



Published in final edited form as:

Behav Brain Res. 2011 March 1; 217(2): 326–336. doi:10.1016/j.bbr.2010.10.038.

Behavioral Differences Between Late Preweanling and Adult Female Sprague-Dawley Rat Exploration of Animate and Inanimate Stimuli and Food

Kiersten S. Smith¹ and Joan I. Morrell²

¹ Laboratory of Genetic Neuropharmacology, McLean Hospital, and Department of Psychiatry, Harvard Medical School, Belmont, MA 02478, USA

² Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102, USA

Abstract

The late preweanling rat has potential as a preclinical model for disorders initially manifested in early childhood that are characterized by dysfunctional interactions with specific stimuli (e.g., obsessive-compulsive disorder and autism). No reports, however, of specific-stimulus exploration in the late preweanling rat are found in the literature. We examined the behavioral responses of normal late preweanling (PND 18-19) and adult rats when presented with exemplars of categorically-varied stimuli, including inanimate objects systematically varied in size and interactive properties, biological stimuli, and food. Preweanlings were faster to initiate specific stimulus exploration and were more interactive with most specific stimuli than adults; the magnitude of these preweanling-adult quantitative differences ranged from fairly small to very large depending upon the stimulus. In contrast, preweanlings were adult-like in their interaction with food and prey. Preweanling response to some stimuli, for example to live pups, was qualitatively different from that of adults; the preweanling behavioral repertoire was characterized by pup-seeking while the adult response was characterized by pup-avoidance. The specific stimulus interactions of preweanlings were less impacted than those of adults by the time of day of testing and placement of a stimulus in an anxiety-provoking location. The impact of novelty was stimulus dependent. The differences in interactions of preweanlings versus adults with specific stimuli suggests that CNS systems underlying these behavior patterns are at different stages of immaturity at PND 18 such that there may be an array of developmental trajectories for various categories of specific stimuli. These data provide a basis for the use of the preweanling as a preclinical model for understanding and medicating human disorders during development that are characterized by dysfunctional interactions with specific stimuli.

Keywords

Late Preweanling; Adult; Objects; Biological Stimuli; Food

Corresponding Author: Kiersten S. Smith, Ph.D., Department of Psychiatry, Harvard Medical School and McLean Hospital, 115 Mill Street, Mailstop #145, Belmont, MA 02474, Tel: (617) 855-2010, Fax: (617) 855-2012, ksmith@mclean.harvard.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

The survival of any individual requires optimal spatial exploration of their environment integrated with adaptive investigation of and appropriate interactions with specific stimuli in the environment. Distinguishing whether specific stimuli within an environment, including food and social opportunities (Barnett, 1958, 1975), have positive value for survival or could cause injury is a critical capacity for an individual as dysfunctional interactions can result in lost benefits and/or increased risk of harm. In humans, disorders such as autism, obsessive-compulsive disorder (OCD), and some neurological disorders (e.g., category-specific agnosia), have dysfunctional interactions with specific stimuli as a pathological hallmark such that there is an inability to appropriately attend to objects and/or people, or perseveratively interact with them. Since these disorders are often initially manifested in childhood (Carter and Pollock, 2000), and various behavioral or pharmacological interventions are prescribed early in life (Muhle et al., 2004; Patel et al, 2007), it is important to have preclinical behavioral models of interactions with specific stimuli that include both young and adult rodents for use in preclinical testing of pharmacological interventions.

We, along with other researchers, posit that the late preweanling rodent has potential as a preclinical model of human childhood relevant to the time period when the emergence, diagnosis, and initial treatment of these various disorders can occur (Andersen et al., 2010; Ryan et al., 2010). Seeking to develop such a model, we here examine the responses of normal late preweanling rats (PND 18-19) and compare them to adults (PND 60) during the investigation of responses to exemplars of categorically different specific stimuli, including inanimate objects varied in size and interactive properties, biological stimuli, and food. No reports of specific stimulus-directed exploration in the late preweanling rat are found in the literature and there are very limited reports of stimulus-directed exploration by rodents of any age (Barnett, 1958; Chitty and Southern, 1954; Laviola et al., 2004; Stansfield et al., 2004). Additionally, the responses of rats of any age to an array of categories of specific stimuli and the impact of the familiarity or novelty of these stimuli have not been systematically studied (Heyser et al., 2003; Stansfield et al., 2004).

Earliest analyses of adult rodent responses to specific stimuli (large inanimate objects) began in the service of limiting rodent pilfering of human food stores (Smith, 1786) and subsequently developed into laboratory-based analyses uncovering the facts that size and novelty of stimuli are important variables regulating the amount and nature of interaction by rats (Barnett, 1958; Besheer and Bevins, 2000; Chitty and Southern, 1954; Ennaceur and Delacour, 1988). Adult rats initially avoid large novel objects, termed neophobia by Barnett (1958), while in contrast small novel objects are initially preferentially investigated and are thus used in recognition memory tasks (Besheer and Bevins, 2000; Bolles and Woods, 1964; Stansfield et al., 2004). Analysis of a limited variety of objects shows that adolescent rats respond to the objects more frequently than adults (Douglas et al., 2003; Laviola et al., 2004; Stansfield et al., 2004), but no studies have examined the responses of younger rats.

Living biological stimuli represent another category of stimuli that are important as these might inflict injury (predator), serve as a potential food source (prey), or if a conspecific might have potential for aggressive, sexual, or parental interactions. While conspecific and prey interactions have been examined in adults, they have only been examined to a limited extent in young (Bolles and Woods, 1964; Muhle et al., 2004; Rosenblatt and Lehrman, 1963; Wiedenmayer and Barr, 1998).

Inanimate food is also an important category of stimuli. While adult neophobic responses to novel food have been extensively documented (Barnett, 1958; Chitty and Southern, 1954;

Weinberg and Smotherman, 1978), less is known about novel food interactions by the postnatal young. Food preferences are shaped during embryonic and postnatal development because the mother's diet affects the flavor of the embryonic fluid (Hepper, 1988; Smotherman, 1982) and her milk (Capretta and Rawls, 1972; Galef and Henderson, 1972; Galef and Sherry, 1973; Le Magnen and Tallon, 1968; Wuensch, 1978). Pups are dependent on lactational milk until weaning but begin to sample and eat solid foods around PND 16 (Galef and Clark, 1972), a process initiated by the social transmission of food information from their mothers during foraging (Galef, 1992; Galef and Clark, 1972; Galef and Kennett, 1987).

We have chosen to examine the PND18 preweanling rat because this the earliest age at which rats possess adult-like integrated locomotor and sensory capacity, and it is prior to the complex process of puberty (PND 28-55) (Ojeda and Urbanski, 1988; Spear, 2000). Since this is the earliest age that rat pups are allowed beyond the maternal nest by their mothers (Barnett, 1958; Galef and Clark, 1972; Pereira et al., 2008; Small, 1899), this suggests, from an evolutionary perspective, that they have developed sufficiently to engage in environmental exploration, and are motivated to do so (Fowler, 1965). Certainly, however, PND 18 rats are not entirely adult-like behaviorally as they are more active during particular segments of the light-dark cycle and have lower anxiety levels than adults (Smith and Morrell, 2007), and further they are still undergoing considerable physiological and neural sculpting (Spear, 2000, for review). Consequently it is not surprising that the late preweanling can be vulnerable to the long-term effects of pharmacologic interventions and behavioral experiences that are different from the effects in adults (Andersen et al., 2002; Bolanos et al., 2003; Carlezon and Konradi, 2004; Patterson et al., 2009). We posit that interactions with specific stimuli will also not be fully mature in the preweanling rat due to these ongoing neurodevelopmental changes.

We posit that the combination of adult-like motor and sensory capacity together with normally lower anxiety levels and a possibly enhanced exploratory motivation may shape the preweanling's behavioral repertoire of exploratory behavior facilitating increased and potentially risky investigation of specific stimuli. We hypothesize that this facilitated behavioral exploration may be a function of the ongoing neural sculpting during this period of development and that the feedback provided by this increased exploration on the developing neural systems may significantly influence the adult behavioral repertoire. Both of these features of the preweanling model add to their potential as a preclinical model of childhood interventions in humans. Here we examined the responses of females only as we have a considerable body of prior data on spatial and environmental exploration and motor capacity in females at these two age groups (Smith and Morrell, 2007, 2008), and because female responses to specific stimuli have been less studied in the literature.

Materials and methods

2.1 Subjects

Subjects were PND 18-19 preweanling (33–40 g) and PND 60 adult (190–225 g) female Sprague-Dawley rats from the animal colony maintained in the Laboratory Animal Facility (LAF) at Rutgers University (Newark, NJ), an institution accredited by the American Association of Accreditation of Laboratory Animal Care (AAALAC). Litters were culled to 12 pups (half each sex) during the first neonatal week and weaned on PND 28.

Subjects were randomly selected same-sex littermates, with a maximum of two adults and two pups tested per litter (Elsner et al., 1986; Olazabal et al., 2002; Wilkinson et al., 2006). Except for animal husbandry, postnatal handling was minimal (Ader, 1968; Andrews and File, 1993; Ivanco et al., 1996; Meaney et al., 1991). All procedures met the standards

approved by the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996) and the Rutgers University Animal Care and Facilities Committee. Although adult female estrous cycle was not assessed with vaginal smears, adult hormonal status could not have impacted the data significantly as our preweanling-adult differences here and previously (Smith and Morrell, 2008) were on a substantially greater scale than the relatively small differences seen in adult females across the estrous cycle.

2.2 General procedures

2.2.1 Behavioral testing in the home cage—Starting two weeks prior to testing, subjects were housed and tested in a behavioral suite of adjacent single-use rooms, and were hand carried between rooms. For 24 hours before testing, subjects were housed in lidded clear Plexiglas boxes (42 cm long × 42 cm wide × 31 cm high) lined with wood chip bedding (Beta Chip, Northeastern Products, Warrensburg, NY), which became their home cage and the testing environment for all tests; 24 h is sufficient for an environment to become a home cage (Montgomery, 1955; Nakagawara et al., 1997). Preweanlings were housed with their dam and siblings, and adults were housed in same-sex sibling pairs. All animals had ad libitum access to food (Formulab Diet 5008, PMI, Nutrition International, Brentwood, MO) and water and were maintained under a 12-hour (h) light-dark cycle (lights on at 0700 h) and a temperature of 22°C. For tests of food novelty and familiarity (see below), all subjects additionally received unadulterated powdered rat chow in the home cage prior to testing.

2.2.2 Time of day of tests—Testing was conducted either when both adults and preweanlings were least active (light phase, 0900 h – 1200 h; 1st quartile) or most active (dark phase, 2000 h – 2400 h; 3rd quartile) in their daily cycle (Smith and Morrell, 2007) to determine whether time of day influenced their responses (Table 1), but chosen such that no preweanling-adult activity differences confounded the outcomes (Smith and Morrell, 2007).

2.3 Stimulus interaction procedures

All of the tests were conducted in an environment to which the subjects were habituated as their home cage; this cage was within a system designed for automated measures of activity. We chose the home cage environment for our stimulus interaction procedures because others have shown that the richest repertoire of responses to specific stimuli occurs in a home or familiar territory (Barnett, 1958; Chitty and Shorten, 1946; Chitty and Southern, 1954; Mitchell, 1976; Wilkinson et al., 2006), regardless of its size (Barnett, 1958; Davis et al., 1948; Nass, 1977; Shorten, 1954). Since novel environments elicit overall high rates of exploration and investigation (Buelke-Sam et al., 1984; Galani et al., 2001), these responses would confound examination of exploration responses to specific stimuli. Since an object already present in an environment before the animal is introduced for testing will be perceived to be part of the environment when the animal is re-introduced (Cowan et al., 1975), stimuli were placed into the home cage in the presence of the subjects. Novel objects were inserted for the first time, and familiar objects were removed and re-placed into the cage to control for the disturbance of inserting an object during the novel object test.

On the day of testing, subjects were transferred from their Plexiglas box home cage to a clean opaque plastic cage for 5 min then returned as single individuals to the home cage testing boxes. Subjects were observed in the home cage for 30 min before introduction of a stimulus. One stimulus was then inserted with minimal disturbance, and subjects were observed for 30 min. Separate subject groups were used for every stimulus examination; therefore subjects were only tested once and were never re-used in any other behavioral paradigms.

2.3.1 Location of stimulus presentation—Using separate subject groups, stimuli were placed either (a) 2 cm from the subject in the subject's preferred corner, a less anxiety-provoking location (Grossen and Kelley, 1972), or (b) in the center of the testing apparatus, a known anxiety-provoking location (File, 1985; Grossen and Kelley, 1972; Lister, 1987). To enhance the anxiety-provoking aspect of the center, center-location tests were done in the light quartile (0900 h – 1200 h) and home cages were illuminated by the overhead fluorescent lighting in the testing room at an average luminance of 34 lumens/m² (Konica Minolta Luminance Meter LS-100, Japan). For preferred corner tests in the light quartile, half of the apparatus was cast into shadow with a cardboard shield (46 cm high × 50 cm wide × 28 cm deep) and half was brightly lit, a version of the black and white box (Crawley, 1981; Crawley and Goodwin, 1980). The luminance was 25 lumens/m² in the light half and 2 lumens/m² in the dark half, a significant difference ($p < .001$) between the two halves.

For environmental consistency, subjects that received stimuli in the preferred corner during the light phase were housed in similar conditions prior to testing, i.e., one half of the cage was shaded and the other remained lit. Testing in the dark quartile (2000 h – 2400 h) was done without the cardboard shield and under red-light conditions. A subject's preferred corner was defined as that corner in which a subject spent >50% of its time during the 30 min prior to stimulus introduction; subject selection of the preferred corner was not dependent on the location of the nest site within the home cage.

2.3.2 Stimulus novelty vs. familiarity—To effectively examine the differences in behavioral responding between novel and familiar objects, it is critical that subjects can make the distinction (Barnett, 1958; Chitty and Southern, 1954). Novel stimuli were operationally defined as those to which subjects had never been previously exposed; familiar stimuli were operationally defined as those that subjects had been exposed to for a minimum of 24 h in the home cage/testing environment. Familiar stimuli were clean and unscented for testing thus avoiding confounds of scenting by cage mates (Sheldon, 1969). Tests with newborn pups and crickets only examined responses to these stimuli when they were completely novel since familiarity with these stimuli leads to induction of maternal behavior (Olazabal et al., 2002; Olazabal and Morrell, 2005; Rosenblatt, 1967) or satiety, respectively, thereby changing the nature of subsequent interactions.

2.3.3 Categories of stimuli

Inanimate objects: Responses to objects that were systematically scaled with respect to the size of the subject were examined. Objects were selected based on either their proportional size (length, weight, and volume) relative to subjects (minimum $n = 15$ per group except for the running wheel, see below) or their interactive properties. These objects were either smaller than, the same size as, larger than, or immensely larger than the subject. An object that produced an auditory stimulus when manipulated and one with a more interactive form were also included. Since the interactive objects were considerably large and prefabricated to specific dimensions (see below), it was not possible to proportionally scale these objects relative to subject size.

Objects were (a) one-third the size of the subject: Wheaton plastic Cryule Vials (2 mL; Wheaton Scientific, Millville, NJ) for preweanlings and Qorpak clear glass round jars (60 mL; Qorpak, Fisher Scientific, Pittsburgh, PA) for adults; (b) same size as the subject: Qorpak clear glass round jars (60 mL) for preweanlings and Qorpak clear glass wide mouth jars (480 mL) for adults; (c) plastic lattice jingle balls with bells (4 cm diameter; Paw Pals IncrediBall, International Pet Supplies, San Diego, CA), which are approximately the same size as the preweanling and one-third the size of the adult; (d) amber transparent polycarbonate Crawl Balls (10.7 cm diameter) with three openings each measuring 6.3 cm

(Bioserv, Frenchtown, NJ), approximately 1.5 times larger than adults and 9 times larger than preweanlings; and (e) an immense object: a stainless steel activity running wheel (325 g; 26.04 cm diameter) that attaches to side of the test box (AccuScan Instruments Inc., Columbus, OH), which is approximately 19 times larger than adults (Familiar, $n = 7$; Novel, $n = 11$) and 128 times larger than preweanlings (Familiar, $n = 11$; Novel, $n = 15$); the wheel is designed to have a minimal friction load and is used for both smaller and larger rodents.

Biological stimuli: Biological stimuli were all novel. Pup-related stimuli were (a) live male and female rat pups 1–3 days old (7.5–8.0 g; 5 cm long) (preweanlings, $n=20$; adults, $n=9$); pup sex did not influence subject response (data not shown); (b) warm postmortem pups (same age and size as live pups) (preweanlings, $n=11$; adults, $n=7$) sacrificed with CO₂ immediately prior to testing and warmed in a Fisher 2-Liter Water Bath (Fisher Scientific, Pittsburgh, PA) to the same temperature as a live pup upon separation from its dam (33°C); and (c) a warm pup-like object (preweanlings, $n=10$; adults, $n=7$), a plastic Cryule Vial, the same size as pups, filled with the charcoal contents of Grabber Mycoal Hand Warmers (Grabber Performance Group, Grand Rapids, MI) warmed to 33°C. The live pup, warm postmortem pup, and warm vial lose 15–18% of their heat at a similar rate by the end of the test session (data not shown); temperatures were measured with a Fisher Digital Thermometer (Fisher Scientific, Pittsburgh, PA).

Prey stimuli were live female crickets (*Acheta domestica*; Fluker Farms, Port Allen, LA). Preweanlings ($n=12$) were presented with 3-week-old nymphs (length 1 cm), and adults ($n=11$) were presented with 6-week-old adult crickets (length 2 cm); nymphs were identical in their prey-like quality except proportionally smaller in size relative to the infants. Prior to use, crickets were housed in micro-isolators in the Rutgers Insectarium on a 16:8 h light:dark cycle (lights on at 0200 h) at 25°C with food (Fluker's Orange Cube, pieces of fruit) and water ad libitum. All testing occurred during the 3rd quartile because both crickets and rats are most active during the dark phase of the daily cycle. Although prey stimuli theoretically also serve as food, we categorized crickets as prey since the response elicited by this particular stimulus is fundamentally different from an inanimate food source (see below).

Food stimuli: This protocol provides a choice between a familiar and a novel food, and thus differs from the other stimulus presentations in the prior sections; this protocol follows a strong precedent and informative approach in the taste and food preference literature (Bunsey and Eichenbaum, 1995; Countryman et al., 2005) and was chosen to facilitate comparison of these outcomes to published reports. Food stimuli presented to subjects (preweanlings, $n=8$; adults, $n=10$) included (a) familiar food, unadulterated powdered Formulab Diet 5008 rat chow (PMI, Nutrition International, Brentwood, MO) and (b) novel food, powdered rat chow flavored with 5% bakers chocolate (Hershey's Cocoa, Hershey Foods Corporation, Hershey, PA) (Burton et al., 2000).

Preweanlings were deprived of food for 2 h and adults for 6 h immediately before testing during the 3rd quartile. We deliberately avoided prolonged food deprivation because this causes severe hunger that overrides and confounds the avoidant responses of the animals which are not in extreme food deprived state; the food deprivation times chosen here induced equivalent patterns of food sampling behavior between the two age groups (Smith and Morrell, 2003). Subjects were simultaneously presented with 5 g each of the novel (5% cocoa-flavored) and the familiar (unadulterated rat chow) foods in the preferred corner in clear plastic petri dishes (Fisherbrand disposable petri dishes 35 × 10 mm) taped to the floor of the home cage to prevent spillage. All other experimental details are as described in the following section.

2.4 Measures of stimulus interaction

Subject interactions with the stimuli were observed directly by one investigator (K.S.S.) and from a corner within the testing room that was not visible to the subjects. Data collected were (a) time to initial contact, defined as the amount of time it took for a subject to initiate contact and investigate a stimulus; (b) number of investigations, that is, number of times a subject approached and investigated a stimulus by sniffing and/or physical contact; and (c) total contact time in which the subject maintained contact with the stimulus. The following additional measures specific to certain stimuli were also recorded. Wheel interaction included the following automated measures generated by the VersaDat software program (AccuScan Instruments Inc., Columbus, OH): (a) number of wheel rotations and (b) time spent inside the wheel. Measures of predatory hunting during interaction with a live cricket were (a) time to catch and kill the cricket after its presentation, (b) total time to eat the cricket, and (c) parts of the cricket eaten. Food (flavored and unflavored rat chow) was weighed before and after the test session.

2.5 Data analysis

In order to fully represent the behavioral repertoires of each individual subject, data analysis was conducted so that two data points per litter are included. However, in this and previous work (Smith and Morrell, 2007) we have also found that the outcomes of the analysis were identical upon re-analysis of data averaging the two subjects per litter so that there was one data point per litter. Data points were rarely eliminated and only if their value was three standard deviations above/below the mean. All data met the requirements for parametric statistical tests, i.e., normal distribution and homogeneity of variance. The significance level was set at $p < 0.05$.

Two-way analysis of variance (ANOVA; Subject Age \times Stimulus) followed by Tukey's post-hoc tests were used to analyze the effect of Time of Day (1st vs. 3rd quartile), Stimulus Location (center vs. corner), and response to novel versus familiar stimuli. A limited number of independent t-tests were then used to identify statistical differences in select comparisons. Proportional object data were first analyzed within each age group with a one-way ANOVA followed by Tukey's post-hoc test and then analyzed between the two age groups by using a two-by-three factor ANOVA (Subject age \times Object). The General Linear Model procedure was used to implement the ANOVA comparisons using the SAS statistical software version 8.2 for personal computers (SAS Institute, Inc., Cary, NC).

3. Results

3.1 Responses to inanimate objects

3.1.1 Influence of time of day and stimulus location on interactions with objects—Adult interactions with an object were influenced by the time of day at which the test took place only in that the time to first contact in adults was slower during their inactive period (significant main effect of Time of Day, $F(1,122) = 8.62$, $p < 0.01$; $t(61) = 2.59$, $p < 0.05$, Fig. 1A). Otherwise the number of investigations and the total time with the object were not impacted by testing in the active versus inactive portions of their diurnal cycle. In contrast, preweanlings interacted with objects faster and more, without regard for time of day. Specifically the time to first contact for preweanlings was not influenced by the time of day at which the test took place such that the time to the first interaction was significantly faster than adults both in the active and inactive periods (significant main effect of Subject Age, $F(1,122) = 17.98$, $p < 0.0001$, Fig. 1A).

Preweanlings further differed from adults in that the number of investigations (significant main effect of Subject Age, $F(1,122) = 19.77$, $p < 0.0001$, Fig. 1B) and the total time with

the object (significant main effect of Subject Age, $F(1,122) = 27.12, p < 0.0001$, Fig. 1C) were both substantially higher regardless of the time of day at which the test took place. All subsequent testing was carried out at a time of day of equal activity for both groups and so that this variable did not confound outcomes.

Placing objects in the center of an environment slowed and decreased the interactions of adults; preweanlings were adult-like in this overall pattern. Specifically, subjects in both age groups took significantly longer to approach an object in the center (significant main effect of Stimulus Location, $F(1,63) = 13.52, p < 0.001$, Fig. 1A), and spent less total time with it (significant main effect of Stimulus Location, $F(1,63) = 14.55, p < 0.001$, Fig. 1C) than when it was placed in the preferred corner. Preweanlings, however, were less impacted by the center placement than adults and were significantly more interactive than adults regardless of object location (significant main effect of subject age for Number of Investigations, $F(1,63) = 6.20, p < 0.05$ and Total Contact Time, $F(1,63) = 19.77, p < 0.0001$, Fig. 1B and 1C).

3.1.2 Responses to three objects that are not influenced by novelty—Adults responded identically to the novel and familiar state of objects that were one-third the subject's size, a subject-sized object, or a crawl ball over the total 30 minute test (Table 2). Preweanlings have the same pattern of responsiveness for these objects. The data for these objects are therefore pooled for subsequent analysis (Fig. 2).

Adults approached a small object one-third their size, a subject-sized object, and a crawl ball within the 30 minute test (Fig. 2A). While preweanlings had an adult-like approach time for the small object, they approached the larger objects considerably faster than adults (significant Subject Age \times Stimulus interaction, $F(2,250) = 8.24, p < 0.001$, Fig. 2A). Adults investigated the crawl ball significantly more than either a small object or a subject-sized object ($F(2,121) = 21.53, p < 0.0001$; Tukey's post-hoc's $p < 0.05$, Fig. 2B). Preweanlings investigated both of the larger objects significantly more frequently than the smaller object ($F(2,129) = 20.10, p < 0.0001$; Tukey's post-hoc, $p < 0.05$, Fig. 2B). Preweanlings differed from adults in that they had a notably higher number of investigations of the large objects (same-sized object and crawl ball) (significant Subject Age \times Stimulus interaction, $F(2,250) = 5.12, p < 0.01$, Fig. 2B). For adults, total contact time was not affected by stimulus size or interactive properties (Fig. 2C) whereas preweanlings spent the most time with the two large objects, a subject-sized object and a crawl ball ($F(2,129) = 9.57, p < 0.0001$; Tukey's post-hoc, $p < 0.05$, Fig. 2C). Contact time with these two stimuli was also significantly greater in preweanlings than adults (significant Subject Age \times Stimulus interaction, $F(2,250) = 7.81, p < 0.001$, Fig. 2C).

3.1.3 Responses to two objects influenced by novelty

Jingle Ball: Novelty substantially shaped subject interactions with an object that had an auditory component, the jingle ball (Table 2). While adults took an equal amount of time to make the first contact with the jingle ball whether familiar or novel, they investigated ($t(26) = -2.93, p < 0.01$) and spent twice as much time ($t(26) = -2.12, p < 0.05$) with a novel jingle ball than a familiar one (Table 2). While preweanlings approached both the novel and familiar jingle ball 5–10 faster than adults (significant main effect of Subject Age, $F(1,68) = 27.73, p < 0.0001$, Table 2), they approached a novel ball three times faster than a familiar one ($t(32) = 3.38, p < 0.01$, Table 2). Unlike adults, preweanlings investigated both novel and familiar balls the same number of times and spent equal amounts of time with both. Lastly, preweanlings spent twice as much time with a familiar jingle ball than adults (significant main effect of Stimulus, $F(1,56) = 4.23, p < 0.05$; $t(26) = -2.36, p < 0.05$, Table 2).

Running Wheel: Novelty also noticeably shaped responses to the largest object used in this paradigm, which is also an object with marked sustainable interactive properties once it becomes familiar. Results of a 2×2 Factor ANOVA (Subject Age \times Stimulus Property) showed significant interactions in the following measures: Overall, both groups contacted the novel wheel more slowly ($F(1,40) = 4.49, p < 0.05$, Fig. 3A), investigated it more ($F(1,40) = 4.21, p < 0.05$, Fig. 3B), spent less time in it ($F(1,38) = 4.37, p < 0.05$, Fig. 3C), and ran less in it ($F(1,40) = 4.37, p < 0.05$, Fig. 3D).

Adults were particularly slow to make their first contact with the novel wheel, which took on average about twice as long as their first interaction with the smaller objects (Fig. 3, Table 2). Adults investigated the novel wheel more than the familiar, but spent less time in it and ran less in it than after it became familiar (Fig. 3B and 3C). In notable contrast, preweanlings approached the novel wheel twice as fast as adults ($t(24) = 2.48, p < 0.05$, Fig. 3A) and interacted with it significantly more than adults as measured by the number of investigations ($t(24) = -4.53, p < 0.0001$, Fig. 3B) and time inside the wheel ($t(24) = -3.26, p < 0.01$, Fig. 3C). This same pattern was also observed with wheel rotations but was not significantly different between the two age groups.

3.2 Response to biological stimuli

3.2.1 Response to pups and pup-like stimuli—Adults and preweanlings first approached the three stimuli at different rates (significant Subject Age \times Stimulus interaction, $F(2,58) = 7.64, p < 0.01$, Fig. 4A). Adults took five times longer than preweanlings to first approach a newborn pup ($t(27) = 3.13, p < 0.01$) and twice as long to approach a warm pup-like object ($t(15) = 1.82, p < 0.05$). In marked contrast, adults were five times faster than preweanlings in their approach to a warm postmortem pup ($t(16) = -2.158, p < 0.05$, Fig. 4A). Adults and preweanlings also differed in the number of investigations of the various stimuli (significant main effects of Subject Age, $F(1,58) = 12.20, p < 0.001$ and Stimulus, $F(2,58) = 3.99, p < 0.05$, Fig. 4B).

While adults investigated all stimuli, preweanlings investigated a live pup twice as many times as adults ($t(27) = -2.70, p < 0.05$) and the warm pup-like object three times more than adults ($t(15) = -2.85, p < 0.05$); preweanlings investigated the warm postmortem pup more but this difference was not statistically significant. Subsequently, adults spent very little time with any of this category of stimuli. While preweanlings spent little time with postmortem pups or warm objects, there was a marked overall adult-preweanling difference in live pup interaction, and much greater contact time with live pups by preweanlings. Preweanlings spent the majority of their test time in contact with live pups, four times longer than adults (significant Subject Age \times Stimulus interaction, $F(2,58) = 7.22, p < 0.01$; $t(27) = -4.36, p < 0.001$ Fig. 4C).

3.2.2 Response to a live cricket—Adults were three times faster in their approach to a live cricket compared to preweanlings ($t(21) = -2.59, p < 0.05$, Table 3). Once contact was established, and while the cricket was still alive, preweanlings investigated the cricket a significantly greater number of times than adults ($t(21) = -2.30, p < 0.05$, Table 3) although the total contact time for both subject groups was very brief, averaging only 30 sec, suggesting briefer and more frequent investigations by preweanlings (Table 3). Two preweanlings and five adults engaged in hunting characterized by the following rapid behavioral sequence: they actively chased the cricket around the cage, caught it, killed it by biting the head off, and then ate it. The remaining animals did not catch and/or kill the cricket within the test period. Although preweanlings took 3.5 times longer to catch and kill the cricket ($t(5) = -2.86, p < 0.05$) and spent more time eating it ($t(5) = -2.89, p < 0.05$, Table 3), their pattern was remarkably organized and followed the same sequence used by

adults. However, preweanlings ate the entire cricket, whereas adults ate only the body, not the head or legs.

3.3 Response to food

Adults contacted, investigated, and ate more familiar than novel food; preweanlings responded similarly on these measures and were hence adult-like in their response pattern to this stimulus. Results of a 2×2 ANOVA showed that both groups spent significantly more time in contact with the familiar than the novel food (significant main effect of Food Stimulus, $F(1,32) = 27.85, p < 0.05$, Fig. 5C) and ate more of the familiar than the novel food (significant main effect of Food Stimulus, $F(1,32) = 4.37, p < 0.05$, Fig. 5D). However, preweanlings differed from adults by approaching both foods four times faster (main effect of Subject Age, $F(1,32) = 10.11, p < 0.05$, Fig. 5A), investigating them more (significant main effect of Subject Age, $F(1,32) = 42.06, p < 0.001$, Fig. 5B), and eating four times more novel food than adults ($t(16) = -2.72, p < 0.05$, Fig. 5D).

4. Discussion

Overall the data demonstrate that preweanlings were generally faster to initially explore and were then subsequently more investigative of and interactive with a wide range of specific stimuli introduced into their environments than adults. In addition to this, preweanling behavioral responses to specific stimuli less impacted by the time of day and stimulus location as suggested by their equal response to stimuli in the least and most active parts of the daily cycle (first and third quartiles, respectively), and the smaller impact of placing stimuli in the center of the environment, a location commonly understood to be anxiety-provoking and hence actively avoided by adults. This result is consistent with our previous work showing that preweanlings can exhibit anxiety-like responses but are normally less anxious than adults (Smith and Morrell, 2007). The impact of novelty on the interaction with a stimulus depended upon the stimulus that was introduced into the home cage, but it was generally more profound in the adult than in the younger rat such that preweanlings more quickly explored novel stimuli and interacted more with them. That said, the preweanlings do have adult-like responses to certain categories of stimuli suggesting that the maturation of the CNS systems regulating food- and predatory hunting-related behaviors occurs earlier than the regulation of systems underlying exploration of other stimuli (e.g., objects).

It can be concluded that, for the most part, the preweanling has an adult-like capacity to discriminate between closely related stimuli, for example among the pup-like stimuli, but then subsequently interacts with the stimuli in a quantitatively, and sometimes qualitatively, different manner from the mature adult. Together, these data suggest that preweanlings have a greater propensity to gather information about certain categories of specific stimuli in their environment compared with adults and that the experiences generated by this normal, increased interaction with stimuli may crucially impact and shape the developing CNS in this period of life.

We and others have suggested that the well-documented phenomena of increased exploration of the environment by the young rat during the peri-weaning period may be based on having a higher level of motivation for environmental exploration than adults (Gerrish and Alberts, 1997, Goodwin and Yacko, 2004; Smith and Morrell, 2007). Based on our current data, we posit that this enhanced motivation for environmental exploration in the preweanling extends to many specific stimuli within the environment. Alternatively, preweanlings might not have memory abilities as keen as those of adults, may lack the ability to form categorical systems for complex stimuli resulting in increased stimulus investigation because they don't remember them, or they may have less sensory capacity than adults thereby requiring more interaction to learn about a stimulus. However, these

interpretations seem unlikely based on studies examining memory function (Roth and Sullivan, 2001) and sensory capacity (Bolles and Woods, 1964; Moorcroft et al., 1971) in developing rodents. We further posit that the greater responsivity of preweanlings to specific stimuli in their environment may in part facilitate the entrainment or establishment of neuronal networks so that preweanlings acquire the adaptive neural connections and response patterns of adults.

The rapid and extensive responses of preweanlings were particularly notable with regard to the larger objects and those objects with interactive properties. After more rapid contact, preweanlings then spent substantially more time in contact with these objects than the adults. While such activity may be a form of object play (Fagen, 1981), this specific type of play behavior has not been characterized in developing rodents. In contrast, adults investigated larger and more interactive objects with greater caution. These data extend and are consistent with prior reports showing that adolescent rats interact with objects more than adults (Douglas et al., 2003; Laviola et al., 2004; Stansfield et al., 2004).

Our tests using three types of objects (small, subject size, and crawl ball) in the novel versus familiar state did not demonstrate any discrimination of novelty, but our testing procedures were different from typical novel object recognition memory test procedures in that those tests are much shorter and present both novel and familiar objects at the same time (Berlyne, 1950; Besheer and Bevins, 2000; Ennaceur and Delacour, 1988). It is possible that for these data, the averaging of exploration of the objects over the full thirty minutes may have obscured any initial response differences, and that the presentation of a single object either in novel or familiar form, as opposed to a test situation where subjects must discriminate between novel and familiar objects, does not facilitate the distinction between object novelty and familiarity.

Furthermore, subjects may have become sufficiently familiar with these specific objects after having been housed with them for 24 hours prior to testing and subsequently found that its interactive properties no longer held their interest thereby resulting in decreased interactions during test. Our tests using the jingle ball and running wheel did however demonstrate marked differences in responses of subjects to their novel versus familiar states demonstrating that both preweanlings and adults can discriminate between novelty and familiarity.

Our findings on the adult responses to the wheel are consistent with Barnett's finding from presenting wild rats with an immense novel object, a food bin, which subjects also initially avoided, a reaction he termed neophobia; this avoidance was overcome as the rats had to interact with the bin as their only food source (Barnett, 1958). Like Barnett's food bin, the wheel is a stimulus with inherent incentive value for rats. In fact we chose the wheel as the immense object in our protocol because others have demonstrated that wheel running is a robust voluntary activity of adult rodents and that this object has positive incentive salience (i.e., interpreted as a rewarding property) revealed once they are familiar with wheel running activity or its after-effects (Lett et al., 2000, 2001; Werme et al., 2000, 2002). Further, our previous work has shown that p18 preweanlings can engage in wheel running at levels similar to the adult (Smith and Morrell, 2007) suggesting that wheel running may also have the same positive incentive salience for them.

Newborn pups as stimuli revealed considerable differences between preweanling and adult responses to specific stimuli. While both groups discriminated all three of the stimuli in this category and rapidly approached a live pup, adults actively avoided the pup after a brief period of exploration while the preweanlings spent the majority of the test period with it. Non-maternal adult rats are known to avoid pups for days (Cosnier and Couturier, 1966;

Mayer and Rosenblatt, 1979; Rosenblatt, 1967). Our data from both groups on the total pup contact time are consistent with previous reports of pup interaction during the initial phases of maternal sensitization (Bridges et al., 1974; Kalinichev et al., 2000; Mayer and Rosenblatt, 1979; Olazabal et al., 2004; Olazabal and Morrell, 2005). These data also provide critical details about how preweanling and adult rats initially approach and investigate newborn pups. The rapid approach by adults to the live and postmortem pup stimulus is consistent with previous reports showing that adult rats readily approach and investigate any conspecific that enters the home territory (Barnett, 1958; Miczek and de Boer, 2005).

Alternatively, given the predatory hunting response of adults to crickets, and since infanticide and pup eating is found among non-maternal rodents (Numan and Insel, 2003) it might be that rat pups held potential as interesting food in their environment. The level of preweanling interaction with pups during the initial approach and investigation further suggests that, like adults, preweanlings will approach unfamiliar conspecifics in the home environment, but the subsequent interaction with live pups, the delayed postmortem pup interaction, and the absolute lack of infanticide by rat juveniles, this behavioral spectrum is qualitatively different from that of adults.

By also testing responses to a warm but dead pup and a warm pup-sized object, we found that heat was not an important factor for interaction with the pup. These data are consistent with the work of others showing that juveniles and adults spend significantly more time in contact with live pups than rubber pup-sized objects (Gray and Chesley, 1984), marbles (Sheehan et al., 2000), or dead pups (Mayer and Rosenblatt, 1979) and extend their findings by controlling for the thermal properties and characterizing the initial response.

Preweanlings and adults also had different initial interactions with another live stimulus, a cricket. This was a rare case in which preweanlings took longer to approach and contact the stimulus than did adults, but then still followed their general pattern and investigated it more than the adults. It is possible that the preweanling is simply less attentive to small new stimuli placed in their cage, as seen with the small inanimate objects, whereas the adults rapidly attended and responded to an intrusion into their home territory. Responses based on innate fear responses seem unlikely as the young did not freeze and remained very active when exposed to the cricket. However, once the initial investigation was completed, the subsequent response to this stimulus exhibited by some of the preweanlings was surprisingly adult-like as both young and adult subjects engaged in predatory hunting. This finding suggests that the animation provided by crickets may serve to focus the attention of the preweanling to therefore respond with a mature innate hunting response. Even though the preweanlings that engaged in hunting took longer to catch and kill the cricket and spent more time eating it, the overall sequence of behaviors was considerably organized and executed in rapid succession similar to that seen in the adults. Adults in this study engaged in hunting behavior identical to what has been documented (Comoli et al., 2003; Ivanco et al., 1996; Kinsley et al., 2006).

We posit that preweanlings spent more time eating the cricket than the adults simply because they ate the entire insect. Since preweanlings were presented with nymphs, which are half the size of adult crickets, it is unlikely that the increased consumption time was attributed to relative subject-to-cricket size differences. Others have reported that adult rats will only eat the torso of the cricket and will avoid the head and rear legs, possibly due to the spiny protrusions on the legs (Ivanco et al., 1996). Our results are consistent with another study showing that young marsupials, specifically adolescent opossums, readily hunt crickets but that their behavior is slightly less developed than the adult (Ivanco et al., 1996).

Preweanlings also responded relatively maturely to novel and familiar foods. Both groups ate significantly less novel food than familiar food, investigated the novel food less, and spent less time in contact with the novel food than the familiar one. The pattern of interactions that adults exhibited is consistent with what has been described in the literature as food neophobia (Barnett, 1958; Chitty and Shorten, 1946; Weinberg et al., 1978). Although food neophobia has not been characterized in preweanling rodents, the adult-like patterns of interaction strongly suggest that the behavior of these young animals can be considered neophobic even though they interacted with the novel food slightly more than the adults did. This avoidance of novel foods during postnatal development may serve a protective function.

It is unlikely that our behavioral results, specifically that both preweanlings and adults ate more familiar food than novel food, were affected by extreme hunger or by food palatability. Our previous work (Smith and Morrell, 2003) and that of others (Countryman et al., 2005) showed that food deprivation is necessary to invoke the approach and consumption of even a familiar food. We deliberately avoided prolonged food deprivation because this causes severe hunger that overrides the normally avoidant responses of the animals. In response to extreme levels of food deprivation, rats will readily eat novel food (Burns et al., 1996; Smith and Morrell, 2003). Subjects in this experiment sampled only the novel food and were given a sufficient amount of both foods during the test to ensure that consumption of the novel food was not due to a need to relieve hunger simply because all of the familiar food had already been eaten. We presented subjects with two equally palatable and nutritious foods because previous studies have shown that foods with higher fat (Hansson, 1973) and/or sugar content (Bhardwaj and Khan, 1978; Khan, 1974) are more palatable to rats and other rodents. Further, our previous work has shown that preweanlings and adults respond similarly by initially avoiding a food containing a high sugar content that is highly palatable to rodents (i.e., Fruit Loops) following modest food deprivation (Smith and Morrell, 2003). Thus the limited sampling of the novel food could not be attributed to decreased palatability or nutritional content.

Taken together, our data show that preweanlings respond to most specific stimuli more quickly and with more interaction than adults, while a more adult-like behavioral pattern is observed when preweanlings are presented with a few select stimuli such as food and in the prey-catching phase of cricket interaction. Certainly, the greatest differences in stimulus-specific exploration between preweanlings and adults were revealed with the pup-like category of stimuli. These data further suggest that stimulus-directed exploration may have different developmental trajectories that are stimulus-specific. For example in the case with the newborn pup stimulus previous reports have shown that pup avoidance emerges by p23 and reaches an adult level before the onset of puberty during early adolescence (p30) (Mayer and Rosenblatt, 1979). Other stimuli that elicited considerable differences in responses between young rats and adults were large interactive objects, particularly an object the same size as the subject, the crawl ball, and an immense object, the running wheel. Stimulus novelty and familiarity with the immense object further revealed age-related behavioral differences.

For the most part, these data demonstrate that young rats explore specific stimuli in the environment in a fundamentally different way than adults during what are normally the days of their first independent forays from the maternally-protected nest. Overall, the preweanlings were less inhibited in their interaction with stimuli. These experiences may place the developing rodent at greater risk for danger but they also significantly shape the developing CNS such that preweanlings acquire the adaptive neuronal connections and response patterns of adults. It is also the case that the greater risk may be mitigated by maternal presence, as the young often accompany the female in her foraging. The greater

sensitivity of preweanlings to some specific stimuli in their environment is important to consider when using stimulus-specific exploration as a tool to assess normal and pathological behaviors in preclinical models of mental health disorders during postnatal development, such as autism and obsessive-compulsive disorder, of which a fundamental characteristic is dysfunctional stimulus-specific interaction.

The fact that most stimulus interactions are quantitatively more robust in the preweanling suggests that CNS systems regulating these relatively refined interactions with the environment may be crucially different at p18 versus p60. Some of these interactions are so extensive that what is in fact a normal range of interactions in the preweanling might be misunderstood as perseverative if considered solely by standards of adult interactions with specific stimuli. For the human condition, our data extends the concept that interventions designed for the adult CNS could be inappropriate for the developing CNS of the young diagnosed with disorders characterized by inappropriate stimulus interactions. In future studies, these data will provide essential points of comparison for determining what constitutes aberrant behavior in transgenic or knockout models of physical and mental diseases such that more effective pharmacologic and behavioral interventions can be designed for humans.

Acknowledgments

This research was supported by Training Grant MBRS S 5 R25 GM 060826-06 from the National Institute of General Medicine and Science and Grant DA 014025 to J.I. Morrell, P.I. The authors thank R.A. Countryman, B. Levin, and R. Ross for their generous assistance with the food paradigm and N. Canteras, S. Fahrbach, and C. Kinsley for sharing their knowledge of predatory hunting and cricket husbandry.

References

- Ader R. Effects of early experience on emotional and physiological reactivity in the rat. *J Comp Physiol Psychol* 1968;66:264–268. [PubMed: 5722035]
- Andersen SL, Arvanitogiannis A, Pliakas AM, LeBlanc C, Carlezon WA Jr. Altered responsiveness to cocaine in rats exposed to methylphenidate during development. *Nat Neurosci* 2002;5:13–14. [PubMed: 11731802]
- Andersen SL, Greene-Colozzi EA, Sonntag KC. A novel, multiple symptom model of obsessive-compulsive-like behaviors in animals. *Biol Psychiatry*. 2010 In Press.
- Andrews N, File SE. Handling history of rats modifies behavioural effects of drugs in the elevated plus-maze test of anxiety. *Eur J Pharmacol* 1993;235:109–112. [PubMed: 8519271]
- Barnett SA. Exploratory behaviour. *Brit J Psychol* 1958;49:289–310. [PubMed: 13596571]
- Barnett, SA. *The Rat: A Study in Behavior*. University of Chicago; Chicago: 1975. (rev. ed.)
- Berlyne DE. Novelty and curiosity as determinants of exploratory behaviour. *Br J Psychol* 1950;41:68–80.
- Besheer J, Bevins RA. The role of environmental familiarization in novel-object preference. *Behav Proc* 2000;50:19–29.
- Bhardwaj D, Khan JA. Sugar preferences of “black rats”, *Rattus rattus* L. *Zool J Linn Soc* 1978;64:41–50.
- Bolanos CA, Barrot M, Berton O, Wallace-Black D, Nestler EJ. Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. *Biol Psychiatry* 2003;54:1317–1329. [PubMed: 14675795]
- Bolles RC, Woods PJ. The ontogeny of behaviour in the albino rat. *Anim Behav* 1964;12:427–441.
- Bridges RS, Zarrow MX, Goldman BD, Denenberg VH. A developmental study of maternal responsiveness in the rat. *Physiol Behav* 1974;12:49–151.
- Buelke-Sam J, Sullivan PA, Kimmel CA, Nelson CJ. Sex and strain differences in the developmental activity profile of the rat tested over clean versus home cage bedding. *Dev Psychobiol* 1984;17:67–77. [PubMed: 6538152]

- Bunsey M, Eichenbaum H. Selective damage to the hippocampal region blocks long-term retention of a natural and nonspatial stimulus-stimulus association. *Hippocampus* 1995;5:546–556. [PubMed: 8646281]
- Burns LH, Annett L, Kelley AE, Everitt BJ, Robbins TW. Effects of lesion to amygdala, ventral subiculum, medial prefrontal cortex, and nucleus accumbens on the reaction to novelty; implications for limbic-striatal interactions. *Behav Neurosci* 1996;110:60–73. [PubMed: 8652073]
- Burton S, Murphy D, Qureshi U, Sutton P, O’Keefe J. Combined lesions of hippocampus and subiculum do not produce deficits in a nonspatial social olfactory memory task. *J Neurosci* 2000;20:5468–5475. [PubMed: 10884330]
- Capretta PJ, Rawls LH. Establishment of a flavor preference in rats: importance of nursing and weaning experience. *J Comp Physiol Psychol* 1972;86:670–673. [PubMed: 4859318]
- Carlezon WA Jr, Konradi C. Understanding the neurobiological consequences of early exposure to psychotropic drugs: Linking behavior with molecules. *Neuropharmacology* 2004;47(Suppl 1):47–60. [PubMed: 15464125]
- Carter AS, Pollock RA. Obsessive compulsive disorder in childhood. *Curr Opin Pediatr* 2000;12:325–330. [PubMed: 10943811]
- Chitty S, Shorten M. Techniques for the study of the Norway rat (*Rattus norvegicus*). *J Mammal* 1946;27:63–78. [PubMed: 21020477]
- Chitty, D.; Southern, HN. *Control of Rats and Mice*. Vol. I–III. Clarendon; Oxford: 1954.
- Comoli E, Ribeiro-Barbosa ER, Canteras NS. Predatory hunting and exposure to a live predator induce opposite patterns of Fos immunoreactivity in the PAG. *Behav Brain Res* 2003;138:17–28. [PubMed: 12493627]
- Cosnier J, Couturier C. Comportement maternal provoqué chez les rattes adultes castrées. *C R Séances Soc Biol Fil* 1966;16:789–791.
- Countryman RA, Orłowski JD, Brightwell JJ, Oskowitz AZ, Colombom PJ. CREB phosphorylation and c-Fos expression in the hippocampus of rats during acquisition and recall of a socially transmitted food preference. *Hippocampus* 2005;15:56–67. [PubMed: 15390165]
- Cowan PE, Barnett SA. The new-object and new-place reactions of *Rattus rattus* L. *Zool J Linn Soc* 1975;56:219–234.
- Crawley JN. Neuropharmacological specificity of a simple animal model for the behavioral actions of benzodiazepines. *Pharmacol Biochem Behav* 1981;15:695–699. [PubMed: 6118883]
- Crawley J, Goodwin FK. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol Biochem Behav* 1980;13:167–170. [PubMed: 6106204]
- Davis DE, Emlen JT, Stokes AW. Studies on home range in the brown rat. *J Mammal* 1948;29:207–225.
- Douglas LA, Varlinskaya EI, Spear LP. Novel-object place conditioning in adolescent and adult male and female rats: effects of social isolation. *Physiol Behav* 2003;80:317–325. [PubMed: 14637231]
- Elsner J, Suter KE, Ulbrich B, Schreiner G. Testing strategies in behavioral teratology: IV. Review and general conclusions. *Neurobehav Toxicol Teratol* 1986;8:585–590. [PubMed: 3785521]
- Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats, 1: Behavioral data. *Behav Brain Res* 1988;31:47–59. [PubMed: 3228475]
- Fagen, R. *Animal Play Behavior*. Oxford; New York: 1981.
- File SE. What can be learned from the effects of benzodiazepines on exploratory behavior? *Neurosci Biobehav Rev* 1985;9:45–54. [PubMed: 2858081]
- Fowler, H. *Curiosity and exploratory behavior*. Macmillan; New York: 1965.
- Galani R, Duconseille E, Bildstein O, Cassel JC. Effects of room and cage familiarity on locomotor activity measures in rats. *Physiol Behav* 2001;74:1–4. [PubMed: 11564445]
- Galef BG. Weaning from mother’s milk to solid foods. *Ann NY Acad Sci* 1992;662:37–52. [PubMed: 1456638]
- Galef BG, Clark MM. Mother’s milk and adult presence: Two factors determining initial dietary selection by weanling rats. *J Comp Physiol Psychol* 1972;78:220–225. [PubMed: 5061998]
- Galef BG, Henderson PW. Mother’s milk: a determinant for the feeding preferences of weaning rat pups. *J Comp Physiol Psychol* 1972;78:213–219. [PubMed: 5061997]

- Galef BG, Kennett DJ. Different mechanisms for social transmission of diet preference in rat pups of different ages. *Dev Psychobiol* 1987;20:209–215. [PubMed: 3582781]
- Galef BG, Sherry DF. Mother's milk: A medium for transmission of cues reflecting the flavor of mother's diet. *J Comp Physiol Psychol* 1973;83:374–378. [PubMed: 4736679]
- Gerrish CJ, Alberts JR. Postsuckling behavioral arousal in weanling rats (*Rattus norvegicus*). *J Comp Psychol* 1997;111:37–49. [PubMed: 9090137]
- Goodwin GA, Yacko H. Emergence of the exploratory motive in rats. *Dev Psychobiol* 2004;45:34–48. [PubMed: 15229874]
- Gray P, Chesley S. Development of maternal behavior in nulliparous rats (*Rattus norvegicus*): Effects of sex and early maternal experience. *J Comp Physiol Psychol* 1984;98:91–99.
- Grossen NE, Kelley MJ. Species-specific behavior and acquisition of avoidance behavior in rats. *J Comp Physiol Psychol* 1972;81:307–310. [PubMed: 5084446]
- Hansson L. Fatty substances as attractants for *Microtus agrestis* and other small rodents. *Oikos* 1973;24:417–421.
- Hepper PG. Adaptive fetal learning: prenatal exposure to garlic affects postnatal preferences. *Anim Behav* 1988;36:935–936.
- Heyser, CJ.; Rosen, M.; Chemero, A. Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience; 2003. Novel object exploration in rodents: not all objects are created equally. [Abstract]. Program No. 89.8. Online
- Ivanco TL, Pellis SM, Whishaw IQ. Skilled forelimb movements in prey catching and in reaching by rats (*Rattus norvegicus*) and opossums (*Monodelphis domestica*): relations to anatomical differences in motor systems. *Behav Brain Res* 1996;79:163–181. [PubMed: 8883828]
- Kalinichev M, Rosenblatt JS, Morrell JI. The medial preoptic area, necessary for adult maternal behavior in rats, is only partially established as a component of the neural circuit that supports maternal behavior in juvenile rats. *Behav Neurosci* 2000;114:196–210. [PubMed: 10718274]
- Khan JA. Laboratory experiments on the food preferences of the black rat (*Rattus rattus* L.). *Zool J Linn Soc* 1974;54:167–184.
- Kinsley, CH.; Bardi, M.; Karelina, E.; Rima, B.; Christon, L.; Friedenber, J.; Sirkin, M.; Chipko, C.; Victoria, L.; Drew, M.; Fyfe, C.; Lambert, K. Neuroscience Meeting Planner. Atlanta: Society for Neuroscience; 2006. Track, attack, consume: pregnancy/parenthood induction of an improved predatory behavioral repertoire and accompanying neural enhancements in the rat. [Abstract]. Program No. 573.20.
- Laviola G, Adriani W, Rea M, Aloe L, Alleva E. Social withdrawal, neophobia, and stereotyped behavior in developing rats exposed to neonatal asphyxia. *Psychopharmacology* 2004;175:196–205. [PubMed: 14985924]
- Le Magnen J, Tallon S. Préférence alimentaire du jeune rat induite par l'allaitement maternel. *C R Séances Soc Biol Fil* 1968;162:387–390.
- Lett BT, Grant VL, Byrne MJ, Koh MT. Pairings of a distinctive chamber with the aftereffect of wheel running produce conditioned place preference. *Appetite* 2000;34:87–94. [PubMed: 10744895]
- Lett BT, Grant VL, Koh MT. Naloxone attenuates the conditioned place preference induced by wheel running in rats. *Physiol Behav* 2001;72:355–358. [PubMed: 11274677]
- Lister RG. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* 1987;92:180–185. [PubMed: 3110839]
- Mayer AD, Rosenblatt JS. Ontogeny of maternal behavior in the laboratory rat: Early origins in 18- to 27-day-old young. *Dev Psychobiol* 1979;12:407–424. [PubMed: 488527]
- Meaney MJ, Mitchell JB, Aitken DH, Bhatnagar S, Bodnoff SR, Iny LJ, Sarrieau A. The effects of neonatal handling on the development of the adrenocortical response to stress: Implications for neuropathology and cognitive deficits in later life. *Psychoneuroendocrinology* 1991;16:85–103. [PubMed: 1961847]
- Miczek, KA.; de Boer, SF. Aggressive, defensive, and submissive behavior. In: Whishaw, IQ.; Kolb, B., editors. *The Behavior of the Laboratory Rat*. Oxford University Press; Oxford: 2005. p. 344-352.
- Mitchell D. Experiments on neophobia in wild and laboratory rats: A reevaluation. *J Comp Physiol Psychol* 1976;90:190–197. [PubMed: 1249271]

- Montgomery KC. The relation between fear induced by novel stimulation and exploratory behaviour. *J Comp Physiol Psychol* 1955;48:254–260. [PubMed: 13252152]
- Moorcroft WH, Lyttle LD, Campbell BA. Ontogeny of starvation-induced behavioral arousal in the rat. *J Comp Physiol Psychol* 1971;75:59–67. [PubMed: 5559218]
- Muhle R, Trentacoste SV, Rapin I. The genetics of autism. *Pediatrics* 2004;113:e472–486. [PubMed: 15121991]
- Nakagawara M, Kubota M, Atobe M, Kariya T. Strain differences in behavioral response to a new environment in rats. *Psychiatry Clin Neurosci* 1997;51:167–170. [PubMed: 9225382]
- Nass R. Movements and home ranges of Polynesian rats in Hawaiian sugarcane. *Pac Sci* 1977;31:35–142.
- National Research Council. Guide for the care and use of laboratory animals. National Academy; Washington, D.C: 1996.
- Numan, M.; Insel, TR. *The Neurobiology of Parental Behavior*. New York: Springer; 2003.
- Ojeda, SR.; Urbanski, HF. Puberty in the rat. In: Knobil, E.; Neill, JD., editors. *The Physiology of Reproduction*. Vol. II. Raven; New York: 1988. p. 1699-1737.
- Olazábal DE, Abercrombie E, Rosenblatt JS, Morrell JI. The content of dopamine, serotonin, and their metabolites in the neural circuit that mediates maternal behavior in juvenile and adult rats. *Brain Res Bull* 2004;63:259–268. [PubMed: 15196651]
- Olazábal DE, Kalinichev M, Morrell JI, Rosenblatt JS. MPOA cytotoxic lesions and maternal behavior in the rat: Effects of midpubertal lesions on maternal behavior and the role of ovarian hormones in maturation of MPOA control of maternal behavior. *Horm Behav* 2002;41:26–138.
- Olazábal DE, Morrell JI. Juvenile rats show reduced c-fos activity in neural sites associated with aversion to pups and inhibition of maternal behavior. *Behav Neurosci* 2005;119:1097–1110. [PubMed: 16187837]
- Patel V, Flisher AJ, Hetrick S, McGorry P. Mental health of young people: a global public-health challenge. *Lancet* 2007;369:1302–1313. [PubMed: 17434406]
- Patterson C, Bouret SG, Dunn-Meynell AA, Levin BE. Three weeks of postweaning exercise in DIO rats produces prolonged increased in central leptin sensitivity and signaling. *Am J Physiol Regul Integr Comp Physiol* 2009;296:R537–R548. [PubMed: 19158409]
- Pereira, M.; Seip, KM.; Morrell, JI. Maternal motivation and its neural substrate across the postpartum period. In: Bridges, RS., editor. *Neurobiology of the Parental Brain*. Academic Press; London: 2008. p. 39-60.
- Rosenblatt JS. Nonhormonal basis of maternal behavior in the rat. *Science* 1967;156:1512–1514. [PubMed: 5611028]
- Rosenblatt, JS.; Lehrman, DS. Maternal behavior of the laboratory rat. In: Rheingold, HL., editor. *Maternal Behavior in Mammals*. Wiley; New York: 1963. p. 8-57.
- Roth TL, Sullivan RM. Endogenous opioids and their role in odor preferences acquisition and consolidation following odor-shock conditioning in infant rats. *Dev Psychobiol* 2001;39:188–198. [PubMed: 11745312]
- Ryan BC, Young NB, Crawley JN, Bodfish JW, Moy SS. Social deficits, stereotypy and early emergence of repetitive behavior in the C57/J inbred mouse strain. *Behav Brain Res* 2010;208:178–188. [PubMed: 19941908]
- Sheehan TP, Cirrito J, Numan MJ, Numan M. Using c-fos immunocytochemistry to identify forebrain regions that may inhibit maternal behavior in rats. *Behav Neurosci* 2000;114:337–352. [PubMed: 10832795]
- Sheldon AB. Preference for familiar versus novel stimuli as a function of the familiarity of the environment. *J Comp Physiol Psychol* 1969;67:516–521.
- Shorten, M. The reaction of the brown rat towards changes in its environment. In: Chitty, D., editor. *Control of Rats and Mice*. Vol. II. Oxford: Clarendon; 1954.
- Small WS. Notes on the psychic development of the young rat. *Am J Psychol* 1899;11:80–100.
- Smith, KS.; Morrell, JI. Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience; 2003. Distinct adult-juvenile responses to novelty correlate to postnatal changes in glutamatergic innervation [Abstract]. Program No. 728.13. Online

- Smith KS, Morrell JI. Comparison of infant and adult rats in exploratory activity, diurnal patterns, and responses to novel and anxiety-provoking environments. *Behav Neurosci* 2007;121:449–461. [PubMed: 17592936]
- Smith KS, Morrell JI. Behavioral responses during the initial exposures to a low dose of cocaine in late preweanling and adult rats. *Neurotoxicol Teratol* 2008;30:202–212. [PubMed: 18276106]
- Smith, R. a Method Hitherto Unattempted: Calculated for the Use of the Gentleman, the Farmer, and the Warrener. Walker; London: 1786. The Universal Directory For Taking Alive and Destroying Rats, and All Other Kinds of Four-Footed and Winged Vermin.
- Smotherman WP. In utero chemosensory experience alters taste preferences and corticosterone responses. *Behav Neural Biol* 1982;36:61–38. [PubMed: 7168731]
- Spear LP. The adolescent brain and age-related manifestations. *Neurosci Biobehav Rev* 2000;24:417–463. [PubMed: 10817843]
- Stansfield KH, Philpot R, Kirstein CL. An animal model of sensation seeking: the adolescent rat. *Ann N Y Acad Sci* 2004;1021:453–458. [PubMed: 15251928]
- Weinberg J, Smotherman WP, Levine S. Early handling effects on neophobia and conditioned taste aversion. *Physiol Behav* 1978;20:589–596. [PubMed: 567356]
- Werme M, Messer C, Olson L, Gilden L, Thoren P, Nestler EJ, Brene S. Δ FosB regulates wheel running. *J Neurosci* 2002;22:8133–8138. [PubMed: 12223567]
- Werme M, Thoren P, Olson L, Brene S. Running and cocaine both upregulate dynorphin mRNA in medial caudate putamen. *Eur J Neurosci* 2000;12:2967–2974. [PubMed: 10971637]
- Wiedenmayer CP, Barr GA. Ontogeny of defensive behavior and analgesia in rat pups exposed to an adult male rat. *Physiol Behav* 1998;63:261–269. [PubMed: 9423968]
- Wilkinson JL, Herman L, Palmatier MI, Bevins RA. Rats' novel object interaction as a measure of environmental familiarity. *Learn Motiv* 2006;37:131–148.
- Wuensch KL. Exposure to onion taste in mother's milk leads to enhanced preference for onion diet among weanling rats. *J Gen Psychol* 1978;99:163–167. [PubMed: 722286]

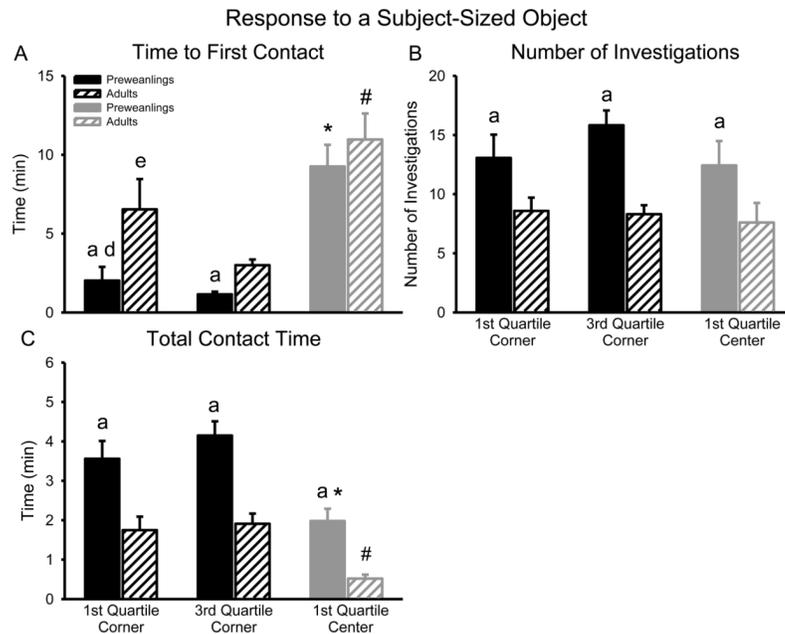


Figure 1.

Responses to a subject-sized object during the least and most active times of the daily cycle in two environmental locations. (A) The time it took subjects to first contact the object, (B) the number of object investigations performed, and (C) the total time subjects spent in contact, which includes active investigation as well as sitting quietly in contact with it. a = preweanlings significantly different from adults. d = preweanlings significant effect of time of day. * = preweanlings significant effect of stimulus location. e = adults significant effect of time of day. # = adults significant effect of stimulus location.

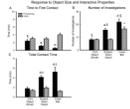


Figure 2.

Responses to inanimate objects of different sizes and interactive value in the 3rd quartile. a = preweanlings significantly different from adults. + = adult response significantly different from all other objects. § = preweanling response significantly different from all other objects. ‡ = preweanling total contact time with the larger interactive objects significantly greater than time with an object one-third their size and a jingle ball.

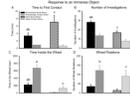


Figure 3.

Responses to a novel vs. familiar super-sized object, the running wheel. a = preweanlings significantly different from adults, same wheel condition. b = preweanling novel responses significantly different from familiar responses. c = adult novel responses significantly different from familiar responses.

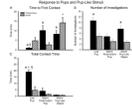


Figure 4. Responses to a newborn pup and pup-like stimuli. All symbols are the same as in Figure 2.

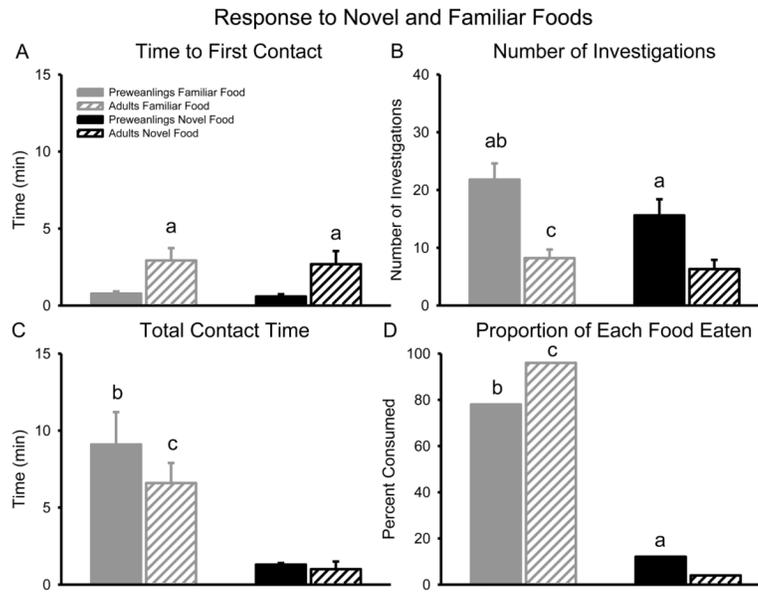


Figure 5.

Responses to novel and familiar foods when both are presented simultaneously after moderate food deprivation. All symbols are the same as in Figure 3.

Table 1

Stimulus Interaction Tests Time of Day and Home Cage Location

Location	Time of Day	Inanimate Objects					Biological Stimuli				Food	
		1/3rd Object (Small)	Same-Sized	Jingle Ball	Crawl Ball	Wheel	Pups	Crickets	Familiar Chow	Novel Chow		
Preferred Corner	Light Phase		X									
	Dark Phase	X		X	X	X	X	X	X			X
Center	Light Phase Only		X									

Table 2Mean (\pm SEM) Subject Responses to Novel and Familiar Inanimate Objects

	Object	State	Preweanlings	Adults	
Time to First Contact (min)	1/3rd Object (Small)	Familiar	2.00 \pm 0.30	2.68 \pm 0.49	
		Novel	3.36 \pm 0.82	3.49 \pm 0.75	
	Jingle Ball	Familiar	1.63 \pm 0.328 ^{*a}	6.62 \pm 1.51	
		Novel	0.54 \pm 0.13 ^a	5.06 \pm 1.23	
	Same-Sized	Familiar	1.11 \pm 0.19	3.24 \pm 0.48	
		Novel	1.21 \pm 0.34	2.52 \pm 0.48	
	Crawl Ball	Familiar	0.58 \pm 0.23	3.62 \pm 0.66	
		Novel	0.52 \pm 0.13	4.43 \pm 0.58	
	Number of Investigations	1/3rd Object (Small)	Familiar	7.78 \pm 1.17	8.59 \pm 0.70
			Novel	9.38 \pm 1.86	7.96 \pm 0.81
Jingle Ball		Familiar	12.43 \pm 2.41	8.79 \pm 1.14	
		Novel	11.83 \pm 1.95	16.57 \pm 2.40 [*]	
Same-Sized		Familiar	17.57 \pm 1.50	8.41 \pm 0.98	
		Novel	12.94 \pm 2.08	8.07 \pm 1.21	
Crawl Ball		Familiar	22.00 \pm 2.61	14.29 \pm 1.84	
		Novel	22.00 \pm 4.13	18.86 \pm 2.95	
Total Contact Time (min)		1/3rd Object (Small)	Familiar	1.46 \pm 0.28	1.44 \pm 0.25
			Novel	2.49 \pm 0.70	1.27 \pm 0.28
	Jingle Ball	Familiar	1.17 \pm 0.21 ^a	0.60 \pm 0.11	
		Novel	1.29 \pm 0.14	1.38 \pm 0.35 [*]	
	Same-Sized	Familiar	4.55 \pm 0.49	2.01 \pm 0.33	
		Novel	3.48 \pm 0.47	1.72 \pm 0.42	
	Crawl Ball	Familiar	5.44 \pm 1.34	1.18 \pm 0.15	
		Novel	4.98 \pm 1.42	1.24 \pm 0.23	

^a preweanlings significantly different than adults;^{*} response to novel jingle ball significantly different than familiar; $p < 0.05$

Table 3Mean (\pm SEM) Subject Response to a Live Cricket

Measures of Stimulus Interaction		
	Preweanlings	Adults
Time to First Contact (min)	3 \pm .72 ^a	1 \pm .17
Number of Investigations	10 \pm 2 ^a	5 \pm 1
Total Contact Time (sec)	28 \pm 6	31 \pm 7
Measures of Predatory Hunting		
	Preweanlings	Adults
Time to Catch/Kill Cricket (min)	7 \pm 3 ^a	2 \pm .36
Total Time to Eat Cricket (min)	5 \pm .57 ^a	3 \pm .37

^a preweanlings significantly different than adults, $p < 0.05$