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Posture effects on spontaneous limb movements, alternated stepping, and the leg extension response in neonatal rats

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Abstract

The development of postural control is considered an important factor for the expression of coordinated behavior such as locomotion. In the natural setting of the nest, newborn rat pups adapt their posture to perform behaviors of ecological relevance such as those related to suckling. The current study explores the role of posture in the expression of three behaviors in the newborn rat: spontaneous limb activity, locomotor-like stepping behavior, and the leg extension response (LER). One-day-old rat pups were tested in one of two postures – prone or supine – on each of these behavioral measures. Results showed that pups expressed more spontaneous activity while supine, more stepping while prone, and no differences in LER expression between the two postures. Together these findings show that posture affects the expression of newborn behavior patterns in different ways, and suggest that posture may act as a facilitator or a limiting factor in the expression of different behaviors during early development.

Keywords

posture; newborn; rat; locomotion; quipazine; anogenital stimulation

Postural control is often viewed as a limiting factor in the developmental emergence of coordinated behavior. For example, in rats the neural mechanisms supporting locomotion begin developing prenatally [1–4]; however, the expression of spontaneous locomotion occurs gradually over the first few postnatal weeks [5]. Although immature rats are able to express some forms of locomotor activity, including immature forms of locomotion like crawling [6], the expression of these movement patterns is limited given the general lack of postural control. In the neonatal rat, postural control is immature and support of the head and trunk must be developed before elevated quadrupedal locomotion can be successfully expressed [5,7]. In this way, posture appears to be a limiting factor in the early expression of motor behavior in rats and its development is fundamental for the execution of more complex locomotion [8].

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An early description of the development of posture in the newborn rat comes from the classic work of Altman and Sudarshan [5]. In their study, they described how the newborn rat undergoes gradual changes in postural control during the first two weeks after birth. During the first postnatal week, the forelimbs come to support the pup's body weight and drive the early expression of locomotor behavior. By the end of the second week the hindlimbs become mature enough to support the weight of the body, and it is at this time that locomotion using all four limbs appears. In this way, the development of posture follows a rostro-caudal gradient, in which maturation of the forelimbs is followed by the hindlimbs [7,9,10]. Additionally, newborn rats must be able to support their heads to be able to express coordinated locomotion. It is not until around postnatal day 5 that rats can perform controlled head lifting [11]. Although some studies suggest that newborn rats are capable of expressing odor-induced crawling before posture control is developed [6,12,13], it is not until after neonates can support their head and trunk that they can perform sustained periods of locomotion [5,7].

The postnatal development of posture happens along with the development of the vestibular afferents and descending pathways in the spinal cord that are necessary for posture control [8,10]. Although pharmacological stimulation of the spinal cord can induce locomotor-like activity in both in vitro studies [e.g., see 14] and in vivo studies in perinatal rats [2,15–17], many projections from the brain to the spinal cord that are necessary for the control of posture and locomotion do not fully develop until the end of the second postnatal week [18]. In addition, electromyography (EMG) recordings in neonatal rats suggest that muscles mature and establish stronger connections by the second week after birth [9,19,20]. For example, motoneurons innervating the ankle extensors are more immature at birth than the motoneurons innervating the ankle flexors [21]. As all of these processes within the sensorimotor and musculoskeletal systems continue to develop in the infant rat, posture becomes stable and controlled, which then allows the pup to express more complex motor behaviors like locomotion [5,8].

Even though lacking stable postural control seems to be the limiting factor for the execution of the adult pattern of locomotion [7], newborn rats can express functional patterns of behavior, as permitted by their capacities during this time, that are necessary for their survival. Research has shown that the perinatal rat is capable of adapting to characteristics and modifications of their surrounding environment and can express coordinated behavior regardless of the immaturity of their motor and sensory systems [15,22–25]. Many of these newborn behavior patterns are expressed in the context of the nest or during maternal-infant interactions, and involve variations in posture.

For example, newborn rats move and adapt their posture during search and location of the nipple. One-day-old rat pups move from a prone to a supine posture under the ventral surface of the mother before they engage in suckling [13,26]. Even so, while newborn rat pups remain supine when they perform oral grasping of their mother's nipple [26], by the second week after birth pups move to a prone posture to engage in suckling [27]. Additionally, Moore and Chadwick-Dias [28] reported that rats may receive anogenital licking from their mothers while in the prone or supine posture. However, pup-directed licking occurs most frequently in the supine posture during the first two postnatal weeks.

These findings suggest that immature animals normally experience variations in posture that facilitate the expression of behaviors of ecological relevance.

Facial wiping, another action pattern expressed by the perinatal rat, has been shown to be modulated by posture. Sensory-induced facial wiping is facilitated when pups are placed in a supine position, but not when in the prone posture [29]. Even more, research has shown that facial wiping can be expressed in an optimal way if the limiting factor of having an immature posture is somehow reduced through experimental manipulation. For example, newborn pups have been tested submerged in water rather than on a hard surface [30], and fetuses have been tested inside the constraints of their own amniotic sac [25], which then allows facial wiping to be expressed.

Importantly, research also has shown that the perinatal rat is sensitive to vestibular information [31]. Newborn rat pups tested after being flown on board the NASA space shuttle during gestation showed alterations in their righting responses when tactile and proprioceptive stimulation was removed, suggesting an effect on their vestibular function [32,33].

All of these findings suggest that variations in posture and vestibular stimulation may influence the early expression of coordinated behavior in perinates. Postural modifications seem to be typical for the expression of behavior within the context of their immediate environment in the nest. However, whether or not posture differentially influences the expression of newborn behavior has not been well studied and systematically assessed. As a result, the aim of the current study was to investigate the role of posture in the expression of three behaviors that are typically expressed within the context of the nest or mother-infant interactions: (a) spontaneous limb activity, (b) locomotor-like stepping behavior, and (c) the leg extension response (LER).

The three behaviors examined here were chosen because they are expressed by the newborn through different routes. The first one, spontaneous forelimb and hindlimb activity, occurs spontaneously in the perinatal rat without any apparent or explicit stimulation [34–36]. The second behavior – stepping – is an example of a drug-induced behavior. Alternated air-stepping behavior induced by quipazine (a 5-HT receptor agonist) is a paradigm that has been effectively used to study locomotor development in the fetal [2] and newborn rat [15]. Although quipazine-induced stepping is examined in the current study, it is well known that newborns express stepping movements on the ventrum of the dam during suckling episodes [26] and also following tail pinch. And finally, the third behavior – the LER – is a sensory-induced behavior normally evoked by maternal licking of the anogenital area in newborns [28], but that can also be induced by artificial stimulation using a vibrotactile device [23].

As discussed above, newborn rat pups adapt their posture during regular activity in the nest and during maternal interactions [26–28]. Development of posture control and postural adaptations at this early age happens while sensory, motor, and neuromuscular processes mature and develop [8]. In particular, the difference in maturation between flexors and extensors may account for posture adaptations from supine to prone in the neonatal rat [26,27], which suggests that the expression of spontaneous activity, stepping, and LER in

these two different postures could vary. In this way, the general hypothesis of this study was that behavior in the newborn rat would be relatively inhibited in the supine posture than in prone, given that at this age innervation of extensor muscles is less mature than innervation of flexor muscles (i.e., making extension more difficult in the supine posture, as limb extension would have to counteract gravity). Rat pups were tested one day after birth in the supine or prone posture to investigate whether posture influences the expression of these behaviors. If posture modulates expression of newborn behaviors, this would suggest that natural variations in posture or vestibular stimulation play a role in the development of behavior, and perhaps the acquisition of mature forms of motor coordination.

GENERAL METHODS

Subjects

A total of 64 rat pups (Sprague-Dawley strain) served as subjects in this study. Dams were housed individually on the day before parturition in standard laboratory cages that remained in the animal colony, where ambient temperature and humidity was controlled. Pups stayed in their home cages in the animal colony until the time of testing. Pups were tested on postnatal day 1 (P1; 24 hrs after birth). All animals were maintained on a 12:12 hr light-dark cycle (lights on at 0700 hr). Food and water were available ad libitum. All procedures involving the care and use of animals were reviewed and approved by the university Institutional Animal Care and Use Committee.

Pups were tested in one experiment only. In each experiment, no more than one pup per condition was used from a single litter. This procedure avoided litter effects.

Testing Apparatus and Behavioral Recording

Subjects were tested inside an infant incubator that regulated humidity and maintained air temperature at 35°C. They were manually voided and then placed inside the incubator 30 min before testing with 2–3 siblings, to allow for acclimation to incubator conditions. After acclimation, the subject pup was gently secured to a soft, vinyl-coated horizontal bar with two thin straps: one strap extended across the abdomen and the second strap extended across the neck. The straps did not interfere with limb or head movements, but held the pup securely and prevented the pup from falling or moving out of camera view.

All pups were initially secured to the horizontal bar in a supine posture. For pups tested in the prone posture, the bar was rotated so that the limbs were pendant and not in contact with a surface. A micro-camera was located inside the incubator and was connected to an outside recording DVD unit. The micro-camera was positioned above subjects in the supine posture, and below subjects in the prone posture, permitting clear view of all the limbs. All test sessions were recorded onto DVD.

Behavioral scoring

Limb activity was scored in multiple viewing passes during DVD playback. Time code impressed during recording permitted identification of individual video frames and synchronization of separate scoring passes. We used an event recorder program

(JWatcher™) to record the category of behavior and time of entry (± 0.01 s). Inter- and intrarater reliability for scoring was 90%.

Data analysis

Limb activity was summarized from scoring records, and differences in limb activity were compared with analysis of variance (ANOVA) tests. Post hoc comparisons used Fisher's protected least significant difference (PLSD). All tests were conducted at the $p < .05$ level of significance, and were performed using StatPlus or StatView 5.0.

EXPERIMENT 1: EFFECT OF POSTURE ON SPONTANEOUS FORELIMB AND HINDLIMB MOVEMENTS

Spontaneous limb activity is commonly expressed during perinatal life before the development of more organized and complex motor patterns such as locomotion [34,35,37]. Any movements that appear to occur without any apparent stimulation are considered to be spontaneous. Research has shown that although spontaneous activity looks random, it is actually quite organized [38] and although variable, it also leads to the expression of coordinated motor patterns in the perinate [39]. In newborn rats, the expression of spontaneous limb movements can be modified in response to sensory feedback produced by changes in proprioceptive feedback [22]. Newborn rats adapt their posture during maternal-infant interactions in the nest [13,26,27], suggesting that the expression of spontaneous limb movements may be affected by postural modifications. The purpose of Experiment 1 was to determine the effects of posture on spontaneous forelimb and hindlimb movements.

Methods

Spontaneous motor activity of P1 rats was examined in one of two postural conditions. Sixteen P1 rats were assigned to testing in the supine ($n=8$) or prone ($n=8$) posture. As there are no reported sex differences in spontaneous motor activity of perinatal rats [38], half of the subjects in each group were male and half were female. After pups were secured in the appropriate testing posture, the test session began. The test session was 30 min in length and recorded onto DVD.

Each instance of forelimb and hindlimb movement, excluding passive movements, was scored during normal speed video playback. A separate scoring pass was made for each limb [38]. Scored movements included high amplitude limb, coordinated behaviors (e.g., occasional stepping or stretching) and low amplitude limb "twitches" (i.e., fast, independent, phasic movements characteristic of sleep) [40]. In this experiment, different categories of movement were not distinguished. Because behavior in the current experiment was not explicitly evoked by the experimenter, all movements were considered "spontaneous" (except for passive movements as described above). Forelimbs and hindlimbs were summarized in six 5-min bins, and analyzed separately using time as a repeated measure.

Results

Forelimbs—For the number of forelimb movements, a two-way repeated measures ANOVA (2 postures $\times$ 6 time bins) revealed a significant effect of posture [$F(1, 15) = 7.10$,

$p = 0.02$], and a significant effect of time [$F(5, 80) = 3.74, p < 0.001$], but no interaction [$F(5, 80) = 1.77, p = 0.13$]. As shown in Figure 1A, forelimb activity was significantly greater in the supine versus the prone posture. Post hoc tests comparing the different time bins showed that forelimb activity was significantly greater at the end of the test session (30-min bin) compared to the first half of the test session (up to 15 min).

Hindlimbs—A two-way repeated measures ANOVA (2 postures x 6 time bins) revealed a significant effect of posture [$F(1, 15) = 8.22, p = 0.01$], a significant effect of time [$F(5, 80) = 7.05, p < 0.0001$], and a significant posture x time interaction [$F(5, 80) = 4.19, p = 0.002$] on the number of hindlimb movements. As with the forelimbs, hindlimb activity was significantly greater in the supine posture. As can be seen in Figure 1B, hindlimb activity changed very little over the course of the test session for pups tested prone, but significantly increased during the test session for pups tested supine. To examine the interaction, a series of t-tests compared the effects of the two postures at each time bin. After the first 5-min ($p = 0.5$), pups tested in the supine posture showed significantly more hindlimb movements than pups tested in the prone posture (all $ps = 0.01$ – 0.04).

In summary, pups tested in the supine posture expressed more spontaneous forelimb and hindlimb movements in comparison to pups tested in a prone posture. Thus posture affected the expression of spontaneous limb activity in newborn rats.

EXPERIMENT 2: EFFECT OF POSTURE ON QUIPAZINE-INDUCED STEPPING OF THE FORELIMBS AND HINDLIMBS

The purpose of Experiment 2 was to investigate the effect of posture on quipazine-induced stepping in one-day-old rats. Research has shown that administration of the serotonergic agonist quipazine induces locomotor-like alternated stepping in neonatal [15,41] and fetal rats [2,16,42]. The effects of quipazine on limb activity have been shown to be blocked by pretreatment with a 5-HT₂ receptor antagonist [43,44]. Recently it was shown that cutaneous and proprioceptive feedback influence quipazine-induced stepping in newborn rats [15]. In Experiment 2, pups were administered quipazine or saline (vehicle control) to examine the effect of posture on forelimb and hindlimb stepping behavior.

Methods

Quipazine-induced stepping behavior of P1 rats was examined in one of two postural conditions. Subjects were 32 P1 rats. Sixteen pups were assigned to testing in the supine ($n=16$) or prone ($n=16$) posture. As we have no evidence for sex differences in quipazine-induced behavioral effects in perinatal rats [2; unpublished observations], half of the subjects in each group were male and half were female. After pups were secured in the appropriate testing posture, the test session began. Following a 5-min baseline, pups received a 50 μ l intraperitoneal injection with a 30-gauge needle: 3.0 mg/kg quipazine maleate (2-(1-piperazinyl) quinoline maleate salt (a serotonergic agonist obtained from Sigma-Aldrich (St. Louis, MO)) or 0.9% (wt/vol) saline (vehicle control). This dose of quipazine previously has been shown to effectively induce stepping behavior in perinatal rats [2,15]. Half of the subjects tested in each posture received treatment with quipazine; the

other half of the subjects were treated with saline. The test session continued for 30 min following drug injection, and was recorded onto DVD.

Data analysis for this experiment was conducted in two phases. In phase one, the frequency of alternated stepping and non-stepping forelimb and hindlimb movements was scored during playback of the 35-min test session (5-min baseline plus 30-min post-injection period). Separate scoring passes were made for the forelimbs and hindlimbs. An alternated step cycle was defined as two consecutive and alternating extensions and flexions of homologous limb pairs; thus the term “step” is used here to indicate a bilateral alternating pattern of coordination [2,15]. Synchronous steps were not scored separately (but rather included in non-stepping behavior), since quipazine does not evoke many synchronous steps in newborn rats [15]. Frequencies of steps and the percentage of steps (as a function of total limb movements) were summarized in 5-min bins and analyzed using repeated measures ANOVA, using time as the repeated factor and posture and drug as independent variables.

For the second phase of data analysis, we examined bouts of stepping behavior in quipazine-treated subjects only. A stepping bout was defined as the set of all consecutive stepping movements in which the interval between successive steps did not exceed a specified criterion [45,46]. The bout criterion was determined by fitting a negative exponential function to the cumulative survivor distribution of inter-step interval lengths, calculated in increments of 0.5 s, and identifying the inflection point where the observed distribution began to systematically deviate from the best-fitting exponential function. A representative plot of such a survivor distribution is shown in Figure 3A. This procedure was applied separately for forelimbs and hindlimbs, and separately for each subject. As is typical in temporal distributions of this kind, the number of short intervals was much more abundant than expected from the exponential curve [36,40]. For nearly all subjects, and both the forelimbs and hindlimbs, the optimal bout criterion identified by this method fell between 2.5 and 4.0 s, with the clear mode at 3.0 s. Therefore, a single bout criterion of 3.0 s was used for all subsequent analyses of bout lengths. Thus, any bilateral alternated step that occurred within 3 s of the preceding step was considered part of the same stepping bout. The number of stepping bouts, the number of steps per bout, and the total duration of stepping bouts was analyzed separately for the forelimbs and hindlimbs using ANOVA tests to compare between posture groups.

Results

Step frequency—In the first phase of data analysis we examined the frequency of stepping behavior. For the number of forelimb steps, there was a main effect of drug [$F(1, 31) = 42.54, p < 0.0001$], posture [$F(1, 31) = 10.42, p = 0.003$], and time [$F(6, 192) = 12.38, p < 0.0001$]. All interactions were significant [posture x drug, $F(1, 31) = 9.99, p = 0.004$; posture x time, $F(6, 192) = 3.43, p = 0.003$; drug x time, $F(6, 192) = 11.63, p < 0.001$; drug x posture x time, $F(6, 192) = 2.86, p = 0.01$]. As shown in Figure 2A, pups treated with quipazine showed significantly more forelimb steps than pups treated with saline, and pups tested in the prone posture showed significantly more stepping than pups tested in the supine posture. To follow-up the 3-way interaction, we examined the effect of posture in each drug group across time. For pups treated with quipazine, significantly more stepping occurred in

the prone versus the supine posture, at all time points following injection of quipazine. For pups treated with saline, there was no effect of posture or time. Forelimb stepping occurred very infrequently in saline-treated pups (Figure 2A).

To account for the amount of stepping relative to total limb movements, we also examined the percentage of limb movements that were engaged in bilateral stepping. For percent forelimb stepping, there was a main effect of drug [$F(1, 31) = 122.7, p < 0.0001$], posture [$F(1, 31) = 4.33, p = 0.05$], time [$F(6, 192) = 35.19, p < 0.0001$], and significant interactions between drug and time [$F(6, 192) = 23.54, p < 0.0001$], and posture and time [$F(6, 192) = 2.39, p = 0.03$]. As can be seen in Figure 2B, percent forelimb stepping increased following injection of quipazine, was higher for pups tested in the prone posture, and remained well above 50% for the entire test session for pups treated with quipazine.

The findings for hindlimb stepping closely mirrored those for forelimb stepping. For the number of hindlimb steps, there was a main effect of drug [$F(1, 31) = 38.09, p < 0.0001$], posture [$F(1, 31) = 13.78, p = 0.001$], and time [$F(6, 192) = 11.15, p < 0.0001$]. All interactions were significant [posture x drug, $F(1, 31) = 9.26, p = 0.01$; posture x time, $F(6, 192) = 3.94, p = 0.0001$; drug x time, $F(6, 192) = 9.45, p < 0.00001$; drug x posture x time, $F(6, 192) = 2.37, p = 0.03$]. As can be seen in Figure 2C, quipazine-treated pups showed significantly more hindlimb steps than saline-treated pups, and pups tested in the prone posture showed significantly more stepping than pups tested in the supine posture. Next we followed-up the 3-way interaction by examining the effect of posture in each drug group across time. For pups treated with quipazine, significantly more stepping occurred in the prone versus the supine posture, at all time points following quipazine injection. For pups treated with saline, there was no effect of posture or time (Figure 2C).

For percent hindlimb steps, there was a main effect of drug [$F(1, 31) = 50.29, p < 0.0001$], posture [$F(1, 31) = 4.35, p = 0.05$], time [$F(6, 192) = 20.73, p < 0.0001$], and significant interactions between drug and time, [$F(6, 192) = 10.44, p < 0.0001$]. As shown in Figure 2D, percent hindlimb stepping increased following injection of quipazine, was higher for pups tested in the prone posture, and as with the forelimbs remained well above 50% for the entire test session for pups treated with quipazine.

Stepping bouts—In the second phase of data analysis we further characterized stepping behavior in quipazine-treated pups. We analyzed parameters of stepping bouts (number of stepping bouts, steps per bout, and total duration of bouts) for the two postures. For the forelimbs, more stepping bouts occurred in the prone posture, but this effect only approached significance, [$F(1, 15) = 4.1, p = 0.06$; Figure 3B]. Additionally, the number of forelimb steps per bout only approached significance [$F(1, 15) = 3.01, p = 0.1$; Figure 3C]. When looking at the total duration of forelimb stepping bouts analyzed, the duration was significantly longer for pups tested in the prone posture [$F(1, 15) = 8.83, p = 0.01$; Figure 3D].

For the hindlimbs, significantly more stepping bouts occurred in the prone posture [$F(1, 15) = 11.13, p < 0.001$; Figure 3B]. The number of hindlimb steps per bout also was significantly higher in the prone posture [$F(1, 15) = 5.5, p = 0.03$; Figure 3C]. For the total

duration of hindlimb stepping bouts analyzed, the duration was significantly longer for pups tested in the prone posture [$F(1, 15) = 14.69, p = < 0.001$; Figure 3D]. Figure 3 shows the parameters of stepping bouts for the forelimbs and hindlimbs, for pups treated with quipazine.

In summary, quipazine promoted more forelimb and hindlimb steps in the prone versus the supine posture. Although the number of stepping bouts and steps per bout were similar across the two postures for the forelimbs, quipazine promoted more stepping bouts and steps per bout in the hindlimbs. Therefore in general, posture affected both the frequency and parameters of stepping behavior.

EXPERIMENT 3: EFFECT OF POSTURE ON THE LEG EXTENSION RESPONSE (LER)

While Experiment 1 explored the effects of posture on spontaneous behavior and Experiment 2 investigated the effects of posture on a drug-induced behavior, in Experiment 3 pups were assessed for postural effects during a sensory-evoked behavior. As a part of mother-newborn interactions, the dam engages in licking of her pups on different areas of the pups' body, including the anogenital (AG) region [47]. Licking of the AG area stimulates urination and defecation in the pup [48] and evokes a behavioral response by the pup known as the leg extension response (LER) [28]. The LER occurs when one or both hind legs extend from a resting position and remain rigid and positioned at a right angle to the trunk of the body, during AG stimulation [23]. While in the natural setting the LER is evoked by maternal stimulation through licking [28], in an experimental setting the LER may be reliably evoked by artificial stimulation using an electronic vibrotactile device [23]. The purpose of Experiment 3 was to investigate the effect of posture on the LER in one-day-old rats.

Methods

LER expression was examined in 16 P1 male rats. Subjects were assigned to testing in the supine ($n=8$) or prone ($n=8$) posture. We tested only males in this experiment because sex differences in LER expression during bouts of AG licking have been reported to occur in immature rats [28]. Since males typically are licked more than females, they would thus have more LER experience, and may be more likely to perform the LER at this young age. One minute after pups were secured in the appropriate testing posture, they were stimulated in the AG region to evoke the LER. The LER was defined as one or both of the pup's hindlimbs moving from a resting position to an immobile, hyperextended position. It was evoked using vibrotactile stimulation: an electronic vibration device (Penthouse Cyber Flicker; 120 Hz) that had a strip of latex (8 mm long; 1.5 mm wide) attached to the tip was applied to the AG region of the pup [23]. Subjects were stimulated in the AG region with the vibrotactile device for 9 seconds. A pilot experiment conducted in our lab indicated that 9 sec of stimulation was generally sufficient for evoking the LER in P1 rat pups, using this method [Cooper, Livesay, Strain, & Brumley, unpublished observations]. The test session was recorded onto DVD using a posterior camera view that was focused on the hindquarters.

Latency and duration of unilateral and bilateral LERs were scored during playback of the test session. These dependent measures were analyzed by one-way ANOVAs with posture as the independent variable.

Results

Seven out of eight subjects per group expressed a bilateral LER, and the same was true for expression of a unilateral LER. Latency to respond to AG stimulation with an LER and the duration of LER were examined for subjects that expressed the response. The ANOVAs yielded no significant differences between postures in the latency to unilateral or bilateral LER (p 's = 0.10 – 0.60), and no significant differences for LER durations (p 's = 0.60 – 0.80). Figure 4 shows the mean latencies and durations for each group. Although not statistically significant, subjects in the prone posture were slightly faster to express a bilateral LER [$F(1, 14) = 3.23, p = 0.10$], as seen in Figure 4A.

In summary, results of Experiment 3 showed no significant differences in the expression of LER between the prone and supine postures.

DISCUSSION

The present study investigated the effect of posture on the expression of three behaviors in the one-day-old rat that are normally expressed within the context of the nest and maternal-infant interactions: (1) spontaneous limb activity, (2) stepping behavior, and (3) the leg extension response (LER). Results confirmed that posture does play a modulatory role in the expression of newborn behavior. However, while posture affected the expression of spontaneous limb activity and stepping behavior, it did not affect the expression of the LER. The general hypothesis of this study was that the supine posture would inhibit or reduce behavior compared to the prone posture, given that at this early age innervation of extensor muscles is less mature than innervation of flexor muscles [21] and thus would make limb extension in the supine posture more difficult for newborns. Instead we found that the relation between posture and behavior is more complex in that the effect of posture was different for the three behaviors examined here.

In Experiment 1, rat pups expressed more spontaneous forelimb and hindlimb activity in the supine posture compared to the prone posture, contrary to our hypothesis. One possibility for this difference in spontaneous movements found between the two postures could be due to differences in sleeping – pups could be sleeping more in one posture than the other and this could be exerting an effect on the expression of their movements. Newborn rat pups spend a lot of time sleeping and specifically in an active sleep state [49, 50], where spontaneous twitching movements are expressed hundreds of thousands of times in a single day [51]. In this sense, because many of the spontaneous movements that were quantified in this experiment were twitching movements (though were not quantified separately from other kinds of movements), it is possible that posture influenced their sleeping behavior. Another possibility is that the supine posture is more arousing for pups and perhaps is activating an attempt at a righting response. Given that vestibular afferents are functional at this age and that perinatal rats are capable of righting themselves from a supine to prone posture [23], pups may have become more behaviorally aroused to try to effect a righting response.

Although the exact reason for the difference in spontaneous activity remains uncertain, the findings here replicate an increase in spontaneous forelimb movements during a half-hour testing period in supine-tested P1 rats that has previously been reported [22].

In the case of Experiment 2, we found the opposite effect than that for spontaneous limb movements: pups treated with quipazine expressed more alternated forelimb and hindlimb steps while in the prone compared to the supine posture. Both flexor and extensor muscle activation is important for the expression of stepping behavior. Because motor neurons innervating the ankle extensor muscles at this age are more immature than those innervating the ankle flexor muscles [21], we expected that stepping in the supine posture would be more difficult for P1 rats, as their weak and immature extensors would have to work even harder than their flexors to counteract gravity to show sustained stepping. Thus, the findings reported here fit our hypothesis. Interestingly, posture did not significantly affect the number of stepping bouts or steps per bout (thus stepping was more sustained and constant) for the forelimbs, but posture did affect these stepping parameters for the hindlimbs. Because the number of hindlimb stepping bouts and steps per bout were lower in the supine posture, it is possible that pups were becoming more fatigued in the supine posture. Additionally, the lack of a significant difference between postures in the forelimbs for stepping parameters may suggest that the forelimbs are mature enough to move at similar rates (or compensate) in both postures despite the challenge of gravity in the supine posture [10]. At this age, postural development has been described to follow a rostro-caudal gradient, where maturation of the forelimbs is followed by the hindlimbs [7,9,10]. Thus our findings here may reflect a relative developmental difference between the forelimbs and hindlimbs.

In comparison to the results of the first two experiments that showed effects of posture on newborn behavior, the results of Experiment 3 did not provide evidence of a postural effect on LER expression. Although expression of the LER requires extension of the hindlimbs, and so we expected the LER to be more inhibited in the supine posture, we did not find support for this. However, there is a critical difference between this experiment and the other two: in Experiments 1 and 2 behavior of subjects was observed during a 30-min test session, whereas in Experiment 3 the expression of LER was evaluated after a single stimulation trial lasting only a matter of seconds. Thus perhaps if pups were tested after a longer period of time spent in the two postures, or after repeated LER trials, a postural difference may emerge. For example, in other studies it has been shown that the LER is influenced by variations in sensory feedback, during which the period of varied feedback occurs over the course of an hour or more [23, 52]. Additionally, it is possible that other adaptations occurred that were not measured here, such as changes in intralimb coordination (i.e., limb angles). Nonetheless, with how we tested and examined the LER here, we did not find support for an effect of posture.

Findings from the present study contribute to our understanding of the role of postural adaptations performed by infant rats during expression of behaviors that form part of their typical experience in the nest. Other reports have shown that rat pups adapt their posture to engage in behaviors that take place in the nest environment, such as those related to suckling [13,26], and during the development of locomotion [5]. However, to our knowledge, this is the first report that has systematically explored the effects of postural variations in the

expression of different behavior patterns in the newborn rat. This study suggests that postural control or the lack of it, can constrain the expression of some behaviors. This limitation depends on each particular behavior, as well as on the physiology of the subject during a particular moment in their development, but can be facilitated when some of these limitations or constraints are controlled or eliminated. For example, in a study by Ferrari et al. [53], premature human newborns were provided with support by an artificial nest made of rolled-up blankets. Newborns in the nest environment performed movements that were not observed in infants that were tested without the support of the artificial nest. Similarly, providing support to the head in newborn rats during olfactory stimulation facilitates the expression of an early form of locomotion [6,12].

The three behaviors investigated in this study not only happen in the nest environment, but also within the constraints of an immature motor system. The expression of behavior within the species-typical natal environment provide experiences that provide the infant with sensory stimulation and feedback that contribute to the strengthening of sensorimotor connections [9,19,20, 54] and of projections from the brain to the spinal cord [18] necessary for the development of postural control. Not only does the expression of action patterns or organized behaviors such as those related to suckling contribute to the development of postural control, but spontaneous motor activity also contributes to neuromotor development. Previous research has shown that spontaneous movements are expressed by fetal and newborn rats and although their expression may seem random, these movements are organized into bouts by mechanisms within the spinal cord [38]. A form of spontaneous motor activity expressed during sleep – twitching movements – has also been found to be controlled by the spinal cord [49] and to provide sensory experience in which the brain is actively engaged [55,56]. As well, spontaneous activity expressed by the newborn rat within the context of the nest could be providing experience at the neural, muscular, and physiological levels that contribute to the development of posture control within the first two weeks after birth [5,7].

In a similar way to the current study, studies with human infants have described the development of postural control as an important aspect for the expression of different behaviors. Human newborns and infants perform movements within the constraints of their own body dimensions and their existing skills. For example, postural adjustments are commonly made to perform coordinated behaviors such as reaching [57,58] and locomotion [59,60]. In infants learning to reach toward objects, posture is considered to be essential for the development of goal-directed reaching movements [61]. As infants develop, arm movements become coordinated and controlled and go from being spontaneous arm extensions to goal-directed reaching movements. For the development of arm coordination, stability, and control necessary for reaching, the infant must develop control of the head [62] and must learn to coordinate head and arm movements when placed in different positions such as sitting or when placed supine [63]. Postural control also plays an important role in each of the motor milestones related to the development of locomotion in human infants such as sitting, crawling, and walking [64–66]. As evidenced in the literature on the development of motor coordination in infants, the head must become stable, muscles must become stronger and their patterns reorganized, and body proportions must be appropriate before motor coordination involved in these behaviors can be performed properly [62, 66].

However, once some of these physiological characteristics in the human infant are developed, motor control continues to be refined through a continuous interplay with postural adjustments that must be performed for the specifications of a particular task within each motor domain [68].

In conclusion, findings from this study are important because they provide information about the role of posture in the expression of ecologically relevant behaviors for the newborn. Depending on their posture, particular types of behaviors may be facilitated, inhibited, or apparently not affected. Variations in posture lead to variations in behavioral expression—the same posture does not affect all behaviors equally. And variations in behavioral expression will lead to variations in sensory feedback. When newborns interact in the nest with their mother and their surroundings (including siblings) and their posture is altered, they adapt their behavior. When they perform these behaviors, they are acquiring experience that will contribute to the acquisition of postural control necessary for the development of motor coordination and complex motor patterns such as locomotion.

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Highlights

- The effect of posture was evaluated on 3 behaviors in newborn rat pups.
- Pups showed more spontaneous activity while supine.
- Pups showed more quipazine-induced stepping while prone.
- Posture did not affect the leg extension response.
- Posture differentially affects different behaviors in newborn rats.

Figure 1. Spontaneous movements of the forelimbs (A) and hindlimbs (B) of newborn rat pups tested in the prone and supine postures. Points show means during the 30-min test session. Error bars depict S.E.M.

Figure 2.

Alternated stepping of the forelimbs and hindlimbs for rat pups tested in the prone and supine postures. Following a 5-min baseline, subjects were treated with quipazine. Top panels (A and C) show mean number of steps and bottom panels (B and D) show mean percentage of alternated forelimb and hindlimb steps. Error bars depict S.E.M.

Figure 3.

Stepping bout activity for quipazine-treated rat pups tested in the prone and supine postures. Panel A shows a log survivor curve of inter-step interval lengths for hindlimb steps summed across the 30-min period post-injection of quipazine, for an individual pup tested in the supine posture. Points depict the best-fitting negative exponential function. Notice how the curve plateaus after 3 sec. This curve is representative of the inter-step interval lengths for the hindlimbs and forelimbs for all quipazine-treated subjects. Thus a single bout criterion of 3 sec was used to define stepping bouts (any steps occurring within 3 sec were considered part of the same stepping bout). Panel B shows the frequency of stepping bouts in the forelimbs and hindlimbs for both postures. Panel C shows the number of forelimb and hindlimb steps per bout. Panel D shows the total duration of forelimb and hindlimb stepping bouts. Error bars depict S.E.M.

Figure 4. Leg extension response (LER) of newborn rat pups tested in the prone and supine postures. Latency (A) and Duration (B) of unilateral and bilateral LERs are shown for each posture group. Error bars depict S.E.M.