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Locomotion And Posture Development In Immature Rats:

Comparison Of Sensory-Enriched Versus Sensory-Deprived Testing Environments

Hillary Erin Swann

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A thesis

submitted in partial fulfillment

of the requirements for the degree of

Master of Science in the Department of Psychology

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Committee Approval

To the Graduate Faculty,

The members of the committee appointed to examine the thesis of Hillary Erin Swann find it satisfactory and recommend that it be accepted.

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September 4, 2012

Michele Brumley, PhD
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RE: Your application dated 9/1/2012 regarding study number 699: Modulation of Action Patterns in Developing Rats

Dear Dr. Brumley:

Thank you for your response to requests from a prior review of your application for the new study listed above.

This is to confirm that your application is now fully approved. The protocol is approved through 9/4/2015 with an annual review at 9/4/2013.

You are granted permission to conduct your study as most recently described effective immediately. The study is subject to continuing review on or before 9/4/2013, unless closed before that date.

Please note that any changes to the study as approved must be promptly reported and approved. Some changes may be approved by expedited review; others require full board review. Contact Patricia Hunter (208-282-2179; fax 208-282-4529; email: anmlcare@isu.edu) if you have any questions or require further information.

Sincerely,

Curt Anderson, PhD
IACUC Chair

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Abstract

Research examining the neurobehavioral development or manipulation of locomotion in rats bases their time points of the emergence of locomotion on a study conducted over 40 years ago by Altman and Sudarshan (1975). However, while the study provided essential normative data, it is not without its flaws. This study sought to provide updated normative data on rat locomotion and posture development in a sensory-enriched and sensory-deprived testing environment. Rat pups were tested daily from postnatal day 1 (P1) to P15. Pups in the sensory-deprived condition were placed in a square, Plexiglas box for a 20-min test period. Pups in the sensory-enriched condition were placed in the same box with 3 siblings and bedding from the home cage to provide sensory stimulation. Overall, development of locomotion and posture did not vary across testing environment. However, pups did demonstrate pivoting and crawling 1 to 3 days earlier than previously reported.

Locomotion and Posture Development in Immature Rats:

Comparison of Sensory-Enriched and Sensory-Deprived Environments

Locomotion involves coordination between motor, sensory, neurotransmitter, and other physiological systems. Rats serve as an excellent model system to investigate the development and mechanisms of locomotion. As with all mammals, the neural mechanisms supporting locomotion begin developing *in utero* in rats (Bekoff & Lau, 1980; Brumley & Robinson, 2005) and continue developing postnatally with further maturation of postural mechanisms (Vinay et al., 2002). Nearly every research article that has examined the developmental trajectory of locomotion in rats, or how this trajectory may be affected by experimental manipulations, base their developmental time points of the emergence of locomotion on studies conducted over 40 years ago, preeminently, the study conducted by Altman and Sudarshan (1975). In fact, according to Google Scholar, over 700 articles examining or manipulating the neurobehavioral development of locomotion in rats cites Altman and Sudarshan (1975). In that paper, the age of the first occurrence of different forms of locomotion (immature patterns such as pivoting, forms of crawling, etc.) in rats are reported in an open field testing environment. While that paper has provided important, normative information on rat locomotion, it is not without flaws. For example, newborn rats were tested at room temperature. Because newborn rats are not very effective at independent thermoregulation and room temperature is cold for a newborn rat, cardiac and respiratory function becomes compromised and thus has the very likely potential to interfere with locomotion (Sokoloff & Blumberg, 1997; Blumberg, Sokoloff, & Kirby, 1997).

The aim of the current study was to a.) provide improved normative data on rat

locomotion and posture development in sensory-deprived conditions and b.) examine locomotion and posture development in a more natural environment that was enriched with sensory stimulation. In addition to testing pups at thermoneutral temperatures, this study examined sex differences in posture and locomotion, and provides much more quantitative information on open-field locomotion than the original Altman and Sudarshan (1975) study. Furthermore, because newborn rats typically develop in the context of a litter and nest, locomotion and posture development was examined in a nest-like environment. Anecdotal observations suggest that locomotion may be expressed earlier in a nest setting (compared to open-field), as pups move and respond to other pups in the litter and may also use other pups to facilitate postural support. To our knowledge, this is the first study to examine the development of locomotion and posture in rats in a nest-like setting[1].

In the following sections, a literature review of the development of locomotion and posture in rats is discussed, followed by problems that can be found in past literature conducted on locomotion and posture development, including discrepancies between studies, the importance of thermoregulation in rats, as well as examining sex differences.. Additionally, the importance of the nest environment in the development of locomotion behavior is discussed. Lastly, the purpose, methodology, findings, and discussion of said findings are discussed.

Development of Locomotion and Posture in the Rat

Movement in the rat begins prior to birth, with coordinated movements first being exhibited *in utero*. Following birth, coordinated behavior gradually increases as the pup

[1] Bolles and Woods (1964) observed locomotion in the home cage from a distance (3 to 4 feet), as well as in a separate testing apparatus, but pooled the data from the different testing conditions and did not run quantitative analyses.

develops with more complex locomotion shown. In the following section, the behavioral developmental of locomotion and posture, as well as the physiological mechanisms involved in locomotion, are discussed.

Behavioral Development of Locomotion

Research has shown that locomotor-like activity can be expressed in the *in vivo* rat fetus. The *in vivo* fetal rat preparation involves anesthetizing the pregnant female from the waist down, placing her body in a warm water bath, making an incision in the abdomen, and gently externalizing the uterus into the water bath (Smotherman & Robinson, 1991). Then fetal behavior is observed and recorded while the fetus remains attached to the placenta and umbilical cord. Findings from these studies have found that spontaneous body and limb movements first emerge at embryonic day 16 (E16) and peak between E19-E21, becoming increasingly coordinated as the day of birth (E22) approaches (Kleven, Lane, & Robinson, 2004; Smotherman & Robinson, 1986; Robinson, Kleven, & Brumley, 2008). Bekoff and Lau (1980) found that fetal rats showed brief bouts of spontaneous alternated interlimb coordination in the forelimbs and hindlimbs one day prior to birth. However, intraperitoneal injection of quipazine, which is a serotonergic receptor agonist, has been shown to evoke more robust and sustained periods of alternated stepping in the rat fetus on E20 (Brumley & Robinson, 2005).

The postnatal emergence of spontaneous locomotion and posture has been established in a seminal paper on the development of locomotion and posture in rats conducted by Altman and Sudarshan (1975). Given that most of the literature on the development of locomotion and posture in rodents cites that original study, a brief review of the emergence of these patterns will be described using the time points established by

Altman and Sudarshan.

Altman and Sudarshan (1975) examined posture and locomotion development in 1,292 Purdue-Wistar rats starting on the day of birth (P0; postnatal day 0). They tested pups daily, for 3 weeks, on an enclosed wooden surface for a 3-minute observation period. Elevation of the head, shoulders, forelimbs, hindlimbs, and the pelvis were reported as indicators of posture. Pivoting, crawling, and walking were reported as indicators of locomotion. Their findings show that pups exhibit shoulder elevation on occasion beginning at P1 (postnatal day 1; 24 hrs after birth), but this is not seen in longer bouts until the end of the first postnatal week when the shoulders are elevated about 50% of the time. Elevation of the forelimbs is directly related to elevation of the shoulders. As the forelimbs are elevated, so are the shoulders. Forelimb elevation starts with the limbs directly under the body and develop until a more distal position from the midline of the body emerges at P7-P8. The ability to support the front portion of the body is present by the end of the first postnatal week; however, the hindlimbs do not exhibit this same maturation. In fact, the hindlimbs are not able to support the weight of the pelvis until P10. According to Altman and Sudarshan (1975), there is a rapid increase in the rate of maturation in the hindlimbs during the second week. At P10-P12, the hindlimbs begin to show elevation and this continues to develop until the entire body is supported and elevated by P15. Head elevation occurs at P8 for short periods of time and gradually these bouts are prolonged. By the end of the third week, rats show head elevation for the entire 3-minute observation period.

As postural control is developing, so is more complex locomotion. The first locomotion pattern to be expressed spontaneously is pivoting at P4-P5. During pivoting,

the forelimbs serve as paddles that propel the animal in a circular pattern while the hindlimbs remain inactive and the pelvis remains anchored on the surface. By the end of the first postnatal week, rat pups engage in pivoting behavior consistently. On P8, the first evidence of crawling behavior is observed. Crawling involves the forelimbs propelling the animal across the surface; however, the hindlimbs are unable to exhibit similar limb coordination and therefore are often dragged behind the animal. On P10-P11, pups exhibit greater control over all limbs and the hindlimbs assist with body propulsion and provide greater traction. This form of locomotion is very immature walking and lacks adult-like coordination. Pups are unable to sustain this immature form of walking for extended bouts until P12-P13 when walking becomes the dominant means of locomotion. While the animal is still not completely coordinated in its movements, the pattern becomes more adult-like once the eyes open at P14-P15. It is at this age that walking becomes more coordinated and more adult-like in appearance.

Physiological Mechanisms of Locomotion

The isolated fetal spinal cord is often used to study the neural mechanisms of locomotion before birth in the rat. Otsuka and Konishi (1974) developed an *in vitro* spinal cord preparation in which the spinal cord is carefully dissected and placed in a bath chamber. Electrodes measure activity from the ventral roots of the spinal cord, which are composed of motor neuron axons that typically would innervate muscle tissue in a live animal. Studies have found that the spinal cord remains functional at variable temperatures, even to extreme temperature ranges; however, optimal temperature for recording and analyzing is between 20-30°C (Sqalli-Houssaini, Cazalets, Fabre, & Clarac, 1991). To evoke locomotion-like activity from the spinal cord, pharmacological agents

are applied to the bath solution bathing the spinal cord. For example, application of NMDA, serotonin, acetylcholine and dopamine have been shown to evoke fictive locomotion from the isolated fetal spinal cord (Cazalets, Grillner, Menard, Cremieux, & Clarac, 1990; Cazalets, Borde, & Clarac, 1996; Kiehn & Kjaerulff, 1996; Iizuka, Nishimaru, & Kudo, 1998; Cowley & Schmidt, 1994a, 1994b). Fictive locomotion is the term used to describe the pattern of alternating ventral root activity between (and within) sides of the spinal cord, which corresponds to alternated activation of limb muscles (i.e., alternating extension and flexion) during locomotion. It is believed that locomotion, and thus fictive locomotion, is produced by central pattern generating (CPG) circuits located within the cervical and thoracolumbar sections of the spinal cord, producing forelimb and hindlimb alternation, respectively.

Studies using the *in vitro* preparation of the fetal rat spinal cord have shown that locomotion CPGs demonstrate transitions in locomotion pattern output across prenatal development (Kudo, Nishimaru, & Nakayama, 2004). Pharmacological activation of the isolated spinal cord using serotonin on E18.5 induces fictive locomotion with an alternating pattern of ventral root activity, while only synchronous activity is found at E14.5-E.16.5, suggesting that overall there is a transition from synchronous to alternated locomotion-like activity closer to the end of the prenatal period (Iizuka et al., 1998; Nakayama, Nishimaru, & Kudo, 2001; Greer, Smith, & Feldman, 1992). This transition from synchronous to alternated fictive locomotion also has been reported to occur following application of NMDA receptor agonists to the bath solution (Ozaki, Yamada, Nishimaru, & Kudo, 1996). Overall, this research has shown that the neural generation and coordination of locomotion-like activity is functional during the late prenatal period.

The Intersection Between Behavior and Physiology

One limiting factor that prevents adult-like locomotion from occurring earlier is posture. Although research has shown that CPGs are functional at birth, motor neurons innervating the limb muscles develop at different rates, affecting the rate at which posture, and therefore locomotion, develop (Vinay, Brocard, & Clarac, 2000). Vinay, Brocard, and Clarac (2000) found that extensor motor neurons are less developed than flexor motor neurons during the first postnatal week. Immature extensor motor neurons prevent the animal from sustaining extended limb positions rather than flexed limb positions. This contributes largely to the lack of walking behavior, which requires extension and flexion of the limbs. Likewise, development also occurs at the level of muscle activity. Westerga and Gramsbergen (1993a) examined the development of the tibialis anterior (TA) and medial gastrocnemius (GM) from the onset of walking (reported as P10) until P42 (adult animals) using electromyogram (EMG) activity. Their findings showed that muscle activity started out low and irregular, but advanced to a more synchronous pattern with increasing levels of activity at P15. Adult-like activity in the muscles is not seen until around P16, which matches when there is a rapid transition in walking gait and stability in animals. Thus, these findings support the suggestion that lack of coordinated locomotion may be attributed largely to a lack of postural stability and poor muscle control.

Additional evidence for posture limiting locomotion during early development comes from research using the air-stepping paradigm. In this procedure, immature animals are securely suspended off the floor in a sling so that they can exhibit limb activity without the restrictions of gravitational forces, weak muscles, and poor postural

control. Previous studies in our lab and others have demonstrated that air-stepping, defined as the limbs moving in alternated, locomotion-like patterns in the air, can be induced in the neonatal rat through activation of the serotonin system and/or dopamine systems (Brumley & Robinson, 2005; Brumley, Roberto, & Strain, 2012; Van Hartesveldt, Sickles, Porter, & Stehouwer, 1991). To evoke air-stepping in rodents with serotonergic stimulation, the 5-HT receptor agonist quipazine is often used (McEwen, Van Hartesveldt & Stehouwer, 1997; Brumley & Robinson, 2005; Brumley, Roberto & Strain, 2012; Guertin, 2004). Using the air-stepping paradigm with postnatal rats, locomotion-like alternated stepping has been reported to occur as early as P0 (McCrea, Stehouwer, & Van Hartesveldt, 1994).

Another potential limiting factor for locomotion expression is body size and growth. For instance, the limbs in developing animals are composed of weaker tissue and bones than adult animals, limiting locomotion expression, as the animal is limited in the amount of force that can be applied to a surface (Carrier, 1996). Similarly, young animals lack the stamina to maintain locomotion for long periods of time (e.g., smaller energy reserves, higher oxygen consumption rates) and therefore cannot engage in sustained bouts of locomotion (Carrier, 1996). In humans, research has demonstrated that infants that have a higher rate of weight gain show decreased stepping behavior compared to leaner counterparts (Thelen, Fisher, & Ridley-Johnson, 1984). Slining and colleagues (2010) found that overweight infants with high subcutaneous fat exhibited longer delays in locomotion development. Overall, these findings suggest that in addition to posture, physical limitations such as body size can influence locomotion development and expression of locomotion in immature animals.

Evoking Locomotor-Like Behavior with Sensory Stimuli

The findings of Altman and Sudarshan (1975) on the development of locomotion in rats are based on observations done in an open-field environment with no experimental presentation of sensory stimuli or pharmacological activation. They do report, however, that pups as young as P4 begin showing walking behavior when placed on a cold surface. Yet under typical circumstances, the development of locomotion does not occur in an open-field, but rather in an environment that contains much sensory stimulation. Thus, other studies have examined the development of locomotion in response to sensory, mainly olfactory, stimulation. Jamon and Clarac (1998) used olfactory stimulation to evoke locomotion in P3-P9 rats. In their experiment, rat pups were exposed to a cotton ball with nest odor or bedding from the nest, which served as an olfactory stimulus, successfully evoking crawling behavior in these animals at earlier ages than found in the Altman and Sudarshan (1975) study (Jamon & Clarac, 1998). Jamon and Clarac (1998) found that the switch to walking[2] occurs at P4, with the belly off the surface at P5-P6. Based on the characteristics of walking established by Altman and Sudarshan, the behavioral pattern found on P3 by Jamon and Clarac could be classified as walking; thus walking occurs in response to an olfactory stimulus over a week earlier than in an open-field environment with no olfactory stimuli present. Additional studies have since shown that crawling can be evoked with other olfactory stimuli, such as amniotic fluid and milk, on P1, which also is a week earlier than the findings of Altman and Sudarshan (1975) (Mendez-Gallardo & Robinson, 2013). Taken together, these studies suggest that sensory stimulation facilitates locomotion expression in immature animals.

[2] Jamon and Clarac reported only walking happening in their experiment; however, their definition of early walking is most comparable to Altman and Sudarshan's definition of crawling.

Pharmacological Activation of Locomotion-like Behavior in Neonatal Rats

In addition to sensory stimulation, pharmacological activation also facilitates locomotion and posture in newborn rats. Swann and colleagues (2016) examined the effects of serotonin stimulation on locomotion and posture in P1 rats. In this study, P1 rat pups were treated with quipazine or saline (vehicle control) and then were placed on a clear, Plexiglas surface for a 30-minute test period. Findings from this study showed that quipazine significantly facilitated locomotion (pivoting, crawling, walking), as well as posture (head elevation, quadruped stance), compared to pups treated with saline. Behaviors not reported by Altman and Sudarshan until the second week of postnatal development, such as crawling and walking, were evoked at P1. Spear and Ristine (1981) also found that quipazine facilitated locomotor behavior in P3 rats when placed a wire, mesh floor. Therefore, these complex, locomotion patterns of behavior can be induced approximately 1-2 weeks earlier following activation of the serotonin system, as compared to spontaneous expression of locomotion.

Thus to summarize: the neural mechanisms of locomotion begin developing prenatally in the rat, with the postnatal expression of locomotion, presumably limited by immature postural control, body size, and lack of stamina. However, pharmacological treatment or presentation of sensory stimuli has been shown to activate locomotor behavior at young ages. Based on these studies, it appears that pharmacological treatment activates neurotransmitter systems and provides an excitatory drive that induces the animal to exhibit more mature locomotion. Likewise, sensory stimulation also initiates movement by providing an attractive stimulus that motivates the animal to respond and exhibit locomotion.

The Need for Replication and Control: Revisiting Past Literature

Discrepancies

Although the study conducted by Altman and Sudarshan (1975) has provided essential normative data for additional studies to build upon, there are discrepancies between findings of that study and other studies that cite Altman and Sudarshan (1975). The studies that cite the original paper have often found different ages at which particular locomotion patterns are first expressed that differ from the original study (see Table 1). In fact, Altman and Sudarshan have developmental time points that differ from an earlier study conducted by Bolles and Woods (1964). In the Bolles and Woods study, Sprague-Dawley rats were observed daily for 36 days. Subjects tested in the home cage were over a wire, mesh floor with nest material (shredded newspaper) while subjects tested in a separate wooden apparatus were placed over Sani-cel[3] and behavior was observed. Based on their observations, Bolles and Woods (1964) found that crawling occurred on P3, head elevation on P4, and walking on P10. In a behavioral measurement test developed by Heyser (2003), it was found that pivoting was shown at P7, crawling at P11, head elevation at P12, and walking at P16. Another study examining the development of locomotion found that crawling and head elevation emerged at P5 and walking at P10 (Geisler, Westerga, & Gramsbergen, 1993). Clearly, these discrepancies cause concern for researchers that are attempting to manipulate the development of locomotion, as well as those that are examining locomotion deficits and thus need normative data for comparison.

It is important to clarify that while these discrepancies may seem minor since they

[3] Bolles and Wood (1964) referred to Sani-cel, a commercial sanitary absorbent material, which is no longer available, but it appears to be ground corncob bedding material.

are a matter of days or a single week, this is a substantial time period in regards to rat development. In fact, a single day for the newborn rat is comparable to an entire month for a human infant (Vinay et al., 2005). While variability occurs within and between litters, the wide range of ages cannot be accounted for by this variability in litters. However, some of the discrepancies between studies may be due to differences in testing (i.e., temperature and sex differences). These issues are discussed next.

Thermoregulation in the Rat

Some of the early studies examining the development of locomotion and posture were conducted prior to research that examined the physical processes and development and maturation of other body systems. This lack of information presents weaknesses in these previous studies that need to be addressed. 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 their testing at room temperature. However, as we now know, it is necessary to consider ambient temperature during behavioral testing because rat pups are unable to effectively self-regulate their body temperatures internally for long periods of time.

In fact, sensitivity to thermal stress occurs as early as during gestation. Typically, pathological effects of thermal stress to fetuses occur when the dam is exposed to hot environments during gestation (Gordon, 1993). Exposure to higher temperatures results in reabsorption of fetuses, fetal death or abnormalities, and impaired brain development (Gordon, 1993). Similarly, exposure to cold environments can also result in pathological effects to fetal rats (i.e., malformations, reabsorptions, etc.), however, it is proposed that

the effects resulting from cold thermal stress is a result of the stress on the dam, not direct effects of the cold exposure to the fetuses (Gordon, 1993). This sensitivity continues following birth, as newborn rat pups are inefficient at independent thermoregulation, gradually developing the ability to regulate body temperature.

Research has shown that neonates' body temperature will closely match ambient temperature following exposure to said ambient temperature (Adolph, 1957). According to Adolph (1957), as rat pups amass fur and fat, the ability to regulate body heat, entirely independent of ambient temperature, does not fully develop until approximately 73 days after birth. However, while still immature, the ability to maintain temperature across a broad range of ambient temperatures is present as early as P15 and continues to develop during the first three to four weeks after birth (Fowler & Kellogg, 1975; Satinoff, 1991). The ability to regulate body temperature is influenced by both the development of neurotransmitters and brain structures. It is largely thought that the anterior hypothalamus is involved in body temperature regulation (Fowler & Kellogg, 1975), as well as the serotonergic system (Ishiwata, 2014).

The disruption of thermal homeostasis impacts normal functioning at multiple levels, including physiological (e.g., metabolism), neural, and behavioral levels. For an extensive review, see Harshaw et al., 2017. Thermoneutral zones are defined as ambient temperature ranges that require the animal to use minimal energy to produce and maintain body heat (Satinoff, 1996). When animals are kept at cold temperatures, they develop at lower rates than those animals that are kept at thermoneutral temperatures (Satinoff, 1991). This affects the development of fur, but also affects neural mechanisms. Horwitz, Heller and Hoffman (1982) found that neural mechanisms show earlier

functioning in pups that are maintained at their thermoneutral temperature. Additionally, and more accurately, cold temperatures significantly impair cardiac and respiratory function (Blumberg, Sokoloff, & Kirby, 1996) and decrease myoclonic twitching, specifically in the limbs and tails of newborn rats (Blumberg & Stolba, 1996). 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 limiting the energy available that can be expended on locomotion in cold environments. Previous research has demonstrated that pups have longer latencies to move from a cold surface to a warm surface and, in general, show less robust responses to thermal cues (Sokoloff et al., 2002). As animals are investing energy to maintain and produce body heat, engaging in locomotion diverts blood into the limbs, resulting in heat loss; therefore, Sokoloff and colleagues suggest that it is beneficial for the animal to limit locomotion to conserve energy and maintain body temperature (Sokoloff et al., 2002). While locomotion might not be directly impaired by exposure to cold temperatures, rat pups might avoid engaging in locomotion to avoid loss of body heat and conserve the energy necessary for BAT thermogenesis. (Previously, it was mentioned that locomotion behavior could be induced with a cold surface and here, it is stated that cold temperatures impair locomotion function. To clarify, in regards to testing temperature, we refer to the ambient temperature that the animal is exposed to during testing, rather than the cold surface which is used as a sensory stimulus to evoke locomotion).

Although Altman and Sudarshan (1975) do call attention to the variation in temperature in the testing room while their study was conducted, they do not address the

potential confounds that could occur because of these variations and lack of regulation of ambient temperature.

Sex Differences

Previous studies have not examined potential sex differences in the development of locomotion and posture behavior. While some researchers have included both males and females in their experimental design (Altman & Sudarshan, 1975; Geisler et al., 1993; Westerga & Gramsbergen, 1990), they have not reported sex as a variable in their statistical analyses. It is important to determine if there is variability that can be attributed to sex differences, which might contribute to discrepancies in findings from previous studies because research has found that other coordinated action patterns in the rat vary across sex. For example, research has found sex differences in male and female rat pups in latency to perform the leg extension response (LER) during the neonatal period. This behavior is characterized by the hyperextension of the hindlimbs in response to maternal-directed anogenital licking (Moore & Chadwick-Dias, 1986). Specifically, male rat pups display a longer and quicker LER response than female rat pups. While 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 hindlimbs could influence the first occurrence of locomotion or postural behaviors—such that males might demonstrate these behaviors slightly earlier than female pups. This idea is exploratory, as previous research has not examined these potential differences in the development of locomotion and posture.

The Role of the Nest in Influencing Rat Pup Behavior

Researchers often test animals, including neonatal rats, in an open-field or other

contrived environments (e.g., a sling used in air-stepping paradigms). However, newborn rat pups born in a laboratory setting develop in a nest structure built with bedding material by the dam. The home nest, in addition to the barriers established by the bedding walls, contains olfactory stimuli (i.e., odor of dam and littermates, urine, milk, etc.), as well as thermal and tactile stimulation through contact with other pups in the litter and the dam. These sensory cues available in the nest can influence behavioral responses of newborn pups, including home seeking behavior (head elevation & crawling), nipple orientation and attachment, and facial grooming behavior.

A study on the ontogeny of home seeking behavior found that newborn rats will begin to consistently choose home nest material at P5, but will move toward and enter the zone containing nest material as early as P2 (Sczerzenie & Hsiao, 1977). Similarly, Polan and Hofer (1998) found that rat pups will show preference for the dam over nest material at P2 when accompanied by a thermal gradient towards the dam and at P4 without a thermal gradient towards the dam. Pups also use maternal olfactory cues to direct them toward the nipple and their attachment to the nipple (Eilam & Smotherman, 1998). By alternating ventroflexion and dorsiflexion of the body, the pup changes from a prone to supine posture, and then uses crawling-like steps to move on the ventrum of the dam to locate and attach to a nipple (Eilam & Smotherman, 1998). The exact combination of olfactory cues that produce both home seeking behavior and nipple orientation and attachment are difficult to tease apart. It is known that the dam produces a pheromone on P14 that attracts the pup (Leon, 1974), however, given that these behaviors are seen as early as P1 (nipple orientation and attachment) and P2 (dam/home seeking), it suggests that the maternal pheromone is not responsible for evoking these behavioral patterns in

early postnatal development, but other cues, such as the texture and odor of the dam's fur influence these behaviors (Polan & Hofer, 1999).

Pups can also use siblings as support to exhibit advanced postural behavior. For example, 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 pups could also use siblings 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 infants' limbs. Overall, it appears that a nest-like environment can influence posture and limb movements in human infants and developing pups.

Therefore, the developmental importance of the nest, and the multitude of sensory stimulation contained within the nest cannot be undermined. Research has consistently shown that rat pups demonstrate more advanced behavior within the nest or when presented with cues found in the nest. The influence of the nest is an important component to take into consideration when examining the normative development of locomotion and postural behavior.

Current Study

The purpose of the current study was to provide normative data on posture and locomotion behavior and development in newborn male and female rats, while controlling temperature and testing conditions. For this study, we examined the

developmental trajectory of locomotion and posture from P1 until P15 in both a sensory-deprived testing environment, as well as a sensory-enriched testing environment (see **Methods**). In both testing conditions ambient temperature was controlled to ensure that subjects were tested at thermonetural temperatures. We also examined sex differences by using balanced numbers of male and female subjects to determine if there are differences between sexes. Overall, we provide increased quantitative and qualitative data on locomotion and posture behavior over the first two postnatal weeks, while controlling possible confounding variables.

The first aim of this study was to examine development of locomotion, and related postural behavior, in an open-field environment and carefully describe the locomotion behavior as it first appears and transitions across the first two postnatal weeks (ages P1-P15). This provides normative, descriptive information on the trajectory of locomotion in a manner similar to previous research (e.g., Altman & Sudarshan (1975)). We hypothesized that behavior would develop in a similar manner as found by other researchers, with less mature behavior appearing first (i.e., pivoting) and more mature behavior (i.e., quadrupedal walking) occurring towards the end of the second postnatal week. Additionally, we hypothesized that by controlling temperature, animals might exhibit locomotion and posture earlier than previously reported by Altman and Sudarshan (1975).

The second aim of the current study was to examine differences in the development of locomotion in a sensory-enriched environment, similar to the home nest, versus a sensory-deprived environment. As previously discussed, differences between open-field testing and the home nest environment can influence patterns of behavior

exhibited by rat pups. Due to the developmental significance of the nest environment, it is important to examine the occurrence of locomotion in a nest-like structure, rather than solely in an open-field testing environment. By creating an artificial nest-like structure, we were able to examine the development of locomotion and posture in a relatively more natural environment. While the artificial nest environment did not have the dam, it provided other cues, such as olfactory stimulation through nest material, tactile and thermal stimulation through siblings that are also in the nest, and lastly, the siblings in the nest could also provide postural support, which may facilitate more advanced behavior. It was hypothesized that locomotion and posture would first occur at earlier ages in the nest-like environment compared to the open-field environment, given that the nest should provide more variable sensory stimulation that can possibly evoke behavior. As far as we are aware, this is the first study to examine locomotion and posture development in both a sensory-enriched and sensory-deprived testing environment.

The third aim of the current study was to determine if there were sex differences in the development of locomotion and posture behavior. Previous research has not discussed any sex differences in locomotion and posture, therefore, this aim was more exploratory and it was hypothesized that males might show a slightly faster developmental trajectory, given that research has shown sex differences in other forms of coordinated action patterns (i.e., LER). The fourth aim of the current study was to examine if body size influenced the occurrence of locomotion and posture. As mentioned previously, research suggests that there are allometric limitations (i.e., weak skeletal system/tissue, lack of stamina) on the development of locomotion and posture. Therefore, it was hypothesized that animals that have a larger body size would exhibit less

locomotion and posture compared to animals with smaller body sizes.

Methods

Subjects

Subjects were P1-P15 Sprague-Dawley rat pups. Adult rats were purchased from Simonsen Laboratories and bred in the Animal Care Facility at Idaho State University. Pregnant females were pair-housed until a couple days prior to birth, when they were then individually housed. Litter size was reduced to 8 pups on P1. Subjects were allowed to remain 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. Any animals that showed signs of distress or other signs of less than good health were not used. Animals were kept on a 12:12 light: dark cycle with food and water available ad libitum. Animal care and use were in accordance with the NIH guidelines and were approved by the ISU Animal Care and Use Committee.

Experimental Design

A total of 64 rats (32 F, 32 M) were used in this study. Subjects were divided into two groups, with an equal number of males and females in each group. Group 1 pups were placed in a sensory-deprived environment during testing and Group 2 pups were placed in a sensory-enriched environment. Pups from each litter (16 litters total) were tested in the two different environments—two pups (1 M, 1 F) in the sensory-deprived environment and two pups (1 M, 1 F) in the sensory-enriched environment. Subjects were tested every day from P1 until P15. All animals in the litter were marked using a non-toxic black marker for identification and to control for handling effects. Due to the

amount of fur growing, in addition to the non-toxic black marker, subjects were also marked on P11 and P12 with black pet hair dye gel (Top Performance). Since animals were tested daily from P1 to P15, the order in which subjects were run was balanced; half of the male subjects and half of the female subjects were tested first.

Subjects assigned to the sensory-deprived condition were tested individually in a clear, Plexiglas box (Figure 1). Subjects assigned to the sensory-enriched condition were placed in the same Plexiglas box with 2 siblings and were tested 2 subjects at a time (for a total of 4 pups in the box). Additionally, bedding and nest material from the home cage were also placed in the box (Figure 2). Box dimensions were adjusted based on pup size. Pups were weighed and measured daily to determine box size. The average for body length of all pups in the litter was calculated and the box was 1.5 to 2 times the size of the average body length. The smallest box was 4" x 4" with 8" walls, increasing in half inch increments with the largest box at 8" x 8" with 8" walls. Behavior was recorded onto DVD and scored during video playback at normal or reduced speed. The testing incubator had two cameras inside that were linked to an external DVD-R device. Recording of the test session began immediately after the subject was placed on the box floor.

Behavioral Testing

Subjects were tested once a day for fifteen consecutive days beginning on P1 (24 hours after birth) and ending on P15. Subjects were tested inside an incubator that functions to maintain humidity (40%) and temperature adjusted for developmental age (see Table 2). All subjects were marked, using a non-toxic black marker or non-toxic pet paint, with a number on their back for identification and tracking purposes. Body mass (g) and body length (mm; crown to rump) were recorded for all subjects. All subjects and

siblings were removed from the dam, manually voided with a small paintbrush, and were placed inside of the incubator 30 minutes prior to testing to allow for acclimation to conditions inside the incubator. Siblings were removed to ensure that daily handling and removal of subjects did not alter maternal care. Following acclimation, subjects were placed in their test environment (sensory-deprived or sensory-enriched). Subjects assigned to the sensory-deprived condition were placed individually in the center of the testing box for a 20-minute test period. Marks placed on the back were used to help track their behavior. Subjects assigned to the sensory-enriched condition were placed in the nest environment (in the box) with 2 siblings (1 M, 1F) and also were observed for 20 minutes. Both the male and female subjects were placed into the nest simultaneously with two siblings. Marks placed on the back were used to keep track of focal subjects in the sensory-enriched environment. All sessions were recorded onto DVD for behavioral coding during video playback. Ambient temperature of the incubator was recorded and nest temperature was measured, using a thermometer gauge, prior to returning subjects to the home cage.

Behavioral Scoring

The 20-minute test session was scored during video playback at normal or reduced speeds using an event recorder program (JWatcher). Behavior was classified into 8 categories: walking, crawling, pivoting, 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 et al. (2016). Walking was defined as a propulsive movement with all four limbs active and the ventrum off of the surface. Crawling was defined as propulsive movement that actively involves the

forelimbs while the ventrum remains in contact with the surface. Pivoting was defined as a propulsive movement where the pelvis remains anchored on the surface while the forelimbs propel the pup in a circular path. Head elevation was defined as any elevation of the head with or without simultaneous propulsive movement. Forelimb elevation was defined as 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 was defined as extension of the hindlimbs with hindpaws in contact with the surface, elevating the hind portion of the body off of the surface. Crawling stance was defined as elevated forelimbs and shoulders only; the pelvis and hindlimbs remain in contact with the surface. Walking stance was defined as elevation of all four limbs, shoulders, and pelvis. Intra-scorer reliability was at 90%. During video playback, the age of eye opening was recorded. Partial eye opening was measured 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). Analysis of variance (ANOVA) tests, paired sample t-tests, and correlation analyses were used to examine locomotion and posture behavior during individual test sessions, as well as trajectories of locomotion and posture development. 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.

First, body mass and body length were compared across each sex/testing condition for each age separately. For these analyses, two-way ANOVAs were used to

compare group means. Independent variables were sex and testing condition. Dependent variables were body mass and body length. Next, ambient temperature in the sensory-deprived condition 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. The independent variable was testing condition and the dependent variable was temperature.

We examined the age of first occurrence of locomotion and posture behavior in each sex/testing environment. Based on the literature, we expected pivoting to precede crawling and crawling to precede walking, by a number of days. In order to establish the first age of occurrence, the presence or absence of each locomotion and posture behavior was quantified and the percentage of subjects that exhibited the behavior on 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 first age of occurrence.

Next, each locomotion and posture behavior was examined across the testing period and compared across testing environment. Repeated measures ANOVAs were used to analyze locomotion and posture behavior. Independent variables were age, sex, and test environment. Dependent variables were duration spent showing pivoting, crawling, walking, head elevation, and walking stance. Due to the low occurrence of some postural behavior independent of locomotion (i.e., forelimb and hindlimb elevation, crawling stance), postural behavior was restricted to head elevation and walking stance for analysis.

Lastly, we examined the relationship between body size (body mass and body

length) and locomotion. To this end, we utilized the ponderal index (Thelen, Fisher, & Ridley-Johnson, 1984) by dividing body mass/body length. The ponderal index provides a measure of leanness, such that an animal with a lower ponderal index is leaner than an animal with a higher ponderal index. Pearson correlations were conducted separately for each locomotion behavior (pivoting, crawling, and walking) and each sex/testing environment group for each age (P1-P15). In addition, the correlation coefficients from P1 to P15 were averaged for each sex/testing condition to capture the overall direction of the relationship between locomotion and body size. Statistical analyses were not performed on averaged correlations, but instead they have been qualitatively described. Fisher's Z-tests were conducted for the averaged correlation values to determine if there were differences between body size and locomotion for each sex/testing condition. Given that there was a low occurrence and variety of postural behaviors, this analysis was restricted to locomotion only.

Missing Data

Approximately 3% of subject data was missing due to video data collection issues and experimenter error. Due to the low quantity of missing data and given that the data was missing at random, missing values were eliminated from all analyses. Initially, mean substitution was used to replace missing values, however, analyses ran on the data set with missing values deleted produced the same significant findings as the substituted values. Therefore, the original dataset was used for all analyses and missing values were eliminated 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 two-way ANOVA 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, $p < .05$), P2 (F (1, 56) = 5.54, $p < .05$), P3 (F (1,60) = 3.46, $p < .05$), P4 (F (1,52) = 7.67, $p < .01$), and P15 (F (1, 60) = 5.18, $p < .05$). Body mass averages are shown in Figure 3A. There was not a main effect of sex on body mass from P5 to P14 (Figure 3A), or an effect of testing condition at any age. For body length on P1 to P15, there were no significant main or interaction effects (Figure 3B).

Testing Environment Temperature

Paired sample t-tests revealed that there was a significant difference between ambient temperature of the sensory-deprived condition and nest temperature of the sensory-enriched condition on P4 (t (13) = -2.66, $p < .05$), P6 (t (13) = -4.32, $p < .001$), P7 (t (15) = -2.45, $p < .05$), P8 (t (15) = -3.87, $p < .01$), P10 (t (15) = -3.15, $p < .01$), P11 (t (15) = -5.01, $p < .001$), P12 (t (15) = -6.31, p .001), P13 (t (15) = -4.51, $p < .001$), P14 (t (14) = -6.79, $p < .001$), and P15 (t (15) = -5.64, $p < .001$), such that the nest temperature was warmer (by 0.9-3.3°C) than the ambient temperature (Table 3). There was not a significant difference between ambient temperature and nest temperature on P1-P3, P5, or P9.

Eye Opening

Heyser (2003) reported eye opening occurring on average at P13, but noted that it

could occur anywhere from P7 to P17. We found that 42 subjects (out of 64 total subjects; 66%) did not show complete eye opening until P15, with 11% of subjects opening their eyes on P14. Additionally, 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 P15, statistical analyses examining potential sex or testing condition differences were not conducted.

Age of First Occurrence of Locomotion

A criterion was established to determine when the majority of subjects exhibited locomotion and posture at an above chance ratio (70% of subjects). For example, a single subject showed walking during the first week of postnatal development; however, walking was not shown by the majority of subjects until the second week of development. Therefore, we examined the age of first emergence of locomotion based on when at least 70% of subjects for that sex/testing condition exhibited the behavior.

Based on the criterion established, 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 showed pivoting on P1, while males in the sensory-deprived condition showed pivoting on P2 (Figure 4). While 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 5). Thus, it appears that crawling emerges either

simultaneously with pivoting or within one to two days after pivoting emerges. 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 one day later on P10. Females in the sensory-enriched environment exhibited walking on P11, while males exhibited walking a day later on P12 (Figure 6).

Overall, the age of first occurrence does not appear to be dependent upon sex or testing condition. Subjects reached 70% criterion for each locomotor pattern within 1-3 days of the first sex/testing condition to reach criterion. While pivoting and crawling were shown at similar time points, walking emerged much later across all sex/testing conditions, as expected.

The Emergence of Locomotion and Posture Behavior from P1-P15

Locomotion Behavior

A two-way repeated measure ANOVA for pivoting duration revealed a significant main effect of age (F (14, 504) = 5.02, $p < .001$). As shown in Figure 7A, pivoting duration fluctuated across the first two weeks of postnatal development. Subjects across sex/testing condition exhibited higher 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, $p < .001$) and remained low until P5. Pivoting duration significantly increased at P6 (t (55) = -2.78, $p < .01$) and increased again from P6 to P7 (t (55) = -2.17, $p < .05$). After P7, pivoting duration decreased gradually and significantly from P12 to P13 (t (63) = 2.78, $p < .01$). There was not an effect of sex or testing environment. 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, 504) = 2.51, $p < .001$). Crawling duration remained consistent from P1 to P3. This range of ages maps onto the age of first occurrence as established by the first occurrence criterion. Crawling duration significantly decreased at P4 compared to crawling duration at P1 (t (55) = 2.88, $p < .01$), as shown in Figure 7B. Following this decrease at P4, crawling duration increased significantly from P5 to P7 (t (63) = -2.13, $p < .05$) and continued to increase in duration from P7 to P9 (t (55) = -2.43, $p < .05$), until it significantly decreased from P10 to P13 (t (63) = 2.86, $p < .01$) (Figure 7B). Again, there was not an effect of sex or testing environment on crawling duration, which appears to follow the criterion data that crawling emerges within the first few days in all sex/testing conditions.

For walking, a two-way repeated measure ANOVA revealed a main effect of age (F (14, 504) = 32.23, $p < .05$) and an interaction between age and condition (F (14, 504) = 4.06, $p < .05$) (Figure 7C). Subjects in the sensory-deprived condition exhibited significantly longer walking durations at P9 compared to P1 (t (27) = -2.40, $p < .05$), as did subjects in the sensory-enriched condition (t (27) = -3.30, $p < .01$; mean walking duration on P1 was 0). Subjects in the sensory-deprived condition approached a significant increase in walking duration at P8 (t (31) = -2.01, $p = .053$), whereas walking duration was still at 0 for sensory-enriched subjects. Interestingly, only males in the sensory-deprived condition met criterion level of walking on P9. Subjects in the sensory-deprived condition displayed increases in walking duration, significantly increasing in duration from P11 to P12 (t (31) = -3.12, $p < .01$) and then from P12 to P13 (t (31) = -2.21, $p < .05$). They then began to decrease in walking duration from P14 to P15 (t (29) =

4.95, $p < .001$). Subjects in the sensory-enriched condition exhibited steady increases in walking duration from P9 to P10 (t (27) = -2.43, $p < .05$), P10 to P11 (t (31) = -3.28, $p < .01$), P11 to P12 (t (31) = -3.43, $p < .01$), P12 to P13 (t (31) = -4.82, $p < .001$), and P13 to P14 (t (29) = -2.78, $p < .01$), as can be seen in Figure 7C. Overall, subjects in the sensory-enriched condition showed shorter walking durations compared to subjects in the sensory-deprived condition at P11 (F (1, 62) = 12.46, $p < .001$), P12 (F (1,62) = 13.88, $p < .001$), P13 (F (1, 62) = 13.66, $p < .001$), and P14 (F (1, 58) = 7.23, $p < .01$).

Postural Behavior

For head elevation, there was a significant main effect of age (F (14, 504) = 21.73, $p < .001$) and a significant interaction between age and condition (F (14, 504) = 13.45, $p < .001$), as shown in Figure 8A. Subjects in the sensory-deprived condition exhibited longer head elevation durations compared to subjects in the sensory-enriched condition at P8 (F (1,62) = 6.92, $p < .05$), P9 (F (1, 54) = 12.39, $p < .001$), P10 (F (1, 62) = 13.78, $p < .001$), P11 (F (1,62) = 21.64, $p < .001$), P12 (F (1, 62) = 27.48, $p < .001$), P13 (F (1, 62)= 44.74, $p < .001$), P14 (F (1, 58) = 27.43, $p < .001$), and P15 (F (1, 62)= 45.48, $p < .001$). Interestingly, head elevation duration significantly increased from P1 to P8 in the sensory-enriched condition (t (31) = 2.14, $p < .05$) and increased again from P11 to P12 (t (31) = -2.36, $p < .05$), P13 to P14 (t (29) = -3.79, $p < .001$), and lastly from P14 to P15 (t (29) = -2.68, $p < .05$) (Figure 8A). Although subjects in the sensory-deprived condition showed relatively longer durations of head elevation, there was not a significant increase in head elevation duration until P9 (t (27) = -3.07, $p < .01$). Following this increase at P9, head elevation duration increased from P12 to P13 (t (31) = -2.23, $p < .05$) and again from P13 to P14 (t (29) = -2.44, $p < .05$).

A two-way repeated measure ANOVA for walking stance duration revealed a main effect of age (F (14, 504) = 4.74, $p < .05$) and an interaction of age and condition (F (14, 504) = 2.74, $p < .05$). For subjects in both testing conditions, walking stance duration remained at or around 0 until P9 and then began to increase in duration. Subjects in the sensory-deprived condition showed significantly longer walking stance durations at P12 compared to durations at P9 (t (27) = -2.64, $p < .05$), with durations remain fairly consistent from P12 to P15 (Figure 8B). Subjects in the sensory-enriched condition showed increases in walking stance duration from P10 to P12 (t (31) = -2.23, $p < .05$) and increases again from P13 to P14 (t (29) = 2.29, $p < .05$). While 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 to those in the sensory-enriched condition on P11 (F (1, 62) = 5.76, $p < .05$) and P14 (F (1,58) = 7.57, $p < .01$).

Relationship between Body Size and Locomotion

Pivoting

Ponderal index and pivoting duration were significantly correlated for females in the sensory-deprived condition at P1(r=-.71, $p < .05$, see Figure 9A), such that as ponderal index decreased pivoting duration increased. Thus, leaner animals engaged in longer durations of pivoting behavior on P1 (Figure 9B), but no other ages. There were nonsignificant correlations between pivoting duration and ponderal index for males in the sensory-enriched and sensory-deprived condition, as well as for females in the sensory-enriched condition across all ages (Figure 10). When examining the averaged correlation from P1 to P15, both males and females in the sensory-deprived condition had an overall

negative correlation between ponderal index and pivoting duration such that as ponderal index decreased pivoting duration increased (Figure 9A and 10A). Males had a stronger correlation (r=-0.21) than females (r=-0.01). Males and females in the sensory-enriched condition, on average, had a positive correlation between ponderal index and pivoting duration, as shown in Figure 10, such that pivoting duration increased as ponderal index increased; however, both of these correlation coefficients suggest a weak relationship (r=0.09, r=0.13). Fisher Z-tests did not reveal significant differences between any of the sex/testing conditions.

Crawling

There was a significant correlation between ponderal index and crawling duration for males in the sensory-deprived condition at P5 (r= -.70, $p < .05$), as well as a significant correlation at P8 (r= .75, $p < .05$), as shown in Figure 11A. Interestingly, at P5, there was a negative relationship between crawling duration and ponderal index such that leaner animals (lower ponderal index) exhibited longer durations of crawling; however, at P8, this relationship reversed so that subjects with a higher ponderal index showed increased crawling duration (Figure 11B & 11C). There was a significant relationship between crawling duration and ponderal index for males in the sensory-enriched condition at P4 (r= .74, $p < .05$), as well as at P6 (r = -.69, $p < .05$) and P8 (r= .65, $p < .05$), as can be seen in Figure 12A. On P4 and P8, males in the sensory-enriched condition with a higher ponderal index engaged in longer durations of crawling; however, at P6, subjects with a lower ponderal index engaged in longer durations of crawling (Figure 12B-D). There were significant correlations on P5 (r= -.68, $p < .05$) and P9 (r= -.64, $p < .05$) for crawling duration and ponderal index for females in the sensory-enriched

condition, such that females with a lower ponderal index exhibited longer durations of crawling behavior, as shown in Figure 13. There were not any significant correlations between ponderal index and crawling duration for females in the sensory-deprived condition at any age (Figure 14).

When examining the averaged correlation coefficients from P1 to P15, males in both testing conditions had an overall negative correlation (sensory deprived: r =-0.02; sensory-enriched: r=-0.04) suggesting that animals with a lower ponderal index exhibited longer durations of crawling; however, the correlation coefficients indicated that this relationship was extremely weak. Females in both testing conditions had an overall positive correlation (sensory deprived: r =0.07; sensory-enriched: r=0.05), such that animals with a higher ponderal index exhibited longer crawling durations. The strength of these relationships were extremely weak based on the averaged correlation coefficient. Fisher Z-tests did not reveal any significant differences between the average correlation coefficients across any sex/testing conditions.

Walking

There was a significant correlation between walking duration and ponderal index for females in the sensory-deprived condition on P7 ($r = -.67$, $p < .05$), such that subjects with a lower ponderal index engaged in longer durations of walking at this age (Figure 15). There were not significant correlations between walking duration and ponderal index for males in the sensory-deprived or sensory-enriched conditions, or for females in the sensory-enriched condition (Figure 16).

The averaged correlation coefficient reflected that males in both conditions (sensory-deprived: r=-0.07; sensory-enriched: r=-0.26) and females in the sensory-

deprived condition (r=-0.16) had an overall negative correlation between ponderal index and walking duration, suggesting that animals with a lower ponderal index engaged in longer walking durations. Again the strength of these relationships was relatively weak. Females in the sensory-enriched condition had a positive correlation between walking duration and ponderal index (r=0.05), such that animals with a higher ponderal index exhibited longer walking durations. Again, the averaged correlation coefficient reflects that this relation is weak. Fisher Z-tests did not reveal any significant differences between the averaged correlation coefficients across any sex/testing conditions.

Discussion

The purpose of this study was to provide improved normative data on the development of locomotion and posture in newborns rats during the first two postnatal weeks, as well as to examine how sensory stimulation may influence locomotion and posture development. Additionally, the present study examined how controlling temperature and sex influenced locomotion and posture development, as well as relationships between body size and locomotion. To this end, rat pups were tested on a daily basis from P1 to P15 in a sensory-deprived or a sensory-enriched condition, controlling ambient temperature and statistically examining potential sex differences.

It was hypothesized that locomotion and posture would develop in a similar trajectory as previously reported by other researchers, such as Altman and Sudarshan (1975), with pivoting being shown first, followed by the emergence of crawling and walking, respectively. Similarly, 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). For the most part, our

hypothesis was supported in 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. Therefore we did not find that pivoting necessarily emerged first followed by crawling, but rather that the pups were capable of exhibiting both behaviors simultaneously 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. Unfortunately, postural stance was seen at such low durations that we were unable to fully explore this hypothesis. However, we did find that head elevation emerged prior to walking stance in some of the subjects (males and females in the sensory-deprived condition), suggesting that we, to some degree, supported the hypothesis that a less mature postural behavior, such as head elevation, would emerge prior to the more mature walking stance.

We also 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. 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 to subjects in the sensory-enriched condition. Anecdotally, subjects in the sensory-enriched condition were huddling with littermates and sleeping much of the time, whereas subjects in the sensory-deprived condition were more frequently actively moving around the testing environment, pausing from rearing and grooming before continue to move around the environment. 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, may have created an environment that limited the need for animals to move around. However, in the sensory-deprived condition, animals were placed on a hard, Plexiglas surface without additional warmth (but they were at thermonetural temperatures) through sibling contact or nest material. 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.

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 et al., 2008). Onset of walking occurred prior to eye opening in the animals, thus longer durations of head elevation 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 cutaneous and proprioceptive feedback received from the stiff substrate. Akay and colleagues (2014) found that coordinated locomotion was negatively impacted in adult mice strains that did not receive proprioceptive feedback from muscle spindle activation. Similarly, Dallman and Ladle (2013) demonstrated that a lack of proprioceptive feedback influenced locomotion during the

early postnatal period, such that P5 and P8 mutant mice that did not have proprioceptive feedback from sensory neurons had impaired righting behavior, pivoting, and increased step duration and height compared to control wild-type mice. Additionally, previous research has shown that P1 rat pups show decreased air-stepping on a stiff substrate when suspended over a Plexiglas substrate (similar to the Plexiglas floor in the current study; Brumley, Roberto, & Strain, 2012). Thus, it is possible that we are seeing a similar avoidance response based on the proprioceptive feedback the pups are receiving. 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. However in the sensory-deprived condition, these alternate substrates are not available. Lastly, adult rats demonstrate exploratory behavior in response to novel environments (Eilam & Golani, 1989; Whishaw, et al., 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). It is likely that the 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 did not elicit exploratory behavior.

We also expected that controlling ambient temperature would result in earlier

locomotion and posture behavior across sex and testing conditions. In general, we found this to be the case. Males and females in each testing condition demonstrated spontaneous pivoting and crawling earlier than previously reported by at least 1-3 days (Table 4; Altman & Sudarshan, 1975; Bolles & Wood, 1964; Geisler et al., 1993; Westerga & Gramsbergen, 1990; Heyser, 2003). Head elevation and walking emerged prior to the age reported by Altman and Sudarshan (1975) by 4 days and 2 days, respectively (Table 4). However, this first emergence was not earlier than the age reported by other researchers (i.e., Bolles & Wood, 1964; Geisler et al., 1993; Westerga & Gramsbergen, 1993b). Interestingly, while 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 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. 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 (Westerga & Gramsbergen, 1990; Geisler et al., 1993), 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 Listar strain rats, and the present study, as well as that by Bolles and Woods (1964), used Sprague-Dawley rats. As discussed previously, 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, the majority of researchers indicate that locomotion and posture do not develop in a strict linear fashion with pivoting disappearing as crawling appears, crawling disappearing as walking emerges, etc., but that these behavioral patterns overlap with a clear and significant change in movement occurring at or around P15 (Altman & Sudarshan, 1975; Westerga & Gramsbergen, 1993b; Geisler et al., 1993; Westerga & Gramsbergen, 1990). Specifically, Altman and Sudarshan (1975) reported that pivoting continues to occur from 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 two 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 wasn't there before P15. At the spinal level, CPGs 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 (Orlovsky et al., 1999; Grillner et al., 2005; Hikosaka, 2007), by incorporating information from the visual (eye opening occurs on or around P15), sensory, and vestibular systems. 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 CPG activity. Thus, even though an animal might be capable of walking, input from these descending pathways to CPGs 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). Regression of polyneural innervation of muscles in the rat (Westerga & Gramsbergen, 1993a, Geisler et al., 1993), 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 previous 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.

The third aim of the study was to statistically examine potential sex differences as previous researchers had included both males and females, but did not examine if their developmental trajectories differed. While we found some sex differences in regards to body mass, these were not consistent across the study period. In regards to locomotion and posture development, we did not find any sex differences. Our hypothesis was centered on research examining sex differences in maternal licking, as well as sex differences in the leg extension response (LER), where sex differences do exist (Moore & Chadwick-Dias, 1986). While 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.

Lastly, we also explored the relationship between body size and locomotion. However, we did not find that there was an overall significant relationship between the two. We classified body size using ponderal index, which provided a measure of the relationship between body mass and body length to determine leanness of the animals. It was expected that trends in human development (e.g., leaner individuals show more locomotor activity) would be repeated here with rats, considering the overlap in locomotion and posture development shared between the species. However, it is important to point out that human infants can be top-heavy and have a higher center of gravity (Adolph & Avolio, 2000), in addition to having heavy legs, which could also contribute to patterns that we see with human infants. While the head is heavy for rat pups, they do not have to lift their heads to demonstrate locomotion behavior such as pivoting or crawling, and even some instances of walking can occur without the elevation of the head. Alternatively, given the high variability in the correlation coefficients we reported here, it is likely that we would not be able to replicate the exact values found and that the relationships could change with a different sample size. From a statistical perspective, we noted relatively weak correlations between body size and locomotion; however, due to our small sample size, it was not possible to achieve a significance level of $p < .05$. If a future study included a larger sample size, we might see this relationship change and more significant patterns between these two variables emerge.

Limitations

The current study is not without limitations. One such limitation was capturing postural behavior. It was originally proposed that additional postural behaviors would be examined, such as forelimb and hindlimb elevation and crawling stance. For that purpose,

the design utilized two camera angles, a top camera angle that came straight down from over the box to capture locomotion and posture behavior and a side camera angle to discriminate postural stances. For subjects in the sensory-deprived condition, capturing postural behavior from the side camera angle was straightforward. However, in the sensory-enriched condition, capturing postural behavior from the side camera angle was difficult due to the amount of bedding and nest material. As the animals burrowed into the nest material, it was difficult to discriminate postural stances. Due to the low occurrence and difficulty to accurately identify posture in the sensory-enriched condition, we eliminated these behaviors from the analyses.

While the sensory-enriched condition 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, in order to conserve body warmth (Alberts, 2007); additionally, 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 rats, including pups, as animals will demonstrate positive thermotaxis (move from cold to warm gradient) and adult rats will press levers to receive warm air when placed in a cold chamber (Weiss & Laties, 1961).

Similarly, pups will demonstrate learned head turning responses to thermal (heat) stimuli (Flory et al., 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 et al., 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, while there were not significant differences, we did see increased durations of locomotion, i.e., 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 the 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, 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, Bresnhan (BBB) Locomotor Rating Scale (Basso, Beattie, Bresnahan, 1995) exist for adult animals, but they have not been validated for use in immature animals.

Conclusions

The development of locomotion and posture requires a dynamic interplay between physiological, neural, musculoskeletal, and sensory systems. The current study sought to further understanding of normative locomotion and posture development in newborn rats across the first two postnatal weeks. Importantly, we found that rat pups were capable of exhibiting pivoting and crawling as early as 24 hours following birth, regardless of sensory stimulation, suggesting that testing animals at ambient temperatures alleviates physiological stress on the animal that might prevent locomotion. It is important to fully understand the developmental trajectory of locomotion and posture behavior, particularly as animal models are utilized to understand and draw parallels with human pediatric research. Experimental manipulations, such as treadmill training or genetic manipulation,

that are used to improve or induce behaviors at earlier time points, depend upon having accurate ages of emergence to be able to have confidence that their manipulation is actually influence locomotion behavior.

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Article	Pivoting	Crawling	Head Elevation	Walking
Altman & Sudarshan (1975)	P4-5	P8	P8	P12
Bolles & Woods (1964)	Not Included	P3	P4	P10
Westerga & Gramsbergen (1990)	Not Included	Middle of 2nd Week	Not Included	P11
Geisler et al. (1993)	Not Included	P5	P5	P10
Westerga & Gramsbergen (1993b)	Not Included	Not Included	Not Included	P10
Heyser (2003)	P7	P11	P12	P16

Table 1. Previous studies examining the first occurrence of locomotion and posture behavior in immature rats.

Figure 1. Testing apparatus used for both the sensory-deprived and sensory-enriched condition, pictured here as used in the sensory-deprived condition.

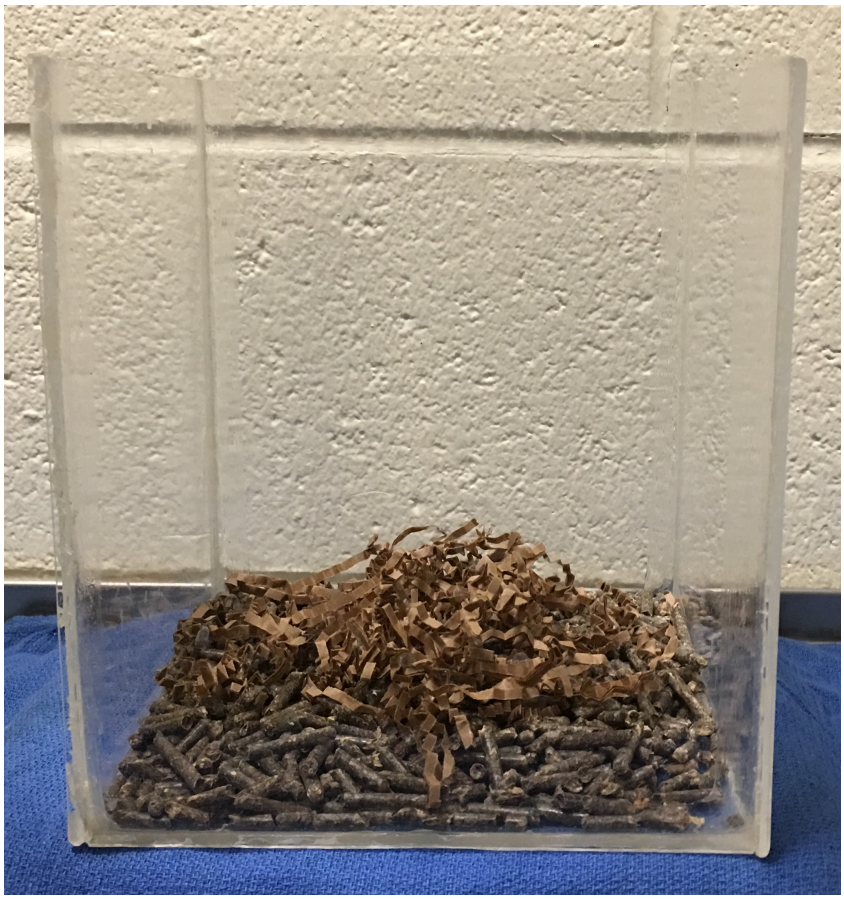

Figure 2. Testing apparatus used for both the sensory-deprived and sensory-enriched condition, pictured here as used in the sensory-enriched condition.

Age	Temperature
P0-P1	35°C
P2-P3	34°C
P4-P5	33°C
P6-P7	32°C
P8-P9	31°C
P10-P11	30°C
P12-13	29°C
P14-P15	28°C

Table 2. Ambient testing temperature for developmental age based on Spiers and Adair (1986).

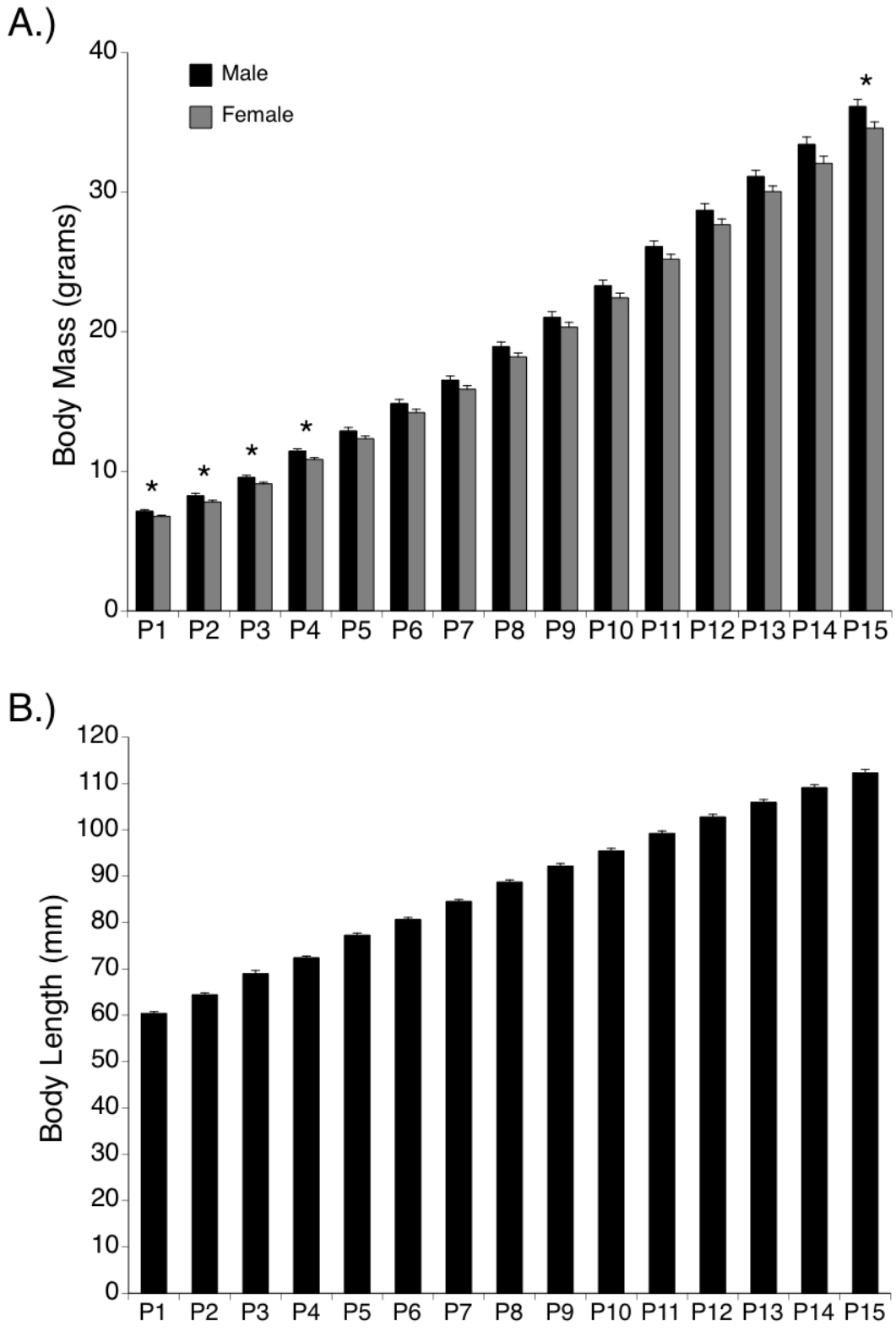

Figure 3. Litter Characteristics. A) Body mass of male and female rat pups for the first two postnatal weeks. B) Body length for the first two postnatal weeks. Bars represent means, vertical lines show SEM, asterisks indicate significance at the $p < .05$ level.

Age	Ambient Temperature	Nest Temperature
P1	34.8±.09	34.7±.33
P2	34.1±.08	34.0±.40
P3	33.8±.10	34.1±.26
P4*	32.9±.10	33.8±.29
P5	33.1±.12	33.6±.26
P6*	32.0±.11	33.4±.35
P7*	32.0±.08	32.9±.37
P8*	31.1±.08	32.5±.43
P9	31.0±.14	32.0±.52
P10*	30.3±.09	31.8±.50
P11*	30.1±.08	32.3±.45
P12*	29.0±.10	32.3±.49
P13*	29.1±.04	32.0±.65
P14*	28.1±.14	31.4±.52
P15*	28.1±.11	31.3±.55

Table 3. Testing temperature for both testing environment conditions. Ambient temperature reflects the temperature of the incubator for both conditions. Nest temperature reflects the temperature measured from the middle of the sensory-enriched condition. Asterisks indicate significance at the $p < .05$ level.

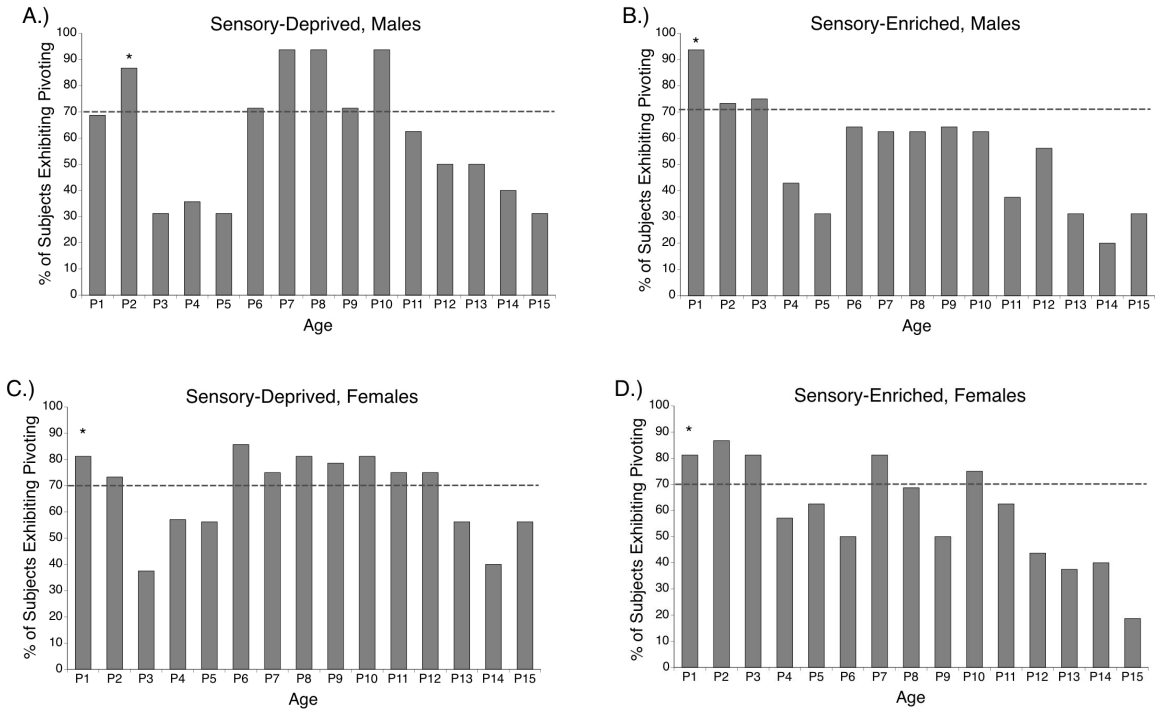

Figure 4. Percent of subjects exhibiting pivoting on P1 to P15 across each sex/testing condition. A) Males in the sensory-deprived condition. B) Males in the sensory-enriched condition. C.) Females in the sensory-deprived condition. D.) Females in the sensory-enriched condition. Bars represent mean percentages, asterisks represent first age of occurrence based on criterion, and dashed line indicates 70% criterion.

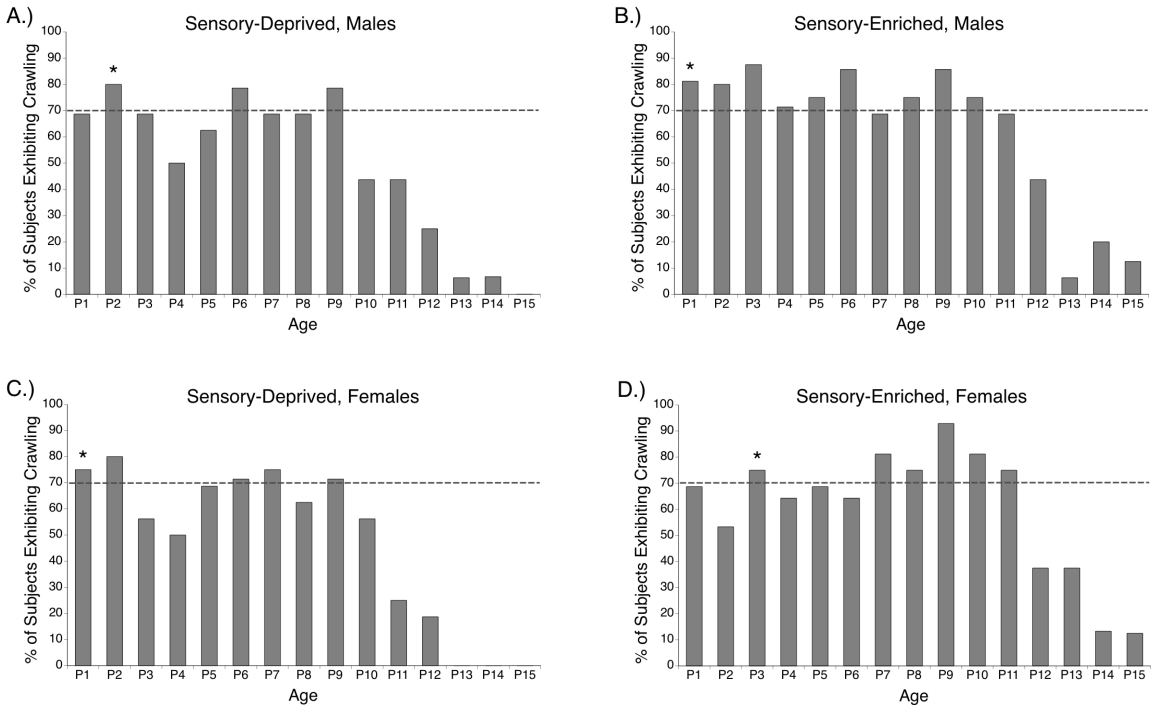

Figure 5. Percent of subjects exhibiting crawling on P1 to P15 across each sex/testing condition. A) Males in the sensory-deprived condition. B) Males in the sensory-enriched condition. C.) Females in the sensory-deprived condition. D.) Females in the sensory-enriched condition. Bars represent mean percentages, asterisks represent first age of occurrence based on criterion, and dashed line indicates 70% criterion.

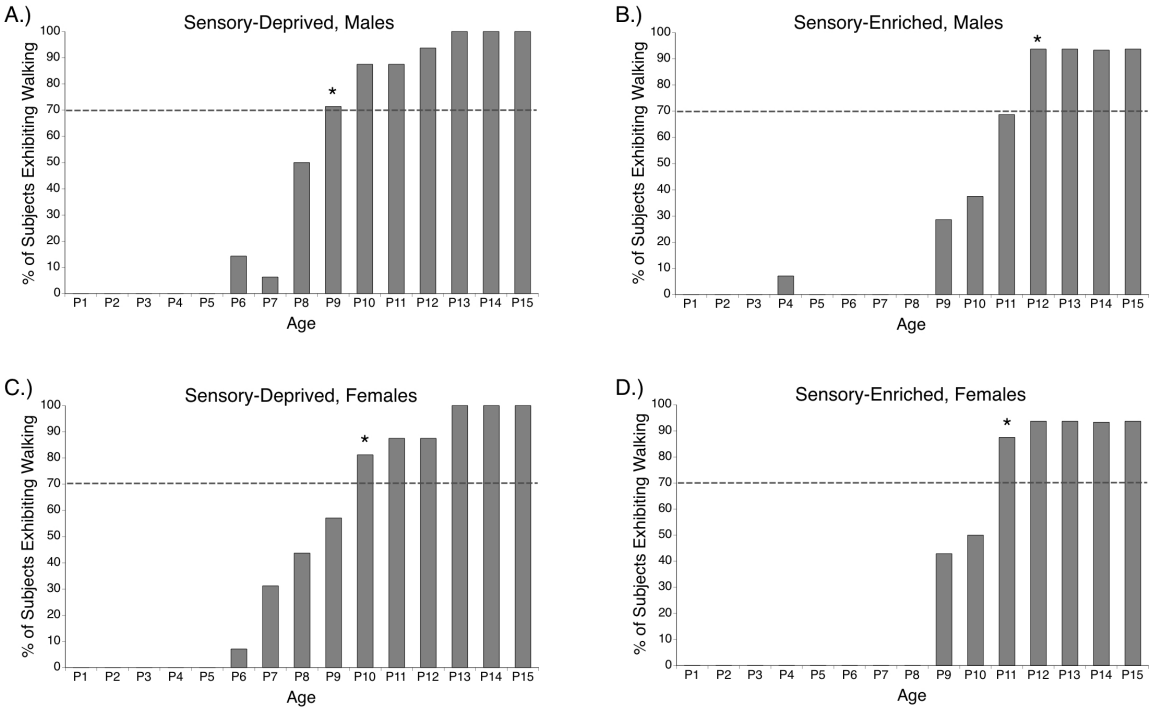

Figure 6. Percent of subjects exhibiting walking on P1 to P15 across each sex/testing condition. A) Males in the sensory-deprived condition. B) Males in the sensory-enriched condition. C.) Females in the sensory-deprived condition. D.) Females in the sensory-enriched condition. Bars represent mean percentages, asterisks represent first age of occurrence based on criterion, and dashed line indicates 70% criterion.

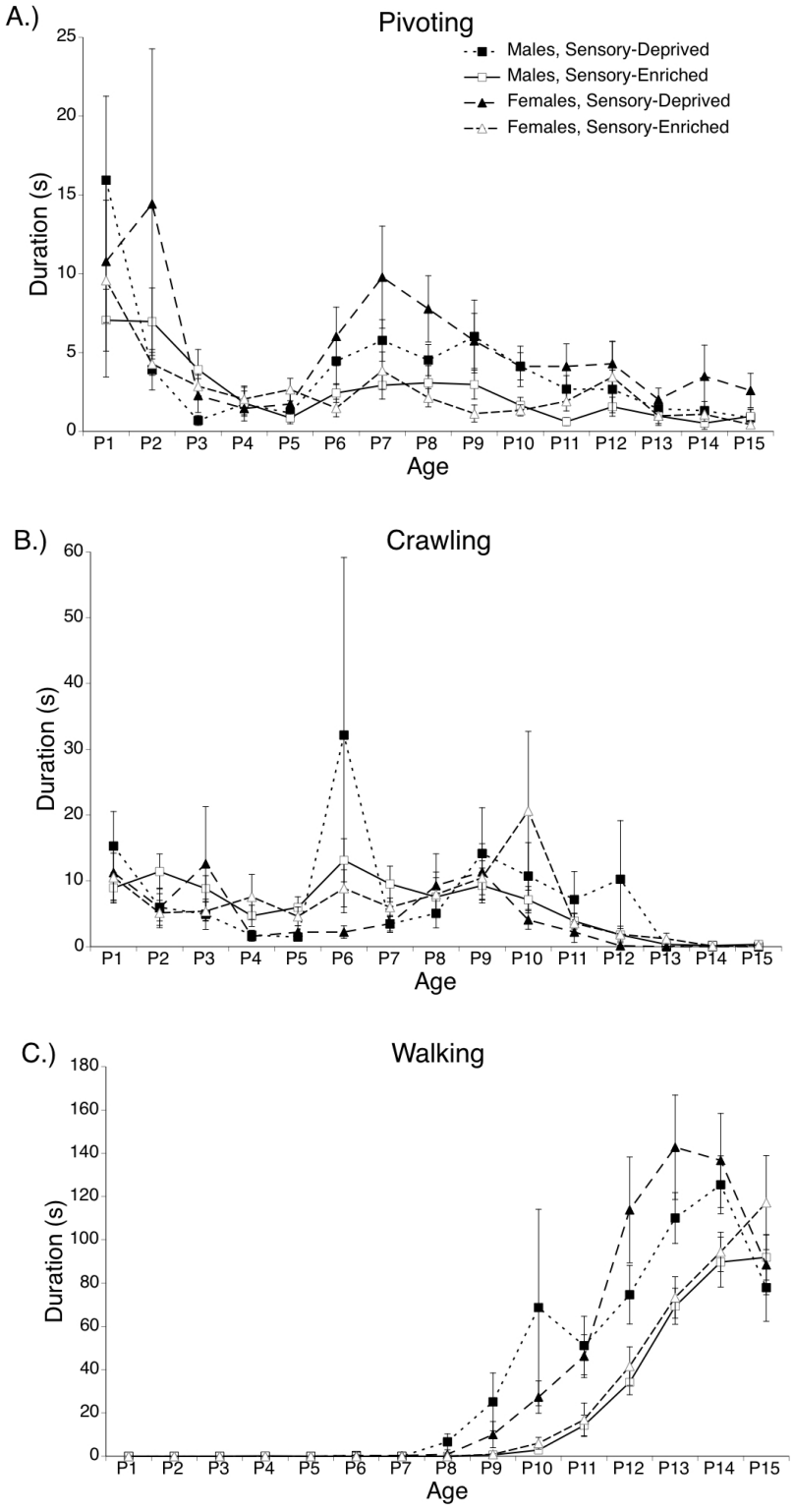

Figure 7. Duration of locomotion over the first two postnatal weeks, as a function of sex and testing condition. A) Pivoting duration. B) Crawling duration. C) Walking duration. Points represent means, vertical lines represent SEM.

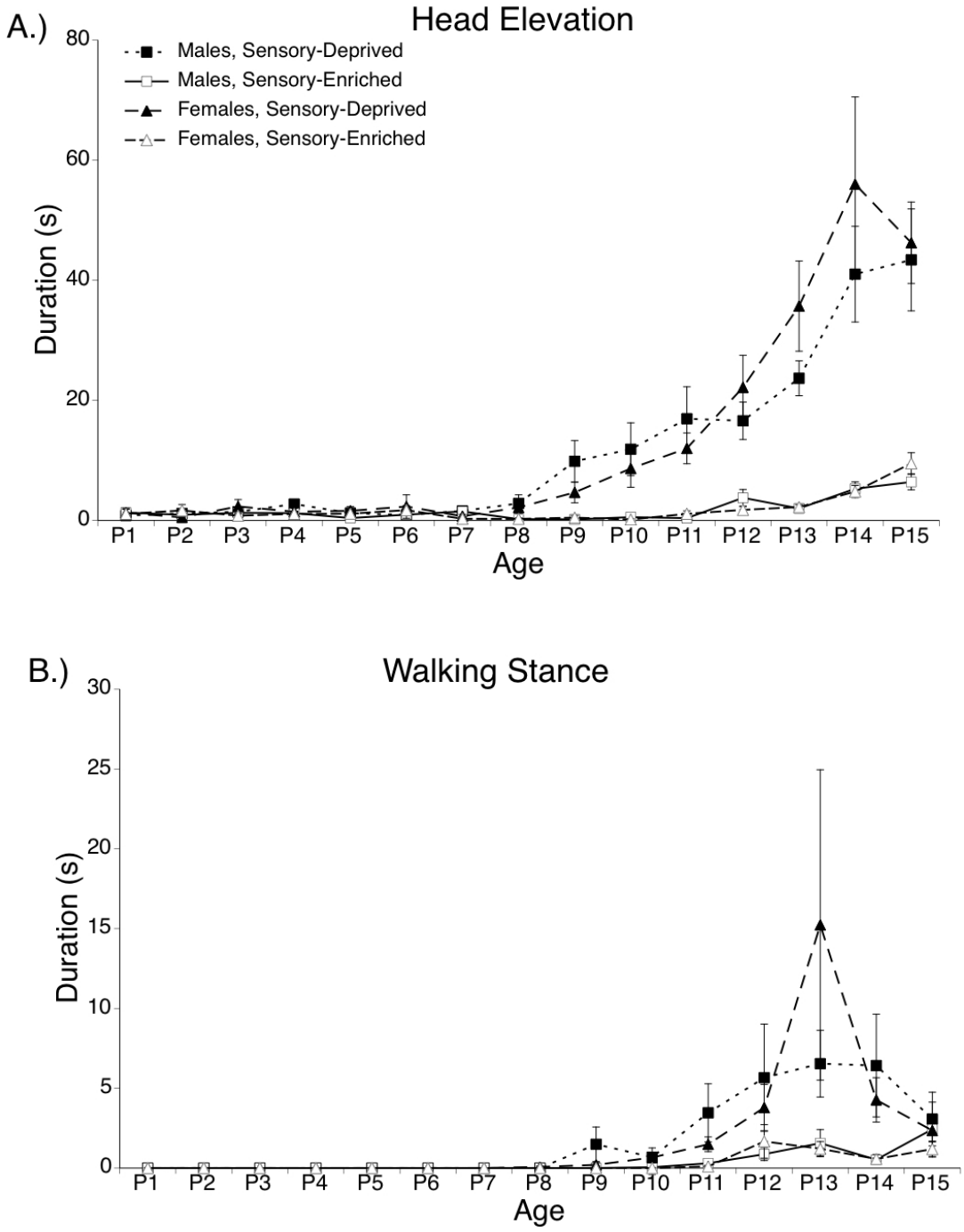

Figure 8. Duration of posture during the first two postnatal weeks. A) Head elevation duration. B) Walking stance duration. Points represent means, vertical lines represent SEM.

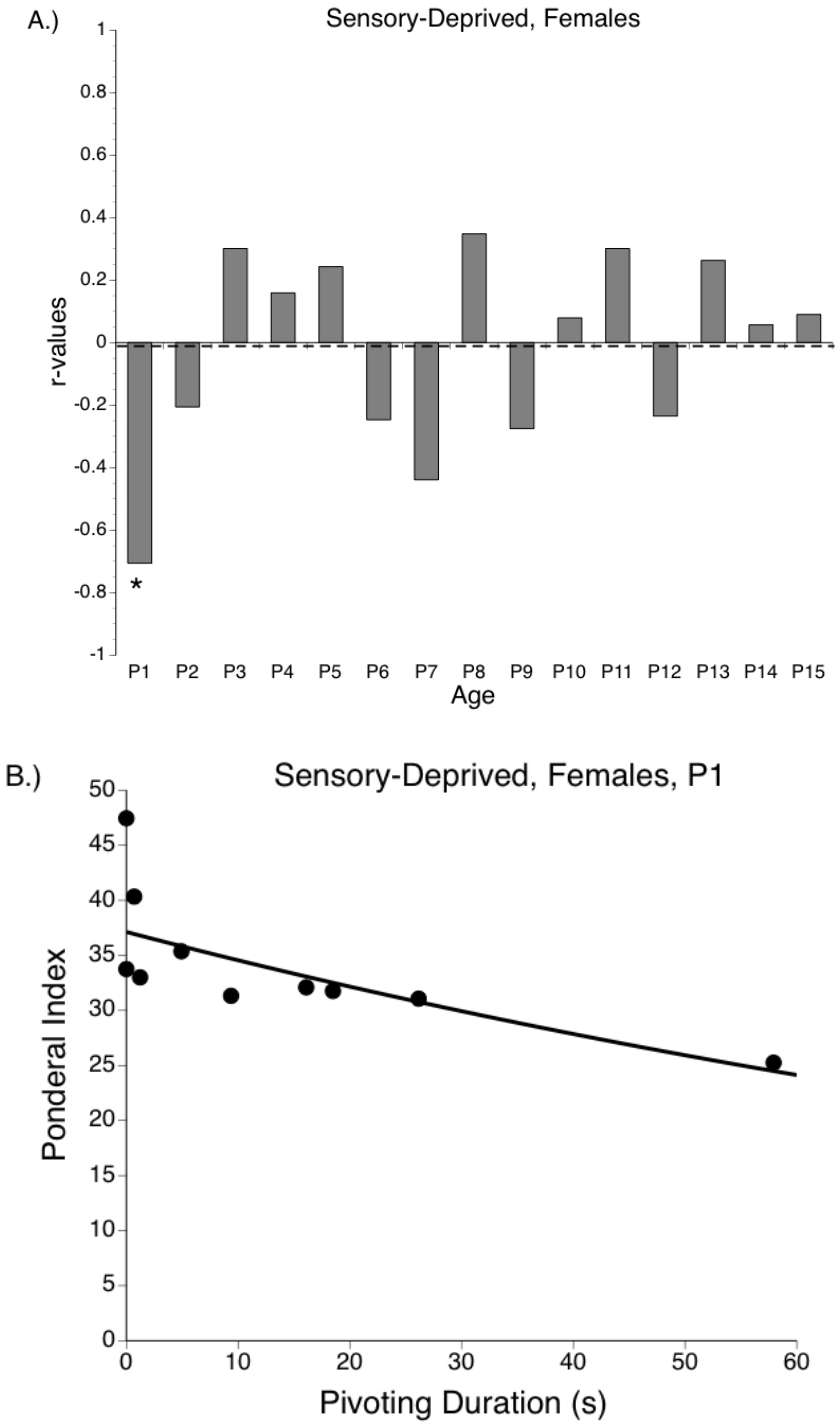

Figure 9. Correlation between pivoting duration and ponderal index for females in the sensory-deprived condition from P1 to P15. A) Correlations for females in the sensory-deprived condition from P1 to P15. Bars represent correlation coefficients, asterisks indicate significance at the $p < .05$ level, and the dashed line represents the average correlation from P1 to P15. B) Ponderal index and pivoting duration for females in the sensory-deprived condition on P1. Points represent individual subject values.

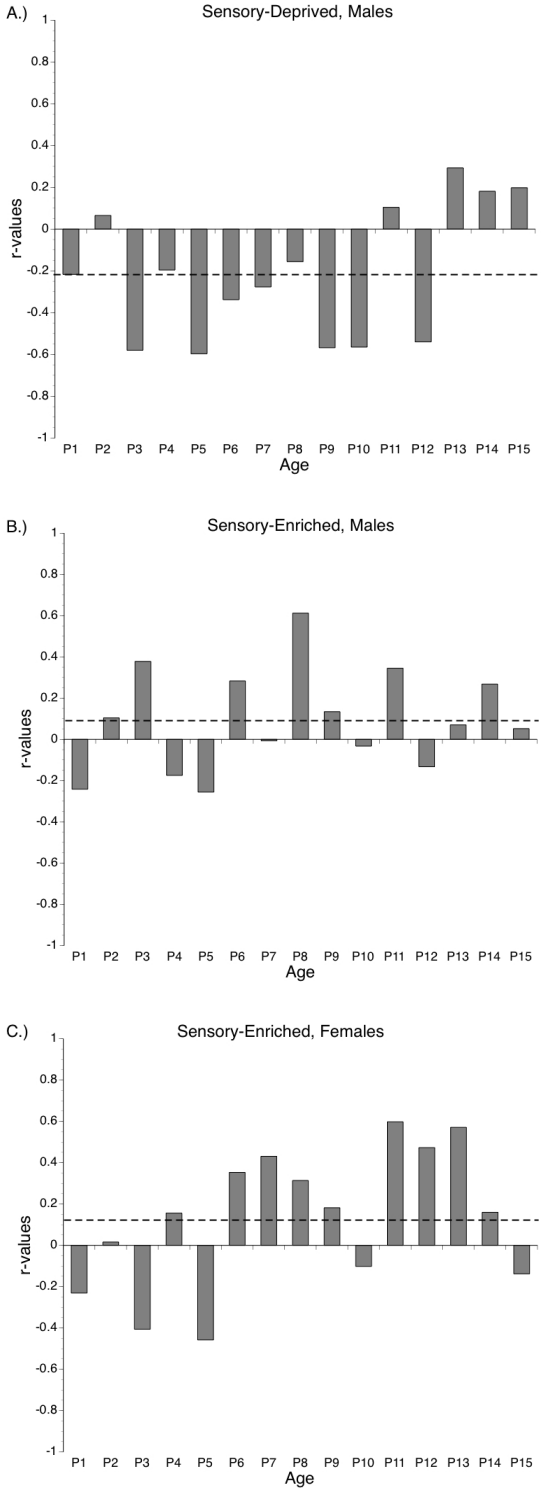

Figure 10. Correlations between ponderal index and pivoting duration from P1 to P15. A) Males in the sensory-deprived condition. B) Males in the sensory-enriched condition. C) Females in the sensory-enriched condition. Bars represent correlation coefficients, dashed lines represent the average correlation from P1 to P15.

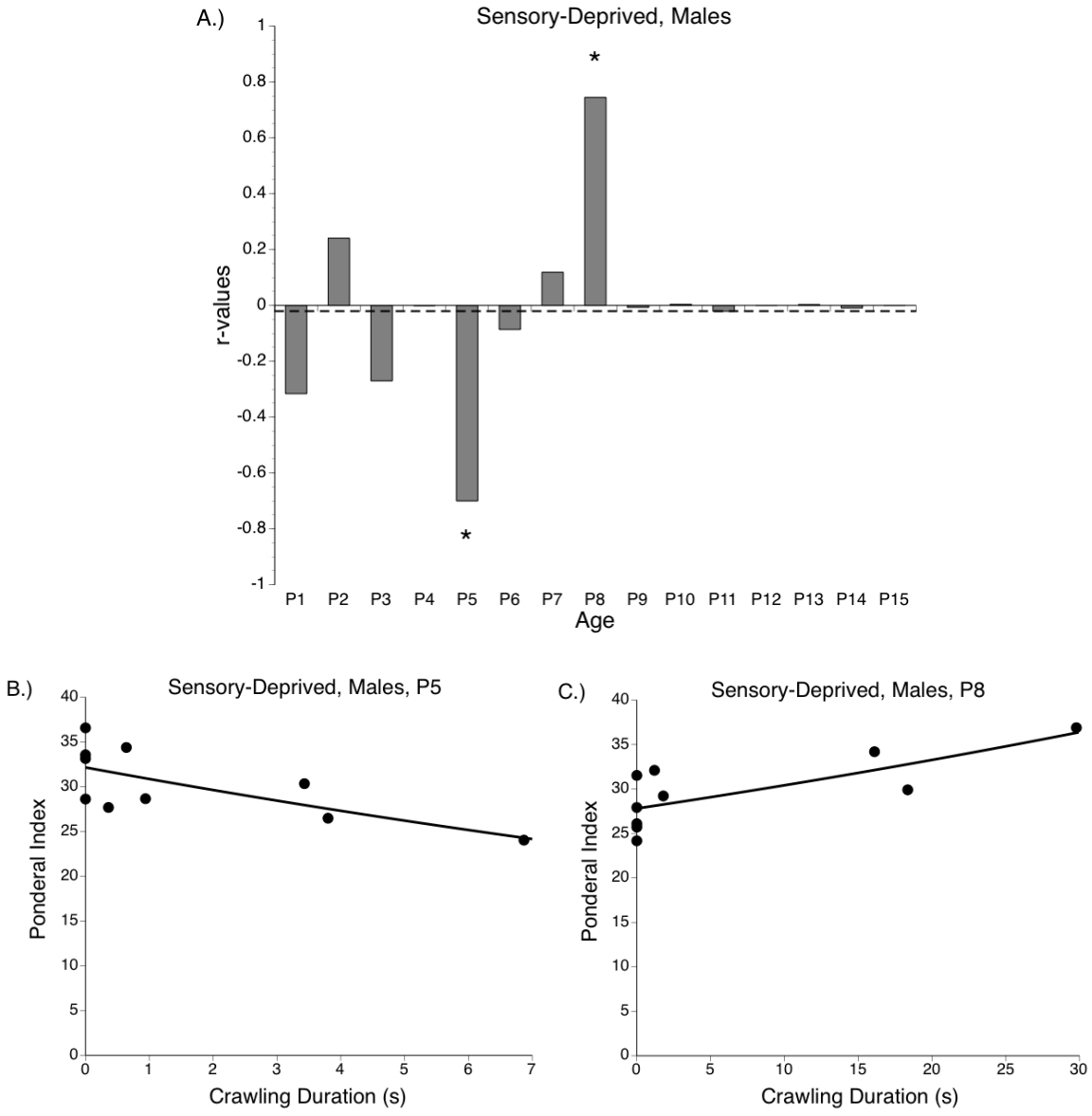

Figure 11. Correlation between crawling duration and ponderal index for males in the sensory-deprived condition from P1 to P15. A) Correlations for males in the sensory-deprived condition from P1 to P15. Bars represent correlation coefficients, asterisks indicate significance at the $p < .05$ level, and the dashed line represents the average correlation from P1 to P15. B) Ponderal index and crawling duration for males in the sensory-deprived condition on P5. C) Ponderal index and crawling duration for males in the sensory-deprived condition on P8. Points represent individual subject values.

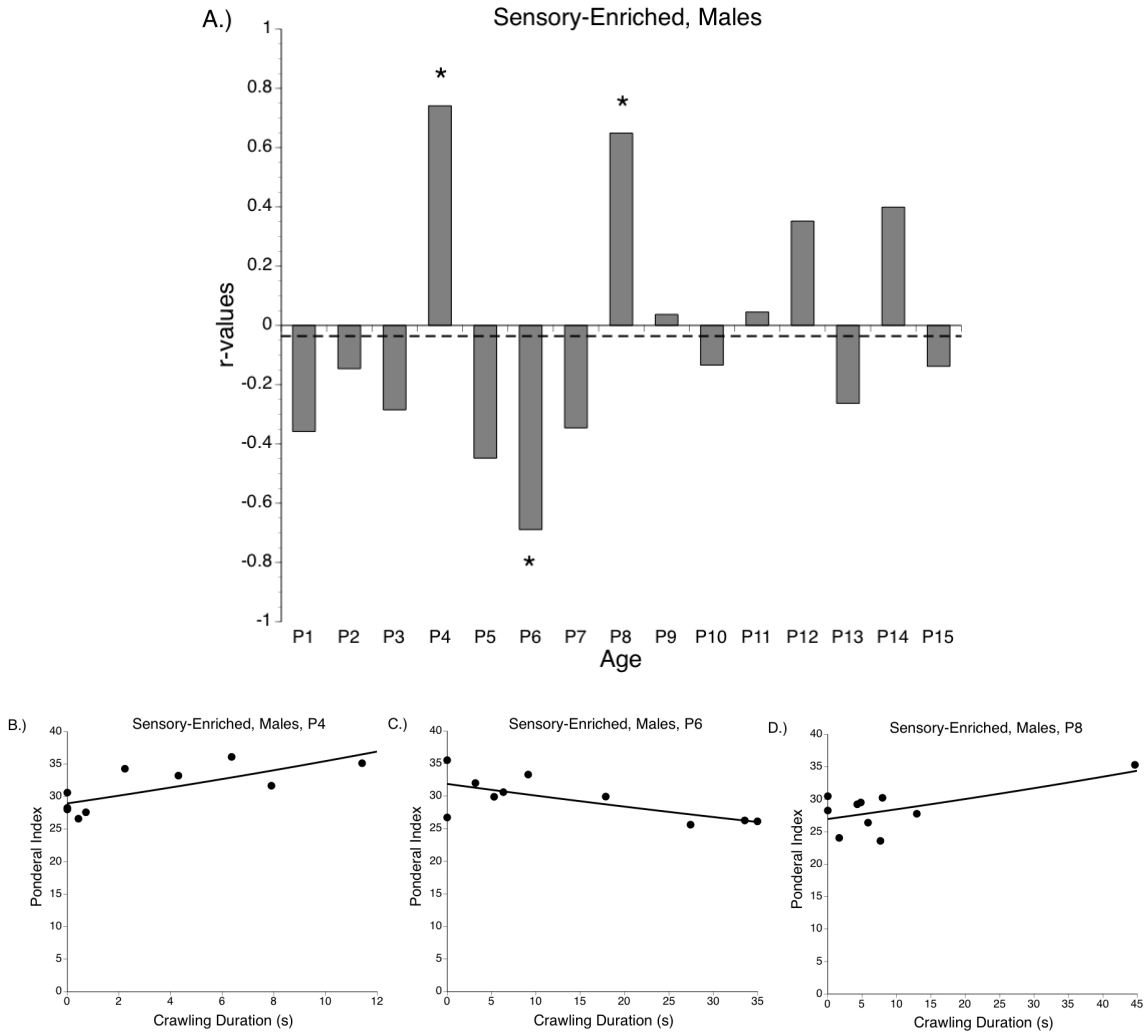

Figure 12. Correlation between crawling duration and ponderal index for males in the sensory-enriched condition from P1 to P15. A) Correlations for males in the sensory-enriched condition from P1 to P15. Bars represent correlation coefficients, asterisks indicate significance at the $p < .05$ level, and the dashed line represents the average correlation from P1 to P15. B) Ponderal index and crawling duration for males in the sensory-enriched condition on P4. C) Ponderal index and crawling duration for males in the sensory-enriched condition on P6. D) Ponderal index and crawling duration for males in the sensory-enriched condition on P8. Points represent individual subject values.

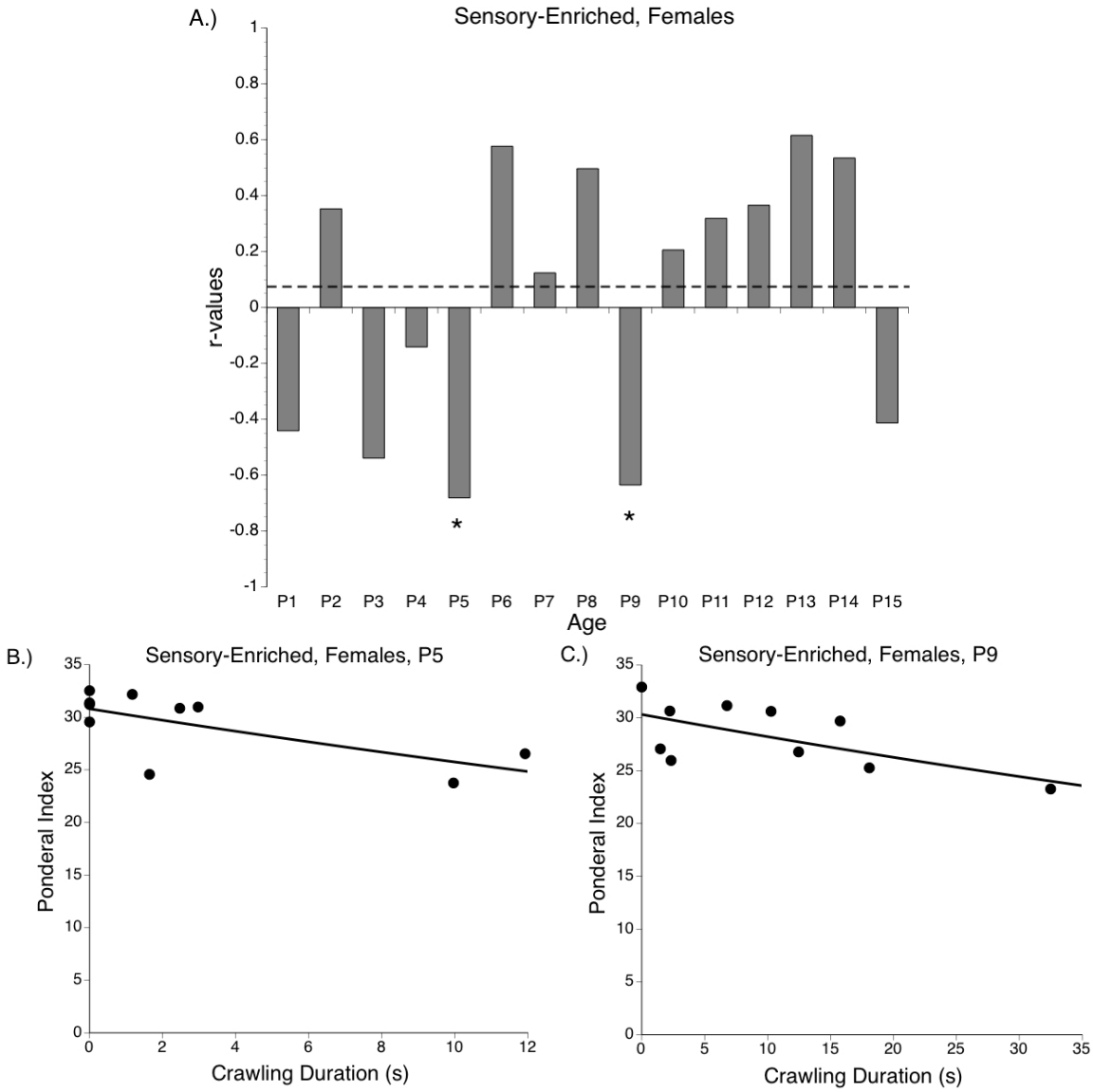

Figure 13. Correlation between crawling duration and ponderal index for females in the sensory-enriched condition from P1 to P15. A) Correlations for females in the sensory-enriched condition from P1 to P15. Bars represent correlation coefficients, asterisks indicate significance at the $p < .05$ level, and the dashed line represents the average correlation from P1 to P15. B) Ponderal index and crawling duration for females in the sensory-enriched condition on P5. C) Ponderal index and crawling duration for females in the sensory-enriched condition on P9. Points represent individual subject values.

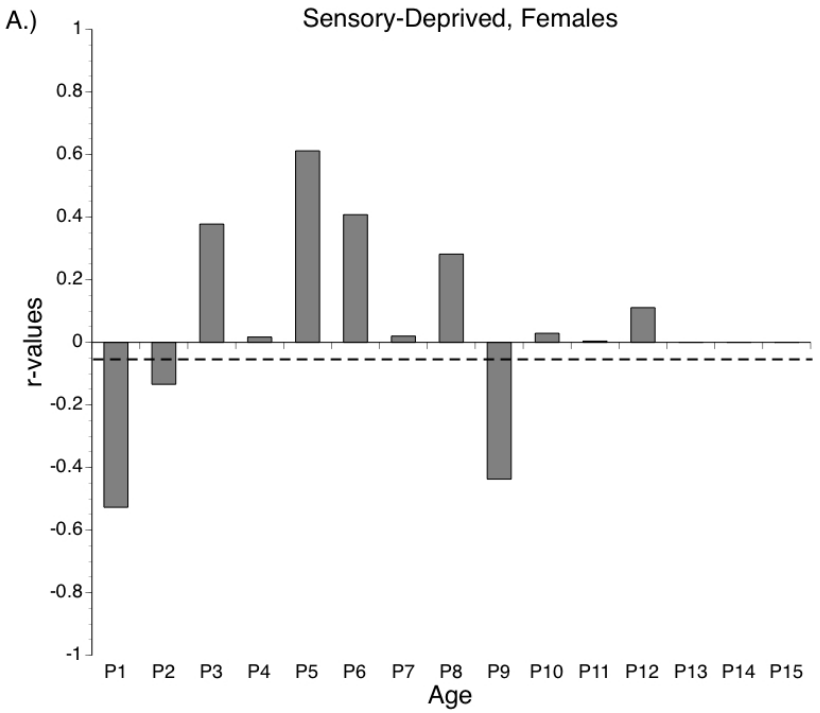

Figure 14. Correlation between ponderal index and crawling duration for females in the sensory-deprived condition from P1 to P15. Bars represent correlation coefficients and dashed line represents average correlation from P1 to P15.

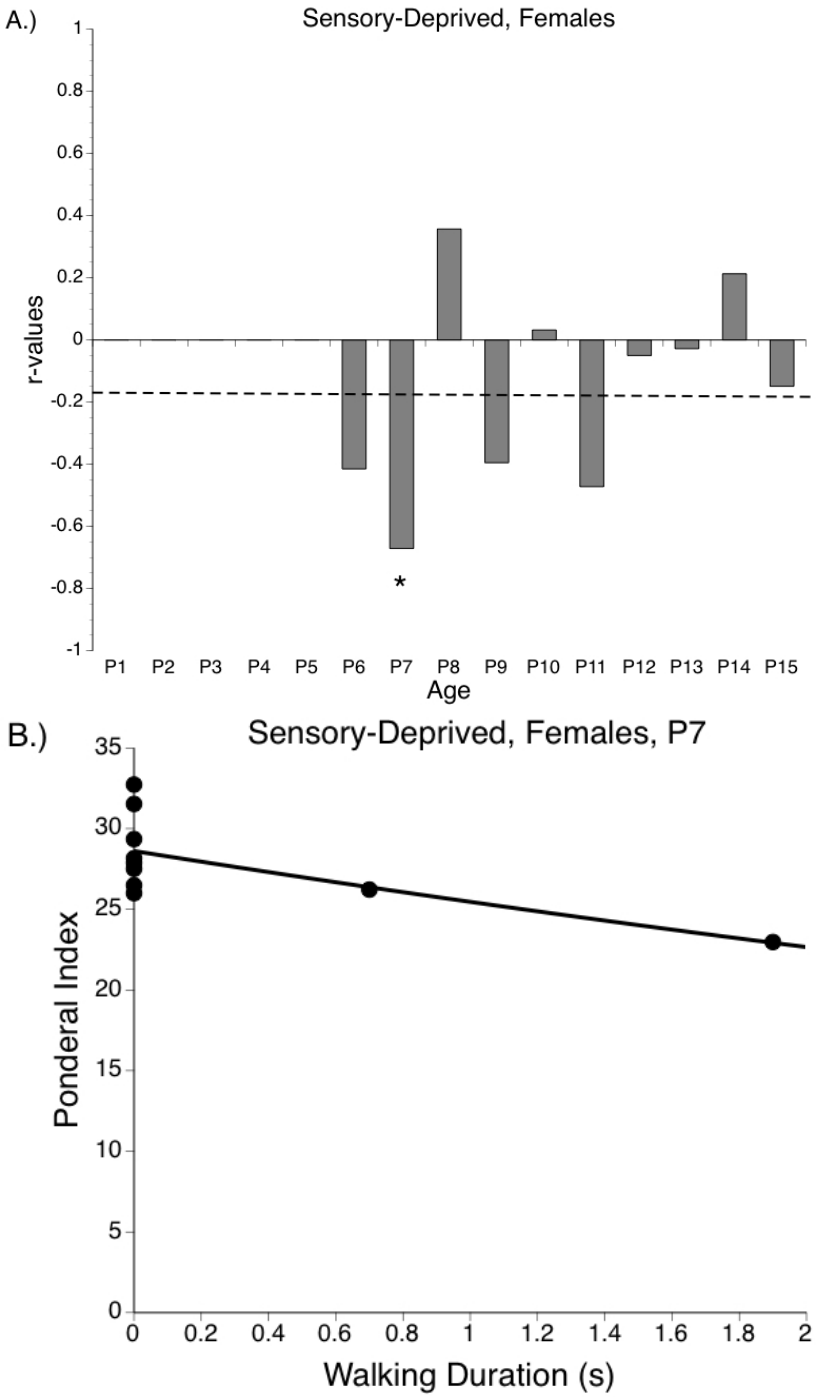

Figure 15. Correlation between walking duration and ponderal index for females in the sensory-deprived condition from P1 to P15. A) Correlations for females in the sensory-deprived condition from P1 to P15. Bars represent correlation coefficients, asterisks indicate significance at the $p < .05$ level, and the dashed line represents the average correlation from P1 to P15. B) Ponderal index and crawling duration for females in the sensory-deprived condition on P7. Points represent individual subject values.

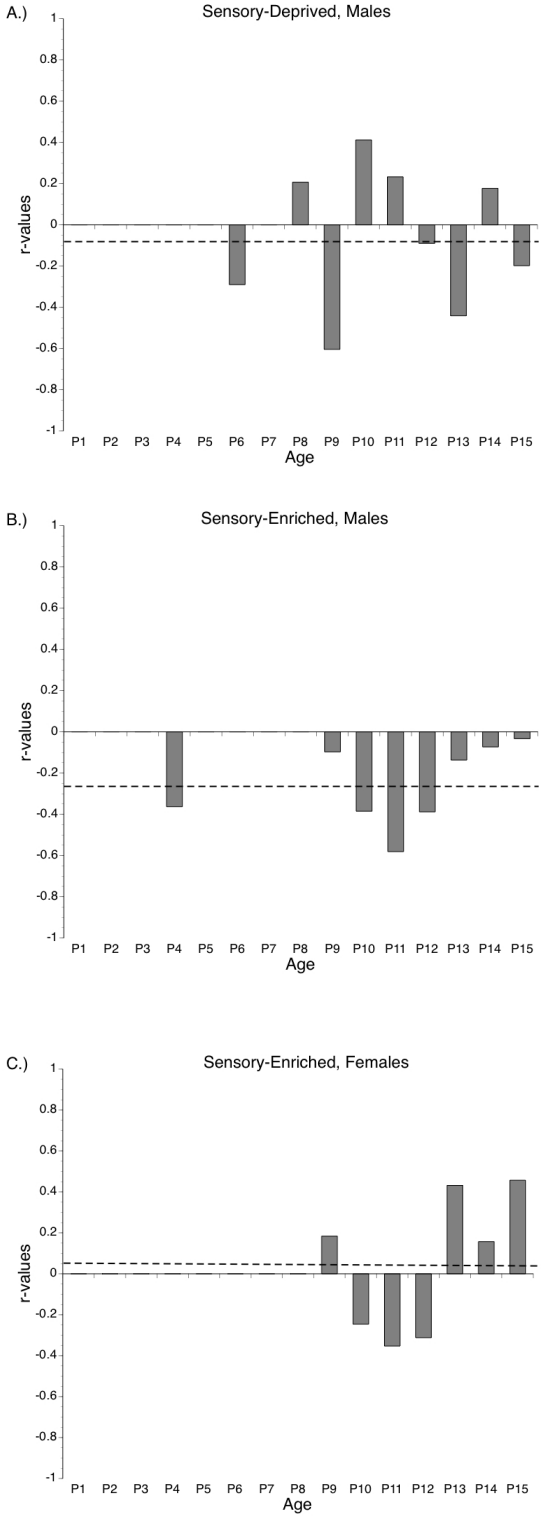

Figure 16. Correlations between ponderal index and walking duration from P1 to P15. A) Males in the sensory-deprived condition. B) Males in the sensory-enriched condition. C) Females in the sensory-enriched condition. Bars represent correlation coefficients, dashed lines represent the average correlation from P1 to P15.

Study	Pivoting	Crawling	Head Elevation	Walking
Present Study	**P1**	**P1**	**P4***	**P10***
Altman & Sudarshan (1975)	P4-5	P8	P8	P12
Bolles & Woods (1964)	Not Included	P3	P4*	P10*
Westerga & Gramsbergen (1990)	Not Included	Middle of 2nd Week	Not Included	P11
Geisler et al. (1993)	Not Included	P5	P5	P10*
Westerga & Gramsbergen (1993b)	Not Included	Not Included	Not Included	P10*
Heyser (2003)	P7	P11	P12	P16

Table 4. Studies examining the first occurrence of locomotion and posture in immature rats, including the current study. Bold indicates the earliest occurrence as reported in our study. Asterisks indicate overlap with previous studies on age of earliest occurrence.