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An ecologically relevant guinea pig model of fetal behavior

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Abstract

The laboratory guinea pig, *Cavia porcellus*, shares with humans many similarities during pregnancy and prenatal development, including precocial offspring and social dependence. These similarities suggest the guinea pig as a promising model of fetal behavioral development as well. Using innovative methods of behavioral acclimation, fetal offspring of female IAF hairless guinea pigs time mated to NIH multi-colored Hartley males were observed longitudinally without restraint using noninvasive ultrasound at weekly intervals across the 10 week gestation. To insure that the ultrasound procedure did not cause significant stress, salivary cortisol was collected both before and after each observation. Measures of fetal spontaneous movement and behavioral state were quantified from video recordings from week 3 through the last week before birth. Results from prenatal quantification of Interlimb Movement Synchrony and state organization reveal guinea pig fetal development to be strikingly similar to that previously reported for other rodents and preterm human infants. Salivary cortisol readings taken before and after sonography did not differ at any observation time point. These results suggest this model holds translational promise for studying the prenatal mechanisms of neurobehavioral development, including those that may result from adverse events. Because the guinea pig is a highly social mammal with a wide range of socially oriented vocalizations, this model may also have utility for studying the prenatal origins and trajectories of developmental disabilities with social-emotional components, such as autism.

Keywords

prenatal behavior; fetal movement; interlimb coordination; restraint stress; cortisol

1. Introduction

Recent interest in delayed effects from early insults, known as Prenatal Programming,[1] Developmental Origins,[2] or Fetal Basis of Adult Disease, suggests a need for longitudinal models that include prenatal observations of behavior. These models would not only link

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early changes in behavioral development with postnatal outcomes, but could also provide important information about mechanisms that underlie the early etiology of these phenomena. More importantly, models that systematically explore the ontogeny of prenatal behavior after insult would also provide translational information for the development of prenatal diagnostics.[3] These diagnostics could lead to early identification and treatment of developmental disabilities, which has been demonstrated to increase quality of life dramatically for affected individuals.[4]

Despite possible benefits, few longitudinal animal models include prenatal observations of behavior. Recent advances in ultrasound technology, however, have made possible the addition of longitudinal study with smaller mammals such as guinea pigs. [5–7] Historically, the laboratory guinea pig (*Cavia procellus*) has proved an excellent model for human development due to numerous commonalities. For example, guinea pigs need folic acid and vitamin C during prenatal development because, like humans, guinea pigs cannot manufacture vitamin C.[8] Guinea pigs have a placental system similar to humans, which has been more extensively studied than that of any other non-human species.[9] Guinea pigs also have a high dependence on social structure.[10, 11] Although not nearly as complex as human language, they exhibit a wide range and type of vocalizations for communication. [12–17] Similar to human attachment, developing guinea pig offspring demonstrate a crucial need for mother/infant bonding, in that pups not only exhibit signs of distress such as vocalization when separated from the mother, but develop symptoms of depression and even physical illness after only a few hours of separation.[18, 19]

As a result of this sensitive nature, Guinea pigs have been an instrumental model in the study of stress, particularly during prenatal development and infancy.[19–24] This sensitivity requires special consideration during potential stress-inducing procedures. For example, the use of ultrasound technology as a non-invasive means to observe behavioral development longitudinally in the fetus often requires both restraint of the pregnant female, and removal of abdominal fur.[6, 7, 25] Restraint is a known stressor for rodents, and in fact is often used as a reliable method to induce stress.[26] Use of such methods with pregnant animals is doubly problematic, because elevated stress can significantly alter both behavior and hormonal development in the fetus.[27]

In order to create a longitudinal model of prenatal development that is free from restraint and/or stress invoking procedures, we combine three features: (a) pregnant IAF hairless guinea pigs, (b) handling and acclimation techniques, and (c) non-invasive ultrasound to visualize fetal behavior. We hypothesize that the use of these techniques with the IAF hairless strain of guinea pig will result in data reflecting clear patterns of behavioral development in the fetal guinea pig, without raising levels of stress in the pregnant female, as evidenced by quantification of salivary cortisol. Based on prior studies with fetal rats and mice,[28, 29] specific patterns of behavior hypothesized include emergence of interlimb coordination as evidenced by increasing rates of synchronous limb movements across development.

2. Material and methods

2.1 Subjects

Subjects ($n=5$) were heterozygous offspring of Institute Armand Frappier (IAF) hairless female guinea pigs (CrI:HA-Hr^{hr}, Charles River Laboratories, Kingston, NY) mated to an NIH multicolored Hartley male obtained from Elm Hill Labs (Chelmsford, MA). The hairless guinea pig was chosen to avoid the stress associated with shaving and/or depilatories during preparation of fur-bearing animals for fetal sonography. First identified from a spontaneous mutation in a colony of Hartley guinea pigs, the IAF hairless strain has been utilized in research for over 40 years. Unlike nude rats and mice, the hairless guinea pig is euthymic and possesses a functional immune system.[30, 31] Developmental and breeding parameters are all nearly identical to the Hartley strain from which they are derived. Preliminary analyses of behavior in adults also reveals few differences between the IAF and Hartley strains.[32] When mated to Hartley males, the heterozygous IAF offspring are indistinguishable from the Hartley background animals. Offspring from this pairing are born with multicolored markings on their fur. These natural patterns of coloration facilitate easy identification after birth without tattoos or tagging.[33, 34]

IAF females were housed in pairs throughout mating, pregnancy, and lactation. Housing was standard polycarbonate (76cm X 56cm) caging lined with recycled shredded office paper. Guinea pigs were provided food (Teklad-7006 guinea pig pellets) and water ad libitum. Cages were maintained on a 12:12-hr light/dark cycle in a temperature (23 C) and humidity-controlled room. Each IAF female was also provided a Polar Tek fleece blanket (Monsanto) sewn into the shape of a tunnel. The fleece tunnel blankets provided the hairless animals an additional source of warmth, a place to hide, and an opportunity for socialization when shared with a cagemate. All females were also provided enrichment as a herd for 1 hr each weekday.[32]

During mating, a single male was placed in the cage with the two females until both females had cycled through a period of estrus. The timing of conception was determined by keeping a daily record of vaginal membrane readings of estrus. In female guinea pigs, the vaginal membrane is typically closed, except during estrus.[35] Prior records charting IAF estrus and delivery in our laboratory were used to determine that the day of conception (Gestational Age 0, GA 0) occurred on the first day of a fully open membrane. Using these metrics, and depending on the size of the litter, the hairless guinea pig generally delivers 2–3 offspring approximately 70 days after conception (Table 1).

At all times, guinea pigs and their offspring were cared for in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.[36] All procedures, husbandry, and care for the subjects in this study were reviewed and approved by the Institutional Animal Care and Use Committee at Wright State University.

2.2 Salivary cortisol

Salivary cortisol samples were collected both prior to and after the ultrasound observations to determine if the ultrasound procedures resulted in elevated stress in the pregnant females. A period of at least 30 min elapsed between pre- and post-sampling. Saliva was collected

using 2–3 cotton swabs per subject (Fisher Scientific), extracted from the swabs by centrifuge, and stored at -80°C until processing. Cortisol levels were determined with an ELISA salivary cortisol kit (Salimetrics, State College, PA). Antibody reactivity for cortisol is 100% according to Salimetrics, and cross-reactivity for endogenous hormones at 100 ng/ml is reported to be $<0.013\%$. [37] Results were analyzed with Statistica software (ver. 10) [38] in a 2-way ANOVA using an alpha set at .05. Gestational Age (GA) and Pre-Post factors were treated as within subjects repeated measures. Effect sizes were calculated using η_p^2 .

2.3 Ultrasound observations

For at least one month prior to mating, female guinea pigs were handled, provided enrichment, [32] and acclimated to the ultrasound testing procedures. Handling included application of lotion to moisturize the skin as needed, a procedure similar to the application of sonography transmission gel during scanning, and daily reading of the vaginal membrane opening to determine estrus. Acclimation involved transporting the guinea pig from an adjacent colony room, collection of a salivary sample, and placement of the female on a padded examination table adjacent to the ultrasound equipment. All equipment to be used during testing was powered on, including monitors and computer equipment for data collection. The ultrasound equipment and software chosen for this study was a Voluson 730 Expert (GE Healthcare, Waukeshaw, WI). The examination table padding was maintained at a temperature of $38 \pm 0.5^{\circ}\text{C}$ (body temperature for the hairless guinea pig) by thermostat control. Females were provided a wheel of Timothy hay (*Pheleum pratense L.*) for enrichment, and as distraction (Figure 1).

During acclimation, guinea pigs were exposed to the scanning procedure, but were otherwise not disturbed except to gently guide them into position in front of the hay wheel. Guiding was accomplished with a single hand using either a gentle nudging motion in the direction of the hay wheel, or by placing a hand as a barrier, thereby preventing movement away from the hay wheel. Females were allowed to consume Timothy hay undisturbed for 10 min prior to the start of sonography. At the end of this 10 min period, warmed ($38 \pm 0.5^{\circ}\text{C}$) Aquasonic 100 ultrasound transmission gel (Parker Labs, Fairfield, NJ) was applied to either the right or left the flank and back of the guinea pig (Figure 1). The side chosen was randomized for each female. Sham scanning procedures involved lightly placing the 9 MHz RNA5–9 transducer probe (GE Healthcare, Waukeshaw, WI) against the flank of the guinea pig, and gently counterbalancing the opposite side with the non-scanning hand. For example, if the right side was to be scanned, the transducer held in the experimenter's right hand would be placed against the right flank of the guinea pig. To counterbalance this light pressure, the experimenter would gently place the free left hand on the left flank of the female. Counterbalancing was necessary, to avoid prodding the guinea pig with the transducer, but was not used as restraint. During scans the female was allowed to move and change postures freely in front of the hay wheel. Vocalizations, kicking, or repeated movements away from the Timothy hay by the female were interpreted as discomfort and the procedures were discontinued. Sham scanning involved making slow circular movements for 20 min, simulating an ultrasound observation of fetal subjects. At the end of the 30 min acclimation period, salivary samples were again collected, the guinea pig was

given a flavored vitamin treat (BioServe, Flemington, NJ), and returned to the adjacent colony room.

Ultrasound observations of fetal behavior were identical to the acclimation scans, except that the scan video was recorded in real time to a DVD recorder. On weeks three and four of gestation both flanks of the pregnant female were scanned, in order to determine the number and placement of offspring. A single fetus was then chosen for behavioral observations from each pregnancy. The uterine placement of the chosen fetal subject (e.g., right horn, left horn, and/or closer to the cervical or ovarian end of the horn) was randomized across the study. For each weekly observation, the same fetal subjects were observed. Both acclimation and fetal observation scans were performed in the afternoon (1–3 pm) on a weekly basis except during the first two weeks of pregnancy. Weeks 1 and 2 of gestation were avoided in order to circumvent any possibility of interfering with embryonic implantation. Females tolerated the procedures well, and only two scans were discontinued due to signs of discomfort in the pregnant guinea pigs.

2.4 Scoring and analysis of fetal movement

Quantification of fetal behavior was done from archival DVD collected during ultrasound scans. Each video was scanned and the first segment with an ipsilateral limb pair (right forelimb and right hindlimb, or left forelimb and left hindlimb) continuously visible for 5 min was selected for scoring. Prior research with direct observation of rats[28] and mice[29] suggested 5 min of video where two limbs were continuously visible to the observer was a sufficient sample for the quantification of Interlimb Movement Synchrony. Interlimb Movement Synchrony is a measure of movement coordination that quantifies the temporal relationship between limb pairs. Movement of each ipsilateral limb was scored on a separate pass of the same video segment, using the JWatcher Video 1.0 event recorder.[39] A movement was operationally defined as any change in orientation of the limb that was independent of body movement. Individual movements were distinguished by changes in direction, or that continued in the same trajectory but were separated by a pause in movement. No distinction was made as to the direction the limb moved. Consequently, synchronous movements include those with the limbs moving both the same and opposite directions. Reliability of coding for movement was determined by comparison of repeated passes of the same video segment. The kappa-adjusted reliability score for the single coder (GAK) was <0.94.

Movements were also quantified separately according to their quality. The mammalian fetus has been observed to exhibit two general types of movements that appear organized as states, similar to those detected during active sleep and awake behavior in infants.[40, 41] In the rodent fetus, these movements are either of low or high amplitude in nature. Low amplitude movements are “twitch-like” and similar to those observed in sleeping infants,[40, 42] while high amplitude movements are smooth, fluid, and coordinated in appearance, reminiscent of awake behavior. Transitions between these two states appear as brief periods of quiescence, and are quantified by measuring the time between the last movement from one state until the first movement of the next state. For example, the time in seconds between the last twitch-like movement and first high amplitude, or from the last high

amplitude movement and the first twitch-like movement. The coding of state was made during a third pass of the 5-min video segment. Prior work with other rodents suggested that 5 min was sufficient to observe multiple state transitions for each subject.

Quantification of Interlimb Movement Synchrony during both states was made through the use of specialized software that interleaves the movements from individual scoring passes of the video, and calculates the temporal lag between successive movements of the limb pairs (ipsilateral forelimb and hindlimb). For each fetal subject, the frequency of synchronous movements with an interval (lag) between limb movements from 0s through 0.5s were quantified. Each of the frequencies in this distribution was then standardized by the rate of movement for each fetal subject. The resulting profiles reflect the relative rates (proportion) of synchronous movement at each interval of time (lag between movements of 0s, 0.1s, 0.2s, 0.3s, 0.4s, and 0.5s). Prior studies have found no utility in quantification of time lags beyond 0.5s.[28]

Statistical analysis of Interlimb Movement Synchrony profiles were performed in separate 2-way MANOVA of High and Low Amplitude movements with Statistica (ver. 10)[38] using the Pillai-Bartlett Trace multivariate test criteria. Both Gestational Age (GA) and synchrony Interval were treated as within subjects repeated measures. Movement and behavioral state organization were analyzed with 2-way ANOVA, with GA and State (High and Low Amplitude) or Limb (Forelimb and Hindlimb) as the within subjects repeated measures. An alpha of .05 was set for all analyses, and post hoc comparisons of significant effects were determined with Fisher's PLSD. Effect sizes were calculated using η_p^2 .

3. Results

IAF hairless females gained a typical amount of weight during pregnancy (Table 1, A) and delivered their offspring after a mean of 69.5 ± 0.922 days (Table 1, B). Ultrasound exposed pups were born healthy, were indistinguishable from non-exposed pups, and exhibited a normal weight gain during the first week (Table 1, C).

3.1 Salivary cortisol

The inter-assay coefficient of variability (CV) across the three ELISA assay plates analyzed was 11.49%, and the intra-assay for within plate CV was 4.20%. Analysis of salivary cortisol levels revealed no significant effects of Gestational Age, $F(6, 28)=1.481$, $p=.224$, $\eta_p^2=.255$; Pre-Post testing, $F(1, 28)=1.808$, $p=.190$, $\eta_p^2=.065$; or GA x Pre-Post interaction, $F(6, 28)=1.044$, $p=.420$, $\eta_p^2=.194$ (Figure 2).

3.2 Limb movement

No movements were observed in any of the subjects the third week after conception. However, fetal movements were observed in all subjects starting the fourth week of gestation. Movement rates increased markedly through week 6, then dropped sharply on the week before birth (Figure 3, top). Analysis of movement rates for forelimbs and hindlimbs revealed a main effect of Gestational Age, $F(5, 24)=7.085$, $p<.001$, $\eta_p^2=.596$; but no main effect of Limb, $F(1, 24)=4.073$, $p=.055$, $\eta_p^2=.145$; or GA x Limb interaction, $F(5, 24)=1.106$, $p=.383$, $\eta_p^2=.187$. Post hoc analyses confirmed a significant elevation of

movement in week 6 compared to all other time points, and a significant reduction in movements on week 9 relative to all other weeks except GA 4.

Rates of limb movement during periods of High or Low Amplitude state revealed a similar pattern with increasing rates through week 6, and reductions the last three weeks of gestation. Additionally, the increases in movement rates were initially more pronounced during high amplitude states across weeks 4–5 (Figure 3, middle). Although both high and low amplitude periods were identified at all times points, the distinction between movement types was qualitatively less pronounced on week 4. Movements observed at week 4 appeared sluggish and slower than at other time points in gestation. Analyses revealed main effects of both Gestational Age, $F(5, 24)=5.167, p=.002, \eta_p^2=.518$; and State, $F(1, 24)=11.568, p=.002, \eta_p^2=.325$; but no GA x State interaction, $F(5, 24)=1.613, p=.195, \eta_p^2=.251$. Post hoc analysis confirmed significant elevation of high amplitude rates on gestational weeks 4 and 5.

3.3 State organization

Similar to prior studies with mice,[29, 43] alternation of high and low amplitude bouts of movement separated by brief periods of quiescence (2–3s) were observed in all fetal subjects. With the exception of week 9, where low amplitude states averaged 2–3 min in length (Table 2), multiple state transitions were observed for all fetuses at each gestational age. A main effect of State was discovered in the quantification of mean duration, $F(1, 24)=18.062, p<.001, \eta_p^2=.429$, with low amplitude periods consistently longer than high amplitude at each week of gestation (Table 2). No main effect was detected for Gestational Age, $F(5, 24)=0.815, p=.551, \eta_p^2=.145$; or GA x State interaction, $F(5, 24)=1.079, p=.397, \eta_p^2=.184$. Likewise, the total proportion of time spent in a low amplitude state was significantly higher at each observational age (Figure 3, bottom), $F(1, 24)=84.495, p<.001, \eta_p^2=.779$, but no effect was distinguished for Gestational Age, $F(5, 24)=1.725, p=.167, \eta_p^2=.264$; or GA x State interaction, $F(5, 24)=1.466, p=.238, \eta_p^2=.234$.

3.4 Interlimb Movement Synchrony

Analysis of Interlimb Movement Synchrony in the fetal guinea pig revealed profiles similar to those observed in prior studies of rats and mice.[28, 29] Low amplitude synchrony, which is characterized by “twitch-like” movements, similar to those observed during active sleep in newborn pups and human infants, increased in frequency from weeks 4–8 before returning to earlier levels the week before birth (Figure 4, top left). At the highest relative frequencies on week 8 the profile of mean synchrony was left-shifted to lower intervals (0 through 0.2s) relative to all other ages. Likewise, MANOVA of low amplitude Interlimb Movement Synchrony revealed a significant main effect of Interval, $F(5, 20)=18.933, p<.001, \eta_p^2=.407$; and Gestational Age x Interval interaction, $F(25, 120)=2.806, p=.039, \eta_p^2=.128$, but no main effect of Gestational Age, $F(5, 20)=1.993, p=.116, \eta_p^2=.293$. Post hoc analyses confirmed significant elevation in relative frequencies at intervals 0 and .1s for weeks 6–8. There was also an additional reduction at the .1s interval for weeks 4 and 9 relative to week 5. Profiles for weeks 5 through 7 also exhibited elevated rates of synchrony for both the .3 and .4s intervals. At the .5s interval, week 6 was found to be significantly elevated relative to all other gestational ages. However, no differences were detected for any age at the .2s interval.

High amplitude synchrony, characterized by smooth coordinated movements analogous to awake behaviors in infants, increased slightly at week 6, before reducing rates at each of the remaining weeks before birth (Figure 4, top right). Similar to the MANOVA results for the low amplitude synchrony, analyses revealed a significant main effect of Interval, $F(5, 20)=10.860$, $p<.001$, $\eta_p^2=.437$; and Gestational Age x Interval interaction, $F(25, 120)=3.942$, $p=.009$, $\eta_p^2=.187$, but no main effect of Gestational Age, $F(5, 20)=1.913$, $p=.129$, $\eta_p^2=.285$. Post hoc analyses confirmed significant differences between various gestational ages for each of the intervals except .5s where no distinction could be made at any age. For the 0s interval, week 6 was significantly elevated from all other ages, and at the .2s interval only week 6 and 9 were found to be different. Intervals .1 and .4 revealed similar patterns, with weeks 4–6 significantly elevated from 7–9, and week 9 significantly depressed relative to all other ages. At the .3s interval, all ages were elevated relative to week 9.

Comparison of high and low amplitude profiles for each week revealed several additional patterns (Figure 4, bottom). During the first week of fetal movement, no differences were found at any interval except 0s. In the subsequent weeks, however, intervals from 0 to .3s exhibited increasing differences between high and low amplitude synchrony through week 8. On week 9 of gestation, low amplitude profiles returned to levels observed in week 4, while synchrony of high amplitude were further depressed.

4. Discussion

Non-invasive observations of fetal behavior in the unrestrained guinea pig produced clear patterns of development, without significant elevation of salivary cortisol levels in the pregnant mother. Quantification of cortisol samples collected were similar to those obtained in prior studies, for non-stressed pregnant females.[32, 44] In contrast, cortisol levels observed for animals subjected to restraint stress are typically several times the levels from this study.[44, 45] This finding suggests the pregnant guinea pigs did not experience stress sufficient to elevate cortisol levels during the observation procedures. Normal cortisol levels also suggest the fetal behaviors observed were reflective of typical non-manipulated development in the guinea pig. Increased levels of cortisol in the pregnant female would be of concern because elevation in steroid hormones has been shown to alter fetal behavior in the form of elevated rates of movement.[27]

As reported by other investigators, the emergence of movement in the fetal guinea pig occurred between weeks 3 and 4.[6, 7] Because movement in the human fetus is generally observed after the 8th week post-conception,[46, 47] gestational week 4 in the guinea pig may be equivalent to the end of the first trimester of human behavioral development. After the onset of movement, rates increase steadily for several weeks, markedly so for high amplitude movements. Conversely, these rates were greatly suppressed just prior to birth. This pattern of increases across gestation followed by a decrease prior to birth has been reported for numerous mammalian species, including humans.[6, 28, 29, 43, 46, 48–51]

State organization, as defined by periods of high and low amplitude movements appeared distinguishable from the earliest time point that movements were observed (week 4). These results suggest the emergence of differentiated state may occur shortly after the onset of

movement, between the third and fifth gestational week in the guinea pig. Analogy to human development is not possible at this time, because post conception weeks 8–20 of human gestation has been poorly investigated for state organization. Lack of state-related studies during this time period may be because past research has concentrated almost exclusively on the emergence of REM sleep which occurs much later in gestation.[52–54] Future studies with frequent sampling of fetal guinea pig behavior between weeks 3 and 5 may reveal a more detailed emergence, potentially informing human research on this earliest origin of state organization.

Interlimb Movement Synchrony, a measure of interlimb coordination during spontaneous movement, also revealed movement patterns of increasing rates followed by decreases prior to birth. Although rising rates of synchrony are observed alongside increases in movement rates, increases in synchronous movement are not merely a bi-product of additional movements. Because relative rates of Interlimb Movement Synchrony are standardized by the movement rates of each individual, elevation in relative rates represents substantial increases in the raw frequency of synchrony. (Figure 4).

Similar to state organization, quantification of Interlimb Movement Synchrony in the human fetus has not been attempted. However, preliminary work with preterm human infants has revealed similar patterns as those observed in the guinea pig, with increasing rates peaking at week 32 post-conception, and decreasing rates through week 40.[48] It is unknown whether these time points would be applicable to typical development in the human fetus or whether they may be shifted in the preterm infant, due to premature birth and movement constraints in the postnatal environment.

Although the specific neural correlates of Interlimb Movement Synchrony are unknown, research with rats and a related measure of bout analysis revealed that synchronous movements were somewhat depressed, but not eliminated after spinal transection. These results suggest both spinal and descending control of spontaneous movements during early development.[55] Similarly, exposure to the antiproliferative methylazoxymethanol (MAM) during active proliferation of the striatum (E17 of a 22 day gestation), resulted in depressed and shifted profiles of interlimb synchrony 3 days later.[56] In the *Pitx3* mouse, where nigrostriatal dopamine neurons fail to differentiate, similar disruptions to the synchronous profiles were also observed.[5, 43, 57] Studies detailing the development of cortical activity and organization in the guinea pig revealed emergence between weeks 4 and 5, with differentiation of the cortical layers taking place between weeks 6 and 7, and descending motor control beginning at the end of week 7.[58–64] These transitions appear to correlate with changes in the synchrony profiles observed in this study (Figure 4). Taken together, the evidence suggests that patterns of Interlimb Movement Synchrony may be a reflection of development and organization in both the brain and spinal cord. Although confirmation of this conclusion requires additional research, the current study does confirm preliminary utility of non-invasive ecologically relevant measures for studying fetal behavior in the guinea pig.

Interlimb Movement Synchrony measures may also hold promise as a potential diagnostic assessment of fetal behavior. The assessment of Interlimb Movement Synchrony requires no

expertise, and very little training to execute. Undergraduate psychology students with no medical training routinely achieve high reliability ratings (.90 or higher) when scoring Interlimb Movement Synchrony from direct observation of both rodents and preterm human infants. Work with these measures in fetal subjects from animal models suggests that data can be collected from as little as 5 minutes[28] of standard 2D ultrasound video of prenatal movement. Real time 4D ultrasound equipment is not essential for quantification of Interlimb Movement Synchrony. This suggests that assessment of Interlimb Movement Synchrony with standard 2D ultrasound may prove beneficial for human diagnostic use in economically depressed areas where only basic equipment may be available.

The guinea pig model in this study is also distinctive in that the behavior of individual subjects can be followed throughout the lifespan from conception through old age, using non-invasive ultrasound prenatally and behavioral testing postnatally. Unlike most other animal models such as rats, mice, sheep, rabbits, or even primates, the current guinea pig model uses no form of restraint, anesthesia, or sedatives at any time during the study. This novel type of longitudinal method in an animal model most closely replicates the non-invasive manner in which human subjects are assessed across development, and holds great potential for future studies in fetal neurobehavioral development.

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Highlights

- IAF hairless guinea pigs were acclimated to ultrasound procedures without restraint
- Behavior of fetal offspring was observed weekly across gestation
- No elevation of cortisol was observed during procedures with pregnant females
- Fetal development of movement coordination was similar to other species studied
- Fetal behavior may be useful in the study of prenatal origins of disability

Figure 1.

Procedural setup for the unrestrained guinea pig female. Photo depicts a pregnant IAF hairless guinea pig on the examination table prepared for sonography of the fetal offspring. The female was allowed to stand or lay on the heated padding (C). The pad was thermostatically controlled to 38 °C, which is body temperature for the guinea pig. Timothy hay (*Pheleum pratense L.*) was supplied ad libitum from a wheel (A) with an open panel for easy access by the female. Body temperature sonography gel (B) was applied to one flank on the female in the approximate area outlined (dotted lines).

Figure 2.

Quantification of IAF hairless guinea pig cortisol across gestation. Bars represent mean salivary cortisol levels collected on cotton swabs both immediately before (Pre-scan) and after (Post-scan) ultrasound observations. No differences were detected in mean cortisol levels between non-pregnant (Gestational Age 0) and any point during pregnancy (weeks 3–9). Likewise, no differences were detected between Pre- and Post-scan samples at any age. Error bars depict the *SEM*.

Figure 3.

Development of movement and state organization in the guinea pig fetus. Bars depict the mean frequency/min of ipsilateral forelimb (white) and hindlimb (black) movements for fetal guinea pigs across weeks 3 to 9 of a 10 week gestation (top). No activity of any type was detected on week 3, but limb movements were observed on week 4, increasing in frequency to a peak of activity at week 6, before returning to low levels the week before birth. Quantification of movements according to states of high amplitude (white) and low amplitude (black) reveal that initial increases in activity levels across weeks 4 and 5 were mainly due to increased rates of high amplitude movements (middle). High amplitude movements are smooth and “coordinated” in appearance whereas low amplitude movements are “twitch-like”, and similar to those observed during active sleep in infants. Points depict the mean rates of limb movements during high amplitude (white) and low amplitude periods. Although frequency of movement was lower at earlier time points during low amplitude periods, the total proportion of time spent in low amplitude state was significantly higher at all ages (bottom). Bars depict the mean proportion of high amplitude (white) and low amplitude (black) states. Errors bars depict the *SEM*.

Figure 4.

Interlimb Movement Synchrony of the guinea pig fetus. Points depict the mean relative frequency of Interlimb Movement Synchrony at each 0.1s interval. Low amplitude movements (left, top) revealed markedly different profiles of synchrony from those of high amplitude (right, top). Low amplitude movements are “twitch-like”, similar to those seen in infants during active sleep, while high amplitude are smooth and “coordinated” in appearance. Although synchrony for both states revealed a developmental pattern of increases followed by decreases, low amplitude profiles display a marked increase across weeks 6–8 before returning just before birth to levels observed at week 4. High amplitude profiles, on the other hand, were elevated mainly at week 6, returning to the lowest levels observed during weeks 7–9. The bottom panels depict the comparison of profiles for Interlimb Movement Synchrony during low amplitude (black) and high amplitude (white) movements at each gestational age. Errors bars depict the *SEM*.

Table 1

Pregnancy data

A. Female IAF weight (g) with [95% Confidence Intervals] ^a				
Pre-pregnancy		Week 1	Week 2	Week 3
765.00 [697.91 – 832.09]		775.80 [700.54 – 851.06]	790.40 [699.92 – 880.88]	806.60 [734.78 – 878.42]
Week 4		Week 5	Week 6	Week 7
805.60 [728.03 – 883.18]		831.80 [747.80 – 915.80]	873.60 [799.10 – 948.10]	932.00 [846.93 – 1017.07]
Week 8		Week 9	Birth	
958.60 [814.25 – 1102.95]		1009.60 [846.44 – 1172.76]	1057.40 [892.12 – 1222.68]	

B. Uterine position				
Fetal Subject	Uterine horn	Number in horn	Total number of offspring	GA at birth (days)
1	Right	1	2	70
2	Left	2	3	68
3	Left	1	1	71
4	Right	2	2	68
5	Right	2	2	67

C. Postnatal growth (g) with [95% Confidence Intervals] ^a				
P1	P3	P5	P7	
92.50 [86.55 – 98.45]	104.33 [90.68 – 117.99]	125.42 [110.94 – 139.89]	148.67 [133.31 – 164.02]	

^aWeight data presented are for group means.

Table 2

Mean Duration of Fetal Low and High Amplitude State [with CI]

Week	Low Amplitude	High Amplitude
4	51.517 [25.112–77.921]	33.427 [16.389–50.465]
5	85.158 [32.453–137.863]	24.999 [14.691–35.306]
6	95.364 [–45.630–236.358]	25.457 [5.747–45.167]
7	135.140 [13.309–256.972]	17.037 [0.551–33.522]
8	58.454 [42.508–74.401]	23.928 [16.023–31.833]
9	132.07 [–17.103–281.250]	12.907 [–2.121–27.934]