

Short report

Behavioral responses to physical vs. social novelty in male and female laboratory rats

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ABSTRACT

Most behavioral tests used with laboratory rodents involve measuring behavioral responses to physical novelty. However, laboratory rodents are often derived from highly social species for which novel social stimuli may induce different levels of fear or curiosity compared to novel physical objects. We hypothesized that behavioral responses will differ in response to novel physical vs. social cues, and that females may show more exploration of social novelty, based on prior studies indicating that females more actively seek social support during duress compared to males. We compared young (55-day-old) Sprague-Dawley rats' responses to an arena filled with novel objects ("physical") or a novel same-sex caged conspecific ("social"). Rats were more active and spent twice as much time in contact with the novel social stimulus compared to novel physical stimuli. Although females were more active than males, females were not particularly more exploratory in the social arena compared to males. The results indicate that a novel social partner (even a caged one with limited ability to interact) elicits more exploration than novel objects for both male and female rats.

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1. Introduction

Many behavioral tests have been developed to measure laboratory rodent responses to novel stimuli. The majority of these tasks measure responses to non-social stimuli, such as time spent in brightly lit or unprotected physical spaces compared to time spent in dark or protected physical spaces (e.g., open field, elevated plus maze, light–dark box; Nadler et al., 2004; Shors and Wood, 1995). However, laboratory rodents are derived from highly social wild animals (e.g., Barnett, 1975; Calhoun, 1963) and thus their social exploration most likely involves complex processes that differ from those involved in the exploration of non-social objects. Further quantification of rodent social exploration would be beneficial, particularly to develop models of human disease processes that are specific to social contexts (e.g., social anxiety, autism; Moy et al., 2007; Bridges and Starkey, 2004). The goal of the current study was to compare rat behavioral responses to social vs. non-social novel stimuli to understand basic differences in exploratory behavior in response to these two different forms of novelty in males and females.

In a variety of species, male and female behavioral responses to the same environmental cues can differ markedly (rats: Fernandes

et al., 1999; mice: Frick and Gresack, 2003; fish: Øverli et al., 2006; humans: Rosenblitt et al., 2001). In rats, females are more exploratory than males in response to novel non-social stimuli whereas the evidence of a possible sex difference in response to social stimuli (usually same-sex) is less clear (e.g., Aguilar et al., 2003; Hughes, 1968; Johnston and File, 1991; Meaney and Stewart, 1979; Reeb and Tang, 2005; Taylor et al., 2000; Varlinskaya and Spear, 2008). The lack of clarity about rat sex differences in response to novel social partners may partially result from different test procedures across studies, with a majority of tests using a dynamic untethered social stimulus whose behavioral repertoire can greatly influence test animal behavior. Thus, in the current study we compared male and female behavioral responses to a caged conspecific to begin to tease apart sex differences in physical vs. social exploratory behavior in rodent models.

We hypothesized that: (1) rats would show different behavioral responses to novel social vs. physical stimuli, (2) females would be more active/exploratory than males, and (3) females would be particularly more exploratory in the social situation compared to males.

2. Methods

2.1. Overall design and sample

Young (55-day-old) male ($n=60$) and female ($n=60$) Sprague-Dawley rats (Charles River Laboratories, Wilmington,

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MA) were individually housed in solid-bottom plastic cages (43.5 cm × 23.5 cm × 20.5 cm) and maintained on a 14L:10D lighting schedule (lights on at 19:00 h) at 21 °C with ~50% humidity. Food and water were available *ad libitum* and cages were cleaned once a week by animal facility personnel trained in animal care and handling. Rats were allowed to acclimate to the laboratory for 2 weeks prior to testing. Throughout the study, rats were handled daily and female vaginal cytology was analyzed to coordinate all female testing during metestrus (Sell et al., 2005). At 10 weeks of age, rats were tested on two novel arenas – half on the novel object arena and half on the novel social arena. Health status of the colony was monitored by testing for 14 common pathogens in ‘sentinel’ rats regularly exposed to soiled bedding of all colony rats; none of these pathogens were identified in the sentinel rats. All methods were approved by The Pennsylvania State University Institute for Animal Care and Use Committee and adhered to methods specified in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996).

2.2. Behavioral response to physical vs. social novelty

Behavioral testing was conducted in the middle of the rats’ active period (4–6 h after lights off) in a non-colony room illuminated with red bulbs providing 6 lx of light at the center of the test arenas. Rats were transported in their home cage from the colony room to the testing room and immediately placed into the novel object or social arena (described below). Both arenas (120 cm × 120 cm × 46 cm high) were designed to be minimally anxiety-provoking to stimulate a range of exploratory responses. The arenas had a clear cover, rats were tested in low light, and the arena floor was covered with clean bedding laced with bedding from colony room cages to provide a complex and familiar rat odor (Cavigelli et al., 2007).

Novel object arena: For this test, a novel rat-sized object (plastic tube, inverted bowl, wire tunnel) was placed into three corners of the above arena (Fig. 1a). Rats were placed into a clean ceramic bowl with 5 cm high walls and lowered into the empty arena corner and video-recorded for 5 min. Rats were removed immediately after testing, feces removed from the arena, and the ceramic bowl rinsed with water and dried for the next subject.

Novel social arena: The novel social arena contained an empty cage and a cage with an unfamiliar rat of similar sex, size, reproductive phase, and age (Fig. 1b; Cavigelli et al., 2007). Unlike the previously-developed ‘social interaction’ test (File and Seth, 2003), we caged the stimulus rat to minimize its influence on the test animal’s response and to make the social stimulus more static and therefore more comparable to objects in the Novel Object Arena. The ‘social’ and ‘empty’ cages (28.5 cm × 18.5 cm × 18 cm high) were constructed of the same materials—a plastic base with wire mesh on the top that allowed for restricted social contact between stimulus and test rats. The stimulus rat was placed in the cage 20 min prior to the first test. The same stimulus rat was used for a day of testing and different rats used across days. Test rats were introduced to the arena, videotaped, and the arena cleaned as previously described.

Locomotion was scored by a trained coder. The arena image was superimposed with an 8 × 8 grid in which each square was slightly wider than the rat; locomotion was quantified as the cumulative number of squares crossed. Latency to novelty was coded as the time from when the rat was first placed in the arena to the time when they first contacted a novel object or the novel social cage with nose or forepaws. In the novel social arena, 3 males never approached novelty – their approach latencies were coded as 310 s. Five other behaviors were recorded: frequency to approach novelty, duration of novelty contact (paws touching

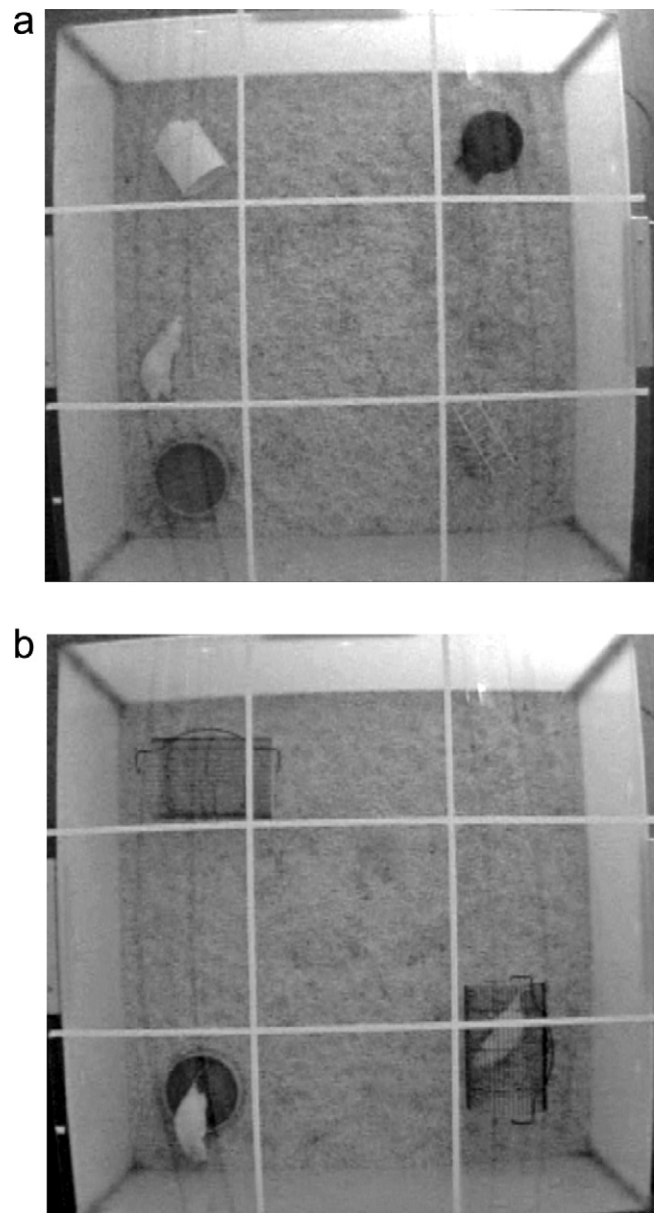


Fig. 1. (a) Novel object and (b) novel social arenas. The novel object arena contains three unfamiliar rat-sized objects near the arena walls; the novel social arena contains an unfamiliar same-sex rat in a cage and a similar but empty cage equidistant from the start bowl. The two rats can interact with one another through the cage bars without injuring one another.

object or cage), frequency of rearing, duration of grooming, and percent time rats spent in the center of the arena. The center was defined as the area more than one rat’s width from the walls of the arena.

2.3. Analyses

Latency to approach novelty, duration of grooming, and the percent time spent in the center of the arena values were not normally distributed and thus were transformed as appropriate to arrive at normal distributions (Sokal and Rohlf, 1995). To compare behavioral responses in the two arenas (within and between sexes), we used the mixed models procedure in SAS (Cary, NC, v9.13). Arena and sex were treated as fixed effects with subject as a random effect, and an arena × sex interaction term included.

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