Bisphenol A Differentially Impacts Neurodevelopment in *Drosophila melanogaster* from Distinct Genetic Backgrounds

Uyen Nguyen, Brendan Tinsley, Aliyah Penn, Lillian Murphy, Yomira Palacios, Alex Ceballos, Chloe Welch, and Kimberly Mulligan, PhD

Department of Biological Sciences, Center for Interdisciplinary Molecular Biology: Education, Research and Advancement (CIMERA), California State University, Sacramento

Introduction

- Our goal is to identify and characterize **gene x environment** etiologies of neurodevelopmental disorders (NDDs), which affect more than 1 in 6 children in the United States
- *Fragile-X mental retardation 1 (FMR1)* causes Fragile X Syndrome and is the most common monogenic cause autism spectrum disorder in humans.
- *FMR1/Fmr1* is functionally conserved from flies to vertebrates and is required for neurodevelopment.
- Bisphenol A (BPA)—used to synthesize plastics and epoxy-resins—is one of the most environmentally ubiquitous chemicals.
- We found that exposure to **BPA impairs both behavioral neuronal phenotypes in *w1118* (control) flies.**
- Remarkably, for all behavioral and neuronal phenotypes examined, **BPA either rescues mutant phenotypes or has insignificant impacts in *dFmr1* mutants.**

Methods and Results

I. Adult Grooming

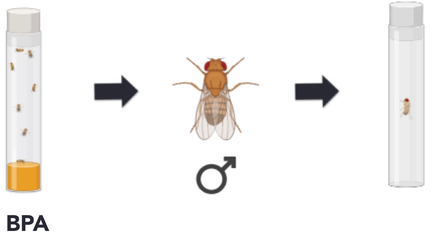


FIG 1: GROOMING ASSAY. Following BPA exposure, male flies were isolated and aged for five days before being aspirated into an observation chamber. Grooming was observed for 5 min.

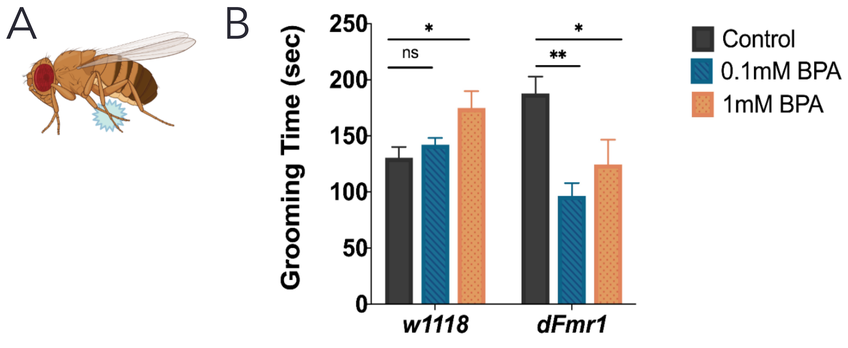


FIG 2. BPA INCREASED GROOMING ACTIVITY IN *w*^{1118} FLIES BUT REDUCED GROOMING IN *dFmr1* MUTANT FLIES.

A. Grooming behavior includes rubbing legs against any body parts.

B. In the control strain (*w*^{1118}) exposure to 1mM BPA, but not 0.1mM BPA, significantly increased the time spent grooming. In *dFmr1* mutant flies, exposure to both 0.1mM and 1mM BPA resulted in a significant decrease in grooming time compared to the unexposed *dFmr1 flies*. (* = P < 0.05; ** = P < 0.01; ns = not significant. n = 8 – 13)

Methods and Results

II. Larval Locomotion

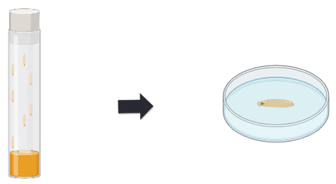
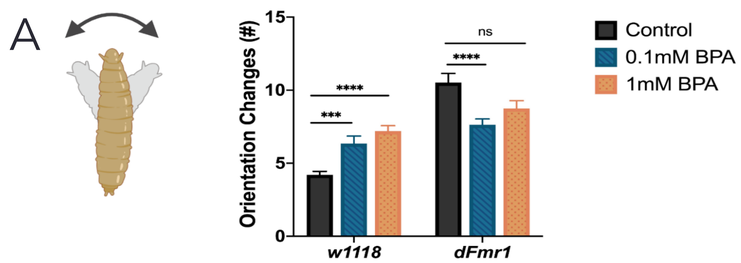


FIG 3. LARVAL LOCOMOTION. Age-matched third instar larvae were collected following exposure to BPA. Larvae were placed on 0.2% agarose. Following a 1-min acclimation period, flies were recorded for 1-min.



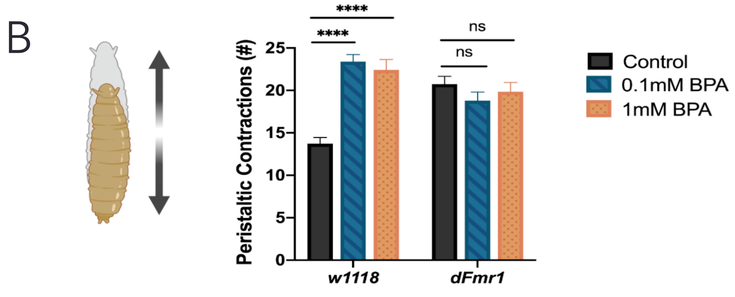


FIG 4. BPA INDUCED HYPERACTIVITY IN *w*^{1118} LARVAE BUT NOT IN *dFmr1* LARVAE.

A. Exposure to BPA increased reorientation events in *w*^{1118} larvae. In contrast, 0.1mM BPA exposure reduced reorientation events in *dFmr1* mutant larvae.

B. Exposure to BPA increased the number of peristaltic contractions in *w*^{1118} larvae. BPA exposure did not significantly affect peristaltic contractions in *dFmr1* mutant larvae.

(*** = P < 0.001, **** = P < 0.0001; ns = not significant. Sample sizes: n = 40-48)

III. Axon Guidance

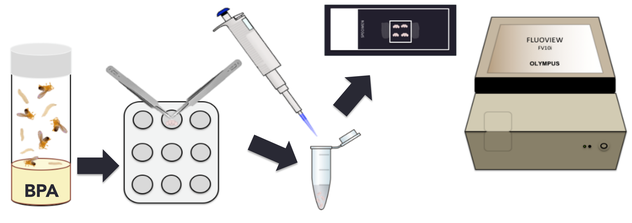


FIG 5. AXON GUIDANCE. Adult brains are dissected, fixed, stained with an anti-FasciclinII (anti-FasII) primary antibody and an AlexaFluor488 secondary antibody, then visualized using confocal microscopy.

Methods and Results

III. Axon Guidance

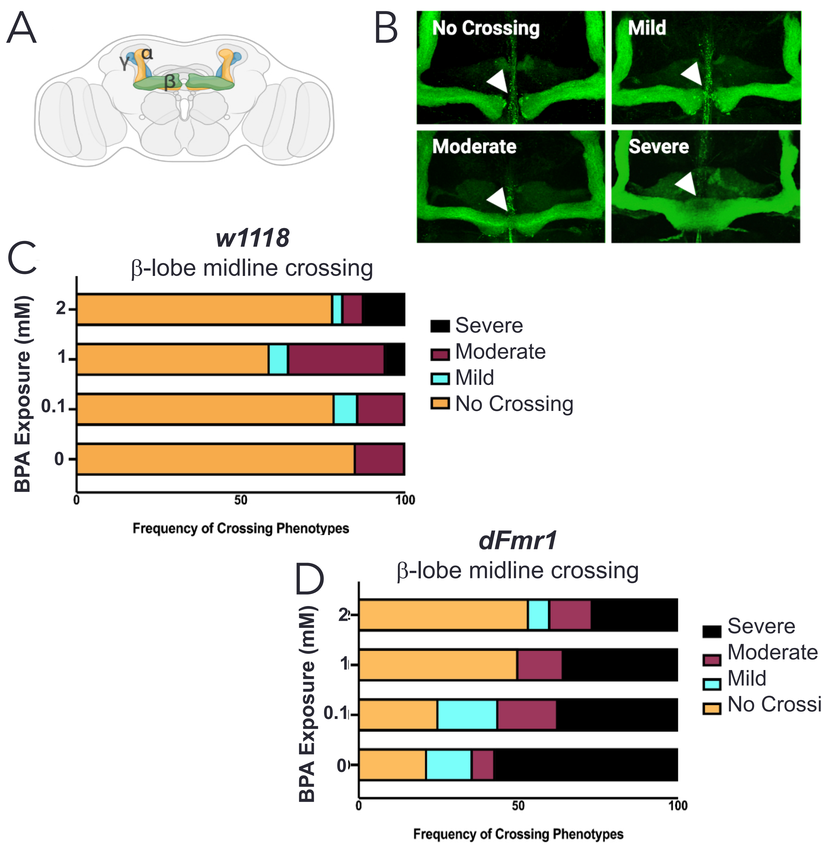


FIG 6. BPA EXPOSURE INCREASED THE FREQUENCY OF β-LOBE AXON GUIDANCE DEFECTS IN *w*^{1118} BRAINS BUT REDUCED THE FREQUENCY AXON GUIDANCE DEFECTS IN *dFmr1* FLIES.

A. The mushroom body, including the α, β, and γ lobes.

B. Anti-FasII (green) stained mushroom bodies with different β -lobe midline crossing phenotypes (no crossing, mild, moderate, or severe crossing).

C. In *w*^{1118} flies, the frequency of midline crossing increased with exposure to 0.1mM and 1mM BPA, but then decreased with exposure to 2mM BPA.

D. In *dFmr1* flies, the frequency of midline crossing was reduced by exposure to 0.1mM, 1mM and 2mM BPA.

(Sample sizes: n = 14 -20)

Acknowledgements

We would like to thank Dr. Thomas A. Jongens at the University of Pennsylvania for the generous gift of the *dFmr1*^{3} *Drosophila*. This work is supported by an NIH SCORE Pilot Award (SC2GM132005-01).

NIH National Institutes of Health

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