Effects of Xenoestrogen on Hypothalamic and Hippocampal Gene Expression

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Introduction

- Bisphenol A (BPA) and Genistein (GEN) are endocrine disrupting chemicals (EDC) which fetuses and infants are often exposed to through placenta and milk[1,2].
- EDCs may increase risk for autism spectrum disorder (ASD) and other neurobehavioral disorders[3,4].
- Other studies have shown BPA and GEN leading to gene expression changes in hippocampus and hypothalamus[5,6].
- To observe the effects of these EDCs we used California mice (*P. californicus*) which demonstrate monogamous pair bonding behavior and biparental care

Hypothesis

Early exposure to BPA or GEN may lead to long standing behavioral and metabolic disturbances that correlate with expression of mRNA and miRNA (miR) in the hypothalamus and hippocampus.

Materials and Methods

Animals and Treatments- Dams were randomly assigned to one of four diets. 1) AAIN 93G diet. 2) GEN diet. 3) "low-dose- LD" BPA. 4) "upper-dose- UD" BPA. Pups were weaned at 30 days and were then put on the CTL-AIN diet. At 90 days testing began.

Behavioral Tests- At 180 days the mice underwent Crawley's sociability and preference for social novelty three-chambered test. Stanford Medicines Behavioral and Functional Neuroscience Laboratory describes the methods of this test[7]. Then the mice were put in a box with a light and microphone. 5 minutes of audio was recorded and analyzed

Metabolic Assessments- The mice were tested in the Promethion continuous measurement indirect calorimetry system for 3 days. Measured parameters included energy expenditure, and food and water intake. Body composition was analyzed with EchoMRI- 1100.

Hippocampal/hypothalamic mRNA and miR Expression- After brain removal, hippocampal and hypothalamic brain regions were micro punched. Samples from both CA1 and CA3 nuclei were collected. Total RNA isolation from these two brain regions was completed. The RNA was quantified by spectrophotometrical analysis. Ten genes and four miR were tested.

Integrative Correlation analyses- We used the mixOmics R package (69) to correlate the behavioral and metabolic outcomes and mRNA and miR expression in selected brain regions. We conducted sparse discriminant analysis with partial least square regression with function 'block.splsda'. The circos plot was generated by using the 'circosPlot' function with correlations calculated by the method described by González, et al[8].

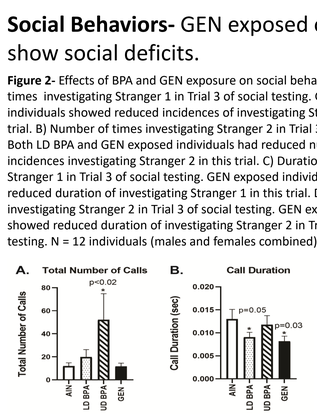
Statistics- Body composition was determined by EchoMRI, all other variables were analyzed using the PROG GLM procedure of SAS. The sources of variation considered were treatment, sex, and treatment × sex interaction. All data are presented as actual means.

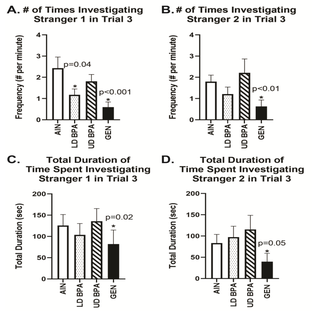
Figure 1 : Apparatus used. A) Crawley's sociability test. Yellow dot indicates where mouse is placed in maze, two arrows represent the mesh cups where novel mice are held. B) Promethion continuous measurement indirect calorimetry system. C) Soundproof box used to capture vocalizations. The arrow represents the avisoft microphone used to collect the vocalizations.

Results

Social Behaviors- GEN exposed offspring show social deficits.

Figure 2- Effects of BPA and GEN exposure on social behaviors. A) Number of times investigating Stranger 1 in Trial 3 of social testing. GEN exposed individuals showed reduced incidences of investigating Stranger 1 in this trial. B) Number of times investigating Stranger 2 in Trial 3 of social testing. Both LD BPA and GEN exposed individuals had reduced number of incidences investigating Stranger 2 in this trial. C) Duration of investigating Stranger 1 in Trial 3 of social testing. GEN exposed individuals showed reduced duration of investigating Stranger 1 in this trial. D) Duration of investigating Stranger 2 in Trial 3 of social testing. GEN exposed individuals showed reduced duration of investigating Stranger 2 in Trial 3 of social testing. N = 12 individuals (males and females combined)/treatment group.



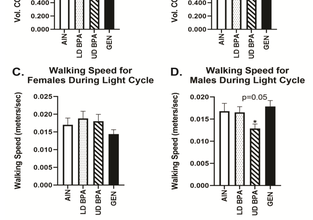


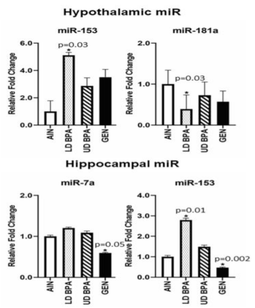
Communication Behaviors- UD BPA exposed offspring show increase in calls

Figure 3. Effects of BPA and GEN on vocalization behaviors. A) Average number of calls. Individuals exposed to UD BPA exhibited greater number of calls when placed in isolation. B) Average duration of calls. LD BPA and GEN exposed individuals showed reduced duration of calls. C) Average number of calls in a burst. UD BPA individuals showed greater number of calls in each burst. D) Average duration of bursts. LD BPA and GEN exposed individuals showed reduced duration of bursts. N = 12 individuals (males and females combined)/treatment group.

Metabolic Assessment- UD BPA exposed offspring show a decrease in walking speed for males during light cycle

Figure 4. RQ value and walking speed during the light cycle. A) Average RQ value for females during the light cycle. LD BPA and UD BPA female showed increased RQ value during the light cycle. B) Average RQ value for males during the light cycle. UD BPA males showed increased RQ value during the light cycle. C) Average walking speed for females during the light cycle. No differences were detected between female groups. D) Average walking speed for males during the light cycle. UD BPA male showed reduced walking speed during the light cycle. N = 6 individuals/treatment group and sex.



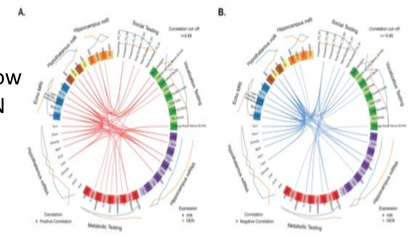


Hippocampal/hypothalamic mRNA and miR expression- LD BPA exposed offspring show increases in hypothalamic and hippocampal miR-153.

Figure 7. Hypothalamic and hippocampal miR expression as determined by qPCR. Significant differences relative to AIN controls are depicted with an asterisk and associated p value. N = 12 individuals (males and females combined)/treatment group.

Integrative Correlational Analysis- How GEN affected offspring differ from AIN offspring

Figure 9. Circos plot correlations for GEN exposed females vs. AIN control females. Correlation value was set to 0.85. Red lines in the center indicate a positive correlation (Panel A); whereas blue lines (Panel B) indicate a negative correlation. Lines on the outside of the circle indicate whether the value was greater in controls (blue) or GEN (orange).



Conclusions and Future Aims

- The findings reveal that BPA exposure can result in dose-dependent affect with the LD tested in the current studies compromising social-communication behaviors, hypothalamic mRNA gene expression patterns, and miR profiles in the hippocampus and hypothalamus to a greater extent than the UD BPA tested.
- The UD BPA resulted in greater number of gene expression changes in the hippocampus.
- The data also suggest that GEN exposure led to similar socio-communication deficits as LD BPA, but gene expression and miR changes in the hypothalamus and hippocampus diverged between these two groups, suggestive that the behavioral impairments detected in these two groups may originate due to different molecular alterations.
- These data are also the first to show that developmental exposure to BPA or GEN can lead miR changes in the brain when measured at adulthood.
- The fact that changes are observed in miR in adult California mice developmentally exposed to a LD BPA suggests that exposure to this EDC may lead to permanent effects on the epigenome
- The integrative correlation analyses reveal that changes in miR patterns, especially miR-153 and miR-181, are linked to alterations in socio-communication behaviors.
- The collective findings reveal cause for concern that developmental exposure of BPA or GEN in monogamous and biparental California mice, and potentially by translation humans, can lead to long standing neurobehavioral consequences.
- Global mRNA profiles will be examined in the future to expand the research that has already been done.

Acknowledgements

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