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Prenatal betamethasone exposure has sex specific effects in reversal learning and attention in juvenile baboons

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Abstract

Objective—We investigated effects of three weekly courses of fetal betamethasone (βM) exposure on motivation and cognition in juvenile baboon offspring utilizing the Cambridge Neuropsychological Test Automated Battery.

Study design—Pregnant baboons (*Papio sp.*) received two injections of saline control (C) or 175 μg/kg βM 24h apart at 0.6, 0.65 and 0.7 gestation. Offspring [Female (FC), n = 7 and Male (MC), n = 6; Female (FβM), n = 7 and Male (MβM), n = 5] were studied at 2.6–3.2 years with a progressive ratio test for motivation, simple discriminations (SD) and reversals (SR) for associative learning and rule change plasticity, and an intra-dimensional/extra-dimensional (IDED) set-shifting test for attention allocation.

Results—βM exposure decreased motivation in both sexes. In IDED testing, FβM made more errors in the SR [mean difference of errors (FβM minus MβM) = 20.2 ± 9.9; P ≤ 0.05], compound discrimination [mean difference of errors = 36.3 ± 17.4; P ≤ 0.05] and compound reversal [mean difference of errors = 58 ± 23.6; P < 0.05] stages as compared to the MβM offspring.

Conclusion—This central nervous system developmental programming adds growing concerns of long-term effects of repeated fetal synthetic glucocorticoid exposure. In summary, behavioral effects observed show sex specific differences in resilience to multiple fetal βM exposures.

Keywords

developmental programming; neurodevelopment; neuropsychological testing; operant conditioning; nonhuman primates; synthetic glucocorticoids

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INTRODUCTION

Fetal life is a period of intensive developmental plasticity during which an orchestrated and interactive pattern of organ development occurs¹. Postnatal survival depends on an adequate level of maturation of key systems such as the lung. In sheep, the prenatal rise in fetal cortisol that occurs in the final twenty days of gestation² has been shown to be essential for terminal differentiation of the fetal lung³. Administration of synthetic glucocorticoids (sGC) to pregnant women threatening premature labor has clearly proven to be beneficial in enhancing survival of offspring by accelerating lung maturation and decreasing the incidence of neonatal morbidity and mortality^{4,5}. However several studies by others and ourselves in rodents, sheep and baboons have shown that fetal exposure to sGC in the dosing regimen administered clinically have unwanted effects on the development of key fetal systems such as the peripheral vasculature^{6–8}, endocrine glands⁹, and brain^{10,11}. Alterations in the trajectory of development are inevitable since the very purpose of the treatment is to produce circulating fetal glucocorticoid (GC) levels that are higher than those appropriate for the current stage of maturation of key fetal organs. There are limited findings on the long-term clinical effects of betamethasone (β M), a common sGC, on neurological development and cognition^{12–19}. Although no rigorous studies have been conducted on GC effects in humans, a recent paper negatively correlates maternal stress and amniotic cortisol with behavior/cognitive outcomes in infants²⁰. To date there have been no studies to evaluate postnatal effects of sGC administration to pregnant non-human primates when given by the intramuscular clinical route and in the doses used clinically.

In this study, we sought to investigate the effects of β M administration on cognitive function and motivation in juvenile baboons exposed prenatally to levels of GC that were inappropriate for the stage of gestation utilizing the Cambridge Neuropsychological Test Automated Battery (CANTAB) system. The behaviors assessed were motivation (progressive ratio task), associative learning (simple discrimination task), rule change flexibility (simple discrimination reversal task), selective attention (intra-dimensional set task) and attentional allocation (extra-dimensional set shift task). These behaviors rely on higher cortical function and normally developed limbic components^{21,22}. Our experimental design based β M administration on National Institute of Child Health and Human Development recommendation for prevention of complications associated with newborn prematurity. The timing of maternal treatment corresponded to the human clinical protocol at weeks 26, 27 and 28 (term 40 weeks). As in the case of human pregnancy GC do not change the length of nonhuman primate pregnancy in contrast to other experimental species such as sheep^{1–4} and thus represent a translatable model to human fetuses exposed to sGC that deliver at term. Although records are difficult to obtain, some authorities indicate that in excess of 50% of human babies who are exposed to inappropriate amounts of GC as a result of this efficacious therapy deliver at term thus enhancing the value of this study^{12,23}.

METHODS

Subjects

All animal procedures were performed in accordance with accepted standards of humane animal care, approved by the Southwest Foundation for Biomedical Research and University of Texas Health Science Center at San Antonio (UTHSCSA) Institutional Animal Care and Use Committee, and conducted in AAALAC, Inc.-approved facilities. Pregnant baboons (*Papio sp*, $n = 25$) 10–15 years of age from the colony maintained at the Southwest National Primate Research Center (SNPRC, San Antonio, TX, USA) were housed in outdoor metal and concrete gang cages, each containing 10–16 females and 1 breeding male. Animals were observed three times per week for evaluation of perineal skin swelling. Gestational age was

calculated, using estimated day of conception (the time of last maximal perineal skin swelling minus 2 days).

Normal baboon gestation lasts 184 days. At 90 days of gestation (dG) (0.5 gestation, 0.5 G), pregnant baboons were weighed, underwent a detailed ultrasound examination, and were placed in individual indoor cages. Baboons were randomized to receive saline (n= 13) or betamethasone phosphate (n=12, Celestan Solubile, Essex Pharma, Munich, Germany) in doses of $175 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ – a weight-adjusted dose equivalent to 12 mg administered to a 70 kg woman. Treatments were administered intramuscularly once daily at 0800 hours at 111, 112, 118, 119, 125, and 126 dG [approximately equivalent to 24 (0.6G), 26 (0.65G) and 28 (0.7G) weeks of human pregnancy]. Mothers delivered spontaneously at full term and offspring were reared with their mother in group housing until young adolescence²⁴. To ensure that testing was conducted at the same stage of post-natal development, offspring (saline Control (C): Female (FC), n = 7 and Male (MC), n = 6 or $175 \mu\text{g}/\text{kg}$ betamethasone (βM): Female (F βM), n = 7 and Male (M βM), n = 5) were transferred to the UTHSCSA in four different cohorts of 5–7 subjects across two years and housed individually in sight of at least one other subject in the UTHSCSA Laboratory Animal Resources facility.

Subjects were behaviorally tested at 2.9 ± 0.03 years of age when males weighed between 7–12 kg and females weighed between 6–9 kg, all being within the normal weight for this age²⁵. Training and testing were conducted between 9 a.m. and 4 p.m., Monday through Friday. If an animal was tested in the morning (9–11:30 a.m.), feeding occurred at 12 and 5 p.m. or if tested in the afternoon (1–4 p.m.), feeding occurred at 9:00 a.m. and 5 p.m. Nonhuman primate chow (2050 Teklad Global 20% protein, 2.7 kcal/g metabolizable energy, Harlan Laboratories, USA) was given no less than 4 hours preceding behavioral sessions and at the end of the day (5 p.m.) with the exception of the progressive ratio task. For the progressive ratio task, each subject was fed 2 hours before the task was administered regardless of when the animal was tested and a second time at 5 p.m. Daily chow rations were calculated prior to training by administering food *ad libitum* over a course of two weeks and measuring consumption. Each subject would then be fed half this amount twice per day over the course of the study (3 months). In this manner feed was adjusted to each individual subject and no refusal to eat was observed. Water was always available and fruit and vitamins supplemented the diet on Monday, Wednesday, and Friday. The light cycle was set with lights on at 7 a.m. and off at 9 p.m.

Equipment/CANTAB Training and Testing

Our use of the CANTAB system for baboon behavioral testing has been described in detail²⁶, also see appendix 1.

Data Analysis

Data are summarized with the mean $\pm$ standard error. Mean contrasts with regard to treatment (Control versus Betamethasone - βM) and sex (Female, F versus Male, M) were performed using either a linear model or a repeated measures linear model with an autoregressive correlation structure to model the association between successive trials. The interaction of treatment and sex was included in all models. All subjects provided data for all tasks except one subject (a male control), which was excluded from participation in the IDED test for failing to reach this stage contemporaneously with the others. SAS Version 9.2 (SAS institute, Cary, NC) was used to conduct analyses; statistical significance was set at $p \leq 0.05$.

RESULTS

Touch-screen training

Analysis of the initial touch-screen training (shrinking program) comparing control and β M groups on percent errors revealed no effect of treatment or sex (data not shown). In contrast, touch-screen training during the moving stimulus task did reveal differences. Analysis of percent errors made (incorrect trials/total trials $\times$ 100) during the moving stimulus sessions revealed a significant treatment effect [mean difference (F β M minus FC) = 18 ± 8.3 ; $P < 0.05$, Figure 1] between the FC and the F β M offspring with the exposed group showing increased percent errors made, but no significant treatment effect among the male subjects. No significant treatment differences were determined for the two-position stimulus touch-screen training program that followed the progressive ratio sessions (data not shown).

Progressive ratio (motivation)

Repeated measures analysis for means of total rewards, total responses or breakpoint per ten sessions did not vary significantly between groups (Figure 2A, data not shown for rewards or breakpoint). However, when treatment groups were pooled, comparison of total responses between control and the β M group revealed a borderline difference ($p = 0.09$), with fewer responses on average in the β M exposed group (Figure 2B).

Simple discrimination and reversal tasks (associative learning and rule change flexibility)

Among controls, the mean number of errors was greater among females than among males in the SD2 task [mean difference (FC minus MC) = 22.9 ± 11.2 ; $P \leq 0.05$] (Figure 3A). In addition, the mean number of errors was significantly increased in the F β M group relative to the M β M group in the SR1, SR2 and SR3 tasks [SR1: mean difference (F β M minus M β M) = 17.6 ± 7.6 ; $P < 0.05$, SR2: mean difference = 52.3 ± 22 ; $P < 0.05$, SR3: mean difference = 31.9 ± 16.6 ; $P = 0.07$] (Figure 3B). The only significant treatment effect was observed in the SR2 task with F β M group showing an increase in mean number of errors made compared with FC group [mean difference (F β M minus FC) = 45 ± 20.1 ; $P < 0.05$] (Figure 3B). There were no significant differences between the MC and M β M groups in the mean number of SD and SR errors.

IDED Test (selective attention and attention allocation)

Analysis of the eight stages of the IDED test revealed no overall treatment difference between the control and β M group (Figure 4). The mean number of errors made by F β M offspring was, however, significantly increased relative to M β M offspring for the SR, CD and CR tests stages [SR: mean difference (F β M minus M β M) = 20.2 ± 9.9 ; $P \leq 0.05$, CD: mean difference = 36.3 ± 17.4 ; $P \leq 0.05$, CR: mean difference = 58 ± 23.6 ; $P < 0.05$]. There were no significant differences between FC and MC, FC and F β M or MC and M β M groups in the mean number of errors made in the IDED test stages.

COMMENT

We have developed a nonhuman primate operant test battery to assess cognitive ability in different physiological states²⁶. There is growing concern from animal models of adverse outcomes following fetal exposure at critical periods of development to levels of GC that are higher than those normally experienced at that stage of development^{5;27–30}. In view of the paucity of information in primates on any developing system, our initial aim was to use the CANTAB system to evaluate specific developmental outcomes among juvenile baboons exposed to sGC in dosing regimens that mimic those to which the human fetus is exposed. Young baboons are one of the best models available for translation to human outcomes because of their genotypic and phenotypic similarity to young human subjects in terms of

fetal growth, postnatal neurodevelopment and cognitive ability^{31–33}. Also, the singly housed subjects were tested in a non-stressful environment in their home cage in view of age matched peers thereby eliminating the confounds accompanying paired housing³⁴. The CANTAB tests are a powerful and well characterized tool in biomedical research for their cognitive diagnostic capacity in human psychological assessment and their adaptability for use with nonhuman primates and other animal species^{35–40}.

Our results revealed an effect of fetal baboon β M exposure on several CANTAB operant tasks. A treatment effect was found between the FC and F β M offspring in the moving stimulus task in which the F β M offspring performed less accurately. This effect suggests impairment at some level (i.e. in anxiety, attention, motor coordination) in the F β M treatment group. Subsequently, no effect was found in the two-position touch-screen training, which suggests intact motor coordination by all groups. Assessing motivation during progressive ratio sessions revealed no overall treatment effect for rewards or breakpoint. For progressive ratio total responses the p-value equaled 0.09 revealing a borderline difference in the β M exposed group responding less than control animals. Results from associative learning, cognitive flexibility (during reversals) and attention based tasks revealed a sex effect in F β M offspring making more errors in the SR, CD and CR tasks during training and testing stages compared with the M β M offspring. A treatment effect was also found in the SR2 task in females. This indicates that the F β M group exhibited impaired reversal learning, increased perseverative behavior and reduced selective attention.

There are several explanations for the potential difference in motivation between the control and β M group in the progressive ratio task. One possibility is that the β M exposed subjects were distractible or hyperactive, i.e. increased displacement behavior, which prevented them from exhibiting the necessary increase in response. Substantiating this argument is evidence indicating that children exposed to sGC from maternal treatment or maternal stress have a higher risk of emotional and behavioral abnormalities including aggressive/destructive behavior, increased distractibility, attention deficit and hyperactivity^{17;41–44} and neuromotor development impairment¹⁸. Female exposed children are more likely to develop behavioral disturbances which are in agreement with our observation of greater effects in females than in males¹⁸. Furthermore, *in utero* effects to the hypothalamic-pituitary adrenal (HPA) axis by sGC exposure may have long lasting ramifications and potentially contribute to behavioral abnormalities^{19;45;46}.

The known effects of prenatal sGC exposure on the developing brain may provide some explanation for the cognitive performance differences. A clinical study found that exposure to β M in fetal life significantly reduces whole brain cortex convolution (an index of surface area) in infants born at or near term⁴⁷. At the subcortical level there is evidence of decreased hippocampal size during adolescence in humans and primates^{48;49} and hippocampal atrophy in psychiatric patients administered multiple courses of sGC⁵⁰. In rhesus monkeys, repeated prenatal β M exposure reduces brain weights at 165 days of gestation⁵¹. Also, prenatal dexamethasone (another commonly administered sGC) administration after a single or multiple injections on days 132 and 133 of gestation in pregnant rhesus monkeys causes a dose-dependent decrease in the number of pyramidal neurons in all hippocampal regions and of granular neurons in the dentate gyrus at 135 and 162 days of gestation⁴⁹ and a 30% reduction in hippocampal size and segmental volume in offspring⁵². Lastly, prenatal stress (maternal acoustic startle for 25% of gestation) has been shown to diminish neurogenesis in the dentate gyrus of juvenile rhesus monkey offspring⁵³.

These effects of GC exposure on neuronal viability could potentially manifest in the behavioral and cognitive deficits observed here. Although the orbital frontal and dorsal lateral prefrontal cortex are important cortical areas in learning simple/reversal

discrimination and attention/set-shifting⁵⁴, respectively, the subcortical area of importance is the hippocampus^{55–58}. Hippocampal involvement in different aspects of learning and memory including simple discrimination tasks has been well documented^{59–63} as well as its involvement in the regulation of anxiety through various neurotransmitter receptor types including GC receptors^{64–69}. Consequently, the participation and convergence of different neurotransmitter systems in this brain area adds to the complexity of the mechanistic processes in this critical brain region¹⁵. In view of evidence that the hippocampal formation is sensitive to sGC treatment, the results of our study indicate the need for further investigation to elucidate the cellular mechanisms of the hippocampus and other brain areas relevant to performance of attention, learning and memory based tasks. There is also need for studies investigating potential differences between sexes of sGC exposure during neurodevelopment. Such studies were not possible in this cohort which we propose to maintain for longitudinal life time studies and thus cannot provide access to brain tissue for histological and biochemical analysis. Finally, the impairments observed in the FβM versus the MβM offspring mainly involved reversal task performance. The prefrontal cortex is important for reversal discrimination suggesting a sex-specific neurodevelopmental effect of βM exposure on this area of the brain. Effects on the prefrontal cortex by βM exposure and more specifically the mechanisms by which this exposure affects female subjects remain unknown.

Reports in various experimental nonprimate animal species support our findings. In guinea pigs, repeated prenatal sGC treatment produces sex-specific effects with females exhibiting hyperactivity in an open-field test and a concomitant decrease in expression of the hippocampal *N*-Methyl-*D*-Aspartate receptor subunit⁷⁰ as well as altered HPA regulation⁷¹. Likewise, young and adult rats born to dams treated with dexamethasone display sex-specific changes in locomotor and habituation activity in an anxiety related behavioral test⁷² and prenatally stressed rats display significantly more depressive and anxious behaviors^{73–75}. Emgard et al.⁷⁶ has shown prenatal dexamethasone exposure inhibits Morris water maze learning, a hippocampal dependent task, possibly by damaging cholinergic neurons. Furthermore, rat and sheep studies show a decrease in neural cell number and size in the forebrain⁷⁷, as well as decreased brain weight and myelination in the optic nerve and corpus callosum following repeated courses of antenatal sGC, respectively^{78;79}. We hypothesize that high βM exposure induced change in the hippocampus and forebrain structures during development causing long-term behavioral and neurological consequences in female offspring.

The possibility exist the sex differences we observed in the treatment group are due to the influence of βM exposure occurring at different critical windows of development in the two sexes rather than a quantitative difference on systems with a similar trajectory of development. These sex specific effects may depend on timing of drug exposure and concurrent neurodevelopmental stage. Accordingly, differential neurodevelopment between sex has been reported in nonhuman primates with cerebral (prefrontal) cortical maturation occurring earlier in males than females⁸⁰. Indeed pre-adolescent human imaging studies confirm whole brain volume is larger in males than females⁸¹. Thus if neuronal maturation is advancing more rapidly in male than in females then βM could potentially impact brain maturation at that stage in the more developmentally advanced males. It is well known that GC halts cellular differentiation and accelerates tissue and organ maturation. Conversely βM exposure in females, which would be lagging developmentally, results in impaired cognition. Brain development consists of many phases including cellular proliferation, migration, differentiation, synaptogenesis and myelination⁸². In our model βM treatment could potentially affect differentiation, synaptogenesis and/or myelination according to the time scale of brain development⁸². This difference in the time line of brain maturation and the timing of βM exposure could potentially explain the sex specific effects we observed. An

alternate explanation of female susceptibility relates to a clinical study which shows increased 11 β hydroxysteroid dehydrogenase 2 activity and cortisol in umbilical artery at term induced by maternal stress in female infants⁸³. However, studies such as fetal or newborn brain imaging are required to characterize the precise stages of central nervous system development that are affected differentially between sexes.

The effects of repeated prenatal β M exposure were sex-specific with female exposed subjects exhibiting impaired performance in the CANTAB operant tasks which are in agreement with clinical studies that show female behavior and cognitive impairments following antenatal sGC treatment^{17;18}. To our knowledge this is the first report of such findings in a nonhuman primate model of learning and attention, although others have administered similar CANTAB tests to rhesus and marmoset monkeys^{58;84;85} with only one of those groups measuring motivation and cognitive ability following prenatal sGC administration⁸⁴. That study reported impaired skilled motor reaching at juvenile age, no effect on motivation but enhanced reversal learning in adolescence with no sex differences after dexamethasone exposure during late gestation. The specific β M treatment used here reveals detrimental neurodevelopment effects in female offspring.

However, the exact reasons for these sex-specific effects are not known. Although speculative, one potential explanation is that male β M exposed offspring show resilience to the *in utero* challenge while female exposed offspring are more vulnerable. Resilience in general refers to a pattern of functioning indicative of positive adaptation in the context of significant risk or adversity⁸⁶. This lack of resilience in females may stem from an imbalance in the mineralocorticoid and glucocorticoid receptor levels in the brain and concomitant HPA axis dysregulation, which have been shown to result in abnormality of cognitive ability among other health concerns⁸⁷. Further work on potential mechanisms is required including evaluation of these receptors in the central nervous system and HPA axis. Additionally, timing of prenatal GC exposure appears to have variable effects on offspring mental development with early (15 week gestation) exposure having positive outcomes and late (37 weeks gestation) exposure having negative outcomes in humans⁸⁸ and varying effects in marmosets⁸⁴. In our model β M administration occurred during late 2nd through early 3rd trimester human pregnancy equivalence, which based on human prenatal stress studies are less likely to cause effects on offspring neurodevelopment⁸⁸. In conclusion, we have shown that repeated administration of prenatal β M causes long-lasting behavioral effects (prenatal programming) that manifest in adolescent age raising many questions on clinical dosing regimens, which as we point out have never been rigorously tested for dose response effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Analysis of touch screen training (moving stimulus task) shows a treatment effect in the Female β M offspring (closed bar, $n=7$) increased percent error versus Female Control (open bar, $n=7$), but no effect between the male groups or between sex within treatment. Male Control (small squares, $n=6$) and Male β M (big squares, $n=5$). Mean $\pm$ SEM; $p<0.05$, *.

Figure 2.

A) Analysis of progressive ratio responses during 10 sessions revealed no differences between treatment or sex. B) Pooled groups showed a borderline difference ($p=0.09$), β M exposed offspring responded less. Female Control (open bar, $n=7$), Female β M exposed (closed bar, $n=7$), Male Control (small squares, $n=6$) and Male β M exposed (big squares, $n=5$). Mean $\pm$ SEM.

Figure 3. A) Simple discriminations 1-5

Figure 3. B) Simple discriminations (6-8) followed by reversals (1-3).

Figure 3.

A) Analysis of errors made during simple discrimination (SD) 1–5 tasks revealed a sex effect with the Female Control (open bars, $n=7$) making more errors than the Male Control (small squares, $n=6$) offspring during SD2 ($p \leq 0.05$, #). Female β M exposed (closed bars, $n=7$) and Male β M exposed (big squares, $n=5$). Mean $\pm$ SEM.

B) Analysis of errors made during simple discriminations (SD) followed by reversals (SR) revealed a treatment effect with the Female β M exposed offspring (closed bars, $n=7$) making more errors than the Female Control (open bars, $n=7$) during SR2 ($p < 0.05$, *), and a sex effect as compared to the Male β M exposed offspring (big squares, $n=5$) during SR1 and SR2 ($p < 0.05$, #), and SR3 ($p = 0.07$, †). Male Control (small squares, $n=6$). Mean $\pm$ SEM.

Figure 4.

Analysis of errors made in the stages of the Intra-/Extra-dimensional Attention Set Shifting test revealed a sex effect between the Female β M exposed (closed bars, $n=7$) and the Male β M exposed (big squares, $n=5$) offspring in the SR, CD, and CR stages ($p \leq 0.05$, #). Female β M offspring made more errors. Female Control (open bars, $n=7$) and Male Control (small squares, $n=5$). Mean $\pm$ SEM.