Prenatal 2g Exposure Alters Placental Expression of Stress-Related Genes

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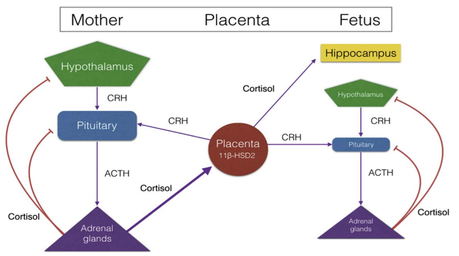
INTRODUCTION

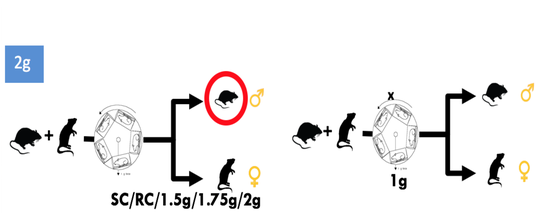
Early life exposure to environmental stressors can cause significant developmental programming effects on offspring within and across generations[5,6]. While effects of prenatal stress (PNS) on later life outcomes have been extensively studied on Earth little is known about the effects of prenatal stress associated with spaceflight[1,2,3]. We previously reported that adult male, but not female, offspring of mid-gestation female rats exposed for 9 days to 2g (2X Earth gravity) showed changes in adult body mass regulation and increased anxiety-like responses to a startle stimulus. We hypothesized that these changes resulted from prenatal stress (glucocorticoid) exposure during gestation. In support of this hypothesis, we established an Unpredictable Variable Prenatal Stress (UVPS) Model ***that successfully mimicked in rat offspring these 2g effects while at the Earth's 1g.*** Here we report that altered gravity reduces placenta expression of 11BHSD2, an enzyme that catalyzes the conversion of inert 11 keto-products (cortisone) to active cortisol thereby regulating access of glucocorticoids to steroid receptors.



The mother's stress axis is intimately related to that of the developing fetus via the placenta. (Image credit: St. Pierre et al. 2015)

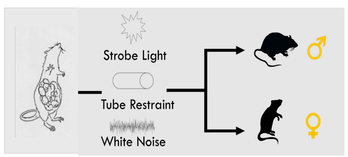
HYPERGRAVITY (HG) PARADIGM

(Image credit: NASA Ames Research Center



Centrifugation Model. Left. To simulate HG, timed pregnant female rats (dams) were exposed to chronic HG (20RPMs) beginning gestational day (G) 11 of their 22-day pregnancy, performed using the 24-foot centrifuge at NASA Ames Research Center. **Right**. HG dams were assigned to either 1.5, 1.75, and 2G; stationary control (1g) or rotational control (1.07g) conditions.

UNPREDICTABLE VARIABLE PRENATAL STRESS PARADIGM



Day / Time	G1 Stressor (min)	G2 Stressor (min)	G3 Stressor (min)
Early : 0600 – 1200	Light (30)	Restraint (60)	Sound (15)
Mid : 1200 – 1800	Sound (60)	Light (15)	Restraint (30)
Late : 1800 – 0600	Restraint (15)	Sound (30)	Light (60)

UVPS Model. Left: *Pregnant dams were exposed daily throughout gestation to a single episode of high-frequency strobe light (3.5 W, 85 mA), tube restraint (PVC tube), and white noise.* ***Right:*** *Each stimulus was presented in an unpredictable temporal regimen, viz, either early, mid or late times across the light/dark cycle for varying durations (15, 30 or 60min).*

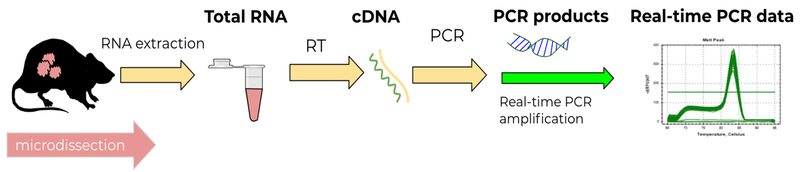
METHODs

For both studies, nulliparous female Sprague-Dawley (SD) rats were bred with SD males. Pregnancy was determined by daily vaginal lavage with the presence of spermatozoa indicating Gestational day 0 (Go) of the rats' 22-day pregnancy.

HG Study. Pregnant dams were continuously centrifuged in standard vivarium cages within enclosures that gimballed out during operation. The centrifuge was stopped for less than one hour daily for veterinary and husbandry activities.

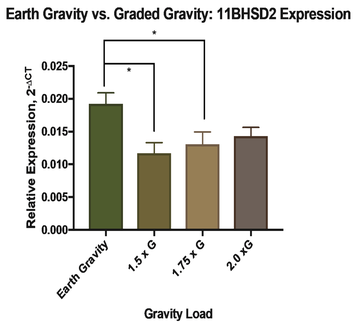
UVPS Study. Stressed and non-stressed dams were housed in separate experimental rooms. Non-stressed dams were handled by experimenters for the same duration each day as pregnant dams.

TISSUE PROCESSING

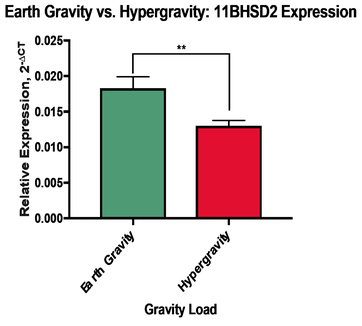


At term, fetal placenta (*Chorion frondosum*) was microdissected, total RNA was extracted and reverse transcription performed to produce cDNA. PCR was performed using Taqman probes for 11BHSD2, DNMT3a, OGT, and IL-6 gene expression.

RESULTS

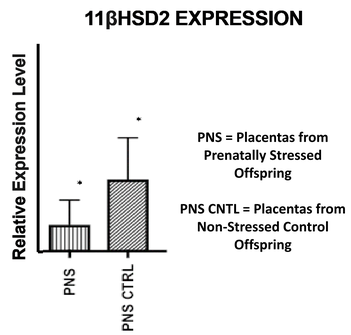


Relative gene expression (calculated as $2^{-\Delta CT}$) of the gene expression for 11βHSD2 in rat placenta: earth gravity versus graded HG. Earth gravity group is comprised of stationary control, rotational control grouped into one experimental parameter (n = 18). Graded gravity breaks each HG condition into individual experimental groups: 1.5, 1.75, and 2.0 x gravity (n = 11, 7, and 6 respectively).

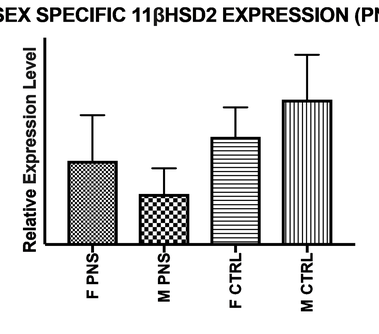


Relative gene expression (calculated as $2^{-\Delta CT}$) of the gene expression for 11βHSD2 in rat placenta: earth gravity versus HG. Earth gravity group is comprised of stationary control and rotational control grouped into one experimental parameter (n = 18). HG groups each experimental, graded HG group into one experimental "HG" group (n = 24).

RESULTS



Relative expression (calculated as $2^{-\Delta CT}$) of the genes for 11βHSD2 in the rat placenta: following prenatal stress. PNS=prenatally stressed rats (n=8); PNS CTRL=control rats from prenatal stress study (n=8).



Relative expression (calculated as $2^{-\Delta CT}$) of the gene 11βHSD2 in the placenta of prenatally stressed rats: male vs female fetuses. F PNS=female fetus, prenatally stressed (n=4); M PNS=male fetus, prenatally stressed (n=4); F CTRL=female fetus, control (n=4); M CTRL=male fetus, control (n=4).

Summary and Conclusions

The healthy development of mammalian offspring is dependent on the integrity of the maternal fetal coupled system. One potential indicator of in utero health is the expression of 11BHSD2 in the placenta. 11BHSD2 has been extensively studied and its role mediating the impact of the maternal stress response on the developing fetus. Our study has shown that the expression of 11BHSD2 was reduced significantly in a hypergravity environment when compared to earth's gravity. We found a similar finding in our PNS study where placentas from dams that were exposed to unpredictable stressors while pregnant showed a significant reduction in 11BHSD2 expression. Our findings demonstrate that hypergravity acts on the maternal system in a similar way as PNS and alters the expression of 11BHSD2.

We previously reported that a sex specific phenotype resulting in PNS adult male offspring of mid-gestation female rats showed changes in adult body mass regulation and increased anxiety-like responses to a startle stimulus, these findings were similar for the male offspring of dams exposed for 9 days to 2g . Collectively, our findings provide evidence that unpredictable, variable stress and HG experienced during fetal life shapes the adult stress axis. Our future studies aim to determine sex-specific differences in placental gene expression that may underlie altered adult male body weight regulation and behavioral outcomes observed in our previous work.

Given the consistencies of our findings in both 11BHSD2 expression and the sex specific phenotype of offspring our lab plans to investigate the role of sex in the expression of biomarkers in the placenta. Currently our lab has validated a protocol to extract DNA from fetal placenta tissue for sex identification to determine the sex of individual placentae by XX/XY genotype. We will use this data to better understand the unique impact of fetal sex and the maternal environment, under variable hypergravity conditions, on fetal outcome.

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