AMERICAN PSYCHOLOGICAL ASSOCIATION

Locomotion and Posture Development in Immature Male and Female Rats (*Rattus norvegicus*): Comparison of Sensory-Enriched Versus Sensory-Deprived Testing Environments

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The aim of the current study was to provide normative data on spontaneous locomotion and posture behavior in developing rats (*Rattus norvegicus*), during the first 2 postnatal weeks. Male and female rat pups were tested daily from P1 (postnatal day 1; ~24 hr after birth) to P15 in a sensory-enriched or sensory-deprived testing environment, which was enclosed in a temperature-controlled incubator. Pups in the sensory-deprived condition were tested individually and placed in a square, Plexiglas box (open-field) for a 20-min test period. Pups in the sensory-enriched condition were placed in the same box with the siblings and bedding from the home cage to provide sensory stimulation that mimicked the home nest. Subjects in this condition were tested two at a time, with an additional two siblings (2 males and 2 females total in box). It was hypothesized that pups in the sensory-enriched testing condition would demonstrate more mature patterns of behavior, given the presence of behavior-activating sensory stimuli in the box. It was found that rat pups exhibited spontaneous pivoting and crawling as early as P1, regardless of sensory stimulation present in the testing environment. These behaviors were shown at least 1 to 3 days earlier than reported in prior studies. Quadrupedal walking occurred as early as P4 but was not reliably expressed until P10/11. These findings suggest that controlling temperature during testing influences the typical age of first occurrence of these behaviors. Finally, there were no sex differences in the duration of locomotion and posture behaviors.

Keywords: motor behavior, neonatal rat, environment, temperature, movement

In rats, as with all mammals, the neural mechanisms supporting locomotion begin developing in utero (Bekoff & Lau, 1980; Brumley & Robinson, 2005) and continue developing postnatally (Vinay et al., 2002). Nearly every research article over the last 40 years that has examined the developmental trajectory of locomotion in rats, or how this trajectory may be affected by experimental manipulations, bases developmental time points of emergence of locomotion on a study published by Altman and Sudarshan in 1975. In fact, according to Google Scholar, over 750 articles examining or manipulating the neurobehavioral development of locomotion in rats cites Altman and Sudarshan (1975). In that seminal paper, the age of the first spontaneous occurrence of different forms of locomotion and posture (i.e., limb elevation, pivoting, crawling, head elevation, and walking) in rats are reported in an open-field testing environment. In general, it is reported that posture and locomotion of the upper body (head and forelimbs) developmentally precedes that of the lower body (hindlimb), as evidenced by pivoting and crawling occurring before full-on quadrupedal walking (Altman & Sudarshan, 1975). The article by Altman and Sudarshan has provided important normative data on locomotion and posture that has enhanced our understanding and knowledge of motor behavior development in a rat model.

However, early studies of locomotion and posture were conducted prior to research that examined physiological processes and development and maturation of body systems. For example, Bolles and Woods (1964) and Altman and Sudarshan (1975) conducted their studies prior to much of the research that examines thermoregulation in newborn rats. Thus, temperature was not a variable that was taken into consideration at the time; both studies conducted testing of immature rats at room temperature. Yet, it is now known that it is necessary to consider ambient temperature during behavioral testing (Harshaw, Blumberg, & Alberts, 2017) because immature rat pups are unable to effectively self-regulate their body temperatures internally for long periods of time. In fact, sensitivity to thermal stress, both acute and chronic exposure, can occur as early as gestation and continues after birth, as newborn rats are inefficient at independent thermoregulation (Blumberg, Sokoloff, & Kirby, 1997; Blumberg & Stolba, 1996; Gordon, 1993; Horwitz, Heller, & Hoffmann, 1982). Newborn rats are capable of using brown adipose tissue (BAT) to produce heat shortly after birth; however, BAT thermogenesis requires the animal to expend high amounts of energy (Cannon & Nedergaard, 2004). Thus, it is

important to test and maintain newborn rats at ambient temperatures that require the animal to use minimal energy to produce and maintain body temperature, or within thermoneutral zones (Satinoff, 1996), when examining neurobehavioral abilities.

In addition, previous studies have not directly examined sex differences in the development of locomotion and posture behavior in rats. Although some researchers have included both males and females in their experimental designs (Altman & Sudarshan, 1975; Geisler, Westerga, & Gramsbergen, 1993; Westerga & Gramsbergen, 1990), they have not reported sex as a variable in their statistical analyses. It is important to determine if there are sex differences, which might contribute to discrepancies in findings from prior studies and contribute to our understanding of neurobehavioral development, and to form a basis of comparison for future studies that examine sex effects in developmental processes. For instance, research has found that there are sex differences in male and female rat pups' latency to perform the leg extension response (LER) during the neonatal period. This coordinated motor behavior is characterized by hyperextension of the hindlimbs in response to maternal infant-directed anogenital licking (Moore & Chadwick-Dias, 1986). Specifically, compared with female rat pups, males display quicker LER latencies and longer LER durations. Although research has not examined if these differences extend to other patterns of motor coordination, it is possible that this additional experience of extension and flexion of the hindlimb could influence the first occurrence of locomotion or postural behaviors—such that males might demonstrate these behaviors slightly earlier than females. This idea is exploratory, as prior research has not examined these potential differences in the development of locomotion and posture.

Researchers often test laboratory animals, including neonatal rats, individually in an open-field or other contrived environment. However, newborn rats born in a laboratory setting develop in a nest structure built with bedding material by the dam. The home nest, in addition to barriers established by the bedding walls, contains olfactory stimuli (i.e., odor of dams and littermates, milk, urine, etc.), as well as thermal and tactile stimulation through contact with other pups in the litter and with the dam. These sensory cues available in the nest can influence behavioral responses of newborn pups by evoking general behavioral arousal, directing specific behavioral responses, or providing biomechanical support. For example, newborn rats use maternal olfactory cues to direct them toward the nipple, and their attachment to it, through a series of ventroflexion and dorsiflexion movements of the trunk and crawling-like steps (Eilam & Smotherman, 1998). Chemosensory cues can also be used to affect the direction of the pup's motor behavior. Mendez-Gallardo and Robinson (2014) demonstrated that rat pups exhibit crawling along a runway in response to both amniotic fluid and milk. Rodent pups also can utilize siblings as biomechanical support to exhibit advanced postural behavior. For instance, Golani and Fentress (1985) examined the development of facial grooming in mice. They found that in huddle positions, mouse pups would make use of their body and limb positions in conjunction with the position of their siblings to perform more advanced facial grooming behavior (e.g., propping their elbows on the back of siblings). Therefore, it would seem to follow that immature rats could also use their siblings as biomechanical support to facilitate locomotion and posture behavior. Similarly, Ferrari and colleagues (2007) placed preterm human infants in an oval

nest composed of rolled blankets and found that the nest promoted smoother movements of the infant's limbs. Overall, it appears that a nest-like environment can influence posture and limb movements in infant rats and humans through behavioral activation and biomechanical support. Therefore, the developmental importance of the nest, and the multitude of sensory stimulation contained within the nest, cannot be undermined for immature mammals. Hence, the influence of the nest is an important component to take into consideration when examining the normative development of locomotion and postural behavior.

The purpose of the current study is to provide normative data on spontaneous posture and locomotion behavior and development in newborn male and female rats, while controlling temperature and examining differences in testing conditions. In this study, the developmental trajectory of locomotion and posture was examined during the first 2 postnatal weeks (P1–P15) in both a sensory-deprived (open-field) testing environment and a sensory-enriched (nest-like) testing environment. In both testing conditions, ambient temperature was controlled to ensure that subjects were tested at thermoneutral temperatures. Our hypothesis was that subjects in the sensory-enriched condition would demonstrate more mature patterns of locomotion prior to subjects in the sensory-deprived condition, given the literature on behavior-activating/directing effects of sensory stimulation discussed previously. In addition, by controlling ambient temperature in both testing conditions, we hypothesized that subjects in both conditions would show earlier expression of motor patterns than previously reported (Altman & Sudarshan, 1975) because they presumably would not have to expend as much energy on physiological heat production. We also used an equal number of male and female subjects to determine if there are differences between sexes in locomotion and posture. Overall, compared with previous studies, we provide increased quantitative and qualitative data on locomotion and posture behavior in rats over the first 2 postnatal weeks, while better controlling for some possible confounding variables.

Method

Subjects

Subjects were Sprague–Dawley rats bred in the Animal Care Facility at Idaho State University. Litter size was reduced to eight pups on P1 (~24 hr after birth). Subjects remained in the home cage with the dam, except during testing. Pups were examined prior to testing to ensure that they had fed recently, as determined by the presence of a milk band on the abdomen, and were in overall good health. Animal care and use were in accordance with National Institutes of Health guidelines and the Idaho State University Animal Care and Use Committee.

Experimental Design and Behavioral Testing

A total of 64 rats (32 female, 32 male) from 16 litters (four subjects per litter) were used in this study. Subjects were tested in one of the two testing environments—two pups (one male and one female) from each litter were tested individually in the sensory-deprived (open-field) testing environment, and two pups (one male and one female) from each litter were tested simultaneously in the sensory-enriched (nest-like) testing environment (Figure 1). All
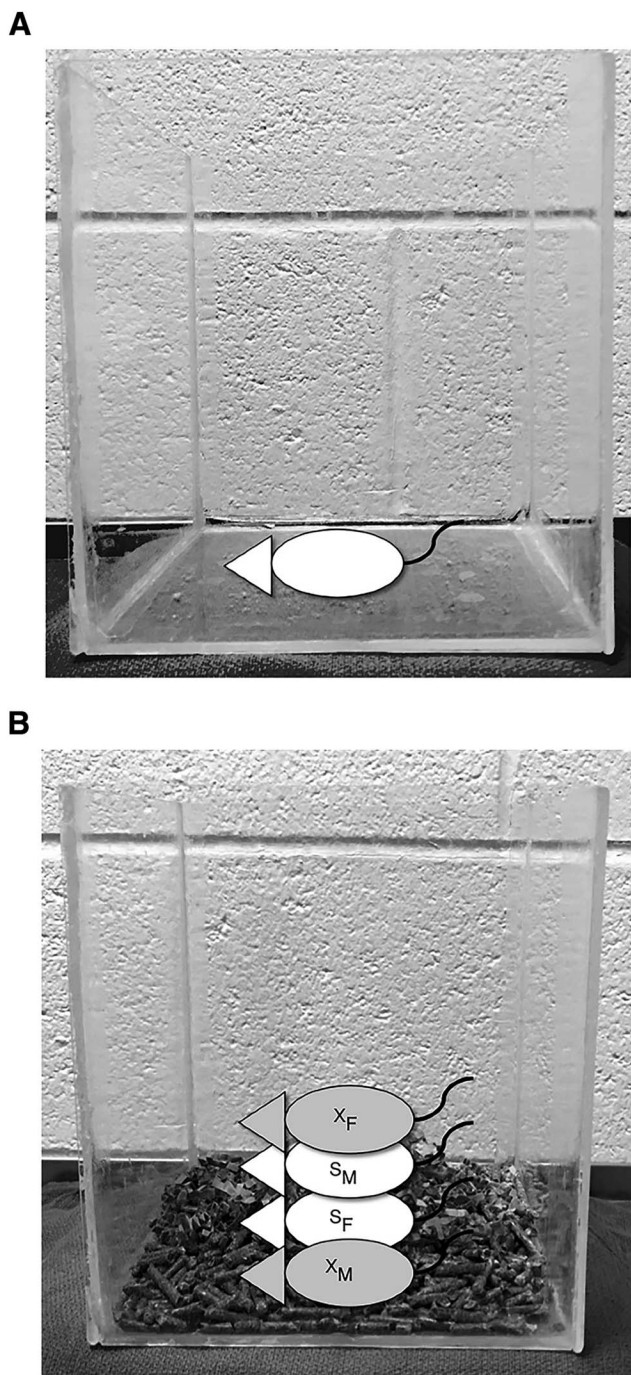
Figure 1. Sensory-deprived and sensory-enriched testing environments, in which rat pups were placed and their spontaneous posture and locomotion behavior were recorded. The box was located inside an infant incubator that controlled temperature and humidity. (A) An empty Plexiglas box was used in the sensory-deprived testing condition. (B) In the sensory-enriched testing condition, nest and bedding material from the home cage and two siblings (X_M, X_F) were placed inside the Plexiglas box as well.

pups in the litter were marked using a nontoxic black marker or nontoxic black pet dye for identification and to control for handling effects. The order in which the subjects were tested in the sensory-deprived condition was balanced; half of the male subjects

and half of the female subjects were tested first. In the sensory-enriched condition, the male and female test subjects were tested together (thus, simultaneously).

Subjects assigned to the sensory-deprived testing condition were tested individually in a clear, Plexiglas box (Figure 1A). Subjects assigned to the sensory-enriched testing condition were placed in the same Plexiglas box with two siblings (one male and one female) and were tested two subjects (one male and one female) at a time, for a total of four pups in the box. In addition, bedding and nest material from the home cage was also placed in the box (Figure 1B) for the sensory-enriched condition, to simulate some aspects of the natal nest. Box dimensions were adjusted based on pup size, but not for pup number, to control for available footage. Pups were weighed and measured daily to determine box size, such that the average body length of all pups in the litter was calculated and the box was 1.5 to two times the size of the average body length. Box size ranged from 4″ × 4″ with 8″ walls, increasing in half-inch increments, to 8″ × 8″ with 8″ walls.

In each testing condition (sensory-deprived or sensory-enriched), the box was located inside an infant incubator that controlled temperature and humidity. Two micro cameras, one capturing a lateral view and the other capturing a dorsal view, were connected to a DVR recording unit outside the incubator, which clearly captured behavior occurring inside of the testing box. The Society of Motion Picture and Television Engineers (SMPTE) longitudinal time-code was impressed on video recordings throughout the test session. Recording of the test session began immediately after the subject was placed on the box floor.

Subjects were tested once a day for 15 consecutive days, beginning on P1 and ending on P15, to capture their spontaneous behavior inside of the testing environment. Individual subjects were tested in the same testing environment each day (e.g., always sensory-deprived or sensory-enriched condition). Body mass (g) and body length (mm; crown to rump) were recorded for all subjects, immediately prior to testing. On each day of testing, pups were manually voided (for standardization purposes) and then placed inside of the warmed incubator in a small plastic dish for 30 min prior to testing, to allow for acclimation to incubator conditions. Following acclimation, subjects were placed in their test environment (sensory-deprived or sensory-enriched) for a 20-min test session. Ambient temperature of the incubator was recorded for each test session, and nest temperature was measured inside the nest of the sensory-enriched condition, using a thermometer gauge. During the test session, siblings from the subject's litter also were removed (and kept warm in an incubator) to ensure that daily handling and removal of subjects did not alter maternal care directed toward individual subjects.

Behavioral Scoring

The 20-min test session inside the box was scored during video playback at normal or reduced speed using an event recorder program which records the category of behavior and time of entry (±0.01s). Behavior was classified into eight categories: pivoting, crawling, walking, head elevation, forelimb elevation, hindlimb elevation, crawling stance, and walking stance. Definitions of locomotion and posture were based on Altman and Sudarshan (1975) and Swann, Kempe, Van Orden, and Brumley (2016) and

are provided in Table 1. Intrarater reliability for scoring was at 90%.

During video playback, the age of eye opening also was recorded. Partial eye opening was defined as one eye being completely opened, with the other eye closed or both eyes being partially opened. Complete eye opening was measured when both eyes were completely opened.

Data Analysis

Data were analyzed using SPSS statistical software (Version 22.0, IBM Corp, Armonk, NY). Analysis of variance (ANOVA) tests and paired sample t tests were used to examine locomotion and posture behaviors. Effect sizes and confidence intervals are reported in the text. To determine effect sizes, the following effect size computations were used: partial-eta squared (ANOVAs, $\eta_p^2 = 0.01$ small, $\eta_p^2 = 0.06$ medium, $\eta_p^2 = 0.14$ large), Cohen's d (t tests, $d = 0.20$ small, $d = 0.50$ medium, $d = 0.80$ large), and Cramer's V (χ^2 tests, $v = 0.10$ small, $v = 0.30$ medium, $v = 0.50$ large, where $df = 1$). When significance was detected, follow-up tests were utilized and are identified in the appropriate results section. A significance level of $p < .05$ was adopted for all tests.

Body mass and body length were compared across each sex/testing condition for each age separately, using a series of two-way ANOVAs. Ambient temperature in the sensory-deprived and nest temperature in the sensory-enriched condition was compared for each age separately. For these analyses, paired-sample t tests were used to compare mean temperatures. Based on the literature (Altman & Sudarshan, 1975), we expected pivoting to precede crawling and crawling to precede walking, by a number of days. To establish the age of first occurrence of specific motor patterns, the presence or absence of each locomotion and posture behavior was quantified, and the percentage of subjects that exhibited the behavior at each age was calculated and plotted. Not all animals demonstrated all behaviors during testing; therefore, we established a criterion of at least 70% of subjects exhibiting the behavior to qualify as the typical age of first occurrence. We determined our criterion level to be above chance levels, by plotting and examining the data to determine when the majority of subjects (>50%) engaged in a behavior. Typically, animals were either at chance levels or near the 70% criterion level. Next, repeated measures ANOVAs were used to analyze locomotion and posture behaviors across the testing period, the first 2 postnatal weeks, and

between testing environments. Dependent variables were duration spent showing posture and locomotion behaviors, as defined in Table 1.

Approximately 3% of subject data were missing due to video data collection issues or experimenter error. Due to the low quantity of missing data and given that the data were missing at random, missing values were eliminated from all analyses using pairwise deletion, for descriptive statistics, criterion percentages, and two-way ANOVAs: number of subjects was 64, except on P2 ($n = 60$), P4 ($n = 56$), P6 ($n = 56$), P9 ($n = 56$), and P14 ($n = 60$), and listwise deletion, for repeated measures ANOVAs and correlation analyses: number of subjects was 40.

Results

Litter and Testing Environment Characteristics

Body mass and body length. A series of two-way ANOVAs for body mass revealed a significant main effect of sex, such that males had greater body mass than females on P1, $F(1, 60) = 6.56$, $p < .05$, $\eta_p^2 = 0.10$, 95% confidence interval [6.81, 7.10], P2, $F(1, 56) = 5.54$, $p < .05$, $\eta_p^2 = 0.09$, 95% CI [7.83, 8.23], P3, $F(1, 60) = 3.46$, $p < .05$, $\eta_p^2 = 0.09$, 95% CI [9.14, 9.52], P4, $F(1, 52) = 7.67$, $p < .01$, $\eta_p^2 = 0.13$, 95% CI [10.94, 11.37], and P15, $F(1, 60) = 5.18$, $p < .05$, $\eta_p^2 = 0.08$, 95% CI [34.68, 36.06]. Body mass averages for males and females are shown in Figure 2A. There was not a main effect of sex on body mass from P5 to P14 (Figure 2A), or a main effect of testing condition at any age. A series of two-way ANOVAs for body length did not reveal any effects of sex or testing condition (Figure 2B).

Testing environment temperature. Table 2 shows ambient temperatures for the sensory-deprived and nest temperatures for sensory-enriched conditions. Paired sample t tests revealed that there were significant differences between ambient temperature of the sensory-deprived condition and nest temperature of the sensory-enriched condition on P4, $t(13) = -2.66$, $p < .05$, $d = -0.71$, 95% CI [-1.58, -0.16], P6, $t(13) = -4.32$, $p < .001$, $d = -1.16$, 95% CI [-2.09, -0.70], P7, $t(15) = -2.45$, $p < .05$, $d = -0.16$, 95% CI [-1.67, -0.12], P8, $t(15) = -3.87$, $p < .01$, $d = -0.97$, 95% CI [-2.31, -0.67], P10, $t(15) = -3.15$, $p < .01$, $d = -0.79$, 95% CI [-2.56, -0.49], P11, $t(15) = -5.01$, $p < .001$, $d = -1.25$,

Table 1

Definitions of Locomotion and Posture Behavior as Originally Defined by Altman and Sudarshan (1975)

| Locomotion and posture behavior	Definition
Pivoting	A propulsive movement where the pelvis remains anchored on the surface while forelimbs propel the pup in a circular path
Crawling	A propulsive movement that actively involves the forelimbs while the ventrum remains in contact with the surface
Walking	A propulsive movement with all four limbs active and the ventrum off the surface
Head elevation	Elevation of the head without simultaneous propulsive movement
Forelimb elevation	Extension of the forelimbs with the forepaws in contact with the surface, elevating the front portion of the body off of the surface
Hindlimb elevation	Extension of the hindlimbs with hind paws in contact with the surface, elevating the hind portion of the body
Crawling stance	Elevated forelimbs and shoulders with hindlimbs and pelvis in contact with surface
Walking stance	Elevation of all four limbs, shoulders, and pelvis
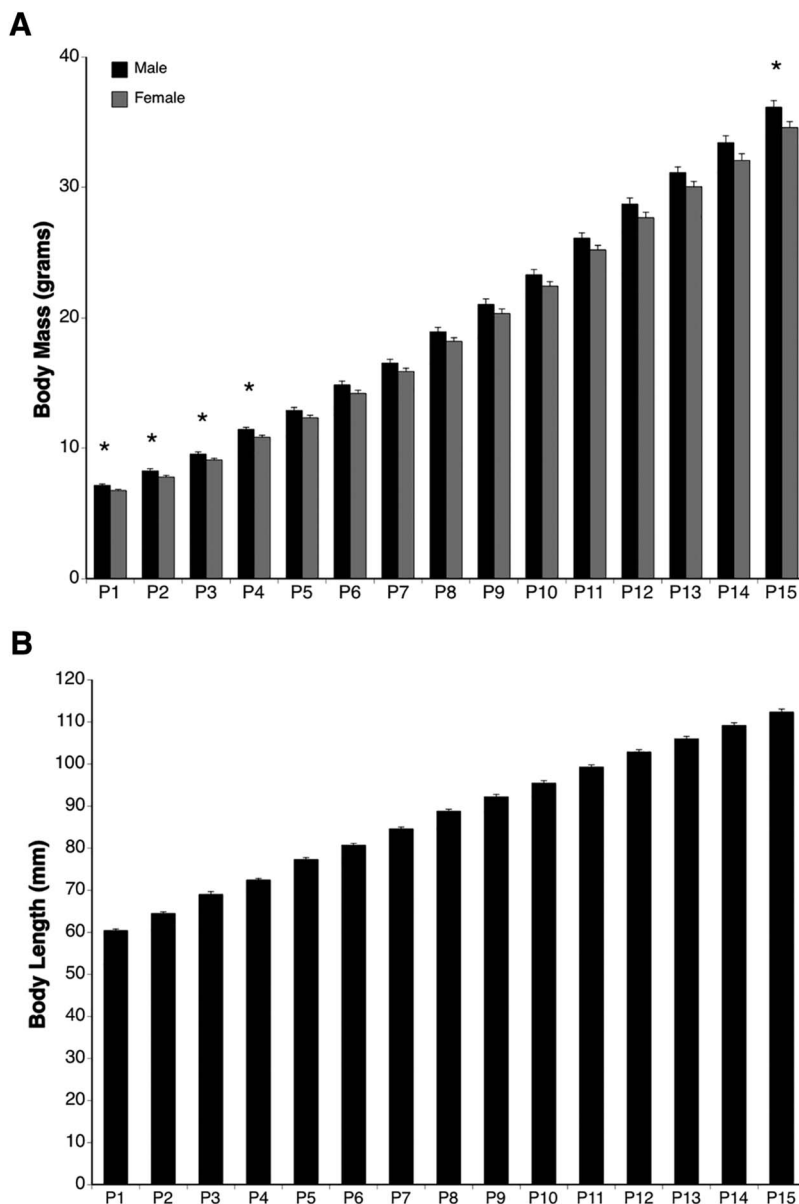
Figure 2. Body mass and length for male and female subjects. (A) Body mass of male and female rat pups for the first 2 postnatal weeks. (B) Body length for the first 2 postnatal weeks. Bars represent means, vertical lines show standard error of the means, and asterisks indicate significance at the $p < .05$ level.

95% CI $[-3.06, -1.23]$, P12, $t(15) = -6.31$, p .001, $d = -1.58$, 95% CI $[-4.47, -2.21]$, P13, $t(15) = -4.51$, $p < .001$, $d = -1.13$, 95% CI $[-4.26, -1.53]$, P14, $t(14) = -6.79$, $p < .001$, $d = -1.75$, 95% CI $[-4.44, -2.31]$, and P15, $t(15) = -5.64$, $p < .001$, $d = -1.41$, 95% CI $[-4.42, -1.99]$, such that the nest temperature was warmer (by 0.9–3.3 °C) than the ambient temperature. There was no significant difference between ambient temperature and nest temperature on P1 to P3, P5, or P9.

Eye opening. Heyser (2003) reported eye opening occurring in rats on average at P13, but noted that it could occur anywhere from P7 to P17. We found that 42 subjects (out of a total 64; 66%)

did not show complete bilateral eye opening until P15, with 11% of subjects opening their eyes on P14. In addition, 6% of subjects only had partial eye opening on P15, and 17% of subjects did not have partial or complete eye opening by P15. Given that the majority of subjects exhibited eye opening on or around P15, statistical analyses examining potential sex or testing condition differences were not conducted.

Typical Age of First Occurrence of Locomotion

A 70% criterion was established to determine when the majority of subjects exhibited locomotion at an above chance ratio. Based

Table 2

Testing Temperature for Both Testing Environment Conditions

Age	Ambient temperature	Nest temperature
P1	34.8 ± 0.09	34.7 ± 0.33
P2	34.1 ± 0.08	34.0 ± 0.40
P3	33.8 ± 0.10	34.1 ± 0.26
P4*	32.9 ± 0.10	33.8 ± 0.29
P5	33.1 ± 0.12	33.6 ± 0.26
P6*	32.0 ± 0.11	33.4 ± 0.35
P7*	32.0 ± 0.08	32.9 ± 0.37
P8*	31.1 ± 0.08	32.5 ± 0.43
P9	31.0 ± 0.14	32.0 ± 0.52
P10*	30.3 ± 0.09	31.8 ± 0.50
P11*	30.1 ± 0.08	32.3 ± 0.45
P12*	29.0 ± 0.10	32.3 ± 0.49
P13*	29.1 ± 0.04	32.0 ± 0.65
P14*	28.1 ± 0.14	31.4 ± 0.52
P15*	28.1 ± 0.11	31.3 ± 0.55

Note. Ambient temperature reflects the temperature of the incubator for the sensory-deprived condition. Nest temperature reflects the temperature measured from the middle of the nest in the sensory-enriched condition. P = postnatal day.
* $p < .05$ level.

on the criterion established, typical age of first occurrence of pivoting varied across sex/testing environment. Males and females in the sensory-enriched condition, as well as females in the sensory-deprived condition, first reliably showed pivoting on P1, whereas males in the sensory-deprived condition first showed pivoting on P2 (Figure 3). Although it was expected that crawling would emerge a few days after pivoting, females in the sensory-deprived condition and males in the sensory-enriched condition exhibited crawling on P1, which was the same day that they demonstrated pivoting. Males in the sensory-deprived condition exhibited crawling on P2; again, the same day that they exhibited pivoting. Females in the sensory-enriched environment showed crawling on P3, which was 2 days after they exhibited pivoting (Figure 4). Thus, it appears that crawling emerges either simultaneously with pivoting or within 1 to 2 days after pivoting emerges.

To determine whether individuals within each group demonstrated both pivoting and crawling on the same day, χ^2 tests were conducted for each condition. For subjects in the sensory-deprived condition, significantly more subjects demonstrated both pivoting and crawling on the day of first occurrence than one pattern, such as pivoting only or crawling only, $\chi^2(1, N = 64) = 4.27, p < .05$, v = 0.26. Similarly for subjects in the sensory-enriched condition, significantly more subjects demonstrated both pivoting and crawling on the day of first occurrence than a single pattern, $\chi^2(1, N = 64) = 6.93, p < .01$, v = 0.33.

For first occurrence of walking, males in the sensory-deprived condition were the first to exhibit walking at P9, followed by females in the same environment exhibiting walking 1 day later on P10. Females in the sensory-enriched environment exhibited walking on P11, whereas males exhibited walking a day later on P12 (Figure 5).

Overall, the typical age of first occurrence does not appear to be dependent upon sex or testing condition. Subjects reached 70% criterion for each locomotor pattern within 1 to 3 days of the first sex/testing condition to reach criterion. Although pivoting and

crawling were shown at similar time points, walking emerged much later across all sex/testing conditions, as expected.

Emergence and Duration of Locomotion and Posture Behaviors From P1 to P15

Initial analyses included sex as an independent variable for measures of locomotion and posture. However, no significant sex effects were found, and thus data from males and females were collapsed for all remaining analyses.

Locomotion. A one-way repeated measure ANOVA for pivoting duration revealed a significant main effect of age, $F(14, 532) = 5.08, p < .001, \eta_p^2 = 0.118$. As shown in Figure 6A, pivoting duration fluctuated across the first 2 weeks of postnatal development. Subjects in both testing conditions exhibited high durations of pivoting at P1, which was the criterion age established for the majority of sex/testing conditions (males in the sensory-deprived condition reached criterion at P2), but significantly decreased at P3, $t(63) = 3.53, p < .001, d = 0.44, 95\%$ CI [3.65, 13.18], and remained low until P5. Pivoting duration significantly increased at P6, $t(55) = -2.78, p < .01, d = -0.37, 95\%$ CI [−3.50, −0.57], and increased again from P6 to P7, $t(55) = -2.17, p < .05, d = -0.29, 95\%$ CI [−4.93, −0.20]. After P7, pivoting duration decreased gradually and significantly from P12 to P13, $t(63) = 2.78, p < .01, d = 0.35, 95\%$ CI [0.46, 2.82]. There was not an effect of testing environment on pivoting duration. Because most subjects met criterion for pivoting on P1, it is not surprising that there were not significant effects of sex or testing condition on pivoting duration.

For crawling, there was a main effect of age, $F(14, 532) = 2.50, p < .01, \eta_p^2 = 0.06$. Crawling duration remained consistent from P1 to P3. This range of ages maps onto the typical age of first occurrence as established by the first occurrence criterion. Crawling duration significantly decreased at P4 compared with the crawling duration at P1, $t(55) = 2.88, p < .01, d = 0.38, 95\%$ CI [2.07, 11.60], as shown in Figure 6B. Following this decrease at P4, crawling duration increased significantly from P5 to P7, $t(63) = -2.13, p < .05, d = -0.27, 95\%$ CI [−3.98, −0.13] and continued to increase in duration from P7 to P9, $t(55) = -2.43, p < .05, d = -0.32, 95\%$ CI [−10.36, −0.99], until it significantly decreased from P10 to P13, $t(63) = 2.86, p < .01, d = 0.36, 95\%$ CI [3.08, 17.40] (Figure 6B). Again, there was not an effect of testing condition on crawling duration, which appears to follow the criterion data that crawling emerges within the first few days in each sex/testing condition.

For walking, a one-way repeated measure ANOVA revealed a main effect of age, $F(14, 532) = 32.16, p < .001, \eta_p^2 = 0.46$, and an interaction between age and condition, $F(14, 532) = 4.05, p < .001, \eta_p^2 = 0.10$ (Figure 6C). Subjects in the sensory-deprived condition exhibited significantly longer walking durations at P9 compared with P1, $t(27) = -2.40, p < .05, d = -0.45, 95\%$ CI [−32.62, −2.52], as did subjects in the sensory-enriched condition, $t(27) = -3.30, p < .01, d = -0.62, 95\%$ CI [−1.22, −0.29]; mean walking duration on P1 was zero. Subjects in the sensory-deprived condition approached a significant increase in walking duration at P8, $t(31) = -2.01, p = .053, d = -0.36, 95\%$ CI [−7.62, 0.06], whereas walking duration was still at zero for sensory-enriched subjects. Interestingly, only males in the sensory-deprived condition met criterion level of walking on P9. Subjects
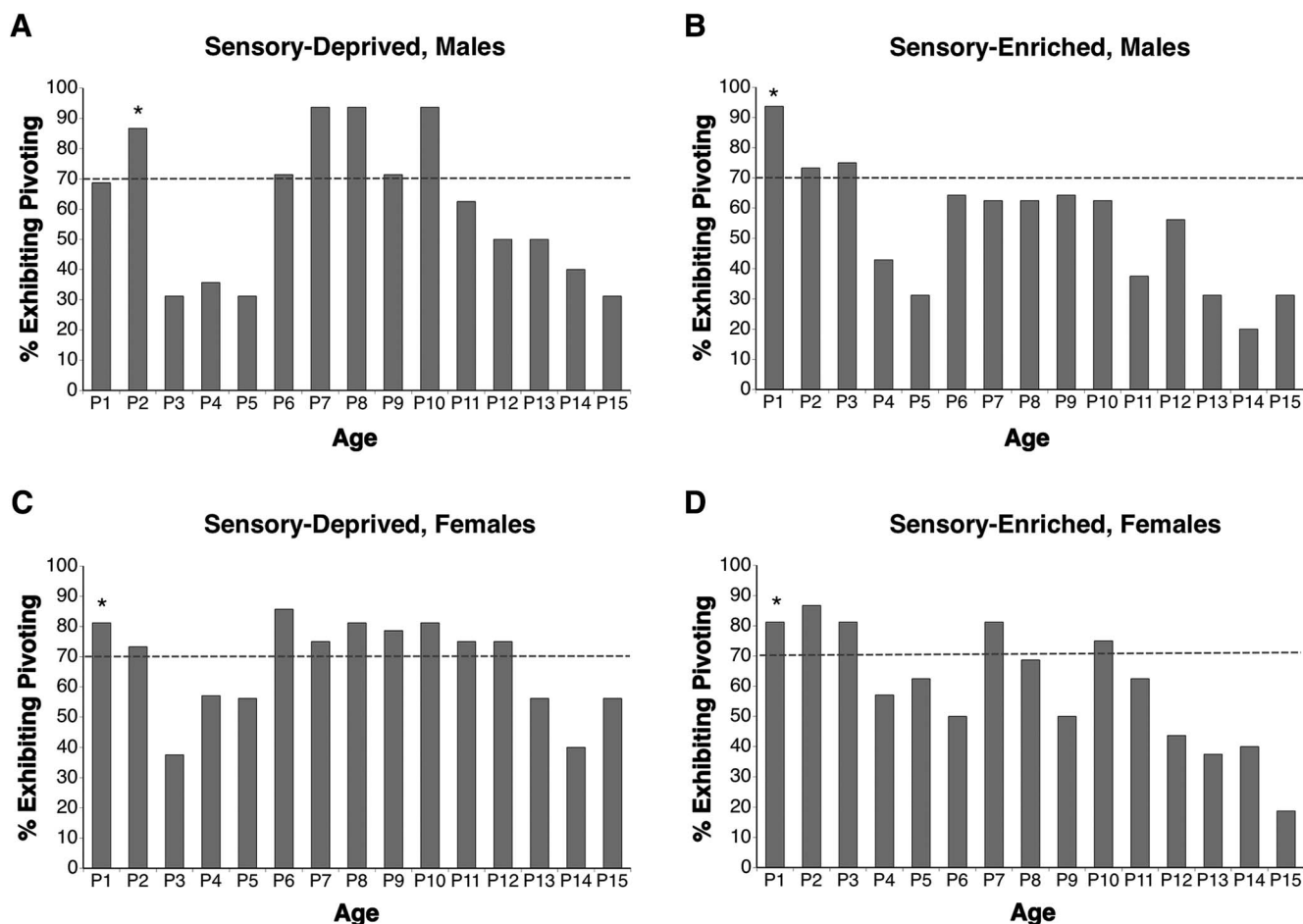
Figure 3. Percent of subjects exhibiting pivoting on Postnatal Day 1 to Postnatal Day 15 across each sex/testing condition. (A) Males in the sensory-deprived condition. (B) Males in the sensory-enriched condition. (C) Females in the sensory-deprived condition. (D) Females in the sensory-enriched condition. Bars represent mean percentages, asterisks represent first age of occurrence based on criterion, and the dashed line indicates 70% criterion.

in the sensory-deprived condition displayed increases in walking duration, significantly increasing in duration from P11 to P12, $t(31) = -3.12$, $p < .01$, $d = -0.55$, 95% CI [-75.30, -15.81], and then from P12 to P13, $t(31) = -2.21$, $p < .05$, $d = -0.39$, 95% CI [-61.90, -2.43]. They then began to decrease in walking duration from P14 to P15, $t(29) = 4.95$, $p < .001$, $d = 0.90$, 95% CI [34.09, 82.04]. Subjects in the sensory-enriched condition exhibited steady increases in walking duration from P9 to P10, $t(27) = -2.43$, $p < .05$, $d = -0.46$, 95% CI [-7.78, -0.66], P10 to P11, $t(31) = -3.28$, $p < .01$, $d = -0.58$, 95% CI [-18.06, -4.21], P11 to P12, $t(31) = -3.43$, $p < .01$, $d = -0.61$, 95% CI [-35.58, -9.02], P12 to P13, $t(31) = -4.82$, $p < .001$, $d = -0.85$, 95% CI [-47.64, -19.29], and P13 to P14, $t(29) = -2.78$, $p < .01$, $d = -0.51$, 95% CI [-34.07, -5.17], as can be seen in Figure 6C. Overall, subjects in the sensory-enriched condition showed shorter walking durations compared with subjects in the sensory-deprived condition at P11, $F(1, 62) = 12.46$, $p < .001$, $\eta_p^2 = 0.17$, 95% CI [22.75, 41.50]), P12, $F(1, 62) = 13.88$, $p < .001$, $\eta_p^2 = 0.18$, 95% CI [50.93, 81.18], P13, $F(1, 62) = 13.66$, $p < .001$, $\eta_p^2 = 0.18$, 95% CI [83.98,

113.76], and P14, $F(1, 58) = 7.23$, $p < .01$, $\eta_p^2 = 0.11$, 95% CI [97.05, 126.08].

Posture. Due to the low occurrence of some postural behaviors independent of locomotion (i.e., forelimb and hindlimb elevation and crawling stance), postural behavior was restricted to head elevation and walking stance for analysis. For head elevation, there was a significant main effect of age, $F(14, 532) = 22.08$, $p < .001$, $\eta_p^2 = 0.37$, and a significant interaction between age and condition, $F(14, 532) = 13.67$, $p < .001$, $\eta_p^2 = 0.27$. As shown in Figure 7A, subjects in the sensory-deprived condition exhibited longer head elevation durations compared with subjects in the sensory-enriched condition at P8, $F(1, 62) = 6.92$, $p < .05$, $\eta_p^2 = 0.10$, 95% CI [0.51, 2.23], P9, $F(1, 54) = 12.39$, $p < .001$, $\eta_p^2 = 0.19$, 95% CI [1.82, 5.76], P10, $F(1, 62) = 13.78$, $p < .001$, $\eta_p^2 = 0.18$, 95% CI [2.64, 7.97], P11, $F(1, 62) = 21.64$, $p < .001$, $\eta_p^2 = 0.26$, 95% CI [4.66, 10.56], P12, $F(1, 62) = 27.48$, $p < .001$, $\eta_p^2 = 0.31$, 95% CI [7.91, 14.24], P13, $F(1, 62) = 44.74$, $p < .001$, $\eta_p^2 = 0.42$, 95% CI [11.78, 20.02], P14, $F(1, 58) = 27.43$, $p < .001$, $\eta_p^2 = 0.32$, 95% CI [18.47, 35.09], and P15, $F(1, 62) = 45.48$, $p < .001$, $\eta_p^2 = 0.42$, 95% CI [20.93, 31.85]. Interestingly,
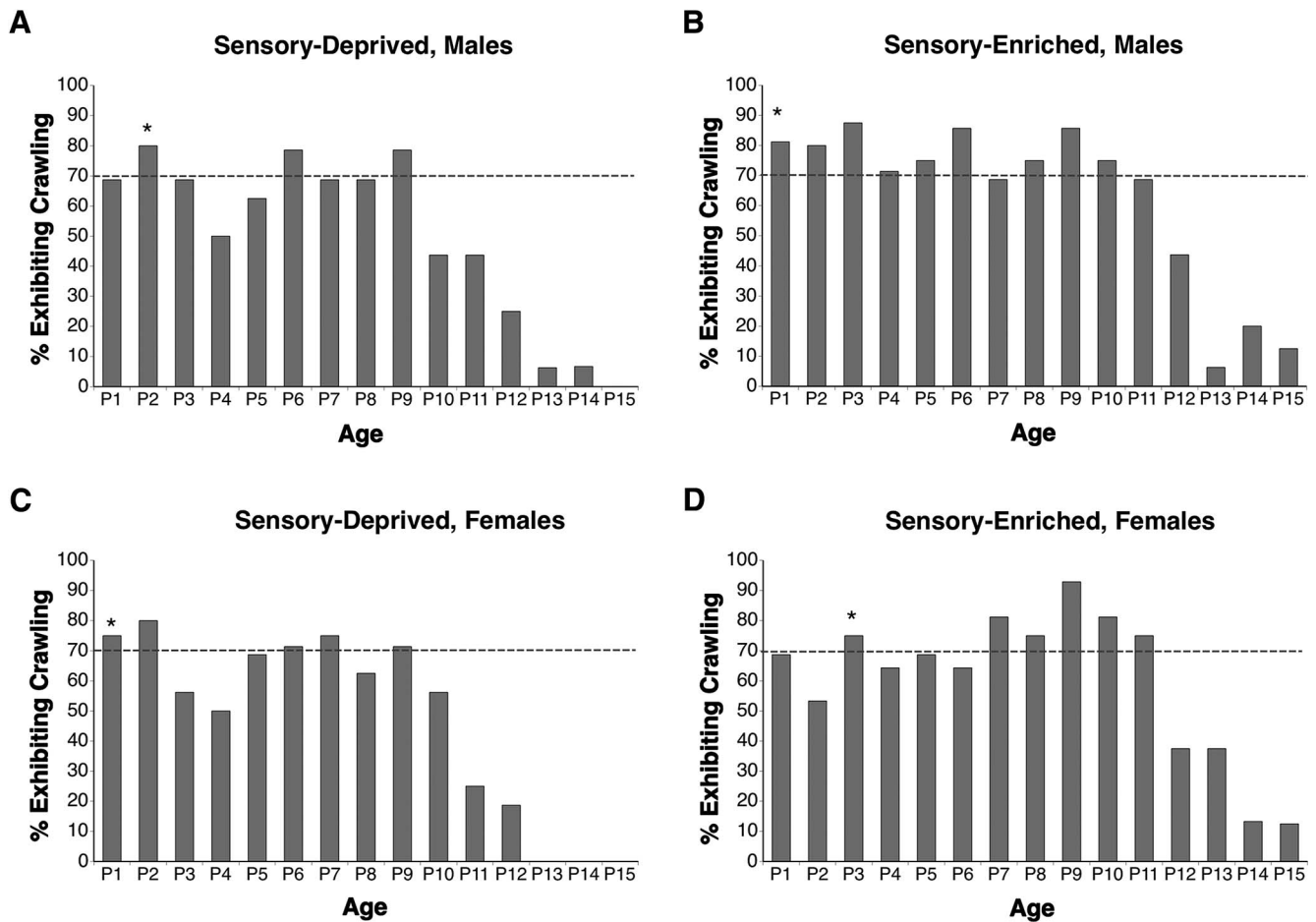
Figure 4. Percent of subjects exhibiting crawling on Postnatal Day 1 to Postnatal Day 15 across each sex/testing condition. (A) Males in the sensory-deprived condition. (B) Males in the sensory-enriched condition. (C) Females in the sensory-deprived condition. (D) Females in the sensory-enriched condition. Bars represent mean percentages, asterisks represent first age of occurrence based on criterion, and the dashed line indicates 70% criterion.

head elevation duration significantly increased from P1 to P8 in the sensory-enriched condition, $t(31) = 2.14$, $p < .05$, $d = 0.38$, 95% CI [0.04, 1.89], and increased again from P11 to P12, $t(31) = -2.36$, $p < .05$, $d = -0.42$, 95% CI [-3.76, -0.27], P13 to P14, $t(29) = -3.79$, $p < .001$, $d = -0.69$, 95% CI [-4.72, -1.41], and lastly from P14 to P15, $t(29) = -2.68$, $p < .05$, $d = -0.49$, 95% CI [-5.74, -0.77], as shown Figure 7A. Although subjects in the sensory-deprived condition showed relatively longer durations of head elevation, there was no significant increase in head elevation duration until P9, $t(27) = -3.07$, $p < .01$, $d = -0.58$, 95% CI [-10.41, -2.06]. Following this increase at P9, head elevation duration increased from P12 to P13, $t(31) = -2.23$, $p < .05$, $d = -0.39$, 95% CI [-19.72, -0.90], and again from P13 to P14, $t(29) = -2.44$, $p < .05$, $d = -0.45$, 95% CI [-34.70, -3.08].

A one-way repeated measure ANOVA for walking stance duration revealed a main effect of age, $F(14, 532) = 4.76$, $p < .001$, $\eta_p^2 = 0.11$, and an interaction of age and condition $F(14, 532) = 2.75$, $p < .001$, $\eta_p^2 = 0.07$. For subjects in both testing conditions, walking stance duration remained at or around zero until P9 and

then began to increase in duration. Subjects in the sensory-deprived condition showed significantly longer walking stance durations at P12 compared with durations at P9, $t(27) = -2.64$, $p < .05$, $d = -0.50$, 95% CI [-7.73, -0.96], with durations remaining fairly consistent from P12 to P15 (Figure 7B). Subjects in the sensory-enriched condition showed increases in walking stance duration from P10 to P12, $t(31) = -2.23$, $p < .05$, $d = -0.39$, 95% CI [-2.39, -0.11], but decreases in duration from P13 to P14, $t(29) = 2.29$, $p < .05$, $d = 0.42$, 95% CI [0.10, 1.76]. Although at most ages, subjects in both conditions showed similar walking stance durations, subjects in the sensory-deprived condition exhibited longer durations of walking stance compared with those in the sensory-enriched condition on P11, $F(1, 62) = 5.76$, $p < .05$, $\eta_p^2 = 0.09$, 95% CI [0.39, 2.28], and P14, $F(1, 58) = 7.57$, $p < .01$, $\eta_p^2 = 0.12$, 95% CI [1.20, 4.69].

Discussion

The purpose of this study was to provide improved normative data on the development of locomotion and posture in immature

Figure 5. Percent of subjects exhibiting walking on Postnatal Day 1 to Postnatal Day 15 across each sex/testing condition. (A) Males in the sensory-deprived condition. (B) Males in the sensory-enriched condition. (C) Females in the sensory-deprived condition. (D) Females in the sensory-enriched condition. Bars represent mean percentages, asterisks represent first age of occurrence based on criterion, and the dashed line indicates 70% criterion.

rats during the first 2 postnatal weeks, as well as to examine how sensory stimulation may influence locomotion and posture expression during development. To this end, rat pups were tested on a daily basis from P1 to P15 in a sensory-deprived or a sensory-enriched environment, controlling ambient temperature and statistically examining potential sex differences.

Altman and Sudarshan (1975) reported that pivoting was shown first by immature rats, followed by the emergence of crawling and walking, respectively. Thus, we expected to find that more immature postures, such as head elevation, would emerge before more mature postures, such as the elevation of the limbs and pelvis (e.g., crawling and walking stance). In general, we did find that more immature locomotion and posture was seen before more mature locomotion and posture. Males and females in both testing conditions demonstrated both pivoting and crawling on or around P1, which was before walking. However, we did not find that pivoting necessarily emerged first followed by crawling but rather that the rat pups were capable of exhibiting both behaviors at an early age. We did find that walking emerged well after the animals were

pivoting and crawling, with pups showing walking on or around P10. Head elevation also emerged prior to walking stance in some of the subjects (males and females in the sensory-deprived condition), suggesting that head elevation emerges prior to the more mature walking stance.

We hypothesized that increased sensory stimulation would evoke earlier locomotion and posture such that subjects in the sensory-enriched condition would demonstrate locomotion and posture earlier than subjects in the sensory-deprived condition. The sensory-enriched condition, in theory, should have provided behavioral activation effects but not necessarily influenced directionality of locomotion trajectory, as well as offered biomechanical support through siblings. Interestingly, we did not find this to be the case. In fact, we actually found that subjects in the sensory-deprived condition showed longer durations of head elevation, walking stance, and walking compared with subjects in the sensory-enriched condition. It is possible that the sensory-enriched environment was overly comfortable for the animals. Sibling contact, in addition to nest material, and warm ambient temperatures
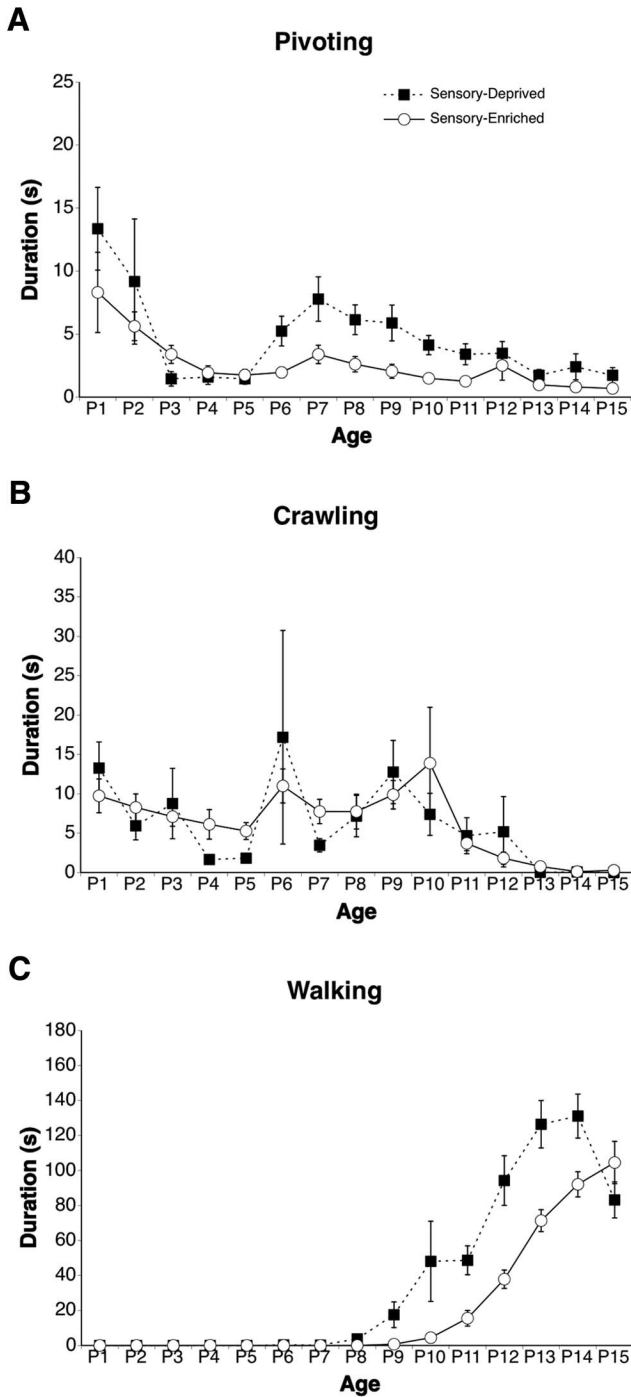
A

Pivoting

B

Crawling

C

Walking

Figure 6. Duration of locomotion over the first 2 postnatal weeks, as a function of testing condition. (A) Pivoting duration. (B) Crawling duration. (C) Walking duration. Points represent means, vertical lines represent standard error of the means.

rial. Therefore, pups in the sensory-enriched condition may have been relatively warmer than pups in the sensory-deprived condition, as indicated by significantly warmer nest temperatures. In addition, although we hypothesized that the sensory-enriched condition would provide activation through chemosensory cues and biomechanical support, it could be that these were not sufficient replications of the sensory cues found in the home nest, thus, influencing the differences in behavior across the sensory conditions. Importantly, we are not able to separate the stimuli in the nest to determine if behavior was the result of activating effects or biomechanical stimulation, but future studies could begin to tease apart the variables to further elucidate our understanding of the role of the nest on locomotion and posture development.

There are a few possibilities that may have led to the differences observed between the two testing conditions. The first is that rats are thigmotactic animals and tend to seek out vertical surfaces. In open-field environments, such as the sensory-deprived environment in the current study, rats spend more time in areas that are surrounded with the largest number of walls (Lamprea, Cardenas, Setem, & Morato, 2008). Onset of walking occurred prior to eye opening in the animals, and thus longer durations of head elevation
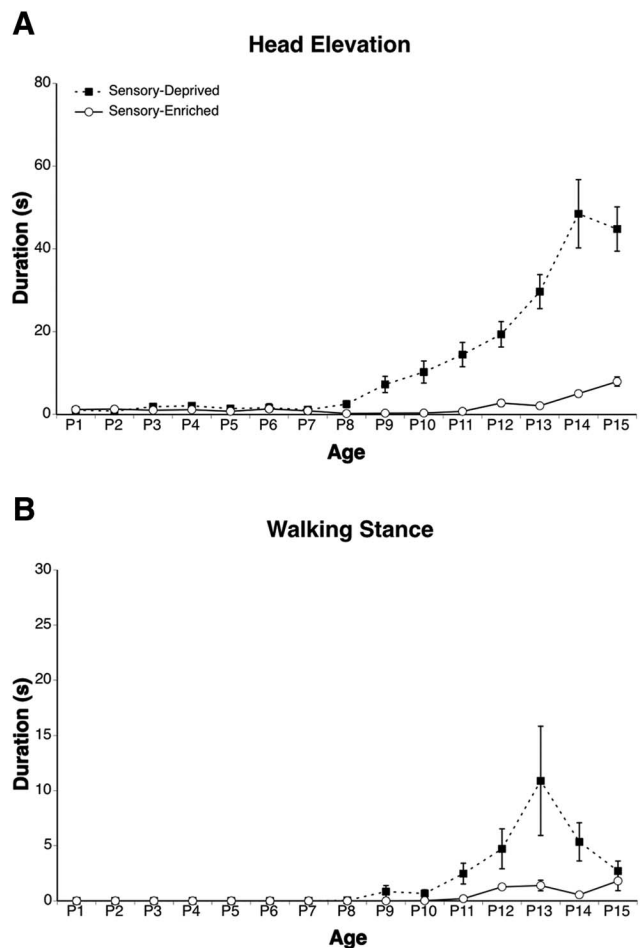
A

Head Elevation

B

Walking Stance

Figure 7. Duration of posture during the first two postnatal weeks. (A) Head elevation duration. (B) Walking stance duration. Points represent means, and vertical lines represent standard error of the means.

may have created an environment that limited the need for animals to move around, as indicated by lower activity durations. However, in the sensory-deprived condition, animals were placed on a hard, Plexiglas surface without additional warmth (but they were at thermoneutral temperatures) through sibling contact or nest mate-

and walking in the sensory-deprived condition could suggest a trial-and-error search for areas that were surrounded by walls (e.g., corners of the square testing environment) and, later, when eyes were open, visually seeking areas that were surrounded by walls. Another possibility is that the Plexiglas surface was an aversive tactile stimulus, and the animals were engaged in exploratory or searching behavior to find a different surface, such as bedding or nest material, to eliminate the sensory feedback received from the stiff substrate. Prior research has shown that air-stepping P1 rats show decreased contact with a Plexiglas floor compared with an elastic floor when a substrate is placed beneath their paws (Brumley, Roberto, & Strain, 2012), suggesting that here we may be seeing a sort of avoidance response on the box floor as well. However, in the sensory-deprived condition, the animal is unable to find a different substrate and continues seeking out a different substrate. In the nest environment of the home cage, the surface of the cage is a stiff substrate; however, if a pup touches the bottom of the cage, by moving around the cage, then they will contact bedding or nest material, other siblings, or the nest. In the sensory-deprived testing condition, these alternate substrates were not available. Furthermore, we did not increase the box size based on the number of pups, thus a limiting factor for increased movement in the sensory-enriched condition could be that there was decreased available footage for the pups to engaging in different patterns of locomotor behavior. Future studies could examine available footage and the effects on motor behavior to determine if this is a significant limiting factor. Lastly, adult rats demonstrate exploratory behavior in response to novel environments (Eilam & Golani, 1989; Whishaw, Gharbawie, Clark, & Lehmann, 2006). This behavior has been described as alternating forward locomotion and stopping, where the animal pauses briefly throughout the novel environment and then also pauses for longer periods of time in areas of the novel environment (Eilam & Golani, 1989). Therefore it may be that subjects in the sensory-deprived condition engaged in exploratory behavior, as they did not have familiar cues and experienced a relatively more novel environment. Subjects in the sensory-enriched condition were surrounded by familiar sensory experiences; therefore the testing condition was not as novel an environment and may not have elicited exploratory behavior.

We also expected that controlling ambient temperature would result in earlier locomotion and posture behavior across conditions, when compared with previous studies. In general, we found this to be the case (Table 3). Males and females in each testing condition demonstrated spontaneous pivoting and crawling earlier than previously reported by at least 1 to 3 days (Altman & Sudarshan,

1975; Bolles & Woods, 1964; Geisler et al., 1993; Heyser, 2003; Westerga & Gramsbergen, 1990). Head elevation and walking emerged prior to the age reported by Altman and Sudarshan (1975) by 4 days and 2 days, respectively. However, this age of first emergence was not earlier than the age reported by other researchers (Bolles & Woods, 1964; Geisler et al., 1993; Westerga & Gramsbergen, 1993b). Interestingly, although the 70% criterion for walking was not met until around P10, 59.7% of subjects displayed walking during the first week of postnatal development, with 7.1% of subjects displaying walking as early as P4. As far as we are aware, this is the earliest reported occurrence of spontaneous walking in the first week of rat postnatal development.

Although we controlled temperature, there are additional factors beyond temperature that may contribute to the similarities and differences in age of first occurrence across studies (Table 3). For instance, the material of the testing surface may influence the behavior exhibited by pups, and researchers across studies have used different surface materials. Altman and Sudarshan (1975) used a wooden surface, Bolles and Woods (1964) tested their subjects on both shredded newspaper and Sani-cel, and we, as well as others (Geisler et al., 1993; Westerga & Gramsbergen, 1990), used a Plexiglas surface (albeit the surface was covered with bedding and nest material in our sensory-enriched condition). Altman and Sudarshan (1975) reported that during the third week of postnatal development, there was a heightened increase in locomotion that was seen on a rough surface, not a slippery surface. However, the Plexiglas used in this study would be considered a slippery surface, and we did not find that there was reduced locomotion on the Plexiglas surface. Another possible contributing variable is rat strain used for testing. Altman and Sudarshan (1975) tested Purdue–Wistar rats, Westerga and Gramsbergen (1990, 1993b) used Hooded Wistar strain rats, and the present study, as well as that by Bolles and Woods (1964), used Sprague–Dawley rats. Differences may also arise from variations in the definitions of patterns of locomotion.

While there are some discrepancies across the studies in age of first emergence of locomotor patterns, the majority of researchers indicate that locomotion and posture do not develop in a strict linear fashion, with pivoting not disappearing as crawling appears, crawling not disappearing as walking emerges, and so forth; instead, these behavioral patterns overlap with a clear and significant change in movement occurring at or around P15 (Altman & Sudarshan, 1975; Geisler et al., 1993; Westerga & Gramsbergen, 1990; Westerga & Gramsbergen, 1993b). Specifically, Altman and Sudarshan (1975) reported that pivoting continues to occur from

Table 3

Differences in Onset of Locomotion Between Current and Past Studies

Study	Pivoting	Crawling	Head elevation	Walking
Present Study	**P1**	**P1**	**P4**[*]	**P10**[*]
Altman & Sudarshan (1975)	P4–P5	P8	P8	P12
Bolles & Woods (1964)	Not included	P3	P4[*]	P10[*]
Westerga & Gramsbergen (1990)	Not included	Middle of second week	Not included	P11
Geisler, Westerga, and Gramsbergen (1993)	Not included	P5	P5	P10[*]
Westerga & Gramsbergen (1993b)	Not included	Not included	Not included	P10[*]
Heyser (2003)	P7	P11	P12	P16

Note. Boldface indicates the earliest occurrence as reported in our study. P = postnatal day.
[*] overlap with previous studies on age of earliest occurrence.

P4/P5 to P15, though pups decrease the amount of pivoting that occurs over development. Geisler and colleagues (1993) reported a similar finding with crawling; crawling occurred on P3 and decreased until it stopped around P9.

If rat pups are capable of showing more advanced locomotion, such as walking earlier on, then why do they continue to use pivoting and crawling (considered more immature forms of locomotion) to move around the environment when they could be walking? Remember that the neural mechanisms involved in locomotion begin developing prenatally. However, the execution of locomotion requires a dynamic interplay of various systems and continued maturation of neural systems, postural control, and the musculoskeletal system during the first 2 postnatal weeks, which very likely influences the behavior of these animals. Previous researchers (Westerga & Gramsbergen, 1990; Westerga & Gramsbergen, 1993a; Westerga & Gramsbergen, 1993b) have indicated that locomotion undergoes a sudden change on P15, characterized by an increase in speed and coordination, suggesting that these systems reach a level of maturation that allows the animal to exhibit adult-like coordination that was not there before P15.

Neurons in the spinal cord produce the basic locomotor rhythm, which can be modulated by sensory input (Grillner et al., 2007). However, it is descending pathways from the brainstem that influence more advanced motor control such as steering, command, and execution of motor programs (Grillner, Hellgren, Ménard, Saitoh, & Wikström, 2005; Hikosaka, 2007; Orlovsky, Deliagina, & Grillner, 1999), by incorporating information from sensory systems (i.e., visual and vestibular). As these pathways are maturing, animals are learning to incorporate multiple levels of information and execute coordinated action patterns. For instance, activation of specific motor programs occurs in the basal ganglia, influencing activation of reticulospinal neurons, which in turn regulate spinal activity. Thus, even though an animal might be capable of walking, input from these descending pathways to spinal centers might influence the selection of pivoting or crawling over walking. Another possible contributing factor is neuromuscular development and its role in posture. Postural control is an important component of locomotion. Although immature patterns of locomotion might require less postural control (say pivoting, which requires head elevation), more mature locomotor patterns typically require more postural control (say walking, which requires head elevation and limb elevation). In addition, regression of polyneural innervation of muscles in the rat (Geisler et al., 1993; Westerga & Gramsbergen, 1993a), reorganization of dendrite bundles in muscles (e.g., soleus muscle; IJkema-Paassen & Gramsbergen, 2005), and formation of dendrite bundles in the spinal cord (Westerga & Gramsbergen, 1990) occur during the transition to adult-like coordination seen on P15. As these changes take place, they likely influence the pattern of locomotion exhibited by animals. However, until these transitions are complete, animals apparently have the capability to exhibit walking but may not engage in this pattern for long durations, as they still do not have the postural stability. In a prior study examining serotonergic stimulation of locomotion in P1 rats (Swann et al., 2016), it was found that quipazine-treated rat pups exhibited higher frequencies of locomotion, but not longer durations, suggesting that longer durations of walking, or even crawling, require substantial postural control, which is not fully developed until around P15.

Another aim of the study was to examine potential sex differences in locomotion and posture, as previous researchers had included both males and females but did not examine if their developmental trajectories differed. Although we found some sex differences in regard to body mass, these were not consistent across the study period. In regard to locomotion and posture development, we did not find any sex differences. Our hypothesis was centered on research examining sex differences in maternal–infant interactions, where sex differences do exist (Moore & Chadwick-Dias, 1986). Although we expected that increased experience with the extension and flexion of hindlimbs via increased LER expression in males might potentially influence locomotion trajectories, it is much more likely that the limiting factor in exhibiting locomotion is the lack of postural control, as well as the necessary maturation of neuromuscular systems. It is not known if there are sex differences in the maturation of descending pathways that influence motor control or initiation of locomotion. The lack of sex differences in behavior reported here may inform future studies examining posture and locomotion mechanisms during the early postnatal period in rats.

Limitations

Although the sensory-enriched testing environment provided a novel component to the current study, we also found that this condition was a limitation to some degree. If the nest provided increased sensory stimulation, which evokes locomotion, then why would these animals show such low durations of activity in the sensory-enriched condition? Rat pups, in the nest, will demonstrate huddling behavior, to conserve body warmth (Alberts, 2007); in addition, the nest material serves as a buffer to prevent heat loss from the nest (Harshaw et al., 2017). Huddling behavior was the most typical behavior that we saw within the sensory-enriched condition, despite controlling ambient temperature. In fact, the temperature of the nest was warmer than the ambient temperature for most testing days. Harshaw and colleagues (2017) suggest that in addition to thermoregulation, BAT thermogenesis also serves social purposes and could serve as a stimulus for other littermates. Warmth is a strong, positive reinforcer for immature rats, as rat pups will demonstrate learned head turning responses to thermal (heat) stimuli (Flory, Langley, Pfister, & Alberts, 1997). In addition to BAT thermogenesis, oxytocin is also involved with thermoregulation, and in fact, the release of oxytocin influences BAT thermogenesis, such that oxytocin-negative mice were incapable of maintaining their body temperature (Kasahara et al., 2013). Oxytocin also has been linked to social interactions, including huddling in rat pups following skin-to-skin contact with the dam (Kojima, Stewart, Demas, & Alberts, 2012). Given that the animals were with the dam immediately prior to testing, elevated oxytocin levels could have led to increased preference for sibling contact through huddling, thus decreasing locomotion duration during testing. In fact, although there were not significant differences, we did see increased durations of locomotion, that is, crawling/walking, once the animals were older and more capable of independent thermoregulation. Overall, the increased warmth of siblings in the sensory-enriched condition, as well as possible release of oxytocin, may have influenced the pups' responses during testing and could explain why more movement is seen in the home cage when pups need to locate the dam and/or siblings.

Another limitation and general concern is in regards to quantifying and describing locomotion and postural patterns of behavior. There is quite a bit of variability in the locomotor patterns and postural stances of these animals, such that there is a level of subjectivity when, for example, a researcher is deciding that the belly is off the surface, and therefore, the subject is walking, but the hindlimbs are passive, and thus it could also be considered crawling. It is of absolute importance that in addition to quantifying behavioral patterns, we also qualitatively describe the behavioral patterns as precisely as possible. Future studies could center on classifying and describing different patterns and variability in pivoting, crawling, and walking, which would help researchers to better describe what behaviors animals are doing. This could lead to improved understanding of how experimental manipulations may influence locomotor and posture development (e.g., is posture, interlimb, or intralimb coordination affected by the manipulation?). There is utility in developing clear definitions of locomotion patterns. For instance, if a researcher is examining the effects of a neuroprotective drug on coordination in spinal-transected rat pups, it would be important to understand if the animal continues to use an immature form of crawling (belly on surface and passive hindlimbs) or if the animal switches to a more complex form of crawling, with active hindlimbs pushing the animal forward, as an indicator of recovery. Standardized assessments such as the Basso, Beattie, and Bresnhan Locomotor Rating Scale (Basso, Beattie, & Bresnahan, 1995) exist for adult animals, but they have not been validated for use in immature animals.

Conclusions

The current study sought to further understanding of normative locomotion and posture development in male and female rats across the first 2 postnatal weeks, while controlling temperature and examining differences in testing conditions. It was found that rat pups were capable of exhibiting pivoting and crawling as early as 24 hr following birth, regardless of sensory stimulation present in the testing environment, suggesting that testing animals at warmer ambient temperatures alleviates physiological stress on the animal that might prevent locomotion. Spontaneous pivoting and crawling behavior occurred at least 1 to 3 days earlier than previous studies. Quadrupedal walking occurred as early as P4, but was not reliably expressed until about P10/11. There were no sex differences in the duration of locomotion and posture behaviors. It is important to fully understand the developmental trajectory of locomotion and posture behavior, particularly as animal models are utilized to draw parallels with human pediatric research. Experimental manipulations, such as treadmill training or genetic manipulations, that are used to improve or induce behaviors at earlier time points, depend upon having accurate ages of emergence of behaviors to be able to have confidence that their manipulation is actually influencing motor behavior and development.

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