Gene/Prenatal Stress Model and Diet Effects on Litter Size in Mice

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INTRODUCTION

- When research examining if diet supplementation of docosahexaenoic acid (DHA) mitigates autistic like behaviors in differing genotypic mice exposed to chronic stress variable was replicated, the question arose of whether there was a significant difference in litter sizes in mice due to interaction of diet, chronic stress condition, and genotype.
- DHA is an Omega-3 polyunsaturated fatty acid that is synthesized by the body through the breakdown of a precursor fatty acid called alpha-linolenic acid.
- Previous studies have found that rodents fed a control diet compared to rodents fed a DHA supplemented diet had no significant differences in litter size (Yi et al., 2012, Haubner et al., 2006, Blum et al., 2007, Yang, et al., 2018).
- Research examining prenatal exposure to chronic stress variables on rodents had mixed results with some studies finding significantly smaller litter sizes (Baker, et al., 2008, Euker & Riegle, 1973, Wiebold et al., 1986) and others finding no significant differences (Pollard, 1985, Lordi et al., 2000).
- SERT regulates the amount of serotonin available in the brain via reuptake. Mice with lower functioning SERT genes are more susceptible to stressful environments due to over reactive neuroendocrine responses (Holmes et al., 2003).
- Chronic stress exposure during gestation can have numerous effects including diminished fertility, reduced number of implantation sites, and intrauterine mortality (Herrenkohl, 1979, Kittinger et al 1980).
- The present study investigates if dietary supplementation of DHA, chronic prenatal stress conditions, and/ or genotype significantly influences litter size in mice.

HYPOTHESIS

Wildtype, non-stressed mice fed a DHA supplemented diet will have larger litter sizes compared to heterozygous knockout, stressed mice fed a control diet.

METHODS

Variables

Genotype: Male homozygous SERT KO mice and wildtype females were bred to obtain heterozygous females. These heterozygous females, along with other wildtype females were paired with wildtype males for mating.

Diet: Dams were randomly assigned to two variable groups: 1) 1% DHA diet, which consisted of control diet with 1% by weight DHA ethyl esters (#103598, Dyets Inc., Bethlehem, PA), 2) control diet only (CTL). The control diet contained no DHA additives, but contained sufficient amounts of alpha-linolenic acid to meet DHA requirements for normal brain function (Domenichiello et al., 2015).

Stress: Generation of a gene/prenatal stress model was replicated from a previous study (Jones et al., 2010). Selected dams were exposed to a one of the six stressors each day with each of the six stressors being presented consecutively over a six-day period. This pattern was repeated approximately 2.5 times.

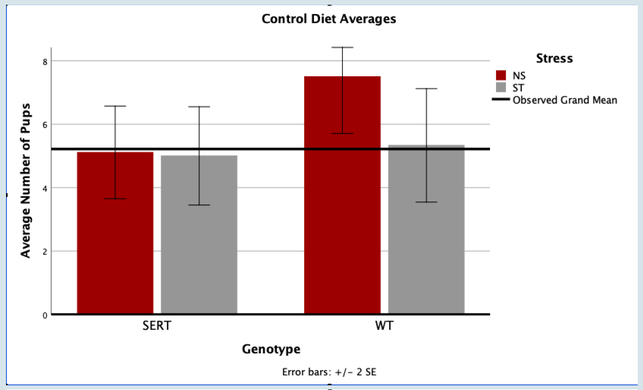
Procedure

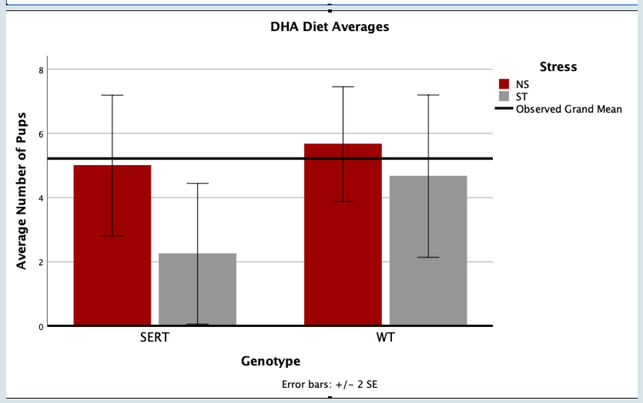
Animals: Two weeks before breeding through gestational period, dams were randomly assigned to a DHA or control diet group. Male and females were paired for mating at 10 weeks old. Once conception was confirmed by the presence of a vaginal plug, dams were separated and placed in clear polycarbonate cages and provided with aspen bedding and nestlet. Dams were randomly assigned to one of eight conditions represented in a 2 × 2 × 2 design of genotype × prenatal stress × diet. On gestational day 6 until parturition, dams were exposed to 1 of 6 chronic stress variables. Animals were kept in temperature and humidity controlled rooms at 25 degrees Celsius on a 12-hour light/dark cycle with food and water available ad libitum. On postnatal day 21, number of surviving pups were noted for each dam.

Statistical Analysis: Statistical analyses were performed using SPSS software (IBM Corp., Somers, NY, USA). Data was analyzed using a 2 x 2 x 2 ANOVA (genotype × prenatal stress × diet).

RESULTS

- All variables consisted of two levels; Genotype (SERT and WT), chronic stress condition (NS and ST), and diet (DHA and CTL).
- The sample included 46 dams. Of these, 21 dams (45.7%) were wild-type and 25 dams (54.3%) were heterozygous, 25 dams (54.3%) where assigned to non-stressed conditions and 21 dams (45.7%) were assigned to stressed conditions, and 29 dams (63%) were fed a control diet whereas 17 dams (37%) were fed a DHA diet.
- There was no significant interaction between the three variables, $F(1,38) = 1.92$, $p = .174$. The total average number of pups for the 46 litters was 5.22 with a standard deviation of 2.366.





DISCUSSION

Hypothesis: Not supported – No significant interactions between diet, chronic stress exposure, and genotype on litter size was found.

Conclusion: Litter size in mice is unaffected by the interactions of genotype, chronic prenatal stress conditions, and diet.

Limitations: The number of dams within each interaction group (WT/NS/CTL, WT/NS/DHA, WT/S/CTL, WT/S/DHA, SERT/NS/CTL, SERT/NS/DHA, SERT/S/CTL, and SERT/S/DHA) is small and may not be large enough to run an accurate three-way statistical analysis to find significant results.

Future directions: The impacts of a supplemental DHA diet and the gene/prenatal stress mouse model on litter sizes is still debated. Future research should be aimed at increasing sample sizes in mouse studies in order to replicate findings with more power.

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