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Predatory Odor Disrupts Social Novelty Preference in Long-Evans Rats

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The present study examined the effects of predatory odor (cat urine) on social novelty preference in Long-Evans rats. Adult male subjects encountered a juvenile conspecific at training, were exposed to either clean cat litter (control) or litter soiled with cat urine (predatory odor), and were tested for social novelty preference. While the predatory odor and control groups did not differ in exploration of the initial conspecific at training or in the investigation of both the novel and familiar conspecifics at test, animals exposed to predatory odor prior to test spent a smaller percentage of their exploration time investigating the novel conspecifics than did controls, suggesting that predator odor is capable of disrupting social novelty preference.

Berlyne (1950) first illustrated that rats spend more time exploring novel objects than familiar ones. After pre-exposure to an empty arena and several initial object training sessions, Berlyne allowed his subjects to freely explore three objects, two of which were identical to the training objects, and a novel object that had not been previously encountered. When comparing the time spent exploring the novel and familiar objects it was seen that the rats spent significantly more time exploring the novel object than the familiar objects, a classic finding suggesting novelty and curiosity as principal determinants of exploratory responses (Berlyne, 1950).

In recent years researchers have come to recognize that similar processes may be involved in the social interactions of animals. Indeed, studies examining the social behavior of rats have shown a preference towards novelty during social interaction as well (e.g., Engelmann, Wotjak,

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& Landgraf, 1995; Perio, Terranova, Worms, Bluthe, Dantzer, & Biziere, 1989; Thor & Holloway, 1982). Terranova, Chabot, Barnouin, Perrault, Depoortere, Griebel, and Scatton (2005), for instance, placed a juvenile rat in an enclosure with an adult rat for a period of 30 minutes. This was followed by a 5-minute test session in which a novel juvenile was introduced as well, and it was found that the adults spent more time exploring the novel juvenile than the familiar one.

A variety of factors have been shown to influence how animals will react to novel stimuli (for review see Anderson, 2006 a, b). For example, many studies have highlighted that stressors possess the ability to impair novel object recognition and preference. Beck and Luine (1999) have demonstrated the ability of restraint stress to significantly impair object recognition memory. Similarly, Baker and Kim (2001) have shown that inescapable restraint paired with tail-shock significantly impairs object recognition memory at a 3 hr retention interval, but not at 5 min. Other work has suggested that chronic stress can result in both short-term and long-term effects, possibly impairing object recognition memory in mice for some time following the cessation of the stressor itself (Elizalde, Gill-Bea, Ramirez, Asia, Lasheras, Del Rio, & Tordera, 2007).

Predatory threat is a natural stressor of animals in the wild, and not surprisingly, predatory odors have been shown to have a significant effect on the behaviors of rodents in the laboratory (for review see Dielenberg & McGregor, 2001; Apfelbach, Blanchard, Blanchard, Hayes, & McGregor, 2005). Morrow, Roth, and Elsworth (2000), for example, exposed male Sprague-Dawley rats to TMT (fox odor) in between training and testing in a novel object recognition task. While the time spent exploring both objects at test was not affected by TMT, the rats that were exposed to the substance did not prefer the novel object to the familiar object at test, suggesting that the presence of predatory odor disrupts the working memory of rats. Zangrossi and File (1992) (employing a damp cloth that had been rubbed at length on a cat) and others (for review see Dielenberg & McGregor, 2001) have suggested that exposure to predatory odor is also capable of reducing general levels of social interaction.

While the effects of stressful predatory odors on novel object recognition have been demonstrated (e.g., Morrow et al., 2000), the influence of predatory odor on novel social recognition has not received as much attention. Given potential differences in the mechanisms underlying these types of tasks (Everts & Koolhaas, 1997), as well as the previous demonstrations that predatory odor is capable of altering general social behavior (e.g., Zangrossi & File, 1992), such an investigation seems warranted.

Thus, in the present study an effort was made to examine the effects of a predatory odor (cat urine in cat litter) on social novelty preference, recognition, and exploration in rats. This question is of interest for several reasons. First, interactions with predators and conspecifics are common aspects of everyday life for animals, and thus there is a need to understand them fully. Additionally, as the effects of predatory odor on novel object recognition are already known, the present study sought to examine whether behavioral response to social novelty would react in similar ways, and perhaps provide some insight into common underlying mechanisms. To achieve these goals, adult male rats interacted with juvenile males for a period of time, were either exposed to urine soiled clumping cat litter or clean un-soiled litter, and were then tested for preferences to interact with the original (familiar) juvenile or a novel juvenile rat with which it had no previous experience.

METHOD

Subjects. Subjects consisted of 24 adult (approximately 70 days of age) male Long-Evans rats. Twelve additional juvenile (approximately 45 days of age) male rats were used to serve as novel and familiar conspecifics. Juvenile conspecifics were employed in order to reduce the likelihood of aggression during social encounters. All subjects were individually housed for 1 week prior to the start of the experiment. This was done in order to enhance the tendency of subjects to explore the conspecifics when later given the opportunity. The juveniles serving as familiar and novel conspecifics were housed multiple animals (4-6) per cage with same-sex littermates throughout the experiment's entirety.

All rats were bred at Saint Joseph's University from breeding pairs originally derived from animals obtained from Taconic Farm Inc. (Germantown, NY). Rats were housed in standard plastic shoebox cages and kept in a room on a reversed 14/10-h light/dark schedule. The rats were maintained on ad lib food and water throughout the experiment's entirety. All procedures were conducted between 12:00 and 15:00 standard military time during the rats' dark cycle of their lighting schedule. All procedures were reviewed and approved by the Saint Joseph's University Institutional Animal Care and Use Committee (IACUC) prior to the start of the study.

Materials. To administer odor, two small plastic 4oz Rubbermaid containers with plastic lids were employed. The lids had 20 small holes drilled in them, approximately 2mm in diameter. These containers were

filled with either urine-soiled or fresh Tidy Cats® Scoop Multiple Cat Instant Action Immediate Odor Control clay cat litter (Nestlé Purina Petcare Company, St. Louis, Mo). The urine-soiled litter was obtained from a multiple-cat home and stored in a sealed freezer bag in a standard electric refrigerator. Litter was stored for up to 2-3 weeks in the refrigerator in an effort to maintain freshness. Over the course of the experiment, the litter held in the Rubbermaid containers was replaced at the start of each testing day, and then replaced again after 6 rats had been run to ensure odor potency. Refrigerated litter was allowed time to return to room temperature prior to use.

The exposure to the predatory (urine soiled litter) or control (fresh litter) odor took place in a standard plastic shoebox cage lined with Alpha-Dri bedding material (a cellulose material that is manufactured by Shepherd Specialty Paper). The bedding was changed after being used to test three successive subjects. The shoebox cage was approximately 47cm (L) X 25cm (W) X 21cm (H), and had a metal wire lid to prevent subjects from escaping.

Social recognition training and testing took place inside the subject's home-cage which was situated on a table with speakers (Dual speakers, model LU43PB) located to the right and left of the cage that provided approximately 60 dB white noise (generated by a Lafayette Instruments Co., White Noise Generator, Model # 15800) in order to mask outside noise that may have interfered with exploration.

Procedure. Subjects (*N*=24) were handled for two consecutive days prior to testing, for five minutes per day. Following the week of isolation, subjects were transported in their home cages to the room in which testing was to occur, and the cage was placed on a table between two speakers providing approximately 60 dB of white noise. The subjects were left in their home cages undisturbed in the novel room for 5min in order to familiarize the animal with the novel environment. Following the contextual familiarization, a juvenile conspecific was placed into the subject's home cage. The conspecific remained in the subject's home cage for a period of 5min that was recorded by means of a video camera (Sony camcorder, model: DCR-TRV260). The camera was secured by a tri-pod stand and placed directly above the testing area. The camera was wired into a Dell PC, and the footage was captured with a motion analysis computer program (EzVideoDV Automated Tracking System, AccuScan Instruments Inc., 2006). This allowed for the total duration (in seconds) of investigative behavior [defined as nosing, sniffing, grooming, "close following" (within

approximately 2cm; i.e., almost touching), or any directed physical contact (cf., Perio, Terranova, Worms, Bluthe, Dantzer, & Biziere, 1989)] initiated by the subject toward the conspecific to be later scored by two trained independent observers whose scores were subsequently averaged.

Immediately following the initial 5min, half of the subjects were exposed to predatory odor. Such subjects were put into a different shoebox containing the Rubbermaid container full of urine-soiled litter and remained there for 5-min. Rats in the control group were treated similarly except the container that they encountered was filled with un-soiled cat litter.

Following litter exposure, subjects were returned to their home cages and underwent social novelty recognition testing. This involved placing the originally encountered conspecific (familiar) as well as a second (novel) conspecific into the subject's home cage. The subject was left in the cage with the familiar and novel conspecifics for a period of 3min. This 3min test period was also recorded and scored as was described previously.

Over the course of the study, subjects were run in squads of 3-5 rats with a single set of unique juveniles serving as conspecifics for each squad. For all of the rats in each squad of subjects, one particular juvenile always served as the novel conspecific and the other always served as the familiar conspecific. Each squad contained an approximately equal number of rats from the predator odor and control groups. The subjects from the control group were always ran prior to those from the stress group, in order to reduce the likelihood that lingering cat odors could alter the behavior of those animals in the control group.

The tails of the subjects and conspecifics were marked with a Sharpie marker prior to training and testing so that they were able to be distinguished from one another in the video. Two independent raters, blind to the meaning of the markings, scored each training and testing session and the pairs of interaction scores from each session were subsequently averaged. There was a high degree of inter-rater reliability as the sets of scores generated by the raters pertaining to the time (in seconds) spent investigating the initial rat at training ($r(21) = .971$, $p < .01$), the time spent investigating the familiar rat at test ($r(21) = .625$, $p < .01$), and the time spent investigating the novel rat at test ($r(21) = .659$, $p < .01$) each displayed significant positive correlations. The averages of the raters' scores on each of these measures were calculated and used to generate a percent novelty preference ([time with novel juvenile rat/time with familiar and novel juvenile rats] x 100) for each rat in the odor and the control groups.

One subject was excluded as a result of experimenter error. Additionally, an exclusion criterion in which any rats differing from the mean time exploring the conspecific at training of the overall sample (irrespective of group) by more than two standard deviations was employed in order to promote uniform training exposure. This criterion resulted in the loss of one additional subject that was excluded from all of the analyses described below. Following this, 11 subjects remained in both the urine odor and control groups (n=11, N=22).

RESULTS

Means and standard deviations of all principal measures are reported in Table 1. An independent samples t-test compared time spent with the initial training conspecific by the urine odor (M = 90.82, SD = 27.01) and control groups (M = 90.36, SD = 23.55), and showed no significant difference between groups ($t(20)$ = -.042, p = .967). An additional independent samples t-test compared the total time spent in exploration of both the novel and familiar conspecifics during testing by the urine odor (M = 68.86, SD = 14.99) and control groups (M = 72.95, SD = 15.81), and also showed no significant difference between groups ($t(20)$ = .623, p = .541).

Table 1. Performance measures of rats in the social recognition task.

Group	**Performance measure**		
	Training: time w/ conspecific	Test: time w/ both conspecifics	Novelty preference
Control			
Mean	90.36	72.95	62.74%
S.D.	23.55	15.81	8.09
Predatory Odor			
Mean	90.82	68.86	56.01%
S.D.	27.01	14.99	6.37

Note. Times are in seconds. Novelty preference = [time with novel juvenile rat/time with familiar and novel juvenile rats] x 100

While both the predator odor (M = 56.01, SD = 6.37; $t(10) = 3.13$, $p = .011$) and the control (M = 62.74, SD = 8.09; $t(10) = 5.22$, $p < .001$) groups both displayed significantly greater than chance novelty preference (percent novelty preference = time with novel juvenile rat/time with both juvenile rats x 100; analyzed with one-sample *t*-tests with test scores of 50%, chance level), an independent samples *t*-test comparing the percent novelty preference of the two groups found that the cat urine odor group had significantly lower novelty preference than the controls ($t(20) = 2.167$, $p = .042$). This difference is depicted in Figure 1.

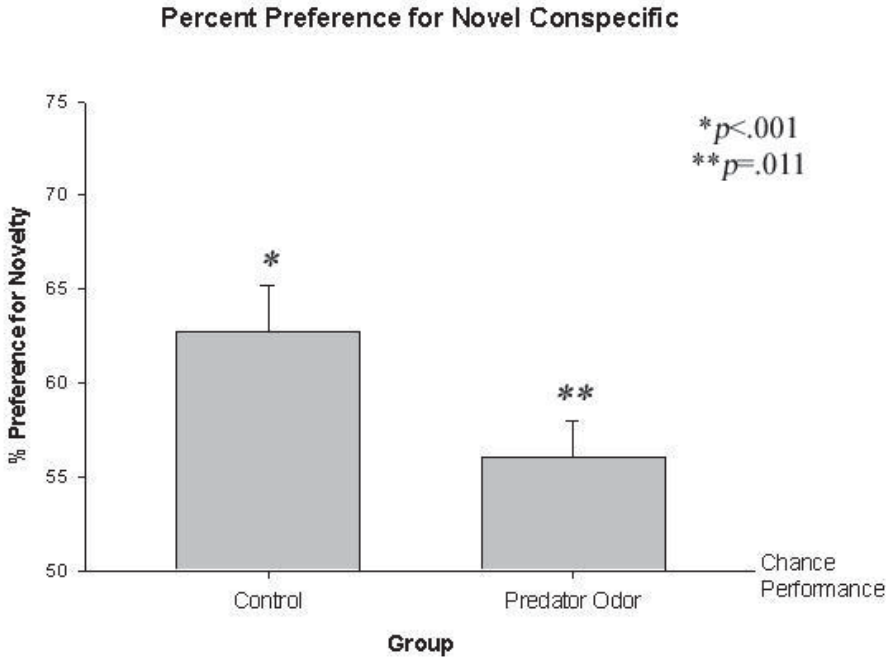

Figure 1. Mean percent preference for the novel conspecific ([time with novel juvenile rat/time with familiar and novel juvenile rats] x 100) of those rats in the control and predator odor groups. Error bars represent *SEM*. *p* values correspond to the outcome of one-sample *t*-tests comparing each group to 50%, chance performance.

DISCUSSION

The present study sought to examine the effects of predatory odor on social novelty preference in rats. Indeed, while both groups displayed a significant preference for exploring the novel conspecfic, there was a greater preference for exploring the novel rat in the control group than in those animals experiencing predatory odor between the training and testing exposures. Importantly, these results do not seem to be the product of overall differences in exploration of conspecifics at training or test.

Indeed, when directly comparing the predator odor and control groups we did not obtain significant differences in the overall time spent with both conspecifics at test. Given this result, and the fact that we employed a percent preference for novelty score which would greatly reduce the potential effect of individual differences in exploration [as opposed to a score which represented absolute novelty preference (in seconds) as is sometimes implemented in novelty preference studies (e.g., Anderson, Karash, Ashton, & Riccio, 2003)], the observed group differences in novelty preference are not likely related to differences in general social exploration at test.

While it was essential in order to properly assess group differences in novelty preference, the lack of general exploratory differences may seem to somewhat contradict those studies that have suggested that exposure to predatory odor is capable of reducing general levels of social investigation (e.g., Zangrossi & File, 1992; for review see Dielenberg & McGregor, 2001). The discrepancies, however, are likely the simple product of significant differences in the general methods employed by studies attempting to answer different questions. Zangrossi and File (1992), for instance, examined social interaction between pairs of animals, in which both members of the pair were previously exposed to cat odor (on a cloth) or one of several additional odors, and found decreased social interaction in animals that had experienced cat odor compared to controls. In contrast, in the present study we measured only exploratory behavior initiated by the subject and directed toward one of two target conspecifics (at test), and only the subject itself had been exposed to the predatory odor (cat urine in litter). Moreover, the previous experience with the familiar conspecific, and subsequent addition of a novel conspecific may have generally encouraged greater exploratory tendencies in the present study.

Importantly, the un-soiled litter of the control group was not itself completely odorless. Indeed, according to the manufacturer's website (http://www.tidycats.com/Products/Scoop/InstantAction.aspx; Retrieved 10 March, 2011) and labels on the product itself, the litter is formulated to

"neutralize" odors. Given that the product itself contains some scent independent of the cat urine, the control group would have also experienced some degree of olfactory stimulation. Thus, it seems that the observed group differences were not likely produced by simply experiencing a novel scent between social recognition training and testing, and that the disruption was due specifically to olfactory stimulation provided by the predatory cue. Similarly, others (Courtney, Reid, & Wasden, 1968) have shown that odor novelty is not a sufficient explanation for the disruption of rat running behavior by cat odor (in this instance, cat odor was produced by allowing a cat to walk through a straight-way prior to the running of subjects).

There are several possible explanations for the observed differences in social novelty preference. Indeed, it could be that exposure to predatory odor distracts the rats in some way, disrupting working memory and causing a lack of recognition of the initial conspecific, making it novel once more, and reducing preference for the novel conspecific during testing. In a similar social recognition procedure, Engelmann (2009) demonstrated interference of recognition memory in mice that encountered a second conspecific between exposure to the training animal and subsequent social recognition choice test between the initial and novel conspecifics. While species differences between rats and mice in social recognition procedures have been documented (e.g., Noack, Richter, Laube, Haghgoo, Veh, & Engelmann, 2010), it seems possible that the predatory odor may be resulting in a similar form of retroactive interference for the rats in the present study. Alternatively, it is a possibility that while under the threat of predation it would be more advantageous to the animal to stay near what is familiar and known to be safe from previous experience, rather than explore a novel conspecific that's not yet known to the animal. Predatory odor could be promoting some degree of neophobia. Future research should attempt to examine further these possibilities.

The obtained data are largely consistent with those of Morrow, Roth, and Elsworth (2000), who exposed male Sprague-Dawley rats to fox odor between training and test exposures in a novel object recognition task. Indeed, they reported that the time spent exploring both objects at test was not affected by predatory odor, and that the rats that were exposed to the substance displayed weaker preference for the novel object at test. Given the generally consistent results, it would seem that predatory odors have similar effects on novelty preference, regardless of which types of stimuli are serving as novel and familiar objects. Interestingly, this occurs despite the differences in the mechanisms underlying novel object and social recognition (Everts & Koolhaas, 1997).

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