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Sensory feedback alters spontaneous limb movements in newborn rats: effects of unilateral forelimb weighting

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Abstract

Perinatal mammals show spontaneous movements that often appear random and uncoordinated. Here we examined if spontaneous limb movements are responsive to a proprioceptive manipulation by applying a weight unilaterally to a forelimb of postnatal day 0 (P0; day of birth) and P1 rats. Weights were calibrated to approximate 0%, 25%, 50% or 100% of the average mass of a forelimb, and were attached at the wrist. P0 and P1 pups showed different levels of activity during the period of limb weighting, in response to weight removal, and during the period after weighting. Pups exposed to 50% and 100% weights showed proportionately more activity in the nonweighted forelimb during the period of weighting, suggesting a threshold for evoking proprioceptive changes. Findings suggest that newborn rats use movement-related feedback to modulate spontaneous motor activity, and corroborate studies of human infants that have suggested a role for proprioception during early motor development.

Keywords

development; infant rat; limb loading; motor activity; proprioception; sensory

Spontaneous motor activity is a ubiquitous feature of early motor development (Corner, 1977; Hall & Oppenheim, 1987; Hamburger, 1963), and recent work in developmental and behavioral neuroscience has argued that spontaneous activity plays a crucial role in the early development of the nervous system (Blumberg, Freeman, & Robinson, 2010). Prior to the expression of more complex and organized patterns of motor behavior, young animals exhibit movements of parts of their body that occur in the absence of obvious or explicit stimulation. Some research has suggested that the repertoire and parameters of spontaneous motor activity may be influenced by the immediate environmental context surrounding the organism (Bradley 1997; Brumley & Robinson, 2010; Smotherman & Robinson, 1986). During perinatal development animals encounter substantial changes in their immediate environment and undergo rapid physical growth. As a consequence, fetuses and neonates are exposed to fluctuations in physical forces acting on the body and experience other biomechanical changes that are critically involved in effective motor control (Brumley & Robinson, 2010). But it is unclear whether or not perinatal animals make use of sensory feedback that results from variation in biomechanically relevant variables, such as limb-loading, to modulate their spontaneous motor output. Hence it is unclear to what degree the proprioceptive information resulting from movement of different body parts is used to monitor performance and adjust the expression of spontaneous motility.

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The goal of the present study was to examine the influence of proprioceptive feedback on spontaneous motor activity by experimentally weighting a single forelimb of neonatal rats. This method of unilateral limb weighting has proven useful in studies of human infant behavior for identifying the importance of proprioception in seemingly disorganized motor activity and for probing the intrinsic dynamics between the limbs during spontaneous kicking. For instance, Thelen, Skala, and Kelso (1987) reported that when 6-week old infants had a weight (that approximated the gain in mass of a single leg between 1 and 2 months of age) attached to one ankle, infants decreased the number of kicks in that leg. Although the number of kicks in the weighted leg decreased, the infants increased the number of kicks in the nonweighted leg. Similarly, when infants 12 to 16 weeks of age were fitted with weights of three different magnitudes, estimating 25%, 50% and 100% of the mass of their calf, the kicking rate of the nonweighted leg increased proportionate to the mass of the weight (Ulrich, Ulrich, Angulo-Kinzler, & Chapman, 1997).

Developmental differences in the effects of unilateral leg weighting on spontaneous kicking behavior also have been reported (Vaal, van Soest, & Hopkins, 2000). Human infants at 6, 12, 18 and 26 weeks of age had an external weight that totaled 33% of their estimated calf mass attached to the right leg during testing. Similar to the findings of Thelen and colleagues, this study found that 6 and 12-week old infants responded to the unilateral weight with an increase in kicks of the nonweighted leg compared to the number of kicks in the weighted leg. Yet, whereas the 6-week old infants did not increase their overall rate of kicking, the 12-week old infants did increase their overall kick rate. The unilateral leg weight did not alter spontaneous kicking behavior in infants 18 and 26 weeks of age. The findings of this study therefore suggest that age-related differences in responsiveness to unilateral leg weighting indicates differences in the motor system that are characteristic of normal development (Vaal et al., 2000).

Taken together, these studies provide evidence that human newborns as young as 6 weeks respond to bilateral asymmetries in leg mass with changes in spontaneous kicking. It appears that these young infants are receiving proprioceptive information from leg movements during the period of unilateral weighting and are using this information to maintain overall levels of kicking and to modulate the relative frequencies of kicks in each leg. Limb weighting studies with human infants have employed paradigms in which kicking behavior was measured during a baseline period and a subsequent period when the weight was attached to one of the legs. In these studies, the baseline and weighted periods each ranged from one to five minutes, and changes in infant kicking are apparent after only brief exposure to the weight condition. Similarly, Thelen (1994) reported 3-month-old infants begin to change their pattern of leg kicking after 4–8 min of exposure to an interlimb yoke. In contrast, perinatal rats show more gradual changes in motor behavior in response to interlimb yoke training over a 30-min training period (Robinson, Brumley & Kleven, 2008). Differences in the rapidity of adjustment of human infants and perinatal rats to interlimb yoke training suggest that rat pups may alter their motor activity in response to limb weighting only over a longer period of exposure to the unilateral weight.

Similar patterns of response of humans and rats to interlimb yoke training, albeit over different time courses, suggest that the motor system of perinatal rats should exhibit functional compensation for weight added to one limb, resulting in systematic, time-dependent changes in spontaneous activity over the period of weighting. Because changes in spontaneous motor activity should occur gradually during the period of limb weighting, a second period of gradual adjustment in motor control may be evident after the weight is removed. Persistent changes in motor behavior have been found in late-gestation fetal rats after exposure to an interlimb yoke (Robinson, 2005; Robinson, Kleven, & Brumley, 2008), and in human infants upon immediate removal of a unilateral leg weight during a brief period of stepping on a treadmill (Lam, Wolstenhome, & Yang, 2002; Pang, Lam, & Yang, 2003).

Finally, our laboratories and others have reported day-to-day behavioral changes and responsiveness to changing biomechanical demands on the motor system during the perinatal period in rats. Robinson, Kleven, and Brumley (2008) showed day-by-day improvements of interlimb yoke training in rat fetuses during the last three days of gestation, suggesting a gradual increase in or the use of kinesthetic feedback to shape motor behavior during late prenatal development. Neonatal rats also appear to show daily changes in responsiveness to the effects of gravity during the first 24 hr after birth. Pups tested on the day of birth (Postnatal Day 0 or P0) have less experience in a postnatal environment, and thus less exposure to gravitational limb-loading, compared to pups tested 24 hr later (P1). Not surprisingly, P1 pups appear to be more responsive to changes in posture than P0 pups (Brumley & Robinson, 2010), and initial posture can have qualitatively different effects on righting behavior during the first 48 hr after birth (Pellis, Pellis & Teitelbaum, 1991). P1 rat pups therefore may show greater responsiveness (i.e., more compensation) to a unilateral limb weight than P0 pups.

To further investigate the effects of differential limb-loading on spontaneous motor activity, the present study manipulated weights applied to a single limb of neonatal rats. Because the forelimbs mature relatively earlier than the hindlimbs during perinatal development (Kleven, Lane, & Robinson, 2004; Smotherman & Robinson, 1986), and newborn rats rely principally on their forelimbs for locomotion (Altman & Sudarshan, 1975; Clarac, Vinay, Cazalets, Fady, & Jamon, 1998), we focused attention on responsiveness of forelimbs to a unilateral limb weight in this study. Based on similar experiments conducted with human infants and relevant behavioral changes reported in perinatal rats, the experimental approach of the present study was designed to address three predictions. (a) Because a unilateral limb weight should increase the load experienced by the weighted limb, we expected rat pups to gradually alter their spontaneous limb activity during a period of limb weighting. (b) Such behavioral compensation should persist and gradually reverse after removal of the added limb weight as the pups readjust to a nonweighted condition. (c) Because pups are likely to modify their response to gravitational cues after 24 hr of postnatal experience, pups tested on either P0 or P1should exhibit developmental changes in responsiveness to limb weighting.

Methods

Subjects

Sixty-four rat pups (Sprague-Dawley strain, Harlan Laboratories) from 17 litters served as subjects in this study. No more than two pups (1 male and 1 female) were selected from each litter on the day of birth (postnatal day 0 = P0) or the day after birth (P1). Thus no more than four pups total were used from each litter. Dams were housed individually on the day before parturition in standard laboratory cages (48 × 20 × 26 cm) 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. 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 followed guidelines established by the Institute of Laboratory Animal Resources (1996) for the National Research Council and were reviewed and approved by the Institutional Animal Care and Use Committee.

At the time of testing, all pups appeared healthy, clean of fetal membranes, and recently fed as indicated by the presence of a milk band across the lower abdomen. Testing occurred on P0 (4–6 hrs after birth; N=32) or on P1 (approximately 24 hrs after birth; N=32). Each pup was assigned to one of four limb weight conditions and was tested at one age only. A sample of 8 subjects was assigned to each age and limb weight condition (2 ages × 4 weight conditions × 8 subjects = 64 subjects total).

Behavioral Recording

Pups were individually tested inside an infant incubator that regulated humidity and maintained air temperature at 35° C. Pups were placed inside the incubator 30-min before testing to allow for acclimation to incubator conditions. After acclimation, each pup was placed in a holding apparatus that maintained the pup in a supine posture. The holding apparatus was composed of a felt surface and three thin, elastic straps that fastened loosely across the pup: one strap fastened across the neck and the other two straps fastened across the upper abdomen (see Fig. 1). The apparatus did not interfere with limb or head movements made by the pup (Blumberg & Lucas, 1994), and permitted clear observation of movements during recording and data collection.

The test session commenced with a 5-min baseline, during which pups were not manipulated. Following the baseline period, a weight bracelet was placed around the circumference of a forelimb at the wrist. There were four conditions of unilateral weighting: a sham control and three levels of weight scaled to approximate 25% (55 mg), 50% (110 mg), or 100% (220 mg) of the mean mass of one forelimb of a P0 or P1 rat pup. The weight bracelets were made from waterproof adhesive tape (3M Health Care) that had lead disks of the appropriate weight glued and evenly distributed across the surface. Figure 1 shows a P1 pup with a 100% weight bracelet attached to the left forelimb. A strip of the adhesive lacking lead weights and scaled in size to the exact parameters of the weighted bracelets (weighing 0.7 mg) was attached to the forelimb of sham pups. The presence of a weight bracelet on the forelimb did not physically interfere with limb movements. The weight bracelet remained on the forelimb for 30 minutes. Application of the weight bracelet to the right and left forelimb was counterbalanced within each litter and across each age. After the period of weighting, the weight bracelet was removed gently from the forelimb and the pup remained in the holding apparatus for another 30 minutes. Removal of the weight bracelet did not cause any visible damage to the forelimb.

A micro-camera was located inside the incubator, positioned above the subject (with both forelimbs in clear view), and was connected to an outside video recording unit. Video recording of the 65-min session included the 5-min baseline, 30-min of limb weighting, and the 30-min post-weight period. After testing, each pup was weighed and placed back in its home cage.

Behavioral scoring

Each instance of forelimb movement, excluding passive movements of the limbs resulting from movements of other parts of the body, was scored during normal speed video playback of the 5-min baseline period and 60-min test session (65-min total). Scored movements included high amplitude limb displacements and coordinated behaviors (e.g., occasional stepping or stretching) and low amplitude limb “twitches” (i.e, fast, independent, phasic movements) (Blumberg & Lucas, 1994). These movements are thought to indicate awake and sleep states in the infant rat, respectively (Blumberg & Lucas, 1996). For the purposes of this study, different categories of movement were not distinguished. All movements were considered “spontaneous” (except for passive movements as described above), as behavior in the current study was not explicitly evoked by the experimenter. Each instance of limb movement was recorded using a custom event-recorder program that preserved the time of entry (± 0.1 s). Each forelimb was scored in a separate viewing pass. Time code impressed during video recording permitted identification of individual video frames and synchronization of separate behavioral scoring passes. Inter- and intrarater reliability for scoring limb movement was > 90%.

Data analysis

The frequency of limb movement was summarized from scoring records in 5-min bins. Differences in forelimb movement frequency were compared with multi-factor analysis of variance (ANOVA) tests, with limb (weighted vs nonweighted) and time (13 5-min bins) treated as repeated measures. Beyond following up significant time effects, additional comparisons of time effects involved planned comparisons of specific pairs of 5-min bins to assess behavioral changes associated with application of the weight (baseline vs. 5-min), adjustment during the period of weighting (5-min vs. 30-min), response to removal of the weight (30-min vs. 35-min), and adjustment during the period after the weighting (35-min vs. 60-min). We also examined relative activity between the limbs: for each subject, activity of the nonweighted forelimb was expressed as a percentage of weighted forelimb activity, and analyzed across the test session (nonweighted / weighted * 100%, within each time bin). Expressing nonweighted forelimb activity as a percentage of weighted forelimb activity was used as a way to normalize activity among subjects, as there is often variability among pups in the absolute frequency of limb activity. Independent variables were age and weight condition. Post hoc comparisons used Tukey-Kramer HSD. Statistical tests were performed using StatView 5.0, and all tests were conducted at the $p < .05$ level of significance.

Results

Initially, an overall four-way ANOVA was conducted with two between-subjects variables (2 age × 4 weight conditions) and two repeated measures (2 limbs × 13 time bins, with time nested within limbs). This analysis revealed the significant main effects of age, $F(1, 56) = 46.61$, $p < 0.0001$, limb, $F(1, 56) = 45.73$, $p < 0.0001$, and time, $F(12, 672) = 6.55$, $p < 0.0001$. Activity was greater in P1 than P0 pups, and greater in the nonweighted limb than the weighted limb. There also were significant two-way interactions between age × time, $F(12, 672) = 5.27$, $p < 0.0001$, condition × limb, $F(3, 56) = 7.68$, $p < 0.001$, and limb × time, $F(12, 672) = 6.77$, $p < 0.0001$, but no other interaction effects were significant. Because the overall four-way ANOVA indicated main and interaction effects involving limbs, we next analyzed activity in the weighted and nonweighted forelimb separately.

Weighted forelimb activity

A three-way repeated measures ANOVA (2 age × 4 weight conditions × 13 time bins) revealed a significant effect of age, $F(1, 56) = 46.95$, $p < 0.0001$), a significant effect of time, $F(12, 672) = 6.65$, $p < 0.0001$, and a significant interaction of these two factors, $F(12, 672) = 5.21$, $p < 0.0001$, on the number of movements made by the weighted forelimb. Examination of the effects of age at each time interval indicated differences between P0 and P1 pups at all time points except at 35-min (ps < 0.01). As shown in Figure 2, activity of the weighted limb was significantly greater on P1 during baseline, throughout the period of limb weighting, and from the 40-min time point until the end of the test session.

Planned comparisons were conducted to characterize temporal changes in weighted forelimb activity over the course of the test session. On P0 there were no significant changes in weighted forelimb activity following application of the unilateral weight (baseline vs. 5-min), between the beginning and end of the period of weighting (5-min vs. 30-min), after removal of the weight (30-min vs. 35-min), or between the beginning and end of the period after weighting (35-min vs. 60 min). On P1 there was no response to application of the weight, but post hoc tests confirmed an increase in weighted limb activity over the course of the weighted period, a decrease in response to removal of the weight, and an increase in activity during the post-weight period.

There were no significant differences among weight conditions on activity of the weighted limb. In particular, there was no evidence that addition of weights of different magnitude depressed limb activity relative to the sham weight condition.

Nonweighted forelimb activity

For the number of movements made by the nonweighted forelimb, a three-way repeated measures ANOVA (2 age × 4 weight conditions × 13 time bins) indicated a significant effect of age, $F(1, 56) = 43.66$, $p < 0.0001$, a significant effect of time, $F(12, 672) = 6.49$, $p < 0.0001$, and a significant interaction of age × time, $F(12, 672) = 4.76$, $p < 0.0001$. Follow-up tests showed that there were significant effects of age throughout the entire test session (ps ≤ 0.01). As shown in Figure 3, activity of the nonweighted forelimb was significantly greater on P1 than on P0.

The effect of time on nonweighted limb activity was the same as for the weighted limb. There were no changes on P0 that corresponded with application or removal of weight, nor was there evidence of adjustment during the weight or post-weight periods. However, on P1 there was a significant increase in nonweighted limb activity over the course of the period of weighting (5-min vs. 30 min), a significant decrease in response to removal of the weight (30-min vs. 35-min), and a significant increase in activity during the post-weight period (35-min vs. 60-min).

As with weighted limb activity, there were no significant differences among weight conditions on nonweighted limb activity. We next analyzed the effects of age, weight condition, and time on the relative activity of the nonweighted forelimb normalized against activity of the weighted limb.

Relative forelimb activity

We analyzed the relative movement frequency of the nonweighted forelimb (i.e. nonweighted forelimb activity as a percentage of weighted limb activity) in a three-factor repeated measures ANOVA (2 ages × 4 weight conditions × 13 5-min bins). There was a significant effect of weight condition, $F(3, 56) = 8.21$, $p < 0.0001$, and time, $F(12, 672) = 9.34$, $p < 0.0001$, as well as a significant interaction between weight condition and time, $F(36, 672) = 1.48$, $p = 0.04$. Other interactions were not significant. Because there was not an effect of age on relative nonweighted forelimb activity, data were collapsed across age for subsequent analyses.

Effects of weight condition occurred at all time bins throughout the period of limb weighting, and at one time point during the post-weight period (at 40 min; all ps ≤ 0.05). Post hoc analyses revealed that immediately after application of the limb weight (at 5-min), pups in the 100% weight group showed proportionately more movements of the nonweighted forelimb compared to pups in the Sham weight group, and the 100% weight group remained elevated compared to Sham subjects throughout the period of weighting. At 10-min,15-min, and 20-min, pups in the 100% weight group also performed relatively more movements of the nonweighted forelimb compared to pups in the 25% weight group, and more than pups in the 50% weight group at 15-min and 30-min. And at 40-min, relative activity of the nonweighted forelimb remained significantly greater in the 100% weight group than in the Sham weight group. In sum, subjects that experienced the 100% weight showed the most consistent differences in relative activity between the forelimbs: throughout the period of limb weighting and the first part of the post-weight period, pups in the 100% weight group showed relatively more activity in the nonweighted versus the weighted forelimb (Fig. 4).

To characterize relative nonweighted forelimb activity for pups in each weight condition, a separate series of one-way ANOVAs examined the effect of time in each weight condition. As can be seen in Figure 4, there was a significant effect of time on relative movement frequency of the nonweighted forelimb in the 50%, $F(12, 180) = 3.18$, $p < 0.001$, and 100%, $F(12, 180) = 5.89$, $p < 0.001$, weight groups. In both the 50% and 100% weight conditions, planned comparisons indicated that relative nonweighted forelimb activity was significantly greater at 5-min than at baseline. However, there was no statistical change in relative nonweighted forelimb activity from the beginning to the end of the weighting period (5-min vs 30-min), from the end of weighting to the beginning of the post-weight period (30-min vs 35-min), or during the post-weight period (35-min vs 60-min). There were no effects of time in the 25% or sham weight groups.

Discussion

The results of this study show that a unilateral forelimb weight altered spontaneous limb activity in P0 and P1 rats. One might have expected that movement of the forelimb would have been dampened by additional mass, resulting in a decrease in activity. However, at each age there were no differences among the weight conditions in the number of movements expressed by the weighted forelimb, suggesting that the motor system compensates for the increased load to maintain a constant level of performance by the weighted limb. Although the weighted forelimb appears to compensate for the increase in load, changes in motor activity were evident in the nonweighted forelimb at the two highest levels of limb weighting (50% and 100% of forelimb mass).

The principal effect of applying weights of varying magnitude to one forelimb of P0 and P1 rat pups was a change in the relative activity of the forelimbs. Pups in the 50% and 100% weight conditions responded to the unilateral weight with a proportionately larger increase in movements of the nonweighted forelimb during the period of limb weighting. The pups in the 100% condition continued to exhibit a proportionately larger increase of movements in the nonweighted forelimb throughout much of the remainder of the test session, albeit at a lower amount in the post-weight versus weighted period. However, the 50% weight group showed reduced levels of relative nonweighted forelimb activity compared to the 100% group at several points after the weight was applied, suggesting that the disparity between forelimbs gradually diminished in the 50% weight group. Because differential forelimb activity did not occur in the sham and 25% weight groups, it appears that the 50% weight (110 mg) is the threshold mass (based on the weights used in the present study) for evoking proprioceptive changes in spontaneous forelimb activity in P0 and P1 rats. However, the 100% weight (220 mg) evokes the greatest and most persistent response.

Our finding that the nonweighted limb showed a disproportionate increase in activity in response to a weight attached to the contralateral limb is a robust response, as it has been reported from multiple laboratories in human infants across a range of ages (6–16 weeks) (Thelen et al., 1987; Ulrich et al., 1997; Vaal et al., 2000). The neonatal rats that served as subjects in the present study are less mature in terms of CNS development than the human infants of any of the preceding studies, being the approximate equivalent of a human fetus during the late second trimester (Clancy, Darlington & Finlay, 2001). Therefore, the response to a weight perturbation appears to reflect intrinsic interlimb dynamics that emerge very early in development, perhaps during the prenatal period (Kleven et al., 2004; Robinson, Blumberg, Lane, & Kreber, 2000).

Another important finding of the study was that P0 and P1 pups showed markedly different levels of activity during the period of limb weighting, in response to removal of the unilateral weight, and during the period after weighting (see Fig. 2 & Fig. 3). In general, P1 pups showed higher levels of activity compared to P0 pups in both the weighted and nonweighted forelimbs across the entire test session. Activity levels remained relatively flat over the course of the test session for P0 pups. On the other hand, P1 pups showed an increase in forelimb activity during limb weighting, a decrease in activity after removal of the weight, and another increase in activity during the post-weight period. Age-related differences in activity may reflect differences in sensitivity to proprioceptive manipulations, arousal, or the amount of postnatal experience within a gravitational environment. P1 pups had spent approximately five times as much time in a gravitational and thus limb-loading environment, compared to P0 pups.

P1 pups showed time-dependent changes in limb activity regardless of weight condition. This may reflect systematic changes in arousal during the test session, but does not rule out responsiveness to the weight bracelet. Although the sham weight bracelet weighed less than 1% of the mean mass of a P0 or P1 forelimb, the presence of the weight bracelet on the limb nonetheless may have provided cutaneous stimulation to the limb. Thus, pups in the sham weight condition may have responded to the presence of a tactile stimulus on the limb. It has been suggested, for example, that changes in the leg movements of E12 chick embryos during ankle restraint may be encoded by cutaneous mechanoreceptors (Bradley & Sebelski, 2000). Given the context in which spontaneous limb movements naturally occur during early motor development for most mammals (i.e., prenatally within the confines of the amniotic sac and postnatally in the nest next to siblings (Alberts & Cramer, 1988)), tactile stimulation is likely to normally co-occur with proprioceptive stimulation during bouts of spontaneous motor activity. Importantly, however, pups in the sham weight condition did not exhibit a differential increase in limb activity of the nonweighted forelimb.

Time-dependent changes in limb activity were only seen on P1, not on P0. Interestingly, P1 pups responded to removal but not application of the limb weight. This suggests that P1 pups may have needed a period of time to adapt to the limb weight, but that this adaptive change in activity level was being actively monitored, as a sudden change in limb mass/ stimulation (removal of the limb weight) had an immediate effect on activity level. Taken together, our findings of differential activity between the weighted and nonweighted forelimb in the 50% and 100% weight groups, and an apparent maintenance of weighted limb activity over the course of the entire test session (regardless of weight condition), is suggestive of a homeostatic regulation of spontaneous limb activity in newborn rats.

Implications for potential neural mechanisms

Our findings of increased overall limb activity in both forelimbs and relatively more movements with the nonweighted limb are similar to the pattern of results obtained by Vaal, van Soest, and Hopkins (2000) with 12-week old human infants. It has been suggested that the corticospinal tracts mediate adaptive changes in limb movement frequencies induced by the limb weighting procedure (Vaal et al., 2000; Vaal, van Soest, Hopkins, & Sie, 2002). However, this is unlikely to be the case in the present study, since the corticospinal tracts innervate the first two cervical segments of the spinal cord at P2 in rats (Schreyer & Jones, 1988).

A simple putative mechanism that could be suggested as responsible for the effects of this experiment is activation of primary afferents in the spinal cord, resulting in greater activity of motor neurons innervating muscles of the forelimbs. If this were the case, we would have expected a local response to the unilateral weight, in the weighted forelimb only. Because we found that activity of the nonweighted limb also changed during the course of the test session, and in a somewhat graded manner (e.g., differential activity of the nonweighted limb was not affected by the sham or 25% weight, was increased with the 50% weight, and was most pronounced with the 100% weight), the effects of unilateral weighting would appear to require circuitry that goes beyond simple ipsilateral reflex arcs.

Since weighting effects were not restricted to the weighted forelimb only, we suggest that sensory regulation of spontaneous limb movements is not merely reflexive but centrally monitored at least at a segmental level of the spinal cord. Evidence for coupling of spontaneous forelimb movements has been reported to emerge 2–3 days before birth in the developing rat (Kleven et al., 2004), and spontaneous motor activity in perinatal rats has been shown to be produced, organized, and regulated by the spinal cord (e.g., Blumberg & Lucas, 1994; Robertson & Smotherman, 1990; Robinson et al., 2000). Spontaneous limb twitches, which are thought to be indicative of active sleep in infant rats, have been shown to be governed by the brainstem in week-old rats (Karlsson, Gall, Mohns, Seelke, & Blumberg, 2005; Kreider & Blumberg, 2000). Thus it is likely that the effects reported in the present study are mediated by the spinal cord and/or brainstem. As we did not directly examine the neural mechanisms of unilateral limb weighting, future studies are required to determine the contributions of the brain and spinal cord to the sensory modulation of spontaneous motor activity in developing rats.

Proprioceptive regulation of spontaneous motor activity

Since the pioneering studies of Viktor Hamburger and colleagues in the 1960s, developmental neuroscience has emphasized the role of the central nervous system in the generation of spontaneous motor activity during perinatal development (Hamburger, 1963; O’Donovan, 1999; Hanson & Landmesser, 2003). The results of this study suggest that proprioceptive feedback from spontaneous movements contributes to the regulation of spontaneous motor activity in neonatal rats, and emphasizes the importance of performance in the perinatal development of motor behavior. Sensory feedback from spontaneous movements has been shown to influence the organization of nociceptive reflex circuits in the spinal cord during early postnatal development (Petersson, Waldenström, Fåhraeus, & Schouenborg, 2003) and the acquisition of different patterns of interlimb coordination in the late gestation rat fetus (Robinson, 2005; Robinson et al., 2008). These findings suggest that experience that accrues from seemingly random, purposeless activity during spontaneous activity can affect the development of functional motor responses (Grillner, 2004; Robinson & Kleven, 2005). Moreover, it has been proposed that sensory feedback from spontaneous movements may guide and tune developing sensorimotor circuits, and permit the construction of an action-based body representation at both a spinal (Schouenborg, 2010) and cortical level (Khazipov & Buzsáki, 2010).

Together with studies involving at-risk human infant populations (Ulrich et al., 1997; Vaal et al., 2002), non-human animal studies (in which longer and more invasive experiments can be conducted) can begin to explore the neural mechanisms for and the role of proprioceptive feedback in regulating spontaneous motor activity during early motor development. Understanding the role of sensory feedback in the ontogeny of motor behavior and the nervous system may be important for developing activity-based rehabilitation treatments (Ulrich, 2010) for infants that exhibit developmental disabilities that affect the motor system (e.g., cerebral palsy, spina bifida, and Down’s syndrome). Because spontaneous movement precedes the appearance of more coordinated action (e.g., locomotion and reaching), earlier diagnosis and intervention may be crucial for ameliorating the long-term consequences of developmental disorders, as well premature birth (Jeng, Chen, Tsou, Chen, & Luo, 2004).

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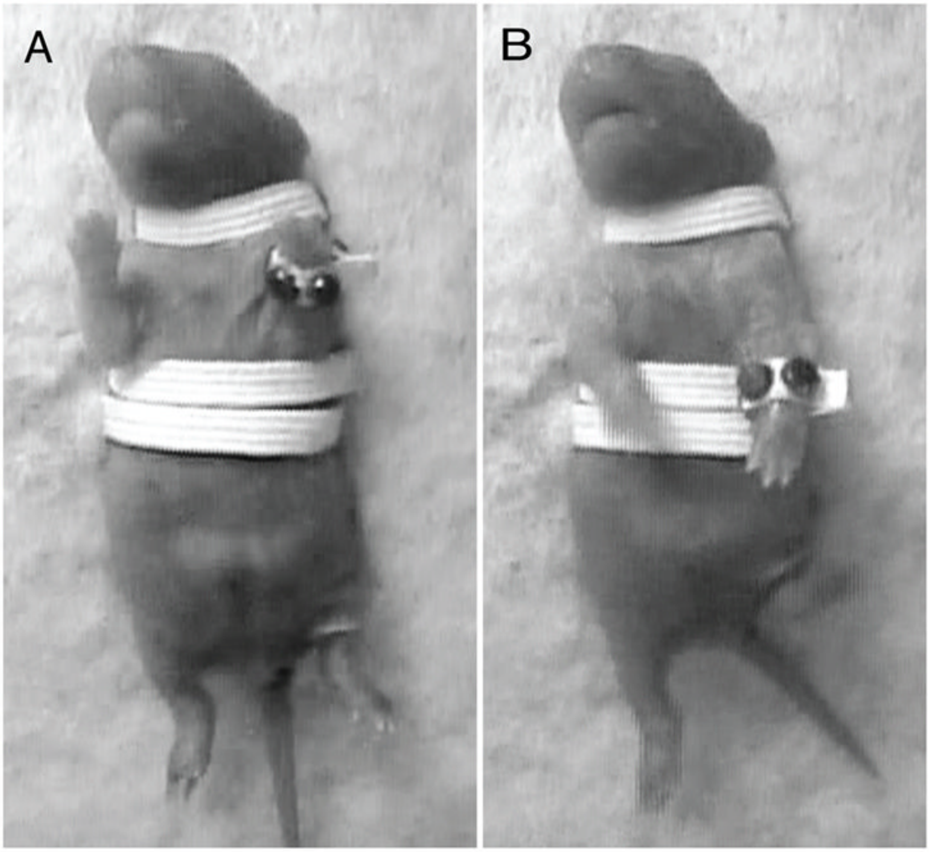

Figure 1.

A newborn rat pup with a unilateral forelimb weight bracelet expressing spontaneous motor activity. Notice that the weight bracelet is attached around the circumference of the left forelimb at the wrist, with lead disks evenly distributed around the limb. The weight bracelet shown is the 100% weight (220 mg). (A) In this video frame, the pup is expressing low amplitude limb twitches, and the limbs are positioned in a relatively relaxed manner. (B) In a subsequent video frame, the pup is engaged in higher amplitude limb movements. Both forelimbs are extending down toward the hindlimbs. Thus, the 100% weight did not prohibit limb movement.

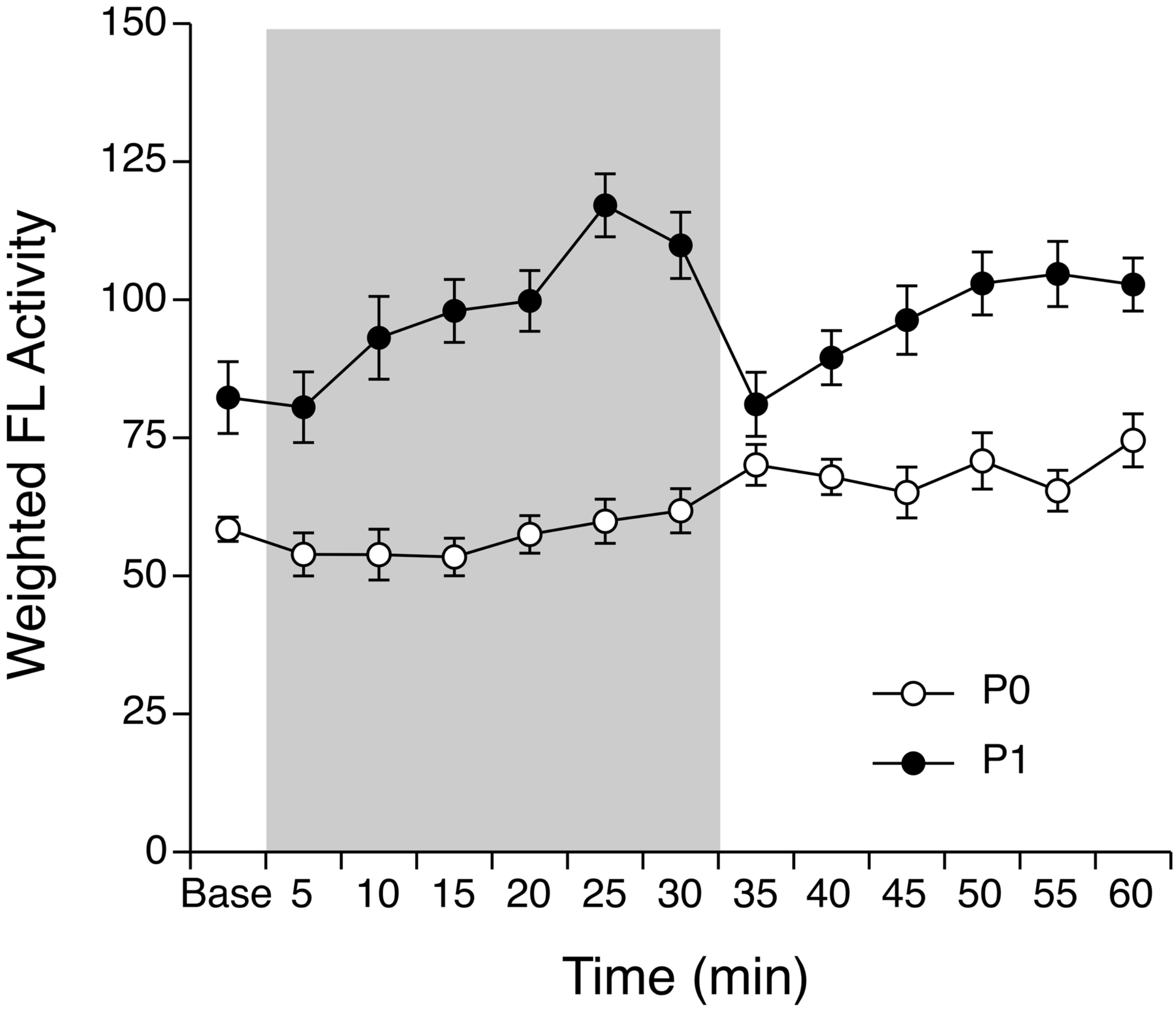

Figure 2.

Activity of the weighted forelimb (FL) for P0 and P1 rat pups during the 65-min test session. In this and subsequent graphs, the shaded region depicts the 30-min period in which a weight was attached to a forelimb. Points show the mean number of limb movements in successive 5-min bins; error bars depict S.E.M.

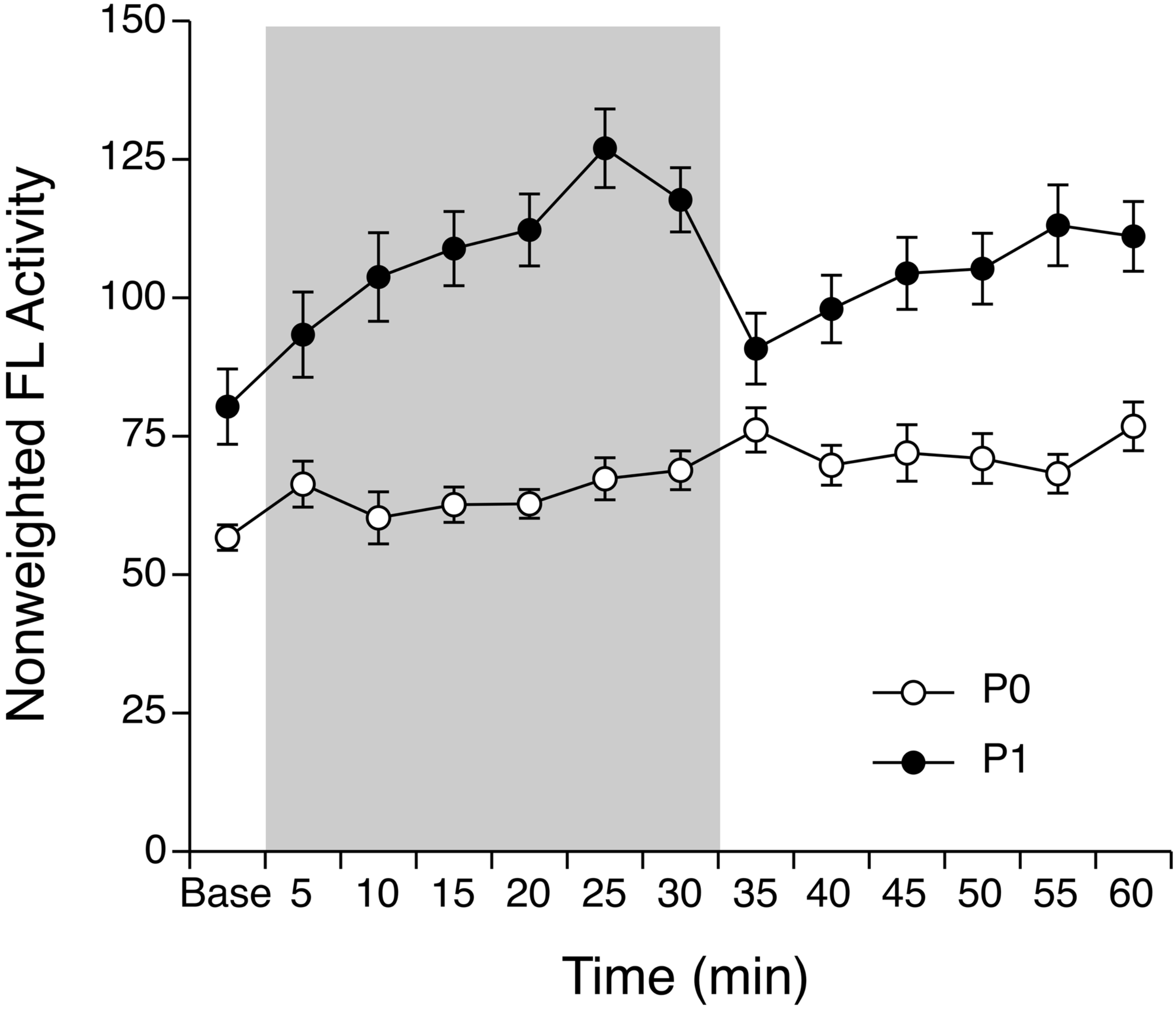

Figure 3.

Limb movement frequency of the nonweighted forelimb for P0 and P1 rat pups. Points show the mean number of movements expressed by the nonweighted limb in successive 5-min bins; error bars depict S.E.M.

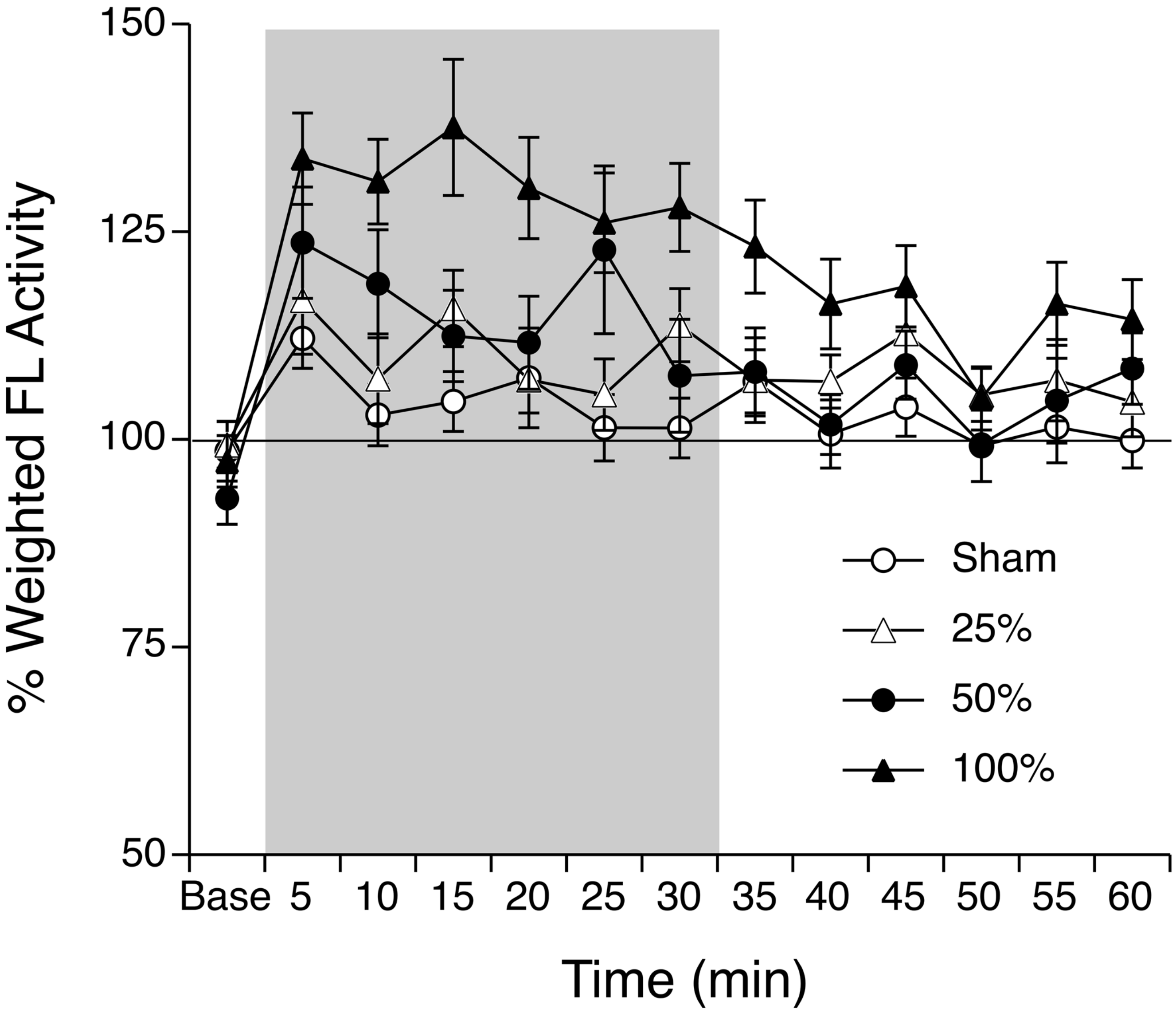

Figure 4.
Relative movement frequency of the nonweighted forelimb for pups in the Sham, 25%, 50% and 100% weight conditions. Activity of the nonweighted forelimb is expressed as a percentage of the weighted forelimb's activity. Points show mean percent activity of the nonweighted forelimb relative to the weighted forelimb in successive 5-min bins; error bars depict S.E.M. Points above the horizontal line (drawn at 100%) represent a disproportionate increase in nonweighted forelimb movements.