Sex-Dependent Cognitive Performance in Baboon Offspring Following Maternal Caloric Restriction in Pregnancy and Lactation

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Abstract

In humans a suboptimal diet during development has negative outcomes in offspring. We investigated the behavioral outcomes in baboons born to mothers undergoing moderate maternal nutrient restriction (MNR). Maternal nutrient restriction mothers ($n = 7$) were fed 70% of food eaten by controls (CTR, $n = 12$) fed ad libitum throughout gestation and lactation. At 3.3 ± 0.2 (mean $\pm$ standard error of the mean [SEM]) years of age offspring (controls: female [FC, $n = 8$], male [MC, $n = 4$]; nutrient restricted: female [FR, $n = 3$] and male [MR, $n = 4$]) were administered progressive ratio, simple discrimination, intra-/extra-dimension set shift and delayed matching to sample tasks to assess motivation, learning, attention, and working memory, respectively. A treatment effect was observed in MNR offspring who demonstrated less motivation and impaired working memory. Nutrient-restricted female offspring showed improved learning, while MR offspring showed impaired learning and attentional set shifting and increased impulsivity. In summary, 30% restriction in maternal caloric intake has long lasting neurobehavioral outcomes in adolescent male baboon offspring.

Keywords

developmental programming, motivation, learning, memory, attention

Introduction

Nutritional insecurity is a global health problem in both developing and developed countries. Moderate reductions in fetal nutrition can occur in teenage pregnancy where the growing mother competes with her fetus for nutrients¹ and in pregnancy in women over 35 years of age in whom uterine and placental blood flow may be reduced.² Results from human epidemiological and animal studies demonstrate that the periconceptional, fetal, and early postnatal nutritional environments modify development of many systems in the offspring including the cardiovascular, metabolic, and endocrine systems.³⁻⁹ These observations have led to the concept of a nutritional basis for the developmental origins of adult disease (developmental programming).^{10,11} Deficient nutrition during pregnancy increases offspring morbidity and mortality as well as predisposes offspring to a wide range of chronic conditions in later life, such as diabetes, obesity, and cardiovascular disease (for review see¹²⁻¹⁴). A proper maternal diet is vital for the development of the central nervous system in offspring.¹⁵ Poor nutrition can have profound effects on the developing brain, especially neurons of the limbic system, which show susceptibility to the insult of malnutrition.¹⁶⁻¹⁹ Epidemiological studies in human offspring demonstrate negative influences of insufficient perinatal nutrients on cognition and behavior.^{14,20-23}

We recently demonstrated major cerebral cortical structural developmental disturbances in the brain of fetuses of baboon mothers administered a 30% global nutrient-restricted diet during pregnancy.²⁴ A limited number of studies of perinatal protein restriction on offspring behavior have been conducted in the rat. Results show inconsistent outcomes—potentially due to differences in models, strains, and conditions under which the experiments were conducted.²⁵⁻²⁹ We have recently shown negative effects of pre and/or postnatal isocaloric protein restriction (50% reduction) in rat offspring motivation and operant conditioning³⁰ as well as disinhibited behavior in the elevated plus maze and an anxious phenotype in the open field.³¹ These results are consistent with the results from earlier

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studies demonstrating modified neuron proliferation in hypothalamic and hippocampal regions observed in rat offspring of dams who underwent protein restriction during gestation and/or lactation.³² Such developmental perturbations are likely to lead to long lasting behavioral abnormalities and cognitive deficits. Complex psychological processes such as motivation, risk assessment, emotional regulation, and learning rely heavily on intact limbic structures and associated neural networks including the hippocampus, amygdala, and areas of the prefrontal cortex.³³

Taken together, these findings are indicative of the behavioral perturbations due to neurodevelopmental modulation subsequent to the insufficient nutrient availability in early life. In light of the structural and functional differences in brain and behavior resulting from maternal nutrient restriction (MNR) during both fetal life and the neonatal period prior to weaning, we investigated the effects of a 30% global MNR during development on motivation, associative learning, attention, and working memory in baboon offspring prior to puberty. Since effects on offspring behavior by substantial reductions in nutrients/protein have been extensively documented in rodents, we sought to investigate the effects of a more moderate global dietary restriction (30%) which potentially more accurately reflects the human nutrition insecurities seen even in developed countries.³⁴ Also by using the baboon model, which has a similar genetic constitution and developmental trajectory to the human, data obtained can be more reliably translated to human development to draw direct comparisons. We hypothesized that moderate global nutrient restriction during pregnancy and lactation would affect the development of the offspring brain in a sex-specific manner as evidenced by reduced motivation and impaired learning, memory, and attention.

Methods

Subjects

All animal procedures were approved by the Texas Biomedical Research Institute and University of Texas Health Science Center at San Antonio Institutional Animal Care and Use Committee, and conducted in AAALAC, Inc–approved facilities. Nineteen nonpregnant female baboons (*Papio* species), 11.5 ± 0.51 years (mean $\pm$ standard error of the mean [SEM]) and of similar morphometric phenotype, maintained at the Southwest National Primate Research Center were selected for the study and housed in outdoor gang cages in groups of up to 16 with a vasectomized male, thereby providing full social and physical activity. They were trained prior to pregnancy to feed in individual cages as described previously.^{35,36} Briefly, at feeding time all baboons passed along a chute and into individual feeding cages. Each baboon's weight was obtained while crossing an electronic scale (GSE 665; GSE Scale Systems, Milwaukee, Wisconsin). Water was continuously available in the feeding cages via individual waterers (Lixit, Napa, California). Following the introduction of a fertile male

Table 1. Maternal and Offspring Morphometrics, FC (n = 8), FR (n = 3), MC (n = 4), MR (n = 4)

	Control, N = 12	MNR, N = 7
Maternal Morphometrics^a		
Age at conception, year	11.1 ± 0.69	12.1 ± 0.58
Weight prepregnancy, kg	13.5 ± 0.58	13.8 ± 0.56
Weight at delivery, kg	14.4 ± 0.61	12.4 ± 0.59^b
Weight at weaning, kg	14.8 ± 0.53	12.8 ± 0.45^b
Offspring morphometrics^a		
Duration of gestation, days	182.3 ± 2.30	183.4 ± 2.13
Birth weight, kg	0.9 ± 0.04	0.7 ± 0.04^c
Age at study, year	3.4 ± 0.22	3.0 ± 0.09
Weight at study, kg	10.5 ± 0.53	8.5 ± 0.59^b

Abbreviations: FC, female control; FR, nutrient-restricted female; MC, male control; MR, nutrient-restricted male; MNR, maternal nutrient restriction; SEM, standard error of the mean.

^a Mean $\pm$ SEM.

^b $P \leq .05$.

^c $.06 < P \leq .08$.

from 30 days of gestation (term 183 days), 12 females were randomly chosen to eat normal primate chow ad libitum (control diet [CTR]—12% energy from fat, 18% from protein, and 69% from carbohydrate consisting of 0.29% glucose and 0.32% fructose; Purina Monkey Diet 5038, Purina, St Louis, Missouri). Seven MNR females were randomly chosen and fed 70% of the feed eaten by the CTR females on a weight-adjusted basis from the time of diagnosis of pregnancy (approximately 30 days of gestation) for the rest of pregnancy and through lactation.³⁷ General details of housing and environmental enrichment have been published.³⁸

All the 19 mothers delivered spontaneously at full term (Table 1) and offspring were reared with their mothers in group housing until weaning when they were moved to a juvenile cage of mixed males and females and maintained on the CTR diet.³⁹ During this period, the morphometric measurements of both mothers and offspring were taken.⁴⁰ The offspring (CTR: female [FC], n = 8 and male [MC], n = 4 and MNR: female [FR], n = 3 and male [MR], n = 4) were transferred to the University of Texas Health Science Center at San Antonio in cohorts of 5 to 7 subjects over a 9-month period and housed individually in sight of all subjects in the Laboratory Animal Resources facility. Subjects were behaviorally tested at 3.3 ± 0.2 years of age when they were within the normal weight range for baboons born at the primate center.⁴¹ Training and testing were conducted between 9 AM and 4 PM, Monday through Friday. On test days, feeding (2050 Teklad Global 20% protein, 2.7 kcal/g metabolizable energy; Harlan Laboratories, Houston, TX, USA) occurred at 12 PM and 5 PM for animals tested in the morning and at 9 AM and 5 PM for animals tested in the afternoon, with the exception of the progressive ratio task. For the progressive ratio task, each subject was fed 2 hours before the task was administered and a second time at 5 PM. Daily chow rations were calculated prior to training by administering food ad libitum over a course of 2 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 such that lights went on at 7 AM and off at 9 PM.

Equipment

Our use of the Cambridge Neuropsychological Test Automated Battery (CANTAB, models 80650/80652**C*; Campden Instruments Ltd, Lafayette, Indiana) system for behavioral testing to assess motivation, associative learning, selective attention, attentional set shifting, and working memory has been previously validated in baboon.^{42,43} The testing station was constructed using an aluminum chassis incorporating an infrared touch screen monitor, a pellet dispenser (model 80209, Campden Instruments Ltd), and pellet trough situated onto a moveable trolley. During behavioral sessions, the testing cage, with the attached testing station, was secured to the home cage allowing the animal to move freely between the 2 cages and permitting access through the bars of the testing cage to manipulate the CANTAB touch screen monitor. The controlling computer (IBM compatible Pentium IV, Campden Instruments Ltd, Lafayette, Indiana) and monitor were connected to the testing station by a 10-m cable and positioned behind a shower curtain in the testing laboratory out of the test subjects' eyesight. The computer controlled the CANTAB software that generated the visual stimuli, task parameters/contingencies, and recorded the number of trials and errors made. The control monitor ran in parallel with the touch screen monitor in order to display the location of the subject's touches in real time to the experimental observer. Two video cameras (Sony DCR-SR40, store.sony.com) were positioned in the room to record the behavior in the home and test cages, while a remote camera (X10 Wireless Technology XC18A, www.x10.com) angled to the touch screen monitor enabled real-time experiment observation of the animal's response.

CANTAB Training and Testing

Reinforcement familiarization. Each subject was acclimated to the testing cage/apparatus for 11 hours across 4 days (sessions of 1 hour, 2 hours, and two 4 hours). For operant training, 1 daily session was administered at the same time of day for each subject. The aim of the reinforcement familiarization task was to train the baboons that the onset of a tone (1 kHz) signaled the availability of reinforcement. This tone was the standard signal for the availability of reinforcement across all tasks. Reinforcement delivery followed a fixed interval schedule (15 seconds). Baboons were trained in 15-minute sessions to retrieve 190 mg banana-flavored food pellets (model F0035, Bio-Serv, Frenchtown, New Jersey) delivered to a pellet trough situated to the lower right corner of the touch screen monitor, until the establishment of reliable noncontingent pellet collection (2 sessions of >75% reward consumption).

Touch Screen Training

Next, subjects were trained to touch a magenta-colored square that filled the entire touch screen in order to obtain pellet reinforcement in the 20-minute training sessions. Small pieces of banana were smeared on the touch screen to prompt the subjects to touch the screen. This incentive was removed once the animals were reliably touching the screen for reinforcements (usually after 3 sessions). Subsequently, a shrinking stimulus program was used such that the magenta-colored square successively decreased in size following performance criteria beginning with the whole screen (24.6 cm × 18.5 cm) to a final square size of 5.8 cm × 5.8 cm—6 different sizes in total. A decrease in stimulus size occurred following every 10th consecutive correct response for reinforcement (a correct response was a touch within the colored square and an incorrect response was a touch to the screen outside the colored square). The dependent variable measured was the percentage of errors made.

Following the shrinking program, a moving stimulus program began in which a magenta-colored rectangle (6.2 cm × 4.4 cm) was presented randomly at 1 of the 9 different locations on the screen per trial for 3 sessions. Lastly, additional touch screen training (2-position) was administered following the progressive ratio sessions (see below). This 2-position stimulus program was conducted using the same stimulus shape as in the moving stimulus program but with the stimulus presented at 1 of the 2 locations on the touch screen per trial, that is left or right of center, for 3 sessions in order to prepare the subjects for the stimulus positions on the screen used in the subsequent simple discrimination (SD) and reversal training. The dependent variable measured was the percentage of errors made.

Progressive Ratio Schedule of Reinforcement

Progressive ratio testing, following touch screen training, measured the subjects' motivation to work for the food pellet reinforcements until the subject ceased to respond (breakpoint). The number of touches on the center of the screen (10 cm²) contingent on reinforcement (2 pellets per reinforcement) increased successively following each subsequent reinforcement earned; the response increment ratio was doubled every eighth reinforcement following the initial increment of 1 for the first 8 reinforcements (1, 2, 3, 4, 5, 6, 7, and 8 = total of 36 responses). Next, the response increment was doubled to 2 for subsequent reinforcements (10, 12, 14, 16, 18, 20, and 22) and on the eighth reinforcement, the increment doubled to 4 and so on. The overall testing time was 30 minutes, but the session terminated early if there were no screen touches for 3 minutes. The dependent variables measured were the number of responses made, rewards earned, and breakpoint (time of session termination due to inactivity).

Simple Discrimination and Reversal Tasks

Following the completion of the progressive ratio and the 2-position touch screen training tasks, SD sessions commenced

to assess associative learning. Initially, 5 SD tasks were administered successively (SD1-5) over sessions. The response criterion was 8 consecutive reinforced trials to complete a SD task. The first SD task (SD1) was administered in 30-minute sessions until the performance criterion was met. Thereafter, subjects were administered 30-minute sessions to complete the remaining SD2-5 tasks. For each SD task, 2 different magenta-colored stimulus shapes were presented simultaneously on the touch screen but only 1 of which was reinforced. Each stimulus pair was unique for each SD task and the reinforced and incorrect stimulus shapes were counterbalanced between and within groups. For each trial, both stimuli were randomly presented to the left and right side on the touch screen and remained on the screen until the subject made a response. Once the subject had responded to the correct stimulus, the incorrect stimulus disappeared from the screen, while the correct one (the positively reinforced stimulus) remained on the screen for an additional 2 seconds during which reinforcement was delivered and the 1-kHz tone sounded. Touching the incorrect stimulus terminated the trial immediately with a 0.2-second 40-Hz tone (punishment tone) followed by a 5-second intertrial interval. After SD1-5 tasks, a sixth SD task was administered followed by a SD reversal (SR) task. In the SR task, reinforcement contingencies were reversed such that the previously reinforced stimulus shape in the SD task became the incorrect stimulus and the previously incorrect stimulus became the positively reinforced stimulus. There were 3 pairs of SD and SR tasks (SD6 followed by SR1, SD7 followed by SR2, and SD8 followed by SR3). In total, there were 8 SD (SD1-8) and 3 SR (SR1-3) training tasks. The dependent variable measured was the errors made per task.

Intra-/Extradimensional Set Shifting

The intra-/extradimensional (IDED) set shifting test sequence consisted of 8 stages: (1) SD, (2) SR, (3) & compound discrimination (CD), (4) CD reversal (CR), (5) intradimensional shift (ID), (6) intradimensional shift reversal (IR), (7) extradimensional set shift (ED) and (8) extradimensional set shift reversal (ER). Session parameters were as follows: 30-minute maximum length, 5-second intertrial interval (for correct or incorrect responses), strict criteria for touch detection, 0-second limited hold for responding to the screen, 2 pellets for reinforcement, reinforcement tone (2-second, 1 kHz), punishment tone (0.2 second, 40 Hz), unlimited number of trials and unlimited amount of time to wait for a response. For each stage, 2 stimuli were displayed on the touch screen monitor with only 1 stimulus being associated with reinforcement. In order to progress through the 8 stages, subjects were required to respond to the correct stimulus for 8 consecutive trials (performance criterion). If a given stage was not completed by the end of a daily test session, the next session began at that stage with the performance criteria reset. Left and right positions of the stimuli were varied by trial in a random fashion. The Cambridge Cognition stimulus set 0 was used with the shape stimuli associated with reinforcement for the SD, SR, CD, CR, ID, and IR stages and

line stimuli associated with reinforcement for the ED and ER stages.

The test sequence began with stage 1 (SD task). When the performance criteria had been achieved, stage 2 (SR task) was presented in which the 2 shapes retained from the SD stage were presented with response contingencies reversed for the stimulus pair. Following performance criteria, stage 3 (CD task) was presented in which shapes retained from the SD/SR stages were combined with superimposed white lines (compound stimuli). For the CD stage, subjects were reinforced for attending to the same exact shape that was reinforced in the preceding SR stage while ignoring the superimposed line stimuli. Lines were associated equally and randomly with both of the shape stimuli. Once the CD task had been completed, stage 4 (CR task) followed. In the CR stage, the response contingencies were reversed so that the previously incorrect shape in the CD stage was now reinforced. Subsequent to the CR stage, a pair of novel compound stimuli were presented in stage 5 (ID task), and once again subjects were reinforced for attending and responding to the correct shape. This stage is labeled the intradimensional shift stage because, despite new examples of shape and line stimuli, the same dimension of the stimulus (shape) remained relevant for reinforcement. A reversal of the ID stage followed with stage 6 (IR task) in which response contingencies were reversed so that the previously incorrect shape in the ID stage was now reinforced. In stage 7 (ED task), a final pair of novel compound stimuli was presented. At this stage, subjects were reinforced for attending to the correct superimposed white line while ignoring the shape. Hence, the ED set shift stage is so labeled because the dimension of the reinforced stimulus changed from shape to line. The sequence finished with stage 8 (ER task), which was a reversal of the ED stage with the response contingencies reversed from the previously incorrect line in the ED stage becoming the reinforced stimulus. The dependent variable measured was the errors made per stage.

Delayed Matching to Sample Task

The delayed matching to sample (DMTS) task parameters were adapted from rhesus monkey protocols and validated by our group in baboons.^{43,44} Subjects were assessed in 30-minute sessions with a maximum of 120 trials. Two banana-flavored food pellets were the positive reinforcement following correct stimulus choice selection. The stimuli were taken from the Cambridge Cognition Pal 0 set that generates 199 283 trial-unique stimuli by enabling the “vary colors” selection which multiplies the number of stimuli (83) by 2401.

Initially, a sample stimulus was presented on the touch screen monitor for 30 seconds during which the subject made an *observing response* which was not reinforced. Observing response latency was the time elapsed between presentation of the sample stimulus and a press on the touch screen monitor. Failure to make an observing response ended the trial followed by a 5-second intertrial interval. An observing response blanked the touch screen contemporaneous with a varying

Table 2. Cognitive Performance Summary

Test	Purpose	Dependent Var	Subtest	Results	P Value	Summary
Progressive ratio Simple discrimination (SD)	Motivation Associative learning	Responses Reinforcements Errors	—	C>R	=.06	Borderline reduced motivation in R
			SD2	MR>MC	=.07	Borderline impaired learning in MR
			SD3	FC>FR	=.08	Borderline improved learning in FR
			SD6	FC>FR	=.05	Improved learning in FR
Intra/extradimensional (IDED)	Attention	Errors	ER	MR>MC	<.05	Impaired attention in MR
Delayed matching to sample (DMTS)	Working memory	Observing latency	—	MC>MR	<.05	Increased impulsivity in MR
		Accuracy (0 seconds)	—	C>R	=.08	Borderline impaired discrimination in R
		Choice latency (1 seconds)	—	MC>MR	<.05	Increased impulsivity in MR

Abbreviations: R, nutrient reduced; FR, nutrient-restricted female; MR, nutrient-restricted male; MC, male control; FC, female control; C, control.

delay interval followed by presentation of choice stimuli for which a *choice response* was required. The correct stimulus and 1 distractor stimulus were simultaneously presented (randomly) at 2 of the 4 corners of the touch screen monitor during choice stimulus presentation. Thus, if the sample stimulus for a given trial was a triangle, the subject had to choose the triangle from the presented choice stimuli, only 1 of which would be the triangle. Subjects were allowed 30 seconds for choice stimulus responding (choice response latency). Five-second intertrial intervals followed both correct and incorrect choice stimuli selection.

The DMTS task consisted of 6 delay intervals chosen randomly and interposed after initial (observing) responses were made to the sample stimuli: 20 trials per delay interval for a maximum of 120 trials. At the outset delay intervals were all set to 0 seconds; delay set 1. When the overall choice accuracy (ie, average accuracy of the 6 delay intervals) was between 60% and 70% for 3 consecutive sessions or 71% to 84% for 2 consecutive session or 1 session equal to 85% or greater, the subsequent delay set was used (0, 0, 0, 1, 1, and 1 = 3 values were set to 0 seconds and 3 to 1 second). In this manner, the following delay sets were incorporated into the DMTS task as subjects demonstrated criterion performance: 0, 0, 1, 1, 2, and 2 seconds; 0, 1, 1, 2, 2, and 4 seconds; 0, 1, 1, 2, 4, and 8 seconds; 0, 1, 2, 4, 8, and 16 seconds; 0, 2, 4, 8, 16, and 32 seconds; 0, 4, 8, 16, 32, and 48 seconds; 0, 8, 16, 32, 48, and 64 seconds; and 0, 16, 32, 48, 64, and 80 seconds. These 10 delay sets were administered with 1 distractor, however not all subjects attained performed criterion to delay set 10. Delay set 6 was assigned as the test set since all subjects mastered this stage. Independent variables measured were performance accuracy, observing, and choice response latencies.

Data Analysis

Data are summarized as mean $\pm$ standard error. For training and each of the 4 tasks we first tested for a treatment effect within a sex. Data were pooled by treatment if no sex

differences were observed. Comparisons were performed using a repeated measures linear model with an autoregressive correlation structure for touch screen training and progressive ratio to model the association between successive trials or a linear model of log for SD, SR, IDED, and DMTS tasks. All subjects provided data for all tasks. SAS Version 9.2 (SAS institute, Cary, North Carolina) was used to conduct analyses; statistical significance was set at $P \leq .05$ designate by *, and borderline effects reported at $P = .06$ to $.08$ designated by #.

Results

Maternal Morphometrics

There were no group differences in maternal weight prior to pregnancy (Table 1). During delivery and weaning, MNR mothers weighed less than CTR as a result of the decreased food availability in pregnancy and lactation. Weaning is a very gradual process in the baboon as the offspring eat more and more solid adult food in the group cage. The proportion of food provided to offspring in the milk by the mothers decreases well before 9 months when the juveniles are completely weaned and separated from the mothers. Thus, mothers gain weight in the total period between delivery and weaning. This weight increase between delivery and weaning was similar in the 2 groups.

Offspring Morphometric Characteristics and Growth

There was no difference in the duration of gestation (Table 1). At the time of behavioral testing, CTR offspring weighed more than MNR offspring.

Summary of Behavioral Results

Cognitive performance results are summarized in Table 2.

Figure 1. A and B, Maternal nutrient restricted offspring ($n = 7$ —male and female combined, closed bars) respond less (A) and earn less reinforcements (B) than control offspring ($n = 12$ —male and female combined, open bars) in the progressive ratio task to assess motivation. Mean $\pm$ SEM; #, $P \leq .06$.

Touch Screen Training

No treatment effects were determined during touch screen training (data not shown).

Progressive Ratio

No treatment effects were determined within sex, so females and males were pooled by treatment. Comparisons of total responses and reinforcements earned show borderline decreases in both number of responses and reinforcements earned with $P = .06$ in MNR compared with control (CTR) offspring (Figure 1A and B). No differences were found for progressive ratio breakpoint (data not shown).

Simple Discrimination and Reversals

Analyses of the 5 SD tasks (SD1-5; Figure 2A) and the 3 SD tasks (SD6-8) following by SRs (SR 1-3; Figure 2B) show an overall effect of treatment in the females (FR make less errors than FC, $P < .05$) and males (MR make more errors than MC, $P < .05$). Post hoc analysis shows the FR offspring make less errors than the FC offspring during the SD3 (borderline effect, $P = .08$) and SD6 ($P = .05$) tasks and the MR offspring make more errors than the MC offspring during the SD2 task (borderline effect, $P = .07$).

Intra-/ExtraDimension Attention Set Shift Test

Comparisons during the different stages of IDED testing reveal different treatment effects by sex. A treatment effect was determined in the males during the ER stage, with MR offspring making more errors than MC offspring ($P < .05$; Figure 3). No treatment effects were observed in females.

Delayed Matching to Sample Task

Analysis of the observing response latencies revealed no effect in females but a treatment effect in males with the MR offspring having shorter latencies in response to initial stimulus presentation compared to MC offspring ($P < .05$; Figure 4A). No treatment effects within the individual sexes were

determined for performance accuracy across delay intervals so females and males were pooled by treatment. A borderline difference was measured in the MNR offspring, being less accurate, following no delay (0 sec) trials than CTR offspring ($P = .08$; Figure 4B). Choice response latency analyses revealed a treatment effect in males, with MR displaying shorter choice response latencies following 1 second delay intervals versus MC ($P < .05$; Figure 4C).

Discussion

The goals of this study were to assess motivation, associative learning, attention, and working memory outcomes in juvenile baboon offspring exposed to a moderate maternal nutrient restriction during fetal life and up to weaning and to determine sex-specific effects. No sex effects were observed in motivation, but pooled data by treatment did show diet-specific outcomes, with MNR offspring displaying a borderline reduction in motivation versus CTR offspring. Outcomes on associative learning during SD training tasks showed different effects of treatment based on sex. Overall, the FR offspring made fewer errors than the FC offspring and the MR offspring made more errors than the MC offspring during SD tasks. The effects observed suggest that both the FC and MR offspring had more difficulties in the SD tasks than FR and MC offspring, respectively.

Learning and attention performance during the IDED test showed no treatment effect in the females. However, in males the MR offspring made more errors in the ER stage as compared to the MC offspring. This treatment effect in males suggests impaired attentional set shifting in the MR offspring. Working memory assessment in the DMTS task revealed decreased observing response latencies in MR offspring compared to MC offspring, indicating a treatment effect in males with no differences in females. These outcomes are indicative of increased impulsivity in the MR offspring. Accuracy in working memory performance during DMTS showed a borderline decrease in the MNR offspring than the CTR offspring following no delay conditions which suggest decreased response control or impaired discrimination in the MNR offspring. A

Figure 2. A, Simple discrimination (SD) tasks (SD1-5). Overall treatment effects were determined in males and females ($P < .05$). Post hoc analyses show borderline differences in female nutrient restricted offspring ([FR] $n = 3$) make fewer errors than female control offspring ([FC], $n = 8$) during SD3 and in male nutrient restricted offspring ([MR], $n = 4$) make more errors than male control offspring ([MC], $n = 4$) during SD2. Mean $\pm$ standard error of the mean (SEM); # $P \leq .06-.08$. B, Simple discriminations (SD6-8) followed by reversals (SR1-3). Overall treatment effects were determined in males and females ($P < .05$). Post hoc analyses show the female nutrient restricted ([FR] $n = 3$) offspring make fewer errors than female control ([FC] $n = 3$) offspring during SD6 task. No differences in male offspring. Male control ([MC] $n = 4$), male nutrient restricted ([MR] $n = 4$), offspring, mean $\pm$ SEM; * $P \leq .05$.

Figure 3. Intra-/extradimension attention set shift test. No differences in female offspring were measured, female control ([FC] $n = 8$), female nutrient restricted ([FR] $n = 3$) offspring. A treatment effect in males was determined, male nutrient restricted ([MR] $n = 4$) offspring make more errors than male control (MC) offspring during the ER test stage. Mean $\pm$ standard error of the mean (SEM); * $P < .05$.

treatment effect was measured in choice response latencies in males with the MR offspring displaying shorter choice response latencies versus MC offspring which agrees with increased impulsivity in the MR offspring.

These behavioral findings contribute to the current knowledge of developmental programming and postnatal motivation and cognition. In regard to motivation for positive reinforcement, prenatally undernourished rats show less appetitive

Figure 4. A, Observing response latency. No differences in female offspring were found, female control ([FC] $n = 8$), female nutrient restricted ([FR] $n = 3$). A treatment effect in males was determined, male nutrient restricted ([MR] $n = 4$) offspring have shorter latencies compared to male control ([MC] $n = 4$) offspring. Mean $\pm$ standard error of the mean (SEM); * $P < .05$. B, Results pooled by treatment show a borderline decrease in accuracy following 0 second delay intervals in the maternal nutrient restriction (MNR) offspring ($n = 7$, male and female combined, closed bars) versus CTR offspring ($n = 12$, male and female combined, open bars). FC ($n = 8$), FR ($n = 3$), MC ($n = 4$), MR ($n = 4$), Mean $\pm$ SEM; # $P \leq .06-.08$. C, Choice response latencies showed no differences in females, FC ($n = 8$) and FR ($n = 3$). A treatment effect in males shows decreased choice response latencies following 1 second delay in MR ($n = 4$) offspring versus MC ($n = 4$) offspring. Mean $\pm$ SEM; * $P \leq .05$.

motivation as shown by exhibiting increased preference for wheel running versus lever pressing for food,⁴⁵ agreeing with our findings of less motivation in the nutrient reduced (R) offspring. Additionally, we have recently reported decreased responsiveness in the progressive ratio test in adolescent rats exposed to an isocaloric protein restriction during gestation and lactation.³⁰ Maternally derived androgens and glucocorticoids increase aversive properties to appetitive stimuli in rat offspring,⁴⁶ and it is significant that we have shown elevated androgens and glucocorticoids in near-term protein-restricted rat dams and hypothesize that similar mechanisms are operative in our pregnant baboon model. We have shown that fetal baboon cortisol is elevated 53% by this degree of global MNR,⁴⁷ although we did not measure androgen or glucocorticoid levels in these subjects after delivery and prior to weaning.

In contrast, severe prenatal protein restriction increases the response for reinforcement in adult male rats.²⁵ This difference may however be the result of different experimental conditions as the amount of protein restriction was 6% casein (75% reduction) isocaloric diet in the rat dams.

The cognitive deficits we report in MR offspring during SD and the ER stage of the IDED test are consistent with the findings in global undernutrition during gestation impaired learning in adult-age rat offspring using concurrent variable-interval schedules.⁴⁸ Similarly adult sheep offspring undernourished during fetal life display cognitive flexibility impairment as assessed by learning speed during reversal tasks in a T-maze.⁴⁹ In addition, perinatal food restriction in male rats impairs spatial learning and memory in the Morris water maze.^{29,50} Performance impairments in differential reinforcement of low rates²⁶

and the radial arm maze²⁷ in rat offspring perinatally protein restricted also support our findings. Although the rodent studies do not precisely reflect the dietary challenges and operant tasks we administered, a commonality exists in our MR offspring exhibiting cognitive inflexibility during several cognitive tasks.

Behavioral results from the DMTS task show that the MR displayed shorter observing response latencies than MC offspring, which means that upon visual stimulus presentation MR responded faster than MC. These results could be interpreted as increased impulsivity by the MR offspring during initial stimulus presentation. However, no overall performance accuracy differences implies that the increased impulsivity is not due to impairment in attention but some other unknown variable, for example loss of response inhibition. There was a borderline decrease in performance accuracy between CTR and MNR offspring following the 0 second delay trials, which suggests impairment in discrimination ability or decreased encoding of positively reinforced stimulus in the MNR offspring. Choice response latency was decreased in MR offspring versus MC following 1 second delay intervals, indicating a treatment effect during stimulus discrimination. Shorter choice response latencies imply increased impulsivity during stimuli presentation following the delay interval. These DMTS task results are consistent with the SD and IDED task results which also show impaired behavior in male offspring of MNR mothers.

In this model, pre- and postnatal maternal nutrient restriction decreased the birth weight of offspring,⁴⁰ which reflects the importance of adequate maternal nutrient intake for normal fetal maturation. This morphometric outcome is consistent with the models of intrauterine growth retardation and fetal programming.⁵¹ The difference in body weight remained evident throughout behavioral testing. We have previously measured an increased anogenital distance in prenatal protein-restricted (protein reduced 50%) rat offspring born to dams with elevated progesterone, corticosterone, estradiol, and testosterone concentrations near term (19 days of gestation).^{52,30} In baboons, we have shown that fetal glucocorticoids are elevated 53% by this degree of global MNR.⁴⁷ Maternal steroids can cross the placenta, and such exposure to transplacentally acquired androgens in fetal life can increase anogenital distance and result in other developmental perturbations.⁵³ Effects of these steroids on fetal development could have a role in the current behavioral findings since human studies report impairment of spatial learning ability in males exposed to excess levels of androgens in utero with no effects on females.^{54,55} There is also abundant evidence of excess levels of glucocorticoids in utero impairing brain development and later behavior in humans and animal models⁵⁶⁻⁶⁷ including baboons.⁶⁸ Although we did not assess postnatal androgen or glucocorticoid levels in offspring, this should not preclude conclusions based on gestational exposure affecting behavioral performance. Evidence does exist of elevated androgens in male rats small for gestational age.⁶⁹ These results also suggest that MR offspring were vulnerable to the perinatal malnutrition, while the FR offspring were more resilient. Resilience in general refers to a pattern of functioning

indicative of positive adaptation in the context of significant risk or adversity.⁷⁰ Perinatal exposure to increased androgens or glucocorticoids could be a potential explanation for these sex-specific effects.

One might hypothesize that sufficient macromolecule components required for normal neurodevelopment resulted in significant structural changes during development and subsequent persistent dysfunction of relevant neural areas in the MNR offspring at the time of testing which could have affected motivation and cognition.^{15,17-19} We recently demonstrated major cerebral cortical structural developmental disturbances in the brain of fetuses from baboon mothers administered a 30% globally MNR diet during pregnancy which if they persist into postnatal life would likely have direct effects on offspring behavioral measures assessed in this study.²⁴ We have also reported that protein restriction during pregnancy negatively impacts normal fetal brain development by changes in maternal lipid metabolism in rats.⁷¹ Thus, rat offspring born to malnourished dams exhibit reduced brain fat such as long chain polyunsaturated fatty acids including arachidonic and docosahexaenoic acid in the brain.⁷¹

Since a lack of nutrients during development causes cognitive and behavioral problems in humans,⁷²⁻⁷⁵ our findings support the need for further controlled studies to elucidate the behavior and underlying brain areas influenced by these nutritional challenges are imperative. These outcomes are potentially due to the insufficient protein (essential amino acids) or fatty acids/lipids available during critical windows of brain development. The 30% global nutrient restriction during pregnancy and lactation in our model should be taken into consideration during interpretation; this model is not isocaloric as are many others used by those attempting to understand the interaction between maternal nutrition and offspring cognition, particularly when rodents are utilized. Hormonal response in both mother and fetus must also be considered together or separately with the malnutrition effects as steroids greatly determine neural proliferation, differentiation, and migration.^{76,77} These differences clearly reflect impairments in motivation and cognition behavior. Important follow-up experiments for detecting levels of proteins in specific neurotransmitter systems and neural system cellular populations are the goals of future studies in determining the mechanisms involved.

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