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Research report

Effect of bisphenol A on *Drosophila melanogaster* behavior – A new model for the studies on neurodevelopmental disorders

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HIGHLIGHTS

- Perinatal exposure to bisphenol A (BPA) leads to behavioral modifications in the *Drosophila*.
- BPA exposure changes the exploratory behavior of the *Drosophila* in the open field assay.
- In BPA-treated *Drosophila*, there was an increase in the grooming episodes, which suggests abnormal repetitive behavior.
- Along with the motor changes, we also observed uncharacteristic social interaction in *Drosophila* exposed to BPA.

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ABSTRACT

Developmental disorders such as autism and attention deficit hyperactivity disorder (ADHD) appear to have a complex etiology implicating both genetic and environmental factors. Bisphenol A (BPA), a widely used chemical in the plastic containers and in the linings of food and beverage cans, has been suggested to play a possible causative role in some developmental disorders. Here, we report behavioral modifications in *Drosophila melanogaster* following early exposure to BPA, which may suggest BPA as an environmental risk factor for the behavioral impairments that are the basis of diagnosis of autism and ADHD. In an open field assay with perinatally BPA-exposed and vehicle-treated control *Drosophila*, different parameters of locomotion (distance traveled, walking speed, spatial movement, mobility, turn angle, angular velocity and meander) were analyzed using the ethovision software. We also examined the repetitive and social interaction behaviors in these flies. In an open field assay, we identified disturbances in the locomotion patterns of BPA-exposed *Drosophila* that may relate to the decision-making and the motivational state of the animal. An increase in repetitive behavior was observed as an increase in the grooming behavior of *Drosophila* following BPA exposure. Furthermore, we also observed abnormal social interaction by the BPA-exposed flies in a social setting. These results demonstrate the effect of the environmentally prevalent risk agent BPA on the behavior of *Drosophila*, and suggest the practicability and the ease of using *Drosophila* as a model in the studies of neurobehavioral developmental disorders.

1. Introduction

The Centers for Disease Control and Prevention (CDC) estimates the prevalence of developmental disorders at 1 in every 6 children [1]. Neurodevelopmental disorders such as intellectual disability, attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), and fragile X syndrome have core abnormal behavioral components that are fundamental to their diagnosis. The core components central to an autism diagnosis consist of abnormal social interactions, impairments in verbal and non-verbal communication as well as repetitive and restricted behavior or interests. In addition, other features are often associated to this triad, such as difficulties with decision-making [2]. A major finding from epidemiologic studies has been the increase in the prevalence of autism in recent years, with about 1 in every 68 children being identified with ASD [3]. With no single identifiable cause linked to autism, the roles of genetic factors as well as oxidative stress, mitochondrial dysfunction, inflammation, and

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immune abnormalities have been reported, leading to a multifactorial model for its etiology [4–12]. Environment is considered an especially strong contributor to the increased prevalence of autism [11–15]. Exposure to maternal infections during critical periods of development, such as prenatal and perinatal periods, has also gained attention in the search for the etiology of autism [16,17]. The increased incidence of autism in certain regions has suggested that there is a link between geography and the genetic predisposition to autism [18–20].

Bisphenol A (4,4′-dihydroxy-2,2-diphenylpropane; BPA) is one of the environmental toxins that has recently received increased attention. BPA is widely utilized in the production of polycarbonate plastics such as drinking bottles, food containers, toys and dental sealants. It is also employed in the production of epoxy resins used in the linings of food and beverage cans. The U.S. Environmental Protection Agency (EPA) recommends the lowest observed adverse effect level (LOAEL) of BPA to be 50 mg/kg body weight/day [21], which is set as the reference dose (RfD) for the maximum acceptable level for daily exposure. BPA and its metabolites are found in the majority of biological fluids, including blood and urine. Analysis by the CDC showed detectable levels of BPA in 92.6% of urine samples in human [22]. BPA is known to cross the placenta [23] and is found in the amniotic fluid, placental tissue, umbilical cord, and fetal serum [24]. Estimated intake of BPA was found to be much higher in bottle-fed infants (1 μg–11 μg/kg body weight/day) than in breast-fed infants (about 0.2 μg–1 μg/kg body weight), thus mandating BPA-free baby bottles [25]. BPA acts mainly as an endocrine disrupting chemical (EDC), with prenatal and postnatal exposure leading to neurodevelopmental disturbances [26,27], along with behavioral changes [28–30]. Many studies have indicated a role of EDCs in the development of ASD and ADHD [31].

Drosophila melanogaster is a widely used model for studies of brain disorders such as Parkinson's disease, Alzheimer's disease, Huntington's disease, fragile X syndrome, and Angelman syndrome [32]. *Drosophila* is a comparatively simple organism with its genome consisting of four chromosomes encoding approximately 14,000 genes [33,34], which is about 50% of the estimated 25,000–30,000 genes in humans [35,36]. Among 59 of the human neurological genes examined, 38 have orthologs in the *Drosophila* genome [37]. A single *Drosophila* gene may serve the same function as multiple related genes of mammals, thus decreasing the redundancy seen in other vertebrate models. Although flies and humans are distinctly different from each other, many molecular processes are conserved between them. The advantage of studying neurobehavioral disorders in *Drosophila* is the presence of genes that are similar to human genes for normal cognitive functions, as a result of phylogenetic conservation of these genes [38,39]. The *Drosophila* model exhibits complex behaviors relevant to humans, including courtship [40,41], circadian rhythms [42], learning and memory [43], aggression [44], grooming [45], and open field exploration [46]. The fly is used as a model in studies because of its compact genome, which has been fully sequenced, and the availability of sophisticated genetic approaches. The *Drosophila* model is also attractive because of its quicker generation time, large number of progeny for better selection, and easy maintenance of the animal model. Nevertheless, *Drosophila* has been underused in the study of complex disorders with abnormal behavioral components because of lack of reliable tests to assess complex behavioral phenotypes relevant to human [47,48].

We combined several behavioral assays evaluating autism-related impairments to examine the effects of exposure to BPA in *Drosophila melanogaster*. Here, we report that exposure to BPA leads to features of repetitive behaviors, abnormal social interaction, and significant impairment in locomotion in an open field arena, a decision-making process. Most importantly, we present the use of *Drosophila* as a prospective model for the study of neurobehavioral disorders with complex gene-environment etiology such as autism.

2. Materials and methods

2.1. Drosophila stocks

Wild-type Oregon-R *Drosophila* stocks were maintained at 25 °C on a standard cornmeal diet (Jazz-mix *Drosophila* food, Fisher Scientific, Pittsburg, PA, USA) under 12 h/12 h light and dark cycle.

2.2. BPA treatment of Drosophila

On the basis of a previously used drug dosing protocol [49], we assumed that a 1-mg fly would consume food equaling 5% of its body weight per day. In our study, the highest BPA dose (1 mM) (>99% purity; Sigma–Aldrich, St. Louis, MO, USA) corresponds to the approximate human LOAEL of 50 mg/kg body weight/day. For the oral administration, the BPA dissolved in dimethylsulfoxide (DMSO) (Sigma–Aldrich, St. Louis, MO, USA) was mixed with recently cooked and cooled standard fly food. Different concentrations of BPA (0.001, 0.01, 0.025, 0.05, 0.1, 0.1, and 1 mM) were used for the social interaction assay, while the higher doses (0.5 and 1 mM) were used for all the other experiments. For all the treatments, the amount of DMSO was kept below 0.1% of the volume added. In the controls (no BPA), only 0.1% DMSO (vehicle) was used. Five virgin female and three male flies (3–5 days old) were mated in vials with the BPA-treated food. The flies were allowed to feed and lay eggs in the treatment vials for 3–4 days, after which the flies were discarded and the vials placed in the incubator. Newly enclosed flies (F1) were anesthetized on ice, and separated according to their gender. The F1 progeny were transferred into fresh vials containing their respective BPA-treated or vehicle control food prior to behavioral testing.

2.3. Behavioral assays

All behavioral experiments were performed between 12 pm and 3 pm (between Zeitgeber time ZT5 – 5 h after the onset of light and ZT8) and in adequate daylight, to reduce variations in performance due to circadian rhythms.

2.3.1. Social interaction

Social interaction was determined in male and female flies by using the social space assay as described by Simon et al. with slight modifications [50]. One day prior to the experiment, flies from each treatment group (n = 30–40) were separated by gender. On the day of the experiment, the flies were allowed to acclimate in the dedicated behavior room (environmentally controlled: 50% humidity, 25 °C) for 1 h before the experiment was initiated. The male and female flies were kept separate for each treatment to avoid interference with courtship behavior. Flies were placed in the same kind of rectangular vertical chambers used by Simon et al. [50]. After the flies spent 20 min exploring and settling in the chamber, a digital image of the chamber with the flies was taken with a camera and exported into iPhoto (Macintosh computer). Image J (NIH software – http://imagej.nih.gov/ij/) was then used to process the image into an 8-bit binary image. The binary image was then imported in a Lispix (NIH image analysis software – http://www.nist.gov/lispix/) program to calculate the distance of the fly to its nearest neighboring fly.