Published in final edited form as:

Behav Processes. 2011 September ; 88(1): 56–59. doi:10.1016/j.beproc.2011.06.006.

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

Sonia A. Cavigelli^{*,a,b}, Kerry C. Michael^a, Sheila G. West^a, and Laura Cousino Klein^a

^aDepartment of Biobehavioral Health, The Pennsylvania State University, Pennsylvania, U.S.A.

^bThe Huck Institutes of the Life Sciences, The Pennsylvania State University, Pennsylvania, U.S.A.

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.

Keywords

rat; behavior; social; non-social; novelty; sex difference

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

*Corresponding Author: Sonia A. Cavigelli, 315 E. Health & Human Development Building, Pennsylvania State University, University Park, PA 16802, U.S.A., Phone: 001-814-863-0210, Fax: 001-814-863-7525, sac34@psu.edu.

(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.

Furthermore, male and female responses were compared. 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 & Sample

Young (55-day-old) male ($n=60$) and female ($n=60$) Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were individually-housed in solid-bottom plastic cages ($43.5 \times 23.5 \times 20.5$ 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 lux 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 (Figure 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 minutes. 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 (Figure 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 minutes 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 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 a sex × arena interaction term included.

3. Results

3.1. Behavioral Response to Novelty

Novel Object vs. Social Stimuli—In the social arena, rats moved 18% more, spent twice as much time in contact with novelty, and reared 30% more than rats in the object arena (Table 1A; all $F's_{1,116} > 18.19$, $p's < 0.0001$). These responses were similar across males and females. Increased contact time in the social arena was accounted for by rats spending more than twice as much time in contact with the social cage than the empty cage in this arena (Table 1B; $F_{1,116} = 105.76$, $p < 0.0001$). Other behaviors did not differ between the novel object and novel social arenas (Table 1A; all p -values > 0.10).

Females vs. Males—In both arenas, females were more active and displayed more exploratory behavior. Females moved 40% more, reared twice as often, approached novelty twice as quickly and frequently, and spent 25% less time in the center of the arena than males (Tables 1A&B; all $F's_{1,116} > 9.64$, $p's < 0.01$). There were no sex differences in how long males and females contacted novelty or groomed in the arenas (Table 1; all p -values > 0.20).

Differential Female and Male Responses to Novel Object vs. Novel Social Stimuli—Males and females showed different behavioral responses overall, but these sex differences were similar across the two arenas. Females were not particularly more active or exploratory in the social arena compared to males, i.e., there were no arena $\times$ sex interactions for any of the behavioral measures (Table 1A; all p -values > 0.05). Interestingly, within the social arena, males and females both spent more time in contact with the social cage than the empty cage, however, in contrast to our hypothesis, males spent proportionally more time in contact with the social cage than did the females (Table 1B; $F_{1,116} = 14.10$, $p < 0.001$).

4. Discussion

Rats moved more, reared less, and spent twice as much time contacting novelty in an arena containing a novel social partner than an arena with novel objects. Females were more active and exploratory than males in both novelty arenas, and females were not particularly more exploratory in the social arena than the males. These results support our first two hypotheses that rats will have different behavioral responses to social vs. physical novelty, and that females will be more exploratory than males, but they do not support our third hypothesis that female rats, compared to males, will be particularly more exploratory in a novel social situation compared to a physical situation.

These results support the hypothesis that fear or curiosity responses are context specific, with some contexts eliciting greater or less fear or curiosity than others. Dissociation of behavioral responses to social vs. physical stimuli has been identified only recently and in limited studies (e.g., Bielsky et al., 2004; Cavigelli et al., 2007 & 2008; Coleman and Wilson, 1998; Egashira et al., 2007; Liu and Smith, 2009; Prather et al., 2001). Several studies have specifically measured mouse ‘sociability’ across strains to develop models of human autism and comparable to the current rat study, nearly all mice strains spent more

time sniffing a cage containing another mouse than an empty cage (Moy et al., 2007 & 2008; Sankoorikal et al., 2006). Overall, few studies have included measures of rodent social behavior, but the current and prior studies show that complex models of altered sociability can be developed with creative use of both social and physical novelty tests. Estimates of social behavior are particularly important to model human conditions that are marked by avoidance of social conditions (e.g. social anxiety, autism; American Psychiatric Association, 2000).

Females in the current study were much more active than males, replicating prior studies with rodents tested in physically novel situations (e.g., Aguilar et al., 2003; Beatty, 1979; Ramos et al., 1997; Ray and Hansen, 2004), and extending this result to novel social conditions. Interestingly, females were not particularly more exploratory in the social test compared to the physical test. At first glance, this may not support the “tend-and-befriend” theory of the female stress responses or prior work with Listar rats (Johnston and File, 1991; Taylor et al., 2000). However, in the current testing situation, rats only had limited access to an unfamiliar and caged social partner, whereas stressed females may prefer social contact with familiar and/or dynamically-interacting uncaged partners (Johnston and File, 1991). A caged conspecific can engage in limited social behavior like male-biased nipping and grabbing but not female-biased affiliative behavior. Thus, future studies should recognize that sex-specific social behaviors may be differentially affected by the method used to present a social partner in a test situation (i.e. cage vs. tether).

Conclusion

Results of this study indicate that rats explore a novel social situation differently than a novel physical situation, and that future studies should consider more measures of social exploration to better quantify rodent behavior and develop models of human conditions in which social exploration is affected.

Acknowledgments

We thank C.E. Barrett, M. Diep, I. Fassasi, A. Jefferson, K. Haskins, C. Matteo, J. McGovern, K. Mehta, J. Patel, J. Quinn, M. Stine, and R. Smull for their assistance with data collection. This research was supported by NIMH R03 MH071406 (to SAC) and an internal Pennsylvania State University grant.

References

- Aguilar R, Gil L, Gray JA, Driscoll P, Flint J, Dawson GR, Giménez-Llort L, Escorihuela RM, Fernández-Teruel A, Tobena A. Fearfulness and sex in F2 Roman rats: males display more fear though both sexes share the same fearfulness traits. *Physiol. Behav.* 2003; 78:723–732. [PubMed: 12782229]
- American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, DSM-IV-TR. American Psychiatric Association; Washington, DC: 2000.
- Barnett, SA. *The Rat*. The University of Chicago Press; Chicago: 1975.
- Beatty WW. Gonadal hormones and sex differences in nonreproductive behaviors in rodents: Organizational and activational influences. *Horm. Behav.* 1979; 12:112–163. [PubMed: 573741]
- Bielsky IF, Hu SB, Szegda KL, Westphal H, Young LJ. Profound impairment in social recognition and reduction in anxiety-like behavior in vasopressin V1a receptor knockout mice. *Neuropsychopharmacology.* 2004; 29:483–493. [PubMed: 14647484]

- Bridges NJ, Starkey NJ. Sex differences in Mongolian gerbils in four tests of anxiety. *Physiol. Behav.* 2004; 83:119–127. [PubMed: 15501498]
- Calhoun, JB. Public Health Service Publication No. 1008. U.S. Department of Health; Bethesda, Maryland: 1963. The Ecology and Sociology of the Norway Rat.
- Cavigelli SA, Stine MM, Kovacsics C, Jefferson A, Diep MN, Barrett CE. Behavioral inhibition and glucocorticoid dynamics in a rodent model. *Physiol. Behav.* 2007; 92:897–905. [PubMed: 17673266]
- Cavigelli SA, Bennett JM, Michael KC, Klein LC. Female temperament, tumor development and life span: Relation to glucocorticoid and tumor necrosis factor alpha in rats. *Brain Behav. Immun.* 2008; 22:727–735. [PubMed: 18155400]
- Coleman K, Wilson DS. Shyness and boldness in pumpkinseed sunfish: individual differences are context-specific. *Anim. Behav.* 1998; 56:927–936. [PubMed: 9790704]
- Egashira N, Tanoue A, Matsuda T, Koushi E, Harada S, Takano Y, Tsujimoto G, Mishima K, Iwasaki K, Fujiwara M. Impaired social interaction and reduced anxiety-related behavior in vasopressin V1a receptor knockout mice. *Behav. Brain Res.* 2007; 178:123–127. [PubMed: 17227684]
- Fernandes C, González MI, Wilson CA, File SE. Factor analysis shows that female rat behaviour is characterized primarily by activity, male rats are driven by sex and anxiety. *Pharmacol. Biochem. Behav.* 1999; 64:731–738. [PubMed: 10593196]
- File SE, Seth P. A review of 25 years of the social interaction test. *Euro. J. Pharmacol.* 2003; 463:35–53.
- Frick KM, Gresack JE. Sex differences in the behavioral response to spatial and object novelty in adult C57BL/6 mice. *Behav. Neurosci.* 2003; 117:1283–1291. [PubMed: 14674847]
- Hughes RN. Behaviour of male and female rats with free choice of two environments differing in novelty. *Anim. Behav.* 1968; 16:92–96. [PubMed: 5639909]
- Johnston AL, File SE. Sex differences in animal tests of anxiety. *Physiol. Behav.* 1991; 49:245–250. [PubMed: 2062894]
- Liu Z-H, Smith CB. Dissociation of social and nonsocial anxiety in a mouse model of fragile X syndrome. *Neurosci. Lett.* 2009; 454:62–66. [PubMed: 19429055]
- Meaney MJ, Stewart J. Environmental factors influencing the affiliative behavior of male and female rats (*Rattus norvegicus*). *Anim. Learn. Behav.* 1979; 7:397–405.
- Moy SS, Nadler JJ, Young NB, Nonneman RJ, Segall SK, Andrade GM, Crawley JN, Magnuson TR. Social approach and repetitive behavior in eleven inbred mouse strains. *Behav. Brain Res.* 2008; 191:118–129. [PubMed: 18440079]
- Moy SS, Nadler JJ, Young NB, Perez A, Holloway LP, Barbaro RP, Barbaro JR, Wilson LM, Threadgill DW, Lauder JM, Magnuson TR, Crawley JN. Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. *Behav. Brain Res.* 2007; 176:4–20. [PubMed: 16971002]
- Nadler JJ, Moy SS, Dold G, Trang D, Simmons N, Perez A, Young NB, Barbaro RP, Piven J, Magnuson TR, Crawley JN. Automated apparatus for quantitation of social approach behaviors in mice. *Genes Brain Behav.* 2004; 3:303–314. [PubMed: 15344923]
- National Research Council. Guide for the Care and Use of Laboratory Animals. National Academy Press; Washington, D.C.: 1996.
- Øverli Ø, Sørensen C, Nilsson G. Behavioral indicators of stress-coping style in rainbow trout: Do males and females react differently to novelty? *Physiol. Behav.* 2006; 87:506–512. [PubMed: 16455115]
- Prather MD, Lavenex P, Maudlin-Jourdain ML, Mason WA, Capitanio JP, Mendoza SP, Amaral DG. Increased social fear and decreased fear of objects in monkeys with neonatal amygdala lesions. *Neuroscience.* 2001; 106:653–658. [PubMed: 11682152]
- Ramos A, Berton O, Mormède P, Chaouloff F. A multiple-test study of anxiety-related behaviours in six inbred rat strains. *Behav. Brain Res.* 1997; 85:57–69. [PubMed: 9095342]
- Ray J, Hansen S. Temperament in the rat: sex differences and hormonal influences on harm avoidance and novelty seeking. *Behav. Neurosci.* 2004; 118:488–497. [PubMed: 15174926]
- Reeb BC, Tang AC. Sex difference in temporal patterns of social interaction and its dependence upon neonatal novelty exposure. *Behav. Brain Res.* 2005; 158:359–365. [PubMed: 15698903]

- Rosenblitt JC, Soler H, Johnson SE, Quadagno DM. Sensation seeking and hormones in men and women: exploring the link. *Horm. Behav.* 2001; 40:396–402. [PubMed: 11673912]
- Sankoorikal GMV, Kaercher KA, Boon CJ, Lee JK, Brodtkin ES. A mouse model system for genetic analysis of sociability: C57BL/6J versus BALB/cJ inbred mouse strains. *Biol. Psychiatry.* 2006; 59:415–423. [PubMed: 16199013]
- Sell SL, Dillon AM, Cunningham KA, Thomas ML. Estrous cycle influence on individual differences in the response to novelty and cocaine in female rats. *Behav. Brain Res.* 2005; 161:69–74. [PubMed: 15904711]
- Shors TJ, Wood GE. Contribution of stress and gender to exploratory preferences for familiar versus unfamiliar conspecifics. *Physiol. Behav.* 1995; 58:995–1002. [PubMed: 8577899]
- Sokal, RR., Rohlf, FJ. *Biometry: The principles and practice of statistics in biological research.* third. W.H. Freeman and Company; New York: 1995.
- Taylor SE, Klein LC, Lewis BP, Gruenewald TL, Gurung RA, Updegraff JA. Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychol. Rev.* 2000; 107:411–429. [PubMed: 10941275]
- Varlinskaya EI, Spear LP. Social interactions in adolescent and adult Sprague-Dawley rats: impact of social deprivation and test context familiarity. *Behav. Brain Res.* 2008; 188:398–405. [PubMed: 18242726]

a **b** **Figure 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.

A) Female and male rat behavioral responses to the novel object and novel social arenas. Values for the novel social arena indicate behavioral responses to both the social and empty cages. B) Female and male behavioral responses to the empty and social cages within the novel social arena. Values indicate means $\pm$ standard deviation.

Table 1

A) <u>ACROSS ARENAS:</u>		NOVEL OBJECT		NOVEL SOCIAL		p	p
Behavior		Female n=30	Male n=30	Female n=30	Male n=30	Arena	Arena * Sex
Locomotion (n)		208 $\pm$ 40	133 $\pm$ 39	224 $\pm$ 40	177 $\pm$ 35	<.0001	ns
Approach Latency (s) ^a		16 $\pm$ 12	34 $\pm$ 41	15 $\pm$ 13	28 $\pm$ 25	ns	<.001
Approach Novelty (n)		26 $\pm$ 6	17 $\pm$ 6	25 $\pm$ 6	20 $\pm$ 7	ns	<.0001
Contact Novelty (s)		44 $\pm$ 12	46 $\pm$ 20	80 $\pm$ 29	87 $\pm$ 21	<.0001	ns
Rear (n)		15 $\pm$ 4	9 $\pm$ 4	12 $\pm$ 4	5 $\pm$ 4	<.0001	<.0001
Groom (s) ^a		7 $\pm$ 7	8 $\pm$ 8	7 $\pm$ 8	9 $\pm$ 11	ns	ns
Percent Time in Center ^a		36 $\pm$ 6	43 $\pm$ 14	35 $\pm$ 9	45 $\pm$ 10	ns	<.0001
<hr/>							
B) <u>IN SOCIAL ARENA:</u>		Empty Cage		Social Cage		p	p
Behavior		Female n=30	Male n=30	Female n=30	Male n=30	Cage	Cage * Sex
Approach Latency (s) ^a		29 $\pm$ 26	60 $\pm$ 37	30 $\pm$ 24	37 $\pm$ 29	ns	<.01
Approach Novelty (n)		9 $\pm$ 3	6 $\pm$ 3	16 $\pm$ 5	14 $\pm$ 5	<.0001	<.01
Contact Novelty (s)		29 $\pm$ 14	20 $\pm$ 12	51 $\pm$ 23	67 $\pm$ 21	<.0001	ns

^aStatistical analyses conducted with transformed values to arrive at a normal distribution.