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Comparison of Infant and Adult Rats in Exploratory Activity, Diurnal Patterns, and Responses to Novel and Anxiety-Provoking Environments

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Abstract

Infant rats emerge from the maternal nest at Postnatal Day 17–18 to have their first critical environmental experiences; they may be particularly sensitive to experiences or experimental interventions that can affect their adult capacity. The authors address open questions on 2 components of normative environmental exploration, locomotor activity and response to anxiety-provoking locations, in Postnatal Day 18 infant and Postnatal Day 60 adult rats. The authors compare diurnal patterns of locomotor activity, wheel running, novel and familiar open-field activity, and 2 measures of anxiety. Infants have an equivalent capacity to adults for locomotor activity and wheel running and a fundamentally adult-like diurnal rhythm, except that they do not anticipate light–dark transitions, are more perturbable at their most somnolent, and are more or less active during specific limited phases than adults. Infants initially have a lower rate of locomotor activity in novel environments and have a greater willingness to be active in anxiety-provoking locations. Such differences may allow enhanced gathering of environmental information by the infant and are important to consider in the design of experiments using infants.

Keywords

infant; adult; exploration; activity; anxiety

In humans, late childhood and adolescence is the period when drug sampling and experimentation first begins (Brown & Tapert 2004; Johnston, O'Malley, Bachman, & Schulenberg, 2005; Spear, 2000; Turner, Mermelstein, & Flay, 2004), when clinical mental health disorders requiring pharmacologic intervention, such as attention-deficit/hyperactivity disorder, emerge (Andersen, 2003; Andersen, Arvanitogiannis, Pliakas, LeBlanc, & Carlezon, 2002), and when the central nervous system (CNS) is readily shaped by many stimuli, often with long-lasting consequences. Ultimately, studies using immature animals may inform us about human disorders with early life origins (e.g., hyperactivity) and may inform the beneficial use of pharmacological treatments. Further, these studies may provide insight into the effects of preadolescent sampling of drugs of abuse (Dahl & Spear, 2004).

Prior to achieving sexual maturity between Postnatal Days (P) 55–60, immature rats are either infants (P1–P28) or adolescents (P28–P55; Ojeda & Urbanski, 1988; Spear, 2000). Infant rats are vigilantly kept in the protective maternal nest by their mothers until around P17–P18 (Barnett, 1958; Galef & Clark, 1972; Pereira & Morrell, 2007; Small, 1899), when they are allowed out into their larger environment. At this point, they have full motor and sensory capacity (Moorcroft, Lyttle, & Campbell, 1971). Experiences at this point readily produce long-

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term effects; enriched environments lead to improved adult cognitive skills (Francis, Diorio, Plotsky, & Meaney, 2002), regular exercise to reduced adult obesity (Patterson & Levin, 2004), and infant exposure to psychostimulants to altered reward responses in adults (Andersen et al., 2002; Carlezon & Konradi, 2004). Others have also examined the impact of pharmacologic interventions during infancy and adolescence (Dahl & Spear, 2004; Spear, 2000; Spear & Brick, 1979). It is likely that susceptibility to long-term changes in this period is facilitated by the notable physiological and neural sculpting also known to be a feature of this stage (for a review, see Spear, 2000).

One of the most fundamental behaviors an organism exhibits is exploration of its environment. Exploration must be protectively avoidant yet properly investigative of novel stimuli that might promote survival, such as food, social opportunities, or shelter (Barnett, 1958, 1975). Exploration includes both spatial exploration and investigation of specific stimuli and is the sum of motor and locomotor activity, emotional drives (motivation, anxiety, and fear), and cognitive capacity (Golani, Benjamini, Dvorkin, Lipkind, & Kafkafi, 2005). We hypothesize that environmental exploration by infants during this first critical period when they are allowed out of the maternal nest is fundamentally different from, possibly greater than, that of adults. We also posit that these differences are a behavioral manifestation of a young organism designed to have higher sensitivity to environmental stimulation. Although these differences in exploration may make the late infant rat more vulnerable to predation in the natural environment, their adaptive value for CNS and behavioral development must be the stronger effect.

The experiments presented here examine two principal components of exploratory behavior, locomotor capacity and the levels of anxiety stimulated by environmental locations. We have deliberately excluded motivational drives based on hunger and questions of stimulus-focused exploration for examination in other experiments (Smith & Morrell, 2003, 2007). Independent of locomotor capacity, fearfulness or anxiety can alter the activity of an animal within an environment, resulting in avoidance, freezing in particular locations, or induction of hyperlocomotor escape (Barnett, 1975). We hypothesize that infants and adults differ in both locomotor capacity and in the levels of anxiety provoked by environmental location and that differences in these components of exploration result in a young organism that explores more than an adult.

Many experimental paradigms examining the effects of drugs of abuse or pharmacological treatments in preclinical rodent models of mental health disorders are based on environmental and stimulus-directed exploration as a measure of CNS capacity (Whishaw & Kolb, 2005). Baseline performance differences between the newly emergent young versus adults must be accounted for if the more complex effects of pharmacological or CNS interventions in the young are to be most usefully administered. As can be seen from the following summary of the current literature, many open questions remain on the normal patterns of activity and anxiety in late infancy, particularly those including a level of detail most useful to current analysis and in comparison with adults.

Observations of rodent activity were published as early as the turn of the 20th century (Small, 1899). There is substantial information for adult rats, including diurnal locomotor activity and open-field activity measures (Joutsiniemi, Leinonen, & Laakso, 1991; Richter, 1922; Shirley, 1928), wheel running patterns (Bauer, 1990; Eikelboom & Mills, 1988; Peng, Jiang, & Hsu, 1980; Stewart, Rosenwasser, & Adler, 1985), rearing (Gregory, 1967; Hughes, Blampied, & Stewart, 1975; Kalinichev, Easterling, & Holtzman, 2002), and grooming (Borchelt, 1980; Jolles, Rompa-Barendregt, & Gispen, 1979; Sachs, 1988). Studies examining young rats focus mostly on adolescents (Barron, Hansen-Trench, & Kaiser, 1996; Campbell & Mabry, 1973; Hastings, Cooper, Bornschein, & Michaelson, 1977; Kalsbeek, de Bruin, Matthijssen, &

Uylings, 1989; Livesey & Egger, 1970), although a few studies have been done in the infant rat.

Simple measures of total activity demonstrate that infants are generally more active in the dark than in the light phase of the daily cycle (Bolles & Woods, 1964; Norton, Culver, & Mullenix, 1975), have considerable locomotor capacity (Moorcroft et al., 1971; Norton et al., 1975), are capable of rearing and grooming (Campbell & Mabry, 1973), and are capable of wheel running (Dalton-Jez & Eikelboom, 2005). Environmental novelty or familiarity significantly influences adult activity (Buelke-Sam, Sullivan, Kimmel, & Nelson, 1984; Galani, Duconseille, Bildstein, & Cassel, 2001), and some infant studies have a limited consideration of this (Buelke-Sam et al., 1984; Eilam & Golani, 1988; Golani et al., 2005; Pappas, Vickers, Buxton, & Pusztay, 1982). Open questions on late infant patterns of exploration remain. What are the essential quantitative and pattern details of the diurnal rhythm? Do the capacities for wheel running and locomotor activity differ? How does time of day alter activity in various environmental locations or the impact of novelty in the activity of infants versus adults?

Measures interpreted as anxiety in rodents (Grossen & Kelley, 1972; Lister, 1990) include certain uses of the open field (Britton & Britton, 1981; File, 1985; Treit & Fundytus, 1988), the black and white box (Costall, Jones, Kelly, Naylor, & Tomkins, 1989; Crawley, 1981), and the elevated plus-maze (Lister, 1987; Pellow, Chopin, File, & Briley, 1985). These methods are frequently used to test the effectiveness of anxiolytics in adults but are rarely used in young rats. Doremus, Varlinskaya, and Spear (2004) reported that adolescents are less anxious than adults, whereas Slawecki (2005) reported adolescents were not different than adults unless stressed. Remarkably, baseline behavior of preweaning infants has not been characterized using these paradigms.

This study provides a vital level of detail on the normative activity of preweaning infants just emergent from the maternal nest and in comparing infant–adult activity and anxiety. We applied all of the frequently used tools of behavioral neuroscience to test our hypothesis that the baseline activity and anxiety levels of the rat at the earliest stage of environmental exploration (P18) are different from adults (P60). Baseline differences in normal activity patterns impact the relative effects of many interventions used in neuroscience to explore the function of the CNS, for example, lesion or pharmacological interventions. Activity has been assessed by analysis of diurnal patterns of locomotor activity and wheel running in the home cage as well as the response to novel and familiar open fields during high and low activity periods of the daily cycle. Anxiety responses were measured with the open-field test and the black and white box test.

Method

Subjects and Housing

Experimental animals included infant females (33–40 g) 18–19 days of age and adult virgin females (190–225 g) 60–70 days of age obtained from our breeding colony maintained in the Rutgers University Laboratory Animal Facility. Rutgers University Animal Facilities are accredited by the American Association of Accreditation of Laboratory Animal Care. The breeding colony consists of the offspring of rats originally purchased from Charles River Laboratories (Wilmington, MA) and is maintained as genetically consistent with their Sprague–Dawley CD strain by regular and frequent purchase of males and females to serve as breeders. All animals were maintained under a 12-hr light–dark cycle (lights on at 0700) and a temperature of 22 °C with ad libitum access to food (Formulab Diet 5008; PMI, Nutrition International, Brentwood, MO) and water. Wood chip bedding (Beta Chip; Northeastern Products, Warrensburg, NY) was used to cover the floors of the cages. Two randomly selected same-sex littermates were used in all behavioral experiments for each age group to reduce the

probability that litter effects influenced experimental results (Elsner, Suter, Ulbrich, & Schreiner, 1986; Olazábal, Kalinichev, Morrell, & Rosenblatt, 2002; Williams, Vorhees, Boon, Saber, & Cain, 2002). Analysis was subsequently based on averaged values of 2 subjects per litter, providing one data point per litter. All procedures used in this study followed the standards approved by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Rutgers University Animal Care and Facilities Committee.

Breeding was done within the Laboratory Animal Facility according to established procedures (Mayer & Rosenblatt, 1998). The day of parturition was considered P0. All litters were culled to 12 pups (half each gender) during the first neonatal week and subsequently housed with the dam until weaning at P28. Except for animal husbandry, postnatal handling was minimal (Ader, 1968; Andrews & File, 1993; Kalinichev et al., 2002; Meaney et al., 1991). Upon weaning, young adult virgin females were group housed in hanging wire mesh cages (40.0 cm long × 36.0 cm wide × 18.0 cm high) until behavioral testing as adults.

One week before testing, subjects were removed from the colony room and housed in opaque plastic cages (44.0 cm long × 23.5 cm wide × 20.0 cm high) within our behavior testing suite; housing and behavioral testing were conducted in separate rooms of this suite. This environment lacked the general disruption found in the animal facility, as it is quieter and more controlled. The day before behavioral testing, subjects were housed for 24 hr in lidded clear Plexiglas boxes (42.0 cm long × 42.0 cm wide × 31.0 cm high) lined with wood chip bedding; these boxes became their home cages and thus were the testing environment for the home cage activity tests. These boxes were inserts that fit within the walls of the automated activity monitoring system described below. Home cages were illuminated by overhead fluorescent lighting in the testing room with an average luminance in each box of 27 lumens/m^2 (Konica Minolta Luminance Meter LS-100, Tokyo, Japan). The average illumination difference between the center and the perimeter of the home cage was minimal (< 3%) and was not statistically significant, $t(6) = -0.45$, $p = .66$. Subjects had continual access to food and water; infants additionally received powdered chow prepared by grinding pelleted food in a Hamilton Beach 72600 FreshChop Food Chopper (Proctor-Silex, Inc., Picton, Ontario, Canada). Adults were housed in sibling pairs, and infants were housed with their dam and littermates. The weight gain by infants and adults was within normal parameters throughout the experiment; all animals used were healthy throughout the experiment.

Measures of Activity in the Home Cage

On the day of testing, subjects (infants, $n = 12$; adults, $n = 14$) were transferred from their Plexiglas box home cage (which had been their home cages for 24 hr, as described above) to clean opaque plastic cages for 5 min and then returned to the home cage boxes but as single individuals without littermates, dams, or cage mates. Additional home cage–test environments were devised by distributing identically scented (soiled) bedding in additional Plexiglas chambers. These home cage boxes were then inserted into the AccuScan (or DigiScan) activity monitoring system (see technical details below). Prior assessment of activity measures verified that the presence of soiled home cage bedding in boxes (one clean with home-scented bedding vs. the one in which subjects were housed in overnight) elicited identical activity patterns (no significant Subject Age × Cage Type × Time interaction), $F(5, 72) = 0.79$, $p = .56$. These home cages were illuminated during the light half of the daily cycle by overhead fluorescent lighting in the testing room with an average luminance in each box of 27 lumens/m^2. The average illumination difference between the center and the perimeter of the home cage was minimal (< 3%) and was not statistically significant, $t(6) = -0.45$, $p = .66$. No lighting was provided during the dark phase of testing.

A 26-hr behavioral profile was obtained for each subject using automated equipment (VersaMax Animal Activity Monitor system, Model VMMAW; AccuScan Instruments Inc.,

Columbus, OH). Testing commenced during the light portion of the light–dark cycle between 0900 and 1500. Locomotor activity was measured by the total distance traveled (centimeters) and the time (seconds) spent in specific locations within the apparatus (center vs. perimeter) using the automated activity monitoring system. The data on activity in the home cage–test environment were collected for 26 hr; data were analyzed for the 24 hr that began 2 hr after animals were reintroduced into the home cage–test environment. Food and water were available during this test; infants were given powdered chow and water sources they could reach and had been previously exposed to while in their home cages with their dams and littermates.

A second group of subjects (infants, $n = 16$; adults, $n = 16$) was tested in the same manner but was also housed with a stainless steel activity wheel (26.04 cm diameter; AccuScan Instruments Inc., Columbus, OH). All testing began at 1300 when subjects were reintroduced into their home cage, as above. One hour later (1400), a running wheel was quietly inserted into the apparatus. Wheel activity, measured by the number of wheel rotations, was recorded for 25 hr; data were analyzed for the 24 hr that began 1 hr after the wheel was introduced. This wheel was designed to have a minimal friction load because of engineering with a single fixed attachment using a high-quality ball-bearing assembly (hand washed to maintain integrity), and so it is used with both rats and mice even though the same wheel (325 g) is used for both smaller and larger rodents. Therefore, the mass-to-friction ratio of the system was not scaled to animal size across the two age groups in our paradigm.

Automated Activity Apparatus

Automated measures of environmental exploration were determined using either the VersaMax Animal Activity Monitor system (described above) or its precursor, the DigiScan Animal Activity Monitor system (Model RXYZCM[8]; Omnitech Electronics, Inc., Columbus, OH). No differences in outcomes resulted from the use of these two systems. Because the VersaMax system allows for analysis of more behavioral parameters, most of the data were gathered with this system. These activity monitors consist of four horizontal sensors and two vertical sensors, each containing 16 infrared beams set 2.5 cm apart. Animal activity was detected and measured automatically by interruption of the infrared beams. Four activity monitors were connected to a VersaMax Analyzer (Model VMA-USB; AccuScan Instruments Inc., Columbus, OH) via interconnecting cables for simultaneous activity recording of up to 4 subjects. The VersaMax Analyzer was connected to a computer (Dell PC) via a USB cable for automated activity data recordings using the VersaMax software package. Each automated apparatus was fitted with a removable clear Plexiglas box (42.0 cm long × 42.0 cm wide × 31.0 cm high) and a cover with 25 air holes (2.5 cm diameter) spaced 5.0 cm apart for ventilation that allowed us to measure subject activity. The details of the environment these provided were altered for each paradigm as described for each experiment.

The VersaMax Animal Activity Monitors and software program automatically recorded and quantified measures of locomotor activity for each subject throughout the testing session and calculated the frequency of behavior in discrete time intervals specified by the experimenter. Only two behavioral frequencies, rearing and grooming, were obtained by manual observation and recording.

Open-Field Tests—Familiar or Novel Environments

Open-field tests were conducted for 30 min either between 0900 and 1200 (the light phase) or between 2000 and 2300 (the dark phase). During the light phase of testing, the boxes were illuminated by the overhead lighting in the testing room, with an average luminance of 34 lumens/m^2, and the illumination difference between the center and the perimeter was < 1% ($p = .82$). Dark-phase testing was conducted under red light conditions; the testing room was

illuminated by two 60-W bulbs, one in an overhead Kodak Darkroom Lamp (Model A; Eastman Kodak Co., Rochester, NY) and the other in a Kodak Adjustable Safelight Lamp (Model B; Eastman Kodak Co., Rochester, NY), each screened by a red glass Kodak Number 2 Safelight Filter (CAT 152 1525; Eastman Kodak Co., Rochester, NY).

Familiar environment—Infants (n = 35) or adults (n = 36) tested in a familiar open field were treated as described above prior to testing. This includes their housing for the 24 hr prior to testing in the Plexiglas box insert home cages. Infants were housed with their dam and siblings for 24 hr; adults were housed with a cage mate, as described above. Just before the familiar home cage activity monitoring, all animals were removed from the Plexiglas insert home cages and were placed in a clean opaque plastic cage. After 5 min, a single subject was returned to the Plexiglas insert home cage in the activity monitoring system, which contained the home cage shavings (from the prior 24 hr), but food and water of the home cage was removed and two clean food pellets were placed in the environment.

Novel environment—Infants (n = 32) and adults (n = 32) tested in a novel open-field environment never had the Plexiglas insert boxes as their home cage environments; they were housed in opaque plastic cages with food and water ad libitum, as described in the first subsection of the Method section. The first time they ever experienced the Plexiglas insert cages of the activity monitoring system was at the time of a single novel open-field test. For this test, the Plexiglas insert cages were used without wood chip bedding, food, or water.

Black and White Box Test

Infants (n = 20) and adults (n = 20) were tested in the clear Plexiglas insert cages that were turned into black and white boxes for a 30-min test session between 0900 and 1200 (the light phase). Prior to testing, subjects were housed in opaque plastic cages as described for novel infants and adults above; the Plexiglas insert cages were entirely novel at the time of test (Crawley, 1981; Crawley & Davis, 1982; Crawley & Goodwin, 1980). To create black and white boxes, each Plexiglas insert cage, identical to that used in activity tests and the open-field test above, was lined with fresh wood chip bedding. One half of the apparatus was shaded with a brown cardboard shield created in our laboratory (46.0 cm high × 50.0 cm wide × 28.0 cm deep), and the other half remained brightly lit by the overhead lighting in the testing room. The luminance was 25 lumens/m^2 in the white half and 2 lumens/m^2 in the black half; the luminance was significantly different between the two halves, $t(6) = -18.75$, $p = .001$.

Measures of Subject Size for Analytic Consideration

The AccuScan VersaMax activity system was originally created to obtain activity measures of adult rodents, with the width of the perimeter margin of the open field correlated to the average width of an adult animal, approximately 6.5 cm wide (R.H. Kant, personal communication, November 10, 2003). The center is thus, by default, the remaining area of the open field that does not correspond to the margin. Furthermore, the system is preset so that activity in the margin is based on these standard measurements. However, the margin zone size can be chosen by using the ZoneMap software program that is part of the system. To compare activity between the different age groups appropriately, we considered the relative size difference between the infants and the adults and subsequently examined activity in the margin and center of the open field that were proportional to animals on the basis of their shoulder width (infant margin = 5.25 cm; adult margin = 6.5 cm).

Statistical Analysis

On the basis of criteria from several studies (Abbey & Howard, 1973; Blumberg, Seelke, Lowen, & Karlsson, 2005; Holson & Pearce, 1992), data were collected from 2 subjects per

litter but were subsequently averaged so that there was one data point per litter. Results from analyses of two data points versus one data point per litter yielded virtually identical outcomes. All data met the requirements for use of parametric statistical tests, that is, normal distribution and homogeneity of variance. Home-cage activity data and open-field data were analyzed using two-way analyses of variance (ANOVAs; Subject Age × Time for home cage activity, and Subject Age × Stimulus Environment for open-field data) and independent *t* tests. Black and white box data were analyzed using a repeated-measures two-way ANOVA (Subject Age × Cage Half) followed by Tukey's post hoc test. A *t* test was used for limited specific comparisons when a significant difference was found for a main effect of subject age or cage half. The level of statistical significance was set at .05. The general linear model procedure was used for the implementation of the ANOVA comparisons. The data were analyzed using the SAS statistical software Version 8.2 for personal computers (SAS Institute, Inc., Cary, NC).

Results

Diurnal Activity Patterns in the Familiar Environment

Fundamental measures: Total distance and wheel rotations—In their home cage, infants covered, on average, 17,402 cm/24 hr, similar to adults at 16,432 cm/24 hr, and performed a similar number of wheel rotations (infants = 1,237 rotations; adults = 1,272 rotations). However, analysis of the patterns across the daily quartiles (first quartile = 0700–1300, second quartile = 1300–1900, third quartile = 1900—0100, and fourth quartile = 0100–0700; see Figures 1B and 1D) revealed quartile-specific infant–adult differences in raw data from both measures: Subject Age × Time interaction, $F(3, 9) = 5.19$, $p < .05$ (see Figures 1A and 1B), and Subject Age × Time interaction, $F(3, 12) = 4.41$, $p < .05$ (see Figures 1C and 1D).

As expected, adults were less active in the light period (first two quartiles) than during the dark period (third and fourth quartiles) by both measures, a difference that was statistically significant for wheel rotations, $t(8) = 2.69$, $p < .05$. Infants were also least active during the first quartile. During the second quartile, infants covered three times more distance than did adults, $F(1, 11) = 6.42$, $p < .05$ (see Figures 1A and 1B), and engaged in significantly more wheel rotations than adults in the second quartile, $F(1, 14) = 5.55$, $p < .05$ (see Figures 1C and 1D). Upon lights out, adults and infants were equally and highly active for the third quartile, whereas in the fourth quartile infant activity levels decreased; they were less active than adults by a factor of 1.6 for both total distance traveled (see Figure 1B) and wheel rotations (see Figure 1D).

Light–dark transition activity patterns—Adults were more active than infants during the hour before and after lights on (0600–0800) and lights off (1800–2000) for both total distance traveled, $F(1, 22) = 11.32$, $p < .01$ (see Figure 1E), and wheel rotations, $F(1, 28) = 18.96$, $p < .001$ (see Figure 1E). During the 0600–0800 period, adults explored the home cage more than infants by a factor of 8.1, $t(11) = 2.82$, $p < .05$ (see Figure 1E), and performed more wheel rotations than did infants, $t(14) = 4.30$, $p < .001$ (see Figure 1E).

Location of activity—Overall, although both groups spent less time in the center than in the periphery of the home cage (infants = 10%; adults = 3%), infants spent more time in the center than did adults by a factor of 4.8, $t(11) = -5.82$, $p < .0001$ (see Figures 2A and 2B). Infants also traveled more in the center, as measured by centimeters, than did adults, a difference of a factor of 1.9, $t(11) = -2.59$, $p < .05$ (see Figure 2C), regardless of time of day (see Figure 2D). A simple calculation of the rate at which subjects explored the center (centimeters per minute) showed that adults investigated the center of the home cage significantly faster than did infants, $t(11) = 2.84$, $p < .05$ (see Table 1). Adults were moving three–four times faster than infants when they were in the center of the apparatus.

Measures of Activity in Novel and Familiar Open Fields at Peak and Nadir of Diurnal Patterns

Locomotor activity—As expected, adults were most active during the third quartile of the daily cycle compared with the first quartile in either the novel or the familiar environment (one-way ANOVA), $F(3, 30) = 9.15$, $p < .001$, Tukey's $p < .05$ (see Figure 3A). Also as expected, adults were most active in the first 10 min of the test and reverted to much less activity by the last 10 min (see Figure 3B). Adult response to a novel environment with increased activity was significant only in the third quartile and mostly due to the initial response of very high activity. The pattern of decreasing activity after introduction into the test chamber was present in both quartiles and environments; familiarity and the light portion of the daily cycle blunted initial and total activity, $t(12) = -3.20$, $p < .01$, and, $t(14) = -6.76$, $p < .0001$, respectively (see Figures 3A and 3B).

Infants were most active during the first quartile compared with the third quartile when placed in the familiar environment and at either time in the novel environment, $F(3, 30) = 6.29$, $p < .001$, Tukey's $p < .05$ (see Figure 3A). Upon introduction into the familiar test chamber, infants had an adult-like pattern of greater initial activity followed by a decline to less activity (see Figure 3B). However, when placed in a novel environment, the pattern was notably different; infants did not exhibit the initial burst of activity. Instead, they explored the novel environment at a constant modest pace.

Several differences in the activity levels of adults and infants in the novel and familiar open-field tests were notable (2 × 4 factor ANOVA), Subject Age × Test Time interaction, $F(3, 60) = 12.70$, $p < .0001$. In the third quartile, adults were more active than infants in the novel environment by a factor of 1.9, $t(10) = 3.97$, $p < .01$ (see Figure 3A), largely because of the initial response of the subjects to the test environments (see Figure 3B). In the first quartile, infants were twice as active as adults in the novel and familiar environments despite the differences in their initial response to the novel environment, $t(18) = -5.11$, $p < .0001$, and $t(18) = -3.00$, $p < .01$, respectively (see Figures 3A and 3B).

Center time—Whereas adults spent only 5%–11% of their time in the center of either a familiar or novel open field—modestly greater in the novel compared with the familiar open field, $t(12) = -4.49$, $p < .001$ (see Figure 3C)—infants remarkably spent up to 44% of their time in the center of these environments, a difference by a factor of 8.8 compared with adults, $F(1, 24) = 86.34$, $p < .0001$ (see Figures 3C and 3D, third quartile data only). Further, infants explored the center of the open field at a much slower rate than did adults, irrespective of test condition or time of day, Subject Age × Test Time interaction, $F(2, 37) = 6.67$, $p < .01$ (see Table 1). For example, adults explored the center of the novel open field during the third quartile seven times faster than did infants, $t(10) = 8.97$, $p < .0001$ (see Table 1).

Rearing—Consistent with time of day and novelty effects in the other measures of activity, adults explored the *z*-axis of the environment (rears) significantly more when placed in a novel environment in the third quartile of the daily cycle compared with a novel environment in the first quartile or a familiar environment, $F(3, 28) = 42.59$, $p < .0001$, Tukey's $p < .05$ (see Table 2). Infants reared more than adults in the familiar environment at both test times but less than adults in the novel environment during the third quartile, $F(3, 56) = 12.40$, $p < .0001$; $t(10) = 2.64$, $p < .05$ (see Table 2). Although infants reared least in the first quartile of their day when placed in a novel environment, this was still marginally more than adults at this time, $F(3, 28) = 4.66$, $p < .01$, Tukey's $p < .05$.

Grooming—Adults groomed more during the third than the first quartile and responded to the novel environment during the dark phase with further increased grooming, $F(3, 28) = 25.52$, $p < .0001$, Tukey's $p < .05$ (see Table 2). Like rearing, infant grooming was lowest in the novel

environment during the first quartile, but this was still more than the comparable adult condition, $t(14) = -2.99$, $p < .01$ (see Table 2). Although infant grooming frequencies in the other conditions were similar to those of adults, infant grooming did not differ across the conditions. Infants groomed with the same frequency as adults in the third quartile, regardless of novelty. Similar to general activity and rearing, infants groomed significantly more than adults in both test environments during the first quartile, a difference that was larger by a factor of 3.2 and a factor of 1.69 in the familiar and novel environments, respectively: Subject Age × Test Time interaction, $F(3, 56) = 5.12$, $p < .01$ (see Table 2).

Activity Patterns in the Black and White Box

Both groups spent significantly more time in the shaded half than in the light half of the box, but infants spent more time than adults in the light half by a factor of 9.6 (two-factor ANOVA with a repeated measure of time): Subject Age × Time interaction, $F(1, 18) = 121.19$, $p < .0001$ (see Figure 4A). In addition, infants also traveled more in both halves than adults, $F(1, 18) = 15.28$, $p < .001$ (see Figure 4B), and explored the center area more than adults, $t(18) = -9.76$, $p < .0001$ (see Table 1). Infants also explored each half at different rates than adults, $F(1, 18) = 111.52$, $p < .0001$ (see Table 1); they explored the dark half faster and the light half slower than adults by a factor of 4 (see Table 1). Infants investigated the center significantly more slowly than did adults, $t(18) = 8.07$, $p < .0001$ (see Table 1). Comparison of the time course of activity from introduction into the chamber to the end of the test showed that, similar to the findings illustrated in Figure 3B, adults showed an initial burst of activity immediately upon being placed in the environment, but infants did not; instead, they engaged in a steady pace of exploration throughout the test.

Discussion

Although late infants have a surprisingly adult-like capacity for activity, they do differ from adults in the daily patterns of their activity and in that they have lower anxiety levels than adults. Infants have an essentially equivalent energy capacity to the adult for locomotor activity and wheel running in familiar environments. They also have a fundamentally adult-like diurnal activity rhythm, with the exceptions that they do not anticipate light–dark transitions, are more perturbable in their most somnolent periods, and are more active in the latter light and less active in the latter dark phases than the adult. Infants initially have a lower rate of locomotor activity in novel environments than do adults and have a notably greater willingness than adults to enter and be active in anxiety-provoking locations. These data suggest that reduced anxiety and adult-like locomotor capacity together result in a late infant more likely to explore certain aspects of its environment more than an adult, including time points during the diurnal phase of the daily cycle when they are less likely to encounter adults. This may be a survival advantage in access to food or avoiding aggressive encounters.

In the laboratory, such infant–adult differences need to be accounted for when the metrics of activity are used to compare differences in the internal state of these two subject groups by measuring some aspect of environmental exploration (Antle & Mistlberger, 2005). Choosing the activity tests and diurnal activity time points that provide unconfounded baseline responses in both groups is critical to accurately measure the immediate effects of lesion and pharmacological interventions across the late infant and adult states. The discussion below suggests the operational utility of this information for experimental design.

In the natural setting, home territory size and activity are determined by resources (D. Chitty & Southern, 1954; S. Chitty & Shorten, 1946; Russell, Towns, Anderson, & Clout, 2005). In our experiments, test chamber size was the determining factor, because tests were either short or with food and water available. Our 24-hr records of unperturbed activity in the home cage and of open-field activity recorded for 30 min in the familiar environment (home cage) accord

with the general summaries of activity levels of infants and adults reported by Moorcroft et al. (1971) with access to food and water. Thus we have, as intended, measured the normative exploratory capacity of both groups absent of major physiological demands. This contrasts with Moorcroft et al.'s finding that adult levels of activity are substantially greater than that of infants when provoked by extreme hunger (24-hr food deprivation; Moorcroft et al., 1971). Our data on ingestion of novel versus familiar foods strongly suggest that Moorcroft et al.'s paradigm used severe food deprivation for infants compared with adults (Smith & Morrell, 2003). A report that P18 infants were 20% less active than adults did not measure the total activity of the infants but rather part of daily activity in a figure-eight maze adjacent to the home cage that subjects entered on a voluntary basis (Norton et al., 1975).

Wheel running measures activity in a manner that is independent of the size of the environment. In adults and adolescents, the diurnal patterns of wheel running are nearly identical to locomotor patterns (Barron et al., 1996; Bauer, 1990; Eikelboom & Mills, 1988; Hastings et al., 1977; Katona & Smale, 1997; Peng et al., 1980; Richter, 1922; Stewart et al., 1985; Werme et al., 2002). Our data demonstrate that P18 infants wheel run in diurnal patterns that are virtually identical to their locomotor patterns. Further, infants can wheel run as much as adults, which accords with a recent finding using a brief test in the late infant (Dalton-Jez & Eikelboom, 2005). We conclude that from P18 onward either of these measures can be chosen depending on the experimental needs, provided that infant–adult differences in exact diurnal patterns and perturbability are taken into consideration. Adults perform wheel running because it is rewarding (Lett, Grant, Byrne, & Koh, 2000; Lett, Grant, & Koh, 2001; Werme et al., 2002; Werme, Thoren, Olson, & Brene, 2000); the increased wheel running over 24 hr generally supports the idea that infants also find wheel running rewarding.

Our data accord with that demonstrating diurnal activity patterns in adults and newly demonstrate that the infants differ in key details from adult patterns. All the differences noted in infant–adult diurnal rhythms are equally apparent with both the locomotor and running wheel measures. Because different groups of animals were used in these two paradigms, these two data sets provide intrastudy confirmation of our primary findings of differences in diurnal rhythm patterns. Although both groups exhibited greater activity in the dark than in the light, infants were active a greater percentage of each 24-hr period than were adults (75% vs. 60% of total time, respectively). During the third quartile, infant activity levels may facilitate their joining of their dams in her search of the environment for resources, which peaks in this period, whereas reduced infant activity in the latter portion of the fourth quartile suggests their need to rest after prolonged activity.

So although our data demonstrate that infants have not yet become adult-like in their diurnal patterns of activity, others have documented that these patterns become adult-like by mid-adolescence (Bolles & Woods, 1964; Joutsiniemi et al., 1991; Norton et al., 1975). An interesting feature of the infant–adult activity pattern difference is our demonstration that infants lack the peri-transition surges in activity characteristic of the adult. These peri-transition surges may be an anticipatory response to changes in foraging opportunities by adults because they typically forage at night (Borbély & Neuhaus, 1978; Evans, 1971; Peng et al., 1980; Richter, 1922; Stewart et al., 1985; Zucker, 1971).

Our data suggest that a specific window of time during the daily cycle is best used to avoid baseline activity surges and abatements of the late infant that are out of synchrony with adult patterns. The period of 2100–0400 is the recommended period for infant–adult comparisons; this is a period of equal activity that avoids the peri-transition periods. In theory, infants and adults could be tested at either of the two periods when their activity levels are similar, 0900–1100 or 2100–0400. However, these are also periods of contrast in the amount of activity, with 2100–0400 being the period of equally greatest activity and 0900–1100 a time of sleeping.

Further, during the first quartile (0900–1100), infants are easily provoked to activity (locomotor activity, rearing, and grooming) by the experimenter's brief handling whereas adults are unperturbed by the same mild disturbance on all three measures in the relative somnolence that characterizes their overall behavior during this time period. Measuring the extent of an effect of an intervention in infants and adults at this particular period may be particularly misleading.

Whether an environment is novel or familiar significantly influences adult rodent activity. The novel open-field environment is a well-established paradigm and is often used as a measure of adult response to novelty or as an open-field test of response to drugs (Bronstein, 1972; Feigley, Parsons, Hamilton, & Spear, 1972; Kabbaj & Akil, 2001; Rowe, Spreekmeester, Meaney, Quiron, & Rochford, 1998; Stead et al., 2006). We found that infants have a different response to a novel open-field environment than adults. Unlike the characteristic burst of initial activity by adults in a novel environment, infants exhibit a measured initial response and start at a pace that they maintain throughout the remainder of the test. The infant response is also unaffected by time of day of testing. Infants rear and groom less in novel compared with familiar environments. Adult and infant locomotor activity is very similar in the familiar open field except that infants are more perturbable in the first quartile. Infants generally rear and groom more than adults in the familiar environment, results consistent with a prior study showing that 3-week-old rats engage in both behaviors more than adults do (Campbell & Mabry, 1973). We recommend careful baseline comparison analysis when using a novel test chamber for infants and comparing the outcomes to adult responses.

Consistent with the literature, our data also confirm that adults were most active in the novel environment compared with the familiar environment (Buelke-Sam et al., 1984; Galani et al., 2001), but our data further demonstrate this is only true in the dark phase. Direct comparison of familiar with novel responses are rare in open-field studies. Although Galani et al. (2001) also found that adults were less active in the familiar environment, we note that our paradigm involved equal disturbance and so avoids the confound that their subjects were undisturbed in the familiar environment and newly introduced into the novel environment. Others have reported that infants younger than we tested respond to a novel environment by initially becoming immobile and then progressively increasing their activity (Eilam & Golani, 1988; Golani et al., 2005). In the P18 infant, we did not find initial immobility but that infants were initially less active than adults at the start of the test session. This was followed by a continued measured pace with no adult-like decline by the end of test. Similar to our findings with the familiar open-field chamber, others have reported that infant activity was increased when home-scented shavings were added; unscented bedding did not mitigate the reduced initial activity (Buelke-Sam et al., 1984; Pappas et al., 1982).

Infants spent more time in and more slowly explored two locations that evoked anxiety-like responses in adults, the center of any environment and the light portion of the black and white box. In the home cage, this difference between the two groups was modest, but the open-field test paradigm really emphasized the expanded center time responses of the infants. Infants spend as much as 40% of their time exploring the center at a measured pace in an open-field test. Whereas the increased center time in infants may be a simple derivative of their increased locomotor activity in the second quartile of the diurnal rhythm, this cannot be the case in the third quartile when the two groups are identically active. No studies on infants have previously included center time analysis, and our adult data of brief center time accords with prior work in the adult and adolescent (Lomanowska, Rana, McCutcheon, Parker, & Wainwright, 2006).

In the black and white box, both infants and adults spent less time in the more anxiety-provoking lighted half, but infants spent nearly 10 times longer in the light half of the box and explored it much more slowly than did adults. Our adult findings accord with prior studies in adult rodents (Crawley, 1981; Crawley & Goodwin, 1980; McHugh, Deacon, Rawlins, &

Bannerman, 2004; Ramos, Berton, Mormede, & Chaouloff, 1997). The youngest rats reported with use of the black and white box in the literature are early adolescents (P28 –P32). These early adolescent rats were reported to either not differ from adults under low-light conditions (Slawecki, 2005) or were less anxious than adults (Doremus et al., 2004). Adolescents were more anxious than adults after brief restraint stress or when tested under bright light conditions (Slawecki, 2005). Further work is needed to determine more clearly when adolescent animals become fully adult-like in their levels of anxiety.

We conducted a preliminary experiment examining infant and adult responses in the elevated plus-maze, another traditional measure of anxiety-like responses in adult rodents. Although our data showed that our adult responses were consistent with previous reports of elevated plus-maze activity in the literature, our data on the infant were inconclusive because the infants fell asleep in the apparatus. We suggest that the plus-maze not be used but that the time in the center of a novel open field and in the two halves of the black and white box can both be used to successfully measure anxiety-like responses during late infancy. Furthermore, because infants are less anxious by all effective measures, this baseline difference from adult responses should be considered when interpreting comparison data and assessing the effects of anxiogenic or anxiolytic treatments in the infant.

The proximal causes for the differences documented across infants and adults are undoubtedly multifaceted and complex. Included among these must be the complex processes of puberty and the subsequent regulation by adult hormone levels. Additional issues to be considered are developmental changes that are independent of hormonally driven or maintained differences, such as those timed by genetic programs for CNS development. Future studies are needed to determine exactly when adult patterns of diurnal activity, novel open-field responses, and anxiety emerge between P18 and P60. Data on some of these time points are found in the work of others on the adolescent rat, as discussed above. These studies serve as the basis for a more detailed determination of which aspect of developmental changes induce the final leap to adult status, including hormones or genetically timed events independent of hormones.

Our data offer information applicable to the infant rat in the natural setting and in laboratory behavioral paradigms that strive to examine the effect of neuropharmacological agents of potential clinical utility. Of course, infant rats are not simply smaller versions of adolescents or adults and the impact of pharmacological treatments on their CNS can only be understood in the context of their developing nervous system and behavioral capacity. The preadolescent rats we used in these studies offer an important and understudied animal model that may provide preclinical information vital to the treatment of the tragedy of substance abuse during childhood and early adolescence and the opportunity to use pharmaceuticals to treat mental health problems in children.

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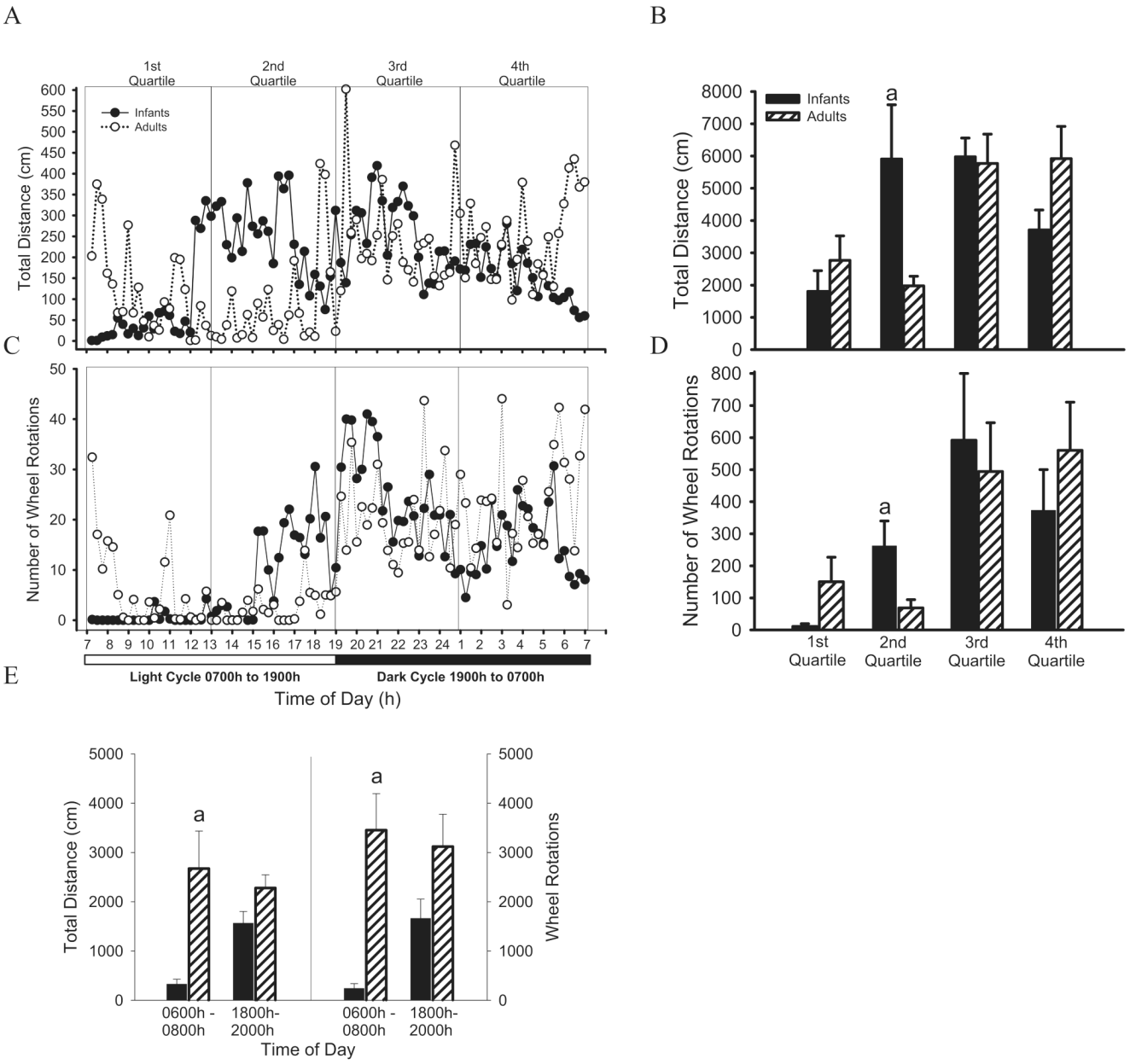

Figure 1.

Infant and adult profiles of home cage activity measured by (A) daily total distance traveled (in centimeters) that was subsequently (B) divided into quartiles. Activity was also measured by (C) number of wheel rotations that were (D) divided into quartiles. (E) Activity patterns were also measured during the light–dark transition periods. a = significantly different from adults.

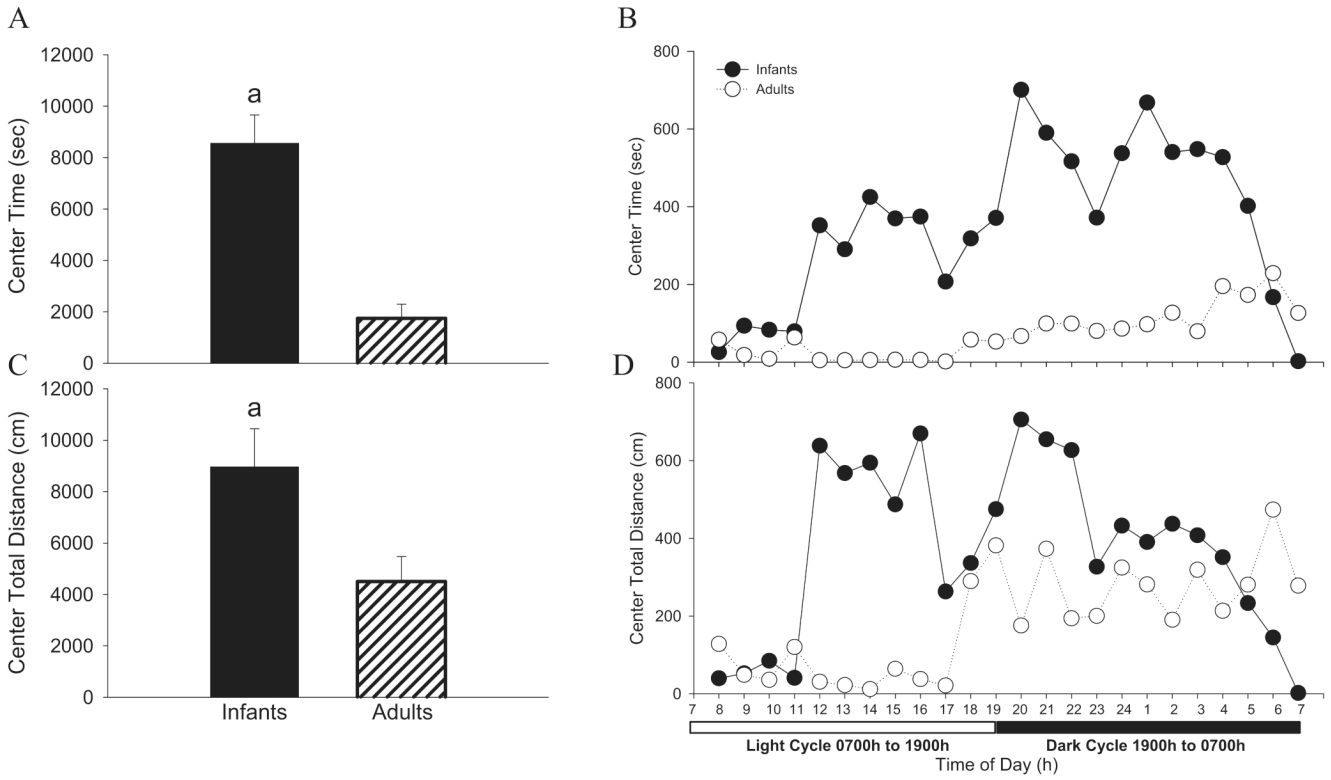

Figure 2.

The overall (A) time spent (in seconds) and (C) the total distance subjects traveled in the center of the home cage (in centimeters) and the patterns of (B) center time (in seconds) and (D) center total distance (in centimeters) during a 24-hr cycle. a = significantly different from adults.

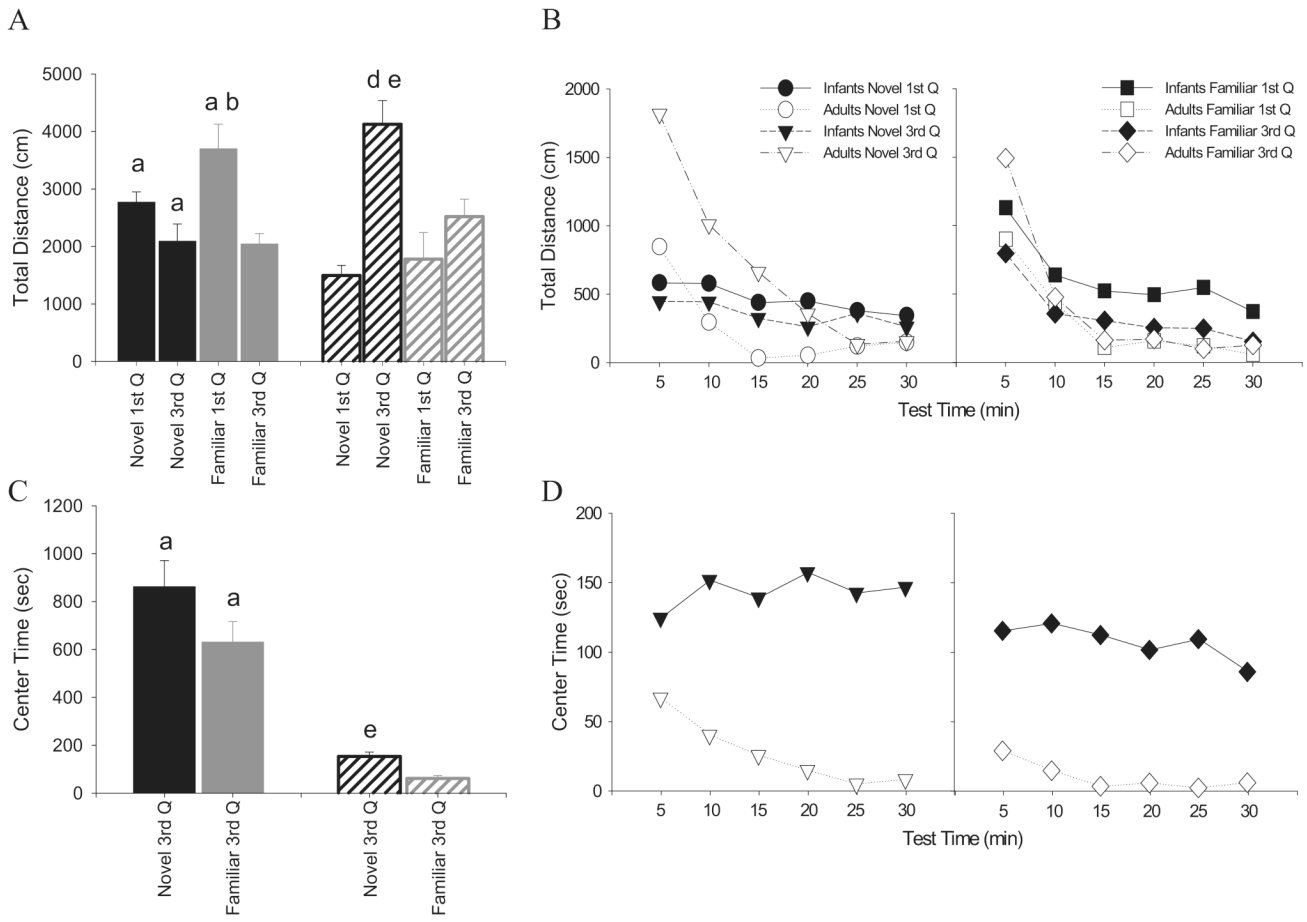

Figure 3.

Average total distance traveled (in centimeters) by infants and adults (A) and patterns of activity during 30-min exposures to either a novel open field or a familiar home cage during the first and third quartiles of the daily cycle (B). Average time spent in the center of the novel open field and the familiar home cage (in seconds) during the third quartile (C) and the corresponding patterns of center time (in seconds) across the test session (D). a = significantly different from adults; b = significantly different from infants in the same test condition at the alternate test time; d = significantly different from adults in the same test condition at the alternate test time; e = significantly different from adults in the alternate test condition when tested at the same time of day; Q = quartile.

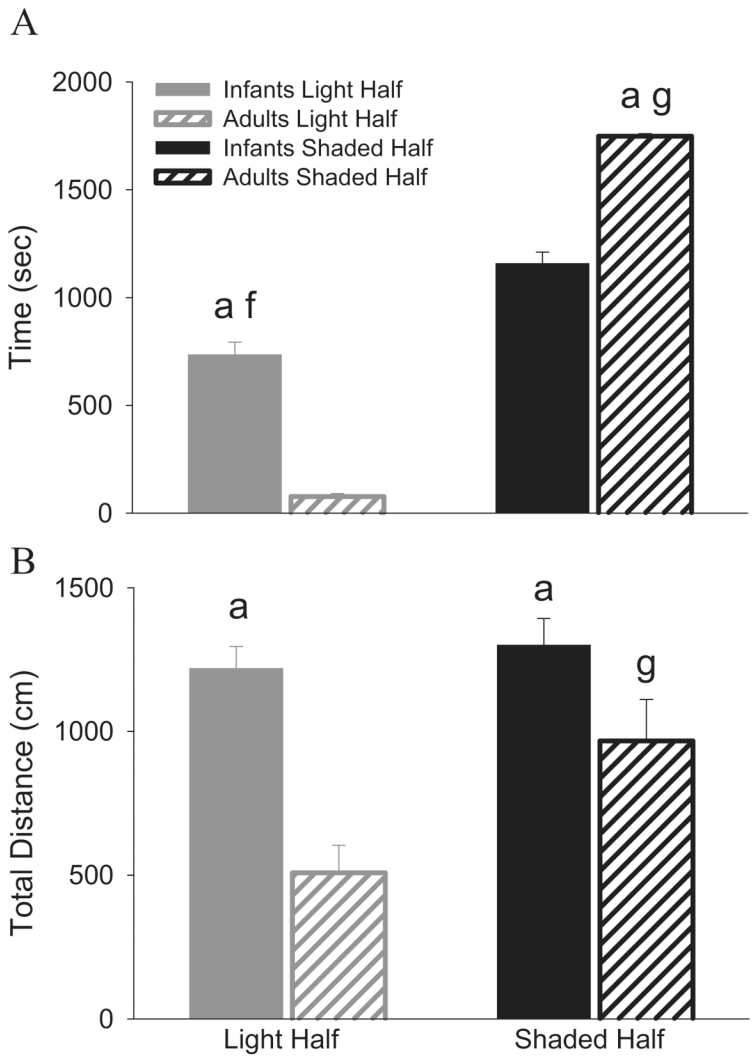

Figure 4.

Infant and adult activity in the black and white box. All testing was conducted during the first quartile. Mean time spent (in seconds; A) and mean total distance traveled (in centimeters; B) in each half during a 30-min test session. a = significantly different from adults; f = infants significantly different in each half; g = adults significantly different in each half.

Table 1

Mean (± SEM) Distance Traveled (in Centimeters) and Exploration Rate (in Centimeters/Minute) for Infants and Adults

Environment	Infants		Adults	
	Distance	Exploration rate	Distance	Exploration rate
Center of home cage, 24 hr	8,968 ± 1,482[a]	60 ± 10[a]	4,500 ± 972	220 ± 51
Center of novel cage				
First quartile	NA	NA	NA	NA
Third quartile	1,075 ± 210	74 ± 10[a]	1,326 ± 115[e]	513 ± 48
Center of familiar cage				
First quartile	925 ± 64[a]	108 ± 19[a]	216 ± 52[d]	375 ± 51[d]
Third quartile	913 ± 85[a]	87 ± 7[a]	603 ± 92	618 ± 31
Black and white box				
Light half	1,219 ± 76[a]	102[a,f] ± 6	508 ± 96[g]	410 ± 34[g]
Shaded half	1,300 ± 93[a]	69 ± 7[a]	967 ± 143	33 ± 5
Center	1,010 ± 52[a]	107 ± 6[a]	243 ± 58	507 ± 49

Note. NA = not applicable.

[a] Significantly different from adults.

[d] Significantly different from adults in the same test condition at the alternate test time.

[e] Significantly different from adults in the alternate test condition when tested at the same time of day.

[f] Infants significantly different in each half.

[g] Adults significantly different in each half.

Table 2

Mean (± SEM) Number of Times Infants and Adults Engaged in Rearing and Grooming

	Rearing		Grooming	
Environment	**Infants**	**Adults**	**Infants**	**Adults**
Novel cage				
First quartile	34[a,c] ± 5	21 ± 3[d]	7 ± 0.7[a]	4 ± 0.6[d]
Third quartile	58 ± 6[a]	83 ± 8[e]	11 ± 1.0	11 ± 0.7[e]
Familiar cage				
First quartile	74 ± 9[a]	21 ± 4	10 ± 1.0[a]	3 ± 0.4[d]
Third quartile	58 ± 9[a]	32 ± 3	9 ± 1.0	7 ± 1.0

[a] Significantly different from adults.

[c] Significantly different from infants in the alternate test condition when tested at the same time of day.

[d] Significantly different from adults in the same test condition at the alternate test time.

[e] Significantly different from adults in the alternate test condition when tested at the same time of day.