The Effects of Dibutyl Phthalate (Plasticizer) on Motor-related Activity

Ephraim Boamah, Class of 2025

Background: Contemporary research highlights theharmful effects of phthalates—industrial chemicals used in plastic products to enhance flexibility—on cellular functions; some of these products are included children's toys, cosmetics, and medical devices (Blount et al., 2000b; Latini2005, as cited in Xie et al. 2019). Phthalates can alter genetic expression for oxidative balance by reducing the production of antioxidants and increasing the accumulation of reactive oxygen species (ROS) (Deng et al., 2020; Ma, 2013). Such an effect can induce toxicity within the nervous system, leading to the accumulation of dark neurons—which are dying and shrinking cells (Gallyas, 2007). Hence, this summer, I investigated how the plasticizer Dibutyl Phthalate (DBP) affected motor activity in a mouse spinal cord. I hypothesized that DBP will induce neurotoxicity in our mouse spinal cord by affecting the Central Pattern Generator (CPG) network in its ability to generate rhythmic movements. As such, I predicted a decrease in the burst amplitude (suggesting a decrease in motor neuron activity), an increase in burst duration and prolonged cycle period (suggesting a decrease excitability in neuronal network between interneurons and moto neurons).

Method: We used spinal cord preparations from neonatal mice 1-5 days old (P1-P5) and recorded motor neuron activity from the ventral roots L2 (responsible for muscle flexion) and L5 (responsible for muscle extension; Acevedo et al., 2016). The mouse spinal cord was perfused with serotonin and the glutamate analog NMDA to produce fictive locomotion (Acevedo et al., 2016). For DBP, three concentrations were used: 10uM, 50uM, and 100uM. To examine the effects of DBP on motor activity, three parameters were measured: burst amplitude (a measurement of the excitability and recruitment of motor neurons), cycle period (the onset of a burst to the onset of the next burst) and burst duration (the onset to the offset of a single burst).

Results: From the L2 ventral root recordings, the data suggests that DBP increased burst amplitude, significantly prolonged cycle period, and a general trend of increase in burst duration. For our L5 data, burst amplitude significantly increased at 100uM and significantly decreased in wash; cycle period significantly increased at 50uM; and burst duration significantly increased at 50uM and 100uM. The data was analyzed using a one-way ANOVA.

Conclusion: This summer, my research on how DBP affects motor activity suggests that DBP induces neurotoxicity within the lumbar central pattern generator network of mammals. For example, I noticed trends and significant changes in burst duration and cycle period. Such changes can be a direct effect of DBP on motor neurons and/or interneurons. Although our results did not confirm our initial hypothesis that DBP will lower burst amplitude, the increase in burst amplitude can potentially be explained by the excitotoxicity generated by DBP. Hence, DBP increased excitation of neurons, leading to recruitment of motor neurons which could then lead to cell death.

Future Directions: First, I hope to increase the period at which to run DBP experiments to determine how excitotoxicity will alter burst amplitude. Second, I will be using Rosmarinic acid—which has anti-inflammatory and antioxidant properties—to pretreat some of the mouse spinal cords to investigate whether the effects of DBP can be reduced. Last, I hope to carry out some intracellular recordings to ascertain at which level of the CPG network (motor neuron and/or interneuron) is DBP having its effects.

Faculty Mentor: Prof. Manuel Diaz-Rios

Funded by the Robert Freedman '87, P'17 and Anne Cirillo P'17 Student Fellowship Program

References

Acevedo JM, Almansa AS, Vergara NM, Cordero LRM, Bou EC, Rios MD. 2016. Caffeine stimulates locomotor activity in the mammalian spinal cord via adenosine A1 receptor-dopamine D1 receptor interaction andPKA-dependent mechanism. Neuropharmacology. 101.490-505. https//doi.org/10.1016/j.neuropharm.2015.10.020.

Deng Y, Yan Z, Shen R, Wang M, Huang Y, Hongqiang R, Zhang Y, Lemos B. 2020. Microplastics release phthalate esters and cause aggravated adverse effects in the mouse gut. Environment International. 161. 1-10. https://doi.org/10.1016/j.envint.2020.105916

Gallyas, F. (2007). Novel cell-biological ideas deducible from morphological observations on "dark" neurons revisited. Ideggyógyászati Szemle, 60(5-6), 212-222.

Ma Q. 2013.Role of nrf2 in oxidative stress and toxicity. Annual Review of Pharmacology and Toxicology.53.401-426. https://doi.org/10.1146/annurev-pharmtox-011112-140320