

# Bisphenol A and Genistein-Induced Changes on miRNA Profiles and Associated Behavioral Changes in California Mice (Peromyscus californicus)

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## INTRODUCTION

- Genistein and Bisphenol-A (BPA) are known xenoestrogens found in soy and soy-based products and plastic food storage containers, respectively (1). These chemicals may also induce epigenetic changes altering gene/protein expression without changing the structure of the DNA itself.
- Example epigenetic changes include alteration of DNA methylation, histone proteins, or affect miRNA expression (2).miRNA can either act inside the cell and target gene promoters, protein coding sequences, mRNA, as shown in figure 2, or outside the cell mediating cell-to-cell communication.
- o In 2016, according to the CDC, 1 in 54 children were affected by ASD, a 9.2% increase from the previously recorded year of 2014 (3).
- Gestational exposure to these compounds may be potential causative factors in increasing the risk for this neurobehavioral disorder.
- To assess whether gestational exposure to these endocrine disrupting chemicals (EDC) can affect the risk for ASD and potential underlying cause in terms of changes in miRNA (miR) patterns, we used Peromyscus californicus as our model as these species are monogamous, highly social, and exhibit biparental care.
- We measured global miRNA patterns and behavioral profiles in those that were developmentally exposed to this chemical compared to their non-exposed counterparts. We are in the process of using an integrative correlation analysis approach to link changes in miR with resulting behavioral alterations.



Figure 1: miRNA pathway of action. Pri-miRNA are primary miRNA; shRNA are small hairpin RNA; pre-miRNA are precursor miRNA. Drosha and GCR8 (DiGeorge Syndrome Critical Region 8) together make the microprocessor complex. In both pathways, pre-miRNA is processed and transported out of the nucleus via a complex of proteins. AGO refers to Argonaute family of proteins, which help produce miRNA-induced silencing complex (miRISC). (2)

### HYPOTHESIS

We predict that California mice offspring exposed perinatally to BPA, genistein, or the combination of the two will show autistic-like behaviors, including cognitive deficits, socio-communication impairments, and anxiety-like behaviors. We also predict that these mice will possess unique miRNA signature patterns in the hypothalamus that may underpin these potential behavioral disruptions.

## MATERIALS & METHODS

- Animals and Treatments: The parental generation was exposed to one of the 5 treatments 2 weeks prior to mating and during gestation and lactation. Five dams were included in each treatment group: for a total of 25 dams (5 dams/treatment x 5 treatments). One male and one female pup were weaned from each dam and randomly selected as test mice. Treatment groups include control (AIN; AIN93G), lowdose BPA (LD-BPA; 5 mg BPA/kg feed weight), high-dose BPA (HD-BPA; 50 mg BPA/kg feed weight), BPA+GEN (5 mg BPA+250 mg GEN/kg feed weight), and genistein (GEN; 250 mg genistein/kg feed weight).
- **Behavioral Methods**: One male and one female offspring from each litter were randomly selected for further behavioral analyses. Behavioral tests timeline is shown in figure 2. The Barnes Maze measures spatial learning and memory; EPM measures stereotypic and anxiety-like behaviors; Crawley's sociability test measures sociability and social novelty; and USVs measures socio-cognitive behaviors. Tests were performed as detailed in William et al. and Moy et al (4,5).
- Met Rack Chamber and EchoMRI: Following all the behavioral test and prior to RNA extractions, mice were placed in a chamber that measured metabolic outputs such as VO2, energy expenditure, and body mass. EchoMRI measured body composition.
- **RNA Isolation Method**: Tissues from test mice were collected and stored in a -80°C freezer. To isolate the RNA, we used Qiagen AllPrep DNA/RNA/miRNA Universal Kit.



## RESULTS

Barnes Maze: F1 exposed to GEN showed cognitive improvement and those exposed to GEN and UDBPA exhibited greater speed in their movement. **B. Speed** 



**Figure 3. Barnes Maze Results.** Data are presented as means ± standard errors. In graph 3A, GEN is significantly different from AIN (control) \*P<.0001. In graph 3B, GEN and UDBPA is significantly different from AIN \*P<.01, \*\*P<.01.

Socio-communicative behavior: Offspring exposed to UDBPA showed reduced social behaviors, as evidenced by unwillingness to engage with a stranger over known mouse.



Figure 4. Barnes Maze Results. Data are presented as means ± standard errors. In graph 3A, no treatment group is significantly different from AIN (control). In graph 3B, UDBPA interaction among strangers 1 and 2 is significantly different from AIN \*P=.06.

EPM: Offspring exposed to all treatment groups exhibited stereotypic behaviors relative to the control counterpart. UDBPA group spent significantly more time inactive compared to the



(control) \*P=.05. In graph 5B, all treatments are significantly different from AIN \*P=.0001, \*\*P=.0005, +P=.0001, + +P= .03. In graph 5C, UDBPA is significantly different from AIN (control) \*P=.02.



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### Met Chamber: F1 Offspring Exposed to GEN or LDBPA Demonstrate Metabolic Changes

A. Total Energy Expenditure



Figure 6. Met Chamber Results. Data are presented as means ± standard errors. In graph 6A, GEN is significantly different from AIN (control) \*P=.01. In graph 6B, GEN is significantly different from AIN \*P=.004. In graph 6C, GEN, LDBPA, and UDBPA is significantly different from AIN with \*P=.01, \*\*P=.03, and +P=.001, respectively.

EchoMRI: Developmental Exposure to EDC Affects Lean Mass



**Figure 7. EchoMRI Results.** Data are presented as means ± standard errors. In graph 7A, GEN+BPA and LDBPA is significantly different from AIN \*P=.05, \*\*P=.01. In graph 7B, all treatments are significantly different from AIN \*P=.01, \*\*P=.03.

## CONCLUSION

- Those exposed to UDBPA showed reduced socio-communicative behaviors.
- F1 exposed to GEN, GEN + BPA, LDBPA, and UDBPA presented with increased autistic-like behaviors, including anxiogenic and stereotypic behaviors.
- Individuals exposed to GEN presented with reduced energy expenditure and oxygen consumption. Both GEN and LDBPA groups had lower body mass. This suggests a dosage dependent effect from BPA and lower energy output from GEN could suggest better metabolic efficiency.
- GEN + BPA and LDBPA exposure increased lean mass in females.
- These data suggest that perinatal exposure to xenoestrogens induces EDC- and sex-dependent differences on various behaviors and metabolic parameters.
- We are currently analyzing the global miRNA-seq results and will then link these biomolecular changes to the EDC-induced phenotypic alterations.

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### • Offspring exposed to GEN had some evidence of improved spatial learning ability.

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