suggests that housing may not have a lasting influence on fear in the young rat.

4. Experiment 3: Effects of social buffering

4.1 Introduction

Rats could encounter predatory threats by themselves or in the presence of other rats, so it is possible that the presence or absence of other rats may modulate how an animal responds to that threat. In our standard model, rats are exposed to the predator odor and tested for conditioned fear with a conspecific. Previous studies have shown that when an animal is presented with a cue (e.g., a light) or context that has been associated with a stressor (e.g., foot shock) the behavioral and neuroendocrine response to that stimulus is attenuated when the animal is tested with another rat (Davitz and Mason, 1955, Kiyokawa et al., 2004, Kiyokawa et al., 2007). This effect has been called "social buffering" and has also been noted for more moderate stressors such as exposing rats to novelty and loud noises (Taylor, 1981, Armario et al., 1983a, Armario et al., 1983b). If social buffering can modulate fearfulness in juvenile rats, then we would predict that rats that are placed in a testing chamber that has been previously associated with a predator odor along with a rat that has never been exposed to a predator odor would show less fear than those tested alone.

4.2 Materials and Methods

4.2.1 Subjects and apparatus

Sixteen male Long-Evans rats were housed in groups of 4 and tested between the ages of 39 and 41 days. An additional 8 rats, also housed in groups of four, were used as unfamiliar partners for testing. Housing conditions for the rats were identical to the preceding experiments except that these rats were housed in a colony room with lights on at 08:00 and off at 20:00. Consequently, testing for these rats was done during the light phase of the light/dark cycle.

4.2.2. Procedure

On the first day (Baseline), each rat was placed individually in the testing chamber described earlier for 7 minutes. On the next day (Conditioning), all rats were exposed to a worn collar that was affixed by an alligator clip to one of the walls of the chamber. As on the baseline day, the rats remained in the chamber for 7 minutes. After all of the rats were tested on this day, the bedding was removed from the chamber and the inside wiped down with alcohol. Fresh bedding was placed in the chamber before testing on the next day. On the third day (Test), half of the rats (n=8) were returned alone to the testing chamber for 7 minutes while the other half of the rats (n=8) were returned to the chamber along with an unfamiliar male partner of the same age. The unfamiliar partner was marked with a Sharpie® permanent marker in order to distinguish between the subject and the partner. Order of testing between the two groups was counterbalanced. The two primary measures used to quantify fear in this study were the amount of time spent in the hide box and the amount of time engaged in risk assessment, as quantified by head-out behavior. Since there was found to be a difference between the groups in the amount of spent hiding, head-out behavior was adjusted as a function of time spent in the

hide box. The number of times the subject rat went in and out of the hide box (transits) was also counted in order to obtain a rough index of overall activity.

4.3 Results and discussion

As can be seen in Figure 5, those rats tested with an unfamiliar partner tended to be less fearful than those tested alone. Rats tested with an unfamiliar partner spent less time hiding, $t(13) = 2.90$, $p < .02$, more time engaged in risk assessment when in the hide box, $t(13) = 2.35$, $p < .04$, and were more active in terms of transits in and out of the hide box, $t(13) = 2.95$, $p < .02$. These data are consistent with a social buffering hypothesis and produced results similar to what has been observed using other models of fear (Davitz and Mason, 1955, Latane, 1969, Taylor, 1981, Armario et al., 1983a, Armario et al., 1983b, Kiyokawa et al., 2004). In particular, juvenile rats will exhibit less conditioned fear to the context in which they had previously experienced the smell of a predator when in the presence of a non-fearful, unfamiliar partner.

5. General Discussion

It is well established that the smell of a predator can have a profound suppressant effect on non-defensive behaviors in rats and mice (Zangrossi and File, 1992a, Adamec and Shallow, 1993, Blanchard et al., 1990a, Blanchard et al., 2001, Dielenberg and McGregor, 2001, Apfelbach et al., 2005) and previous work from this laboratory has shown that brief exposure to cat odor also results in substantial unconditioned and conditioned suppression of play behavior among juvenile rats (Siviy et al., 2006). We have also recently found that neonatal handling during first two weeks of life can attenuate the conditioned suppression of play in young rats (Siviy and Harrison, 2008), suggesting that early experiences in the nest can have an impact on how a rat will deal

with a predatory threat when older. Given the extent to which our findings with young rats are intertwined with a socially vigorous and affectively positive behavior (i.e., rough-and-tumble play), the present series of experiments attempted to sort out how some of the social variables associated with cat odor-induced reductions in play among young rats may be modulating the magnitude of these effects.

As summarized in Table 1, these findings suggest that the extent to which a juvenile rat will respond to cues associated with a predator threat can be modulated by social variables. While some of these effects can be subtle, they tend to be in the same direction and suggest that positive social experiences may attenuate fear or at least modify how that fear may be expressed in the young rat. For example, housing young rats in isolation can be quite stressful (Von Frijtag et al., 2002, Arakawa, 2003, Weiss et al., 2004) and rats housed in chronic isolation engaged in less risk assessment from within the confines of a hide box than socially housed animals during the first few days of extinction (Experiment 1). Although this could be viewed as a less adaptive response in that these animals would not be in a good position to monitor any continued threat, increased vigilance among the socially housed rats did not have an impact on subsequent playfulness so the adaptive significance of this difference is unclear.

In Experiment 2, there were minimal differences between rats that had previously played in the testing context prior to predator odor exposure compared to rats that had not experienced play in the testing context. However, the one significant effect that was observed in this experiment would be consistent with less fear among those rats that had experienced affectively positive social experiences (i.e., play) in the testing context. In particular, rats that had previously played in the testing context hid slightly less in the

presence of a predator odor than those without similar experience. Finally, the results of Experiment 3 showed that being placed with another rat in a context previously associated with a predator odor resulted in less hiding, more risk assessment, and more activity than when tested alone. Taken together, these data are consistent with a working hypothesis that social factors can modulate the behavioral responsiveness towards predator cues in juvenile rats.

Experiment 3 was the most direct test of a social buffering hypothesis and the results are comparable to those obtained in other studies using a similar paradigm but with different fear-inducing stimuli (Davitz and Mason, 1955, Latane, 1969, Taylor, 1981, Armario et al., 1983a, Armario et al., 1983b, Kiyokawa et al., 2004, Kiyokawa et al., 2007). The results extend these earlier findings by showing that social buffering can also occur when the fear-inducing stimulus is a predator cue. The pattern of changes noted for those rats tested with a partner may be especially informative as to how the behavioral strategies of these animals are being modified by this particular type of social influence. These rats are hiding less and moving in and out of the hide box more often. However, when in the hide box they are more likely to be in a head-out posture, indicating an elevated level of risk assessment. This pattern of results is similar to what has been reported with benzodiazepines. For example, benzodiazepines tend to decrease hiding, increase approach towards the source of cat odor, and increase risk assessment (Blanchard et al., 1990b, Zangrossi and File, 1992b, Dielenberg et al., 1999). Like benzodiazepines, social contact may shift the behavioral strategy of these rats from one of hiding and avoidance to one of active investigation towards the potential threat.

The rats from the "buffer" group in Experiment 3 were tested with another rat that had never been exposed to a predator odor. This may be a critical factor in determining the magnitude of the social buffering effect as testing with a similarly fearful rat is not as effective at social buffering as when testing is done with a non-fearful rat (Kiyokawa et al., 2004). This was not directly assessed in the present study, although previous work from our laboratory (Siviy et al., 2006, Siviy and Harrison, 2008) and the first experiment from the present study use a model where both rats of a pair are exposed and tested under the same conditions. By design, all rats that are tested in these studies for predator odor-induced fear are being tested with another fearful partner. Nevertheless, it may be informative if future studies assess social buffering to a predator odor with a partner that has also been made fearful through exposure to a predator odor but independently of the rat being tested.

The exact underlying mechanism(s) accounting for social buffering effects have yet to be fully delineated, yet there is evidence that physical contact may be a critical factor (Wilson, 2000, Wilson, 2001, De Vries et al., 2003). Future studies should address the extent to which specific types of social contact, such as play (Wilson, 2001, Pellis and Pellis, 2007), can account for the modulatory effects being observed with a predatory threat. There is also evidence that different profiles of social buffering can be obtained by manipulating when the social contact occurs (Kiyokawa et al., 2007). For example, rats that are pair-housed immediately after fear conditioning (tone followed by footshock) and then tested alone 24 hours later still showed freezing in response to the conditioned stimulus (CS), although they did not exhibit a hyperthermic response to the CS as did control rats. On the other hand, rats that were housed alone after fear conditioning and

tested with a non-stressed partner showed no freezing but still had the hyperthermic response to the CS. Pair-housing combined with paired-exposure reduced both the behavioral response (freezing) and the autonomic response (hyperthermia) to the CS. These data, along with the current set of results, highlight the extent to which the type of social cue may have a significant impact on how a social buffering response unfolds.

Most of the robust effects that have been noted with social variables have been associated with behavior during testing for contextual fear conditioning. This is in marked contrast to studies with benzodiazepines, where anxiolytic effects tend to be more robust when tested in the presence of a predator odor (Dielenberg et al., 1999) or direct threat (Blanchard et al., 1989, Blanchard et al., 1990b, Blanchard et al., 1998). With the exception of the modest difference observed with hiding in Experiment 2, there were no differences among any of the groups when directly exposed to a worn cat collar. This might suggest that the response to a more immediate threat is less sensitive to modulation by an animal's social history and/or social experiences. However, social variables may become more of a factor in determining how an animal behaves once the threat is no longer imminent.

Dealing effectively with the threat of predation 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 present may be as detrimental to ultimate survival as being too bold in the face of a clear threat to safety (Dielenberg and 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. While the present series of experiments focused mostly on proximal cues (e.g., presence of another

rat) and ongoing social factors (e.g., housing condition), previous work from this laboratory (Siviy and Harrison, 2008) has also shown that fearfulness towards a predator threat during the juvenile period may be modulated by more distal cues experienced during the first few weeks of life. As described earlier, handled rats showed less of a conditioned suppression of play than did non-handled controls. 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, Meaney, 2001, Champagne et al., 2003a, Champagne and Curley, 2005, Zhang et al., 2005) and maternal care may provide an anticipatory cue as to the type of environment into which an animal has been born (Meaney, 2001, Champagne and Curley, 2005). In particular, increased maternal licking and grooming is thought to reflect or mimic the behavior of a mother that lives in an environment where resources are abundant and readily available. 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. So while rats born into this environment may still need to be vigilant to possible predator threats, this type of environment may favor an early return to playfulness once any immediate threat has passed.

The quality of maternal care early in life can lay down an emotional foundation that an animal can then take into the juvenile period and carry through to adulthood (Zhang et al., 1998, Meaney, 2001, Champagne and Curley, 2005, Holmes et al., 2005). As the young rat begins to venture away from the natal group, other social influences can begin to have an impact as well. Play behavior becomes particularly prominent between the period of weaning and puberty and there is evidence that play and other forms of

active social contact could factor into the strength of social buffering effects (Wilson, 2000, Wilson, 2001, De Vries et al., 2003). So while the mother provides the initial emotional framework, social interactions with conspecifics may continue to modify and mold the affective architecture that the animal will use as it navigates through its world. How these experiences actually translate into specific behavioral changes in the responsiveness to a predator threat seem to depend upon the type of interaction and the developmental period during which that interaction occurs. For example, neonatal handling was found to reduce the conditioned suppression of play while leaving risk assessment relatively unaffected (Siviy & Harrison, 2008). On the other hand, housing conditions during the juvenile period (social or isolate) had no impact on the conditioned suppression of play while affecting risk assessment. A future challenge will be to gain a better understanding as to how exactly the behavioral topography resulting from a predator threat is being modified by the various types of social influences that can occur over the course of development.

When presented with an anxiety-provoking situation, such as returning to an environment where a predator odor was previously experienced, how an animal will respond seems to depend at least in part on both distal and proximal social cues. Positive social cues, such as high levels of maternal care experienced early in life (distal cues) and/or the presence of a non-fearful conspecific (proximal cues), seem to yield a lower expression of fear and may lead to a more rapid behavioral recovery (i.e., extinction) as long as the threat is no longer imminent. Conversely, not having these positive social cues may hamper this recovery and may lead to prolonged fear even though the threat is no longer present. We may see an analogous situation in humans as stressors and lack of

social support early in life tends to have an adverse effect on later mental health (Maughan and McCarthy, 1997, Repetti et al., 2002, Nemeroff, 2004, Fox et al., 2005, Fox et al., 2007). Consequently, studying the impact of social interactions prior to puberty may have direct relevance for understanding those factors contributing to the etiology of anxiety disorders both in childhood and adulthood.

In one of the more significant insights into mammalian brain evolution, Paul MacLean (MacLean, 1990) stressed how the social bond is pivotal for understanding the evolution of mammals. Given the importance of social bonds in the lives of mammals, it is perhaps not surprising that young rats may take advantage of various social cues to help determine the extent to which caution should be used in a given situation. The comfort that comes from positive social interactions may not only help promote social bonding and cohesion, but may also provide animals with added coping mechanisms for dealing with those situations that can lead to fear and anxiety. When these positive social cues are not there the result could be, among other things, an exaggerated response to anxiety-provoking stimuli. But when these cues are present, the animal may be better able to keep anxiety at bay and continue functioning in an adaptive manner.

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Table 1. A summary of social effects on fear to a predator odor in juvenile rats

Manipulation	***Measures***	***Direct exposure to cat odor***	***Contextual fear conditioning***	***General conclusions***
Neonatal handling (Siviy & Harrison, 2008)	Play Risk assessment	No effect	Handled rats show less conditioned suppression of play but still show increased risk assessment	Handling decreases fearfulness when tested in a context previously associated with cat odor.
Housing (Experiment 1)	Play Hiding Risk assessment	No effect	Socially housed rats show more risk assessment. No differences on reductions in play or on hiding.	Socially housed rats may show more adaptive response to putative threat by monitoring any continued risk.
Prior social experience with test chamber (Experiment 2)	Hiding Risk assessment	Rats that previously played in context hid slightly less	No effect	Prior social experience with context has minimal effect on fearfulness.
Social buffering (Experiment 3)	Hiding Risk assessment Overall activity	Not tested	Rats tested with partner showed less hiding, more risk assessment, and more activity.	Less fearful when exposed to context with unfamiliar partner. Consistent with social Buffering.

Figure Captions

Figure 1. The testing apparatus used in the present series of experiments. The image to the left shows a view of the main chamber taken from above with the exterior of the hide box in the upper left. The image to the right shows a view from inside the hide box. The outside chamber is illuminated only by 2 X 25W red light bulbs. The inside of the hide box is not illuminated but has an infrared-sensitive camera inside.

Figure 2. Mean (± SEM) number of nape contacts and pins for socially housed and isolate housed rats across days of testing. C = conditioning day when a worn cat collar is present; E1 – E7 = extinctions days on which no collar is present.

Figure 3. Mean (± SEM) percentage of total time spent engaged in head-out behavior. Abbreviations are the same as in Figure 2.

Figure 4. Mean (± SEM) percent of time spent hiding (top) and engaged in a head-out posture (bottom) on the conditioning day and test day in those rats that either had prior play experience in the testing context (Play) or did not have any prior play experience in the testing context (Alone). The horizontal dashed lines refer to baseline values for the combined group.

Figure 5. Mean (± SEM) percent of time hiding, percent of time engaged in a head-out posture when in the hide box, and the number of transits in and out of the hide box for rats either tested alone or with an unfamiliar partner. Testing was done on the day after exposure to a worn cat collar. The unfamiliar partner was not exposed to any cat odor.

Figure(s)

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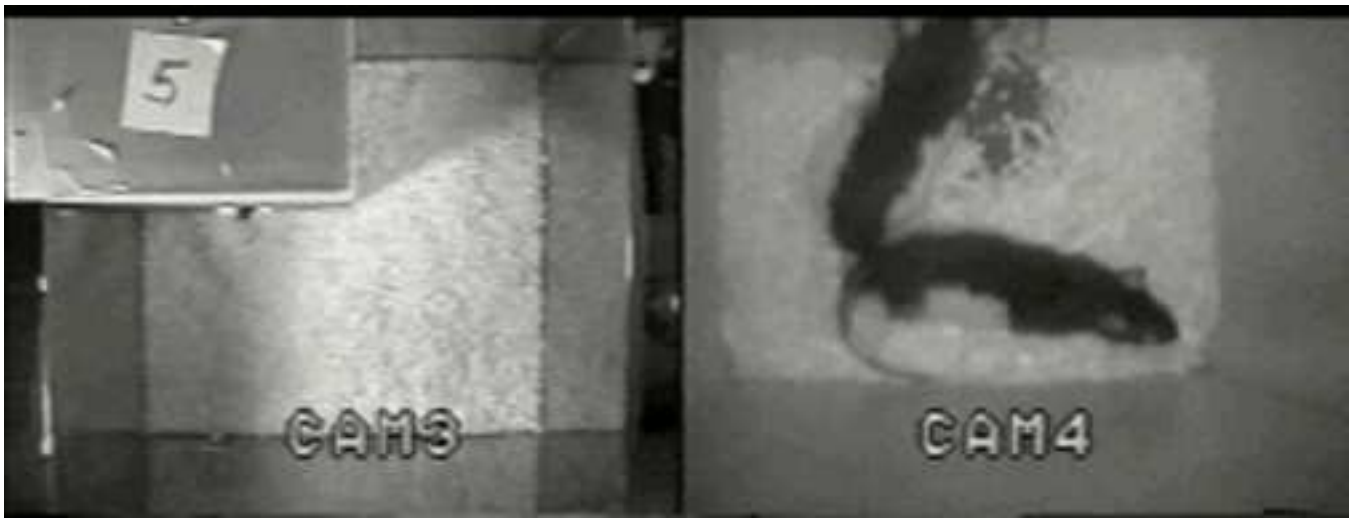

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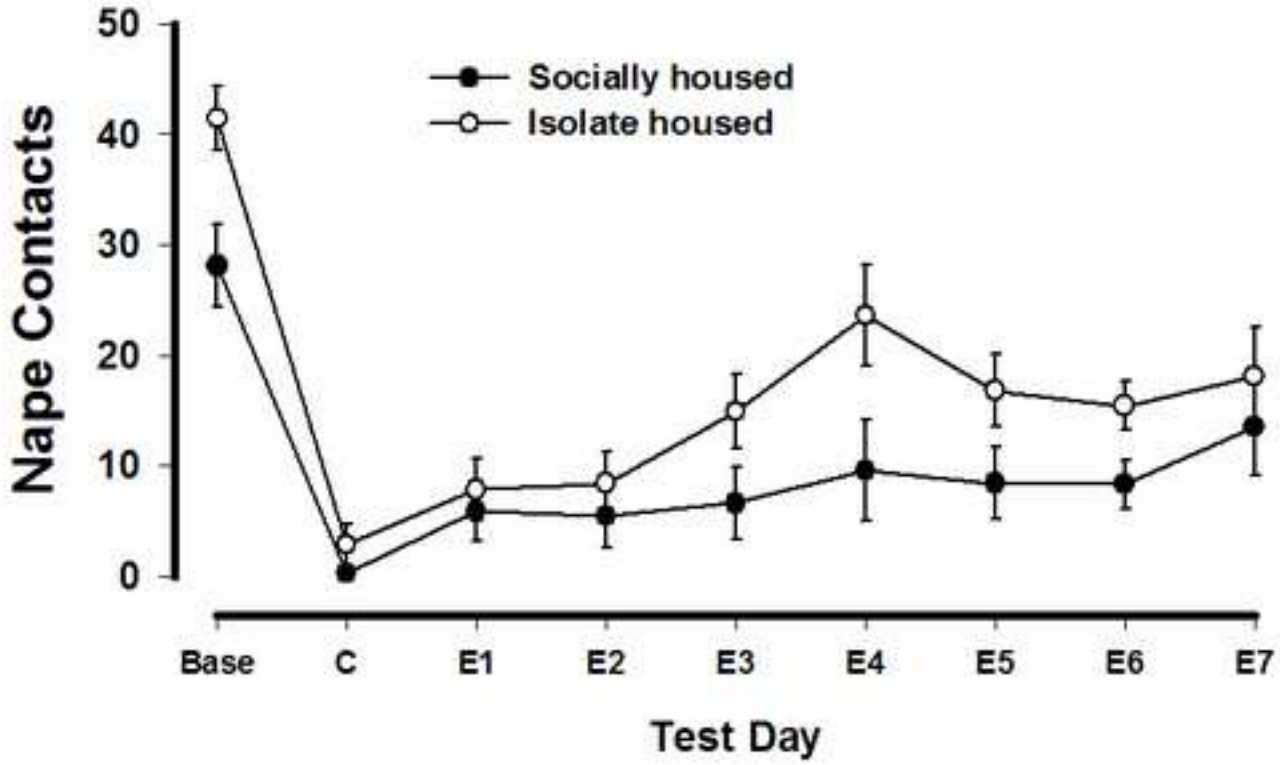

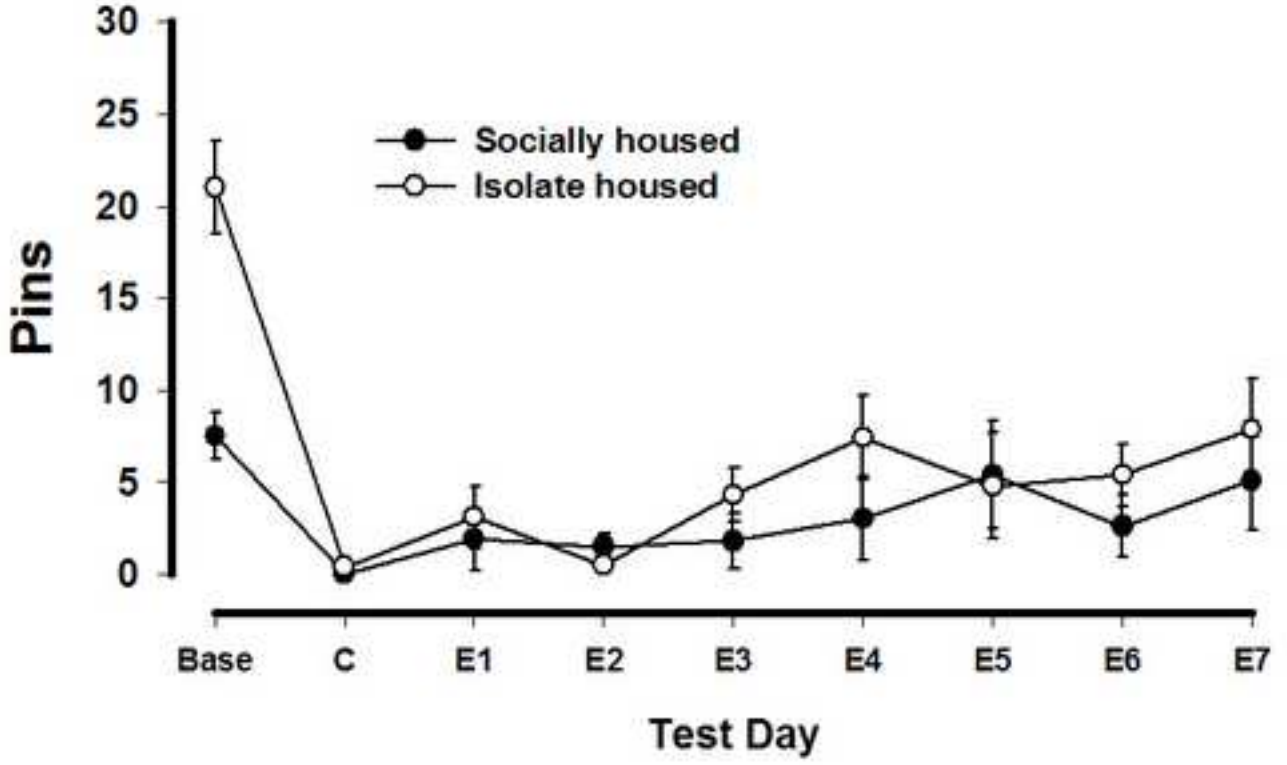

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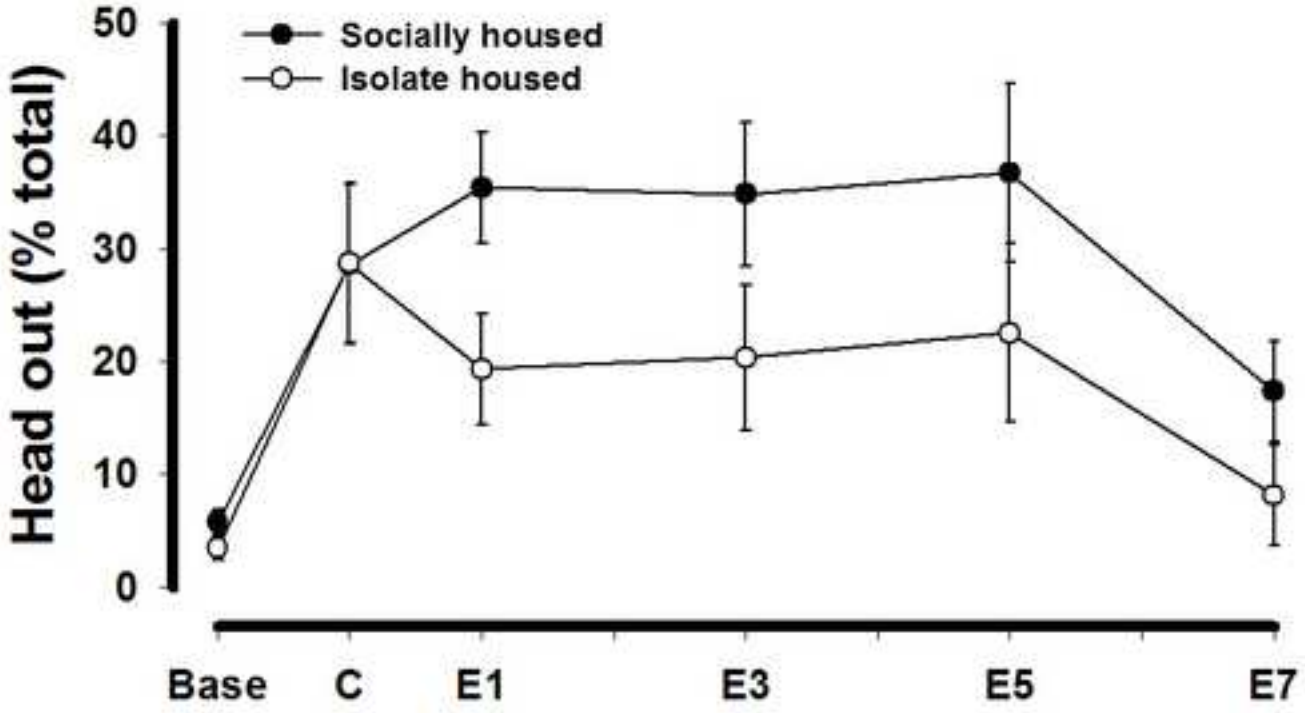

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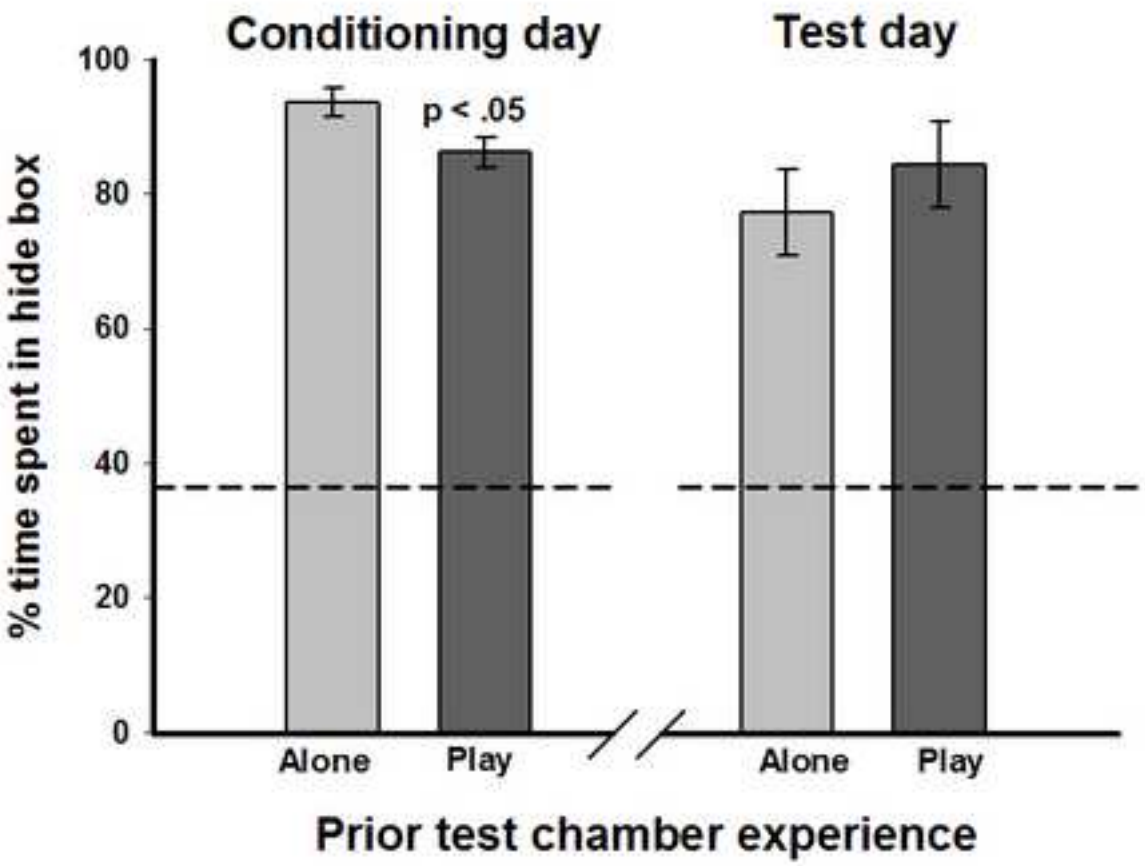

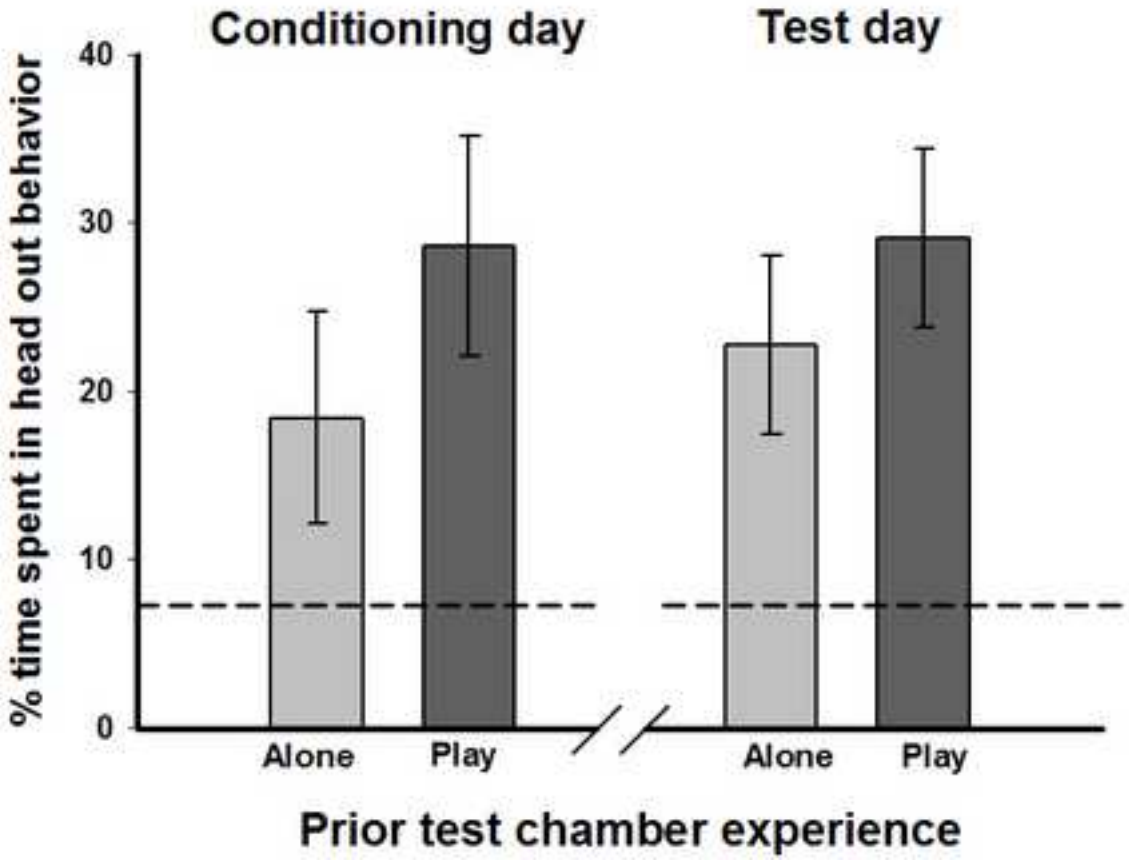

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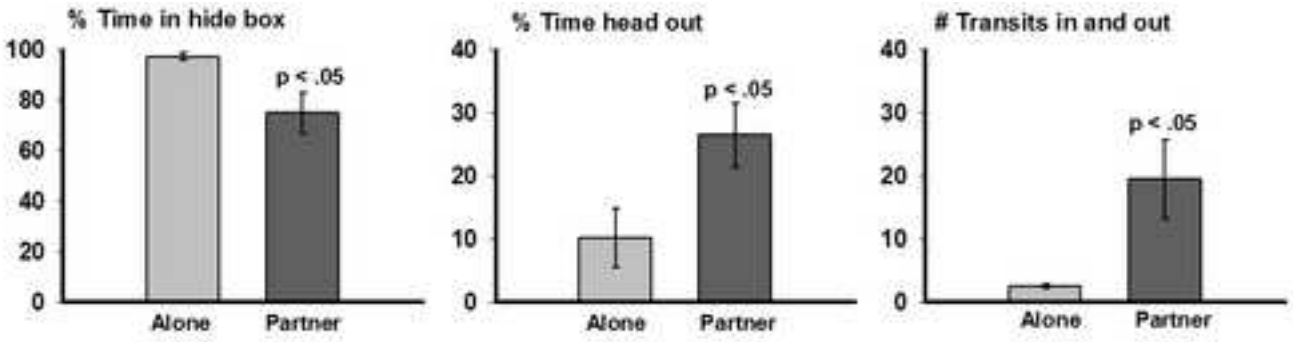
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