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Prenatal behavior of the C57BL/6J mouse: A promising model for human fetal movement during early to mid-gestation

Gale A. Kleven

Department of Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC

April E. Ronca

Department of Obstetrics and Gynecology, and Department of Neurobiology and Anatomy, Wake Forest University School of Medicine, Winston-Salem, NC

Abstract

The study of fetal neurobehavioral development in genetically altered mice promises a significant advance in our understanding of the prenatal origins of developmental disabilities in humans. Despite their importance, little is known about fetal neurobehavioral development in mice. In this study, we observed prenatal behavioral patterns of the C57BL/6J mouse, a common background strain for genetically-altered mice, and report their similarity to those observed in the early-to-mid gestation human fetus. Fetal offspring from pregnant C57BL/6J dams were observed on the day before birth (E18 of a 19-day gestation). Scoring and analysis of fetal movement included Prechtl's Method for Qualitative Assessment, Interlimb Movement Synchrony, a measure of the temporal relationship between movements of limb pairs, and Behavioral State, quantified through detailed analysis of high and low amplitude limb movements. With the exception of fetal breathing movements, all categories and patterns of behavior typically reported in the early-to-mid gestation human fetus were observed in the C57BL/6J mouse fetus. Our results suggest that behavioral analysis of fetal C57BL/6J mice may yield important new insights into early to mid-gestation human behavioral development.

Keywords

Fetal behavior; prenatal movement; mice

A major hindrance to prenatal neurobehavioral assessment is the inaccessibility of the fetus. Although real time or 4-D ultrasonography holds immense promise in revealing hidden domains of the prenatal world (Kurjak *et al.*, 2006), temporal resolutions are currently insufficient for visualizing short duration movements (Salihagic-Kadic *et al.*, 2005), and ethical considerations limit experimentation with human subjects in general (DiPietro, 2005). In contrast, animal models commonly used to study fetal neurobehavioral development, such as the sheep and rat, are not hampered by this limitation. A wide range of

prenatal behaviors have been observed and quantified in these species (Hall & Oppenheim, 1987; Kleven *et al.*, 2004a; Natale *et al.*, 1981; Robinson *et al.*, 2000; Ronca *et al.*, 1994), including cyclicity and behavioral state (Corner, 1977; Robertson & Bacher, 1995; Ruckebusch *et al.*, 1977), responses to sensory stimuli (Narayanan *et al.*, 1971; Robinson *et al.*, 1995; Ronca *et al.*, 1996), responses to pharmacological manipulations (Cheng *et al.*, 1993; Derks *et al.*, 1997; Smotherman & Robinson, 1995), behavioral alterations following neural insult (Kleven *et al.*, 2004b), and even learning before birth (Chotro *et al.*, 1991; Hepper, 1988; Mickley *et al.*, 2001; Robinson & Kleven, 2005). The existing data suggest that the forms and patterns of movement, cyclicity, behavioral states, and evoked and learned responses are similar to those observed in human fetuses (Nathanielsz, 2006).

Animal models employing genetic manipulations offer immense promise for advancing our understanding of developmental disabilities, but have yet to be undertaken in the study of prenatal neurobehavioral development. These types of studies require direct access to the mouse fetus, the species in which genetic modifications are most readily available. Previous investigations into fetal behavior suggest similarities between the mouse and other rodent species (Coppola *et al.*, 1997b; Kodama & Sekiguchi, 1984; Schiffman & McHale, 1990). However, these studies were limited to simple movement frequencies, and none were undertaken in the C57BL/6J mouse (*Mus musculus*), a common background strain for genetically altered mice. Thus, there is a significant void in our knowledge of prenatal behavior in the C57BL/6J strain in particular and in contemporary mouse models in general. In the present study, we analyzed spontaneous prenatal behavior in C57BL/6J fetal mice using three different measures: 1) The method of Qualitative Assessment of General Movement developed by H. F. R. Prechtl (de Vries *et al.*, 1982; Einspieler *et al.*, 2004), 2) Interlimb Movement Synchrony, derived from detailed quantification and analysis of limb movement and coordination (Kleven *et al.*, 2004a), and 3) Behavioral State, based on detailed analyses of high and low amplitude movements and quiescence (Blumberg *et al.*, 2005; Karlsson *et al.*, 2005; Seelke *et al.*, 2005). This recent work, connecting myoclonic twitching, extraocular muscle activity, and brain functioning, has demonstrated that alternating periods of low and high amplitude movement of the infant rat represent changes in behavioral state similar to quiet and active sleep in the human infant. Collectively, the detailed quantification of spontaneous fetal mouse behavior obtained by the measures used in this study provides an important translational link for prenatal neurobehavioral studies spanning from the mouse to the human fetus.

Methods

Subjects

Fetal subjects ($n=5$) were the offspring of 5 pregnant C57BL/6J mice (*Mus musculus*). To insure correct gestational ages, adult female mice were time mated to males during a 4-day breeding period. During breeds, mice were housed together in polycarbonate cages (3:1, female:male) with cob bedding and a small amount of Tek Fresh (Harlan Teklad) to provide nesting material and environmental enrichment. Food and water was provided *ad libitum* and cages were maintained in temperature- and humidity-controlled rooms (12:12-h light/dark cycle). Conception (E0) was determined each morning by either positive vaginal

smears or the presence of a sperm plug. Successfully mated females were immediately separated from breeding males, and group housed with other females until the day of testing (E18 of a 19 day gestation).

At all times, mice were cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). All procedures, husbandry, and care for the mice in this study were reviewed and approved by the Wake Forest University Health Sciences Animal Care and Use Committee.

Preparation of subjects

Pregnant mice were prepared for fetal observations during the light cycle between 10:00 am and 4:00 pm on the last day of gestation (E18). Under brief isoflurane anesthesia, 10 μ l of 100% ethanol was injected into the spinal canal of the pregnant female between the first and second lumbar vertebrae. This procedure produces irreversible spinal anesthesia, eliminating sensation in the lower half of the body, and has been used extensively in fetal rodent research (Coppola *et al.*, 1997a; Robinson & Smotherman, 1992b; Smotherman *et al.*, 1984). To allow direct access to the unanesthetized fetal subjects, the female was secured in a Plexiglas holding device and immersed to chest depth in a buffered isotonic saline bath of Locke's solution maintained at 37.5 ± 0.5 °C. The uterus was externalized into the bath through a midline laparotomy, after which the female was left undisturbed for 20 min to ensure that both the dam and fetal subjects had fully recovered from general anesthesia.

Following recovery and acclimation, a single fetal subject was externalized from the uterus into the saline bath. The chorionic and amnionic membranes were removed and the subject was allowed to rest unrestrained in a curved holding device (Figure 1). Spontaneous activity of the fetal subject was recorded (15 min) on digital video media for later playback and scoring. The camera was adjusted so that the fetal subject nearly filled the field of view. Fetal attachment to the placenta and uterus by the umbilical cord was maintained and monitored throughout the observation period for signs of distress or deterioration. Fetal well being, as evidenced by bright pink coloration and the absence of hypoxic behavior, was also monitored during observations. Smotherman and Robinson (1988; Robinson & Smotherman, 1992a) have reported detailed descriptions of these stereotypical responses to acute hypoxia after umbilical cord occlusion in a number of rodent species. They describe the hypoxic response as a period of suppressed activity followed by a flurry of behaviors unique to hypoxia and which are not seen during nonmanipulated observations (Smotherman & Robinson, 1986). These behaviors are readily distinguished from typical fetal activity and include: (a) lateral trunk curls, often vigorous and alternate from side to side in a rhythmic manner, (b) simultaneous extensions of both forelimbs above the head, which are held in this position, and (c) rostral head extensions, in the form of a "tossing" movement.

Categorical analysis of spontaneous fetal behaviors

In order to fully describe and quantify the range of behaviors exhibited by the C57BL/6J mouse fetus, a categorical quantification of behaviors based on Prechtl's Qualitative Method of Assessment for the human fetus and infant (de Vries *et al.*, 1982; Einspieler *et al.*, 2004) was performed. Using the 15 min video sessions, fetal mice were observed at half speed, and

frequencies of the following behaviors entered into the JWatcher Version 1 (Blumstein *et al.*, 2006) event recorder: (a) Startle, a movement involving the entire body where during a period of quiescence the limbs jerk outward and are then brought in to the trunk (b) General Movement, typically defined as a series of variable speed movements involving all body parts, but lacking a discernable pattern, (c) Hiccup, a rhythmic spasm of the diaphragm, (d) Breathing movement, slow diaphragmatic movements typically associated with inhalation and exhalation, (e) Head flexion, which are head movements that bring the lower jaw toward the chest, (f) Head rotation, where the head moves from side to side (g) Jaw opening, (h) Mouthing, which is any suckling or swallowing movement including licking, and (i) Paw-face contact. An additional two categories not typically part of the qualitative assessment, but observed in both rat (Robinson, 1989) and human fetuses (de Vries *et al.*, 1982), were measured. They are: (j) Stretching, where multiple limbs are extended and the body is held briefly in an elongated position and (k) Yawning, which typically occurs during stretching and involves an exaggerated jaw opening movement which is held briefly before the mouth is closed.

Quantitative analysis of spontaneous limb movement

Fetal limb movements were scored using the same video observations collected for the categorical analysis of fetal behavior. Limbs were scored one at a time from normal-speed playback (30 fps) using Version 1.0 of JWatcher event recorder (Blumstein *et al.*, 2006), and viewed on a 32" diagonal screen Panasonic monitor. With this scoring setup, fetal mouse subjects were observed under magnification that resulting in a crown-rump length in excess of 50 cm. For each limb, the digital video was cued to the same frame, in order to insure synchronization of the event records. Limb movements were defined as any movement of the limb, without regard to direction or duration. Each event was recorded at the start of a limb movement. For limbs that were moving continuously, a new event was recorded if there was a pause between changes in direction. For example, alternations of the hindlimbs in a stepping pattern typically result in multiple events, because small pauses can be observed during each full extension of a hindlimb.

Prior work with fetal rats and preliminary observations of fetal mice also revealed discrete changes in movement activity of the limbs that was qualitatively different and similar to descriptions of state transitions reported in the infant rat (Blumberg & Lucas, 1996). To distinguish potential differences in movement frequency and limb coordination during different behavioral states, the movement of each limb was also coded for one of two qualities. Low amplitude movements, occurring only during periods of low muscle tone and thought to indicate active sleep (Karlsson & Blumberg, 2002; Karlsson *et al.*, 2005; Seelke *et al.*, 2005), were defined as myoclonic twitch-like movements. High amplitude movements, conversely, were smooth seemingly coordinated movements, during which upper body muscle tone was observed. These high amplitude movements include stretching and peddling movements of the limbs at any velocity, and are thought to indicate awake-like behavior. Scoring reliability was established separately for forelimbs and hindlimbs, with an intra-rater correlation 0.95 between multiple event files of the same limbs.

Event files were prepared for data analysis with computer software written by the first author. Scored files for the individual limbs were combined to produce forelimb and hindlimb pairs. The lag time between successive movements of limbs within the pair was then calculated and rounded to the nearest 0.1 s. These intervals of synchronous limb movements were quantified in bins of 0.1 s from 0.0 s (perfect interlimb synchrony) to 0.5 s (one half second lag between right and left limb movement). Prior work using this measure in fetal rats revealed the majority of synchronous movements to be clustered within this time period (Kleven et al., 2004a). Because levels of activity vary widely from subject to subject, each interval of interlimb synchrony were also standardized by movement rates of the same limb pair producing relative rates of synchronous movement. The resulting profiles depict the proportion of movement synchrony occurring at each of the 0.1 s intervals. Prior developmental work with fetal rats has demonstrated that these methods of quantification and analysis provide detailed information about the temporal organization of fetal movement across development that is not effected by differences in rates of movement, thereby facilitating comparisons between groups and with other studies.

The resulting profiles of interlimb movement synchrony for forelimbs and hindlimbs were analyzed in a 2-way repeated measures ANOVA, with limb pairs and synchrony intervals treated as repeated measures. Synchrony profiles for high and low amplitude movements were analyzed separately by forelimbs and hindlimbs in 1-way repeated measure ANOVAs, with the synchrony intervals treated as repeated measures. Post hoc pair wise comparisons of significant main effects and interactions were analyzed using Fisher's PLSD. Effect sizes were calculated using η^2 (Kennedy, 1970). An alpha of $p < 0.05$ was set for all statistical tests which were run using StatView software Version 5 (SAS Institute Inc., 1998).

Analysis of fetal state

Analyses of state were compiled from event files of the spontaneous movement scoring, using the high and low amplitude data from the combined left and right forelimb event files for each subject. Forelimbs were chosen because movement rates in this limb pair greatly exceeded (nearly double) those observed in the hindlimbs. Preliminary observations of spontaneous movement in the fetal mouse revealed patterns of behavior similar to those reported for infant rats (Blumberg & Lucas, 1996; Karlsson et al., 2002; Seelke et al., 2005), therefore, we adapted this method of defining state-like movements according to the presence or absence of myoclonic twitching. Periods of continuous high amplitude or low amplitude movements were quantified, as were the periods of quiescence separating the last high amplitude movement and the first low amplitude movement, and the transition separating the last low amplitude movement and the first high amplitude movement. Means corresponding to state frequency, duration, proportion, and interstate intervals were analyzed in a series of 1-way ANOVAs, with state as a repeated measure. Follow up pair wise comparisons of significant main effects and interactions were analyzed using Fisher's PLSD. Effect sizes were calculated using η^2 (Kennedy, 1970). An alpha of $p < 0.05$ was set for all statistical tests which were run using StatView software Version 5 (SAS Institute Inc., 1998).

Results

Categorical analysis of spontaneous fetal behaviors

With the exception of breathing movements, which were not observed in any of the fetal subjects during the 15 min observations, all other categories of behavior were observed in one or more subjects. Hiccups and yawns were also rare, and only a few subjects (3 and 2 respectively) exhibited these behaviors. Conversely, startles, mouthing (suckling and swallowing movements), and paw-to-face contacts were observed routinely at rates of 1-8 per minute, and General Movements occurred at frequencies in excess of 100 for all subjects. Figure 2 depicts the frequencies of the various behaviors for each subject.

Quantitative analysis of spontaneous limb movement

Fetal subjects exhibited limb movements throughout the 15 min. observation period, with rates of spontaneous forelimb movements per min occurring at nearly twice those of the hindlimbs, (50.79 ± 6.0 and 28.72 ± 3.2). However, two very distinct and qualitatively different types of activity were observed. The first, low amplitude movements, were characterized by bouts of twitch-like movements, with multiple limbs moving in quick succession. Often these bouts were of large enough magnitude that they caused the fetus to change position. Similar descriptions of twitch-like bouts of movement have been reported in both fetal and infant rats (Robinson *et al.*, 2000). The second type of movements were of high amplitude, and were involved in behaviors such as stretching and alternated peddling movements of the limbs. These rates of high amplitude movements exceeded those of low amplitude movements in both limb pairs (Figure 3). Significant main effects both between limb pairs (forelimbs and hindlimbs), $F(1, 4) = 452.79$, $p < 0.0001$, $\eta^2 = 0.20$, and movement types (high and low amplitude), $F(1, 4) = 19.14$, $p = 0.01$, $\eta^2 = 0.59$, were seen, as was a significant interaction, $F(1, 4) = 14.14$, $p = 0.02$, $\eta^2 = 0.02$.

Synchronous movements between limb pairs during both low and high amplitude movements were a ubiquitous feature of fetal mouse behavior. Although rates of synchrony were greatly reduced in the hindlimbs relative to the forelimbs (Figure 4, top), standardization of these frequencies by movement rates of the same limb pairs revealed only a slight reduction in hindlimb synchrony rates when accounted for by differences in movement rates between the forelimbs and hindlimbs (Figure 4, bottom). Overall analyses of Interlimb Movement Synchrony revealed main effects of both limb pairs, $F(1, 4) = 58.46$, $p = 0.002$, $\eta^2 = 0.05$, and synchrony intervals, $F(5, 20) = 39.44$, $p < 0.0001$, $\eta^2 = 0.70$, but no significant interaction between the two factors. Forelimb and hindlimb pairs were analyzed separately, to determine if high and low amplitude movements contribute differentially to the overall synchrony profiles. Neither limb pair analysis revealed main effects of movement type. However, an interaction between intervals and movement type was seen in both the forelimbs, $F(5, 20) = 4.48$, $p = 0.007$, $\eta^2 = 0.11$, and hindlimbs, $F(5, 20) = 3.61$, $p < 0.02$, $\eta^2 = 0.11$. Post hoc analyses revealed the source of interactions to be at the 0.4 s and 0.5 s intervals for both limb pairs, with elevated rates of high amplitude synchronies at these longer intervals (Figure 5). This result is not surprising considering that high amplitude movements include rhythmic alternating peddling movements observed in

both forelimbs and hindlimbs that are not seen during low amplitude movements, which in contrast are very stereotyped in manner.

Because the descriptions and criteria for both General Movements and Interlimb Movement Synchrony during high amplitude movements are similar, comparison tests were run to determine if the rates of General Movement for subjects were correlated with their frequency of Interlimb Movement Synchrony during high amplitude movement. No correlation was found between either forelimb or hindlimb synchrony and with General Movement frequencies. An inspection of the frequency rates of these three measures revealed that although synchrony rates were observed at a higher rate in forelimbs than hindlimbs for every subject, General Movement rates were not as consistent (Figure 6). Figure 7 details, in a single subject, the time line of frequencies for the three measures during high amplitude movement. Most notable is the last 5 minute period, when Interlimb Synchrony and General Movements occur in isolation from one another, suggesting that although they both occur during high amplitude movements, these two measures are assessing different aspects of the same movement patterns.

Quantification of fetal behavioral state

Changes in fetal activity quantified as low and high amplitude were observed to cycle in a pattern that was identical across subjects, and similar to that reported in infant rats (Karlsson *et al.*, 2002). During the 15 minute observation period, fetal subjects spent the greatest percentage of time engaged in low amplitude activity, $55\% \pm 11$, followed by high amplitude activity, $25\% \pm 14$. With periods of quiescence and brief transitions between bouts of the two types of movement, these behavioral sets alternated equally throughout the observation period an average of 12 ± 2 times. Figure 8 depicts the relative proportion of each of the four components arranged clockwise, representing the cyclical nature of organization observed. Despite this regularity in the transitions from one type of movement to another, there were also differences observed between the individual subjects, with some exhibiting longer periods of high amplitude behavior, and others spending more time in quiescence (Figure 9). The transitions from low amplitude to high amplitude were very short, with a mean of 1.3 ± 0.75 s, and were typically preceded by a visible increase in muscle tone. Conversely, transitions from high to low amplitude were longer, 19.5 ± 14.2 s, during which time relaxation of fetal limbs were observed just before the start of the next bout of twitch-like low amplitude movements (Figure 10). There were no significant differences between the occurrence rates of the four behavioral state categories. However, analysis of differences in the mean duration of state was statistically significant, $F(3, 12) = 17.51$, $p = 0.0001$, $\eta^2 = 0.78$.

Discussion

This study represents the first detailed quantification of spontaneously occurring behaviors in the C57BL/6J mouse that are directly relevant to those observed in the human fetus. The results reported herein are also the first application of the widely accepted measure of General Movements developed by H. F. R. Prechtl to fetal rodent behavior.

Our analyses revealed relative rates of occurrence strikingly similar to those seen in the human fetus (Figure 2). In this study, General Movements occurred at the very highest levels, and at nearly twice the rate of startles, corresponding to the relationship reported during the second trimester in humans (Kurjak *et al.*, 2006). Similarly, higher rates of mouthing (sucking and swallowing movements) and paw-to-face contact were seen in the mouse fetus, relative to head flexions and rotations, as has also been reported for human fetuses during mid-gestation (de Vries *et al.*, 1985). Behaviors that are infrequently observed in the human fetus, such as stretching and yawning, were also seen at the very lowest rates in the mouse fetus.

Limb movement in the fetal mice (Figure 3) was twice that reported in previous studies with rats (Kleven *et al.*, 2004a; Robinson *et al.*, 2000; Ronca *et al.*, 1994), and even higher than those observed with 4-D ultrasonography in the second trimester human fetus (Kurjak *et al.*, 2005). Due to the higher metabolic rate of the mouse (Kleiber, 1947), this finding is not unexpected. Moreover, the relative organization of these movements are typically recognized as more informative than their actual rates, which tend to vary widely from subject to subject (D'Elia *et al.*, 1998; DiPietro *et al.*, 2002; Nijhuis *et al.*, 1999).

Although most categories of human fetal behavior typically reported in qualitative analyses were observed in comparable rates in the mouse fetus, fetal breathing movements were pointedly absent. There are several possible explanations. One explanation is that fetal breathing movements in humans are typically visualized with 2-D ultrasound, where the internal structures such as the diaphragm are easily viewed, as opposed to the superficial view afforded direct observation of the mouse fetus. Similarly, observations of the human fetus with 4-D ultrasound have also failed to identify fetal breathing movements, reportedly because of either the superficial scanning of 4-D versus 2-D ultrasound, or the low temporal resolution of the 4-D technology, which is unable to capture movements less than a few seconds in length (Salihagic-Kadic *et al.*, 2005). With ability to record movements to a resolution of 0.1 s, the latter explanation is unlikely relevant to the current study.

One other likely explanation has to do with the temporal organization of fetal breathing movements. Because these behaviors typically occur in clusters, often with long periods intervening between breathing episodes (de Vries *et al.*, 1985; Kobayashi *et al.*, 2001), it is possible that the 15 minute observation period selected for this study was too short to obtain data for this measure. Future mouse studies with longer observation periods or the use of 2-D ultrasound to visualize fetal breathing movements may shed light on their absence from this and other studies of direct observation in fetal rodents.

Profiles from detailed analysis of Interlimb Movement Synchrony in the mouse fetus revealed a characteristic pattern similar to those seen in rats (Kleven *et al.*, 2004a) and preterm human infants (Key *et al.*, 2002). The combination of tightly coupled synchrony in the forelimbs, evidenced by elevated rates at intervals 0.0 s through 0.3 s along with slightly more disperse rates in the hindlimbs (Figures 4-5), was a pattern seen 2-3 days before birth in the rat fetus, and prior to 30 gestational weeks in preterm human infants. Because only one prenatal age was observed in the mouse, and data are not available for the human fetus and infant prior to 28 gestational weeks, a more precise gestational comparison of the mouse

and human interlimb synchrony data is not possible at this time. Currently, studies are underway to collect interlimb movement synchrony at younger ages in the mouse fetus.

Because both Interlimb Movement Synchrony and General Movements can be observed during periods of high amplitude movements, it may be tempting to speculate that these two measures are in some way related. However, the detailed way in which every individual limb movement is scored and quantified for synchrony, makes comparison with General Movement frequencies difficult. The one consistency across all subjects was that the total Interlimb Movement Synchrony for both forelimbs and hindlimbs during high amplitude movements was higher than the total frequency of General Movements. However, the degree of disparity between these two measures differed for each subject (Figure 6), and there was also no systematic relationship detected, as evidenced by a lack of correlation, when comparing Interlimb Movement Synchrony and General Movement frequencies (Figure 7).

The absence of a correlation is likely due to the different methodologies and temporal resolution used for each measure. For example, in Interlimb Movement Synchrony movements are scored at the onset of limb movement, and intervals between the onset of successive movements > 0.5 s are not quantified, because prior work has demonstrated their occurrence is typically not different from randomized data (Kleven et al., 2004a). However, General Movements, by definition, often occur across seconds, with multiple limbs in motion at any one time. Because individual limb movements are not quantified separately, each General Movement constitutes a bout of movement that may include varying numbers of individual limb movements, and consequently, varying frequencies of Interlimb Movement Synchrony. As evident in Figure 9, some occurrences of General Movements and Interlimb Movement Synchrony even appear to be mutually exclusive. Together with the data in these studies, these differences suggest that the calculation of Interlimb Movement Synchrony and tally of General Movements are distinctly different approaches to quantifying spontaneous movement in the developing fetus. Additionally, because the underlying neural substrates contributing to the movement organization is unknown for either of these two measures, it is possible they may each be assessing different aspects of movement organization, and by association, different neural circuitry. Experimental manipulations addressing this issue would greatly enhance our understanding of what specifically is being measured by General Movements and Interlimb Movement Synchrony.

Analyses of fetal state also revealed an interesting pattern of fetal behavior, with alternating periods of activity, quiescence, and myoclonic twitching. These are highly suggestive of sleep-wake states described in studies of infant rats (Seelke et al., 2005), and the human fetus and infant (Groome *et al.*, 1997). These bouts (Figure 10) were significantly shorter in length than those typically reported in late gestation human fetuses and in older infant rats, and are suggestive of early cyclical periods of low and high motor activity that have been observed in the human fetus at approximately 18-20 weeks gestation (Groome *et al.*, 1992; Salihagic-Kadic et al., 2005). However, the organization of these behavioral states was also strikingly similar to more detailed description and correlation to physiological measures reported in the newborn infant rat (Seelke et al., 2005). Bouts of high activity, suggestive of awake behavior, were followed by a period of quiescence or quiet sleep accompanied by

visible relaxation of the fetus, after which an active sleep-like state in the form of myoclonic twitching was observed, followed by a quick transition of less than 1-2 s returning to high amplitude awake-like behavior (Figures 8 -10). Although claims of exact correspondence to awake behavior and active and quiet sleep cannot be substantiated without electrophysiological data, it appears clear that state-like or proto-state behaviors similar to those of the infant rat and human fetus are present before birth in the mouse, despite their highly altricial developmental status. Such findings open the door to experimental studies of fetal state in the mouse corresponding to an early and under-examined period of development in the human fetus.

These findings also raise the question as to what exact time point(s) during human gestation the observed fetal mouse behavior corresponds. Many researchers still subscribe to comparative “rules of thumb” proposed by Dobbing and Sands (1979) which suggests that the fetal period of rodent development corresponds to roughly the first two trimesters in the human fetus. These estimations are primarily based on an alignment of brain growth spurt and myelination between rats in the first 10 postnatal days and the third trimester of human fetal development. These dates are then extrapolated back across development. However, comparing the behaviors observed in this study with the time frame of occurrence those same behaviors were reported for the human fetus suggests a slightly narrower period of development. In humans, the latest emerging behaviors observed are suckling and swallowing movements (de Vries et al., 1982). Because these behaviors do not emerge until the 12th-13th weeks of human gestation, but were also observed in this study of fetal mice, it is possible that the behavior of the mouse fetus on day 18 of gestation corresponds to human behavioral development somewhere between the 13th and 28th weeks. Having only examined one gestational age in the mouse, however, the definitive answer to this question is beyond the scope of the present study. A comparative examination of behavioral patterns in the fetal mouse across earlier gestational ages may aid in determining more exacting correspondence between fetal mice and humans.

Taken together, these findings extend our current understanding of fetal movement in rodents to the mouse, and move towards validating the fetal mouse as an important model for neurobehavioral assessment of the human fetus during early to mid-gestation. Our results are particularly meaningful in that they open the door to new translational studies of genetically altered mice. Given the wide availability of various mouse models of developmental disability, this study sets the stage for uncovering genetic and epigenetic mechanisms involved in both normal and abnormal fetal neurobehavioral development.

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Figure 1.

Pictorial for direct observation of the mouse fetus on E18. The unanesthetized fetal subject rests freely within a curved holding apparatus (A), while submerged in a warm isotonic saline bath. Littermates of the subject are also visible through the transparent wall of the externalized uterus (B). At all times, the fetus remains tethered to the pregnant female through the umbilical cord, and placental connection within the uterus is maintained throughout the observation period. Scale bar depicts 5 mm.

Figure 2.

Frequency rates of behaviors during the 15 minute observation period for each of the fetal subjects. Behaviors which were observed at higher frequencies are depicted on a separate scale (top) from those that were observed less frequently (bottom). Each symbol represents the frequency of an observed behavior for an individual fetal subject.

Figure 3.

Mean high and low amplitude movement rates for both the forelimbs and hindlimbs of fetal subjects on the day before birth. Because these two types of movements are mutually exclusive, rates for each type are calculated based on the frequency of occurrence within periods of either high amplitude or low amplitude movement. Further quantification of these movement types by duration and proportion of observation time are depicted in Figures 8 and 10. Error bars depict the Standard Error of Measure (*SEM*).

Interlimb Movement Synchrony

Figure 4.

Total interlimb synchrony rates of forelimbs and hindlimbs. Both graphs depict the same data. The top graph shows mean frequencies of rates per minute, and the bottom shows the same data after standardization by movement rates. Profiles of forelimbs and hindlimbs quantify the distribution of frequencies of movement synchronization at each of the 0.1 s intervals. Error bars depict the Standard Error of Measure (*SEM*).

Synchrony by Movement Type

Figure 5.

Mean interlimb movement synchrony for forelimbs and hindlimbs by type of movement (high and low amplitude). Profiles are standardized by activity level of the limb pair, and expressed as rates per minute at each of the 0.1 s intervals. Each data point represents the relative rate of movement synchrony for the corresponding interval of time (e.g. 0.1 s, 0.2 s, etc.). Synchrony intervals represent the lag in time between movements within a limb pair. Error bars depict the Standard Error of Measure (*SEM*).

Frequency of GM and Synchrony

Figure 6.

Comparison of General Movement and frequency of interlimb synchrony during high amplitude movements for both forelimbs and hindlimbs for each subject. Bars indicate the frequency quantified for each of the three measures over the 15 min observation period.

Figure 7.
Time line of General Movements and Interlimb Movement Synchrony for forelimbs and hindlimbs over the observation period from a single subject. Marks indicate occurrences across the 15 minutes.

Behavioral State

Figure 8.

Depiction of the mean relative proportion of fetal behavioral states observed. Categories are arranged in order of occurrence, with a mean frequency of 12 ± 2 cycles per 15 minute observation period. Progression from state to state is shown in a clockwise manner.

Figure 9.

Time line of movement frequencies for all four limbs of each fetal subject across the 15 minute observation period. Lower marks depict the temporal organization of the low amplitude movements, while upper marks represent high amplitude movements.

Fetal State Durations

Figure 10.

Bars depict the mean durations for low and high amplitude behavior and the quiescence periods in between. Error bars depict the Standard Error of Measure (*SEM*).