The effects of plasticizer Dibutyl Phthalate on mammalian spinal locomotor activity

Ephraim Boamah, Class of 2025

Introduction

Plasticizer phthalates are industrial additives used in plastic products to enhance the malleability of plastic products. Phthalates are used in children's toys, medical devices, food packaging and many more. When consumed, phthalates generate reactive oxygen species (ROS), which are unstable oxygen molecules that induce oxidative stress and trigger cellular apoptotic processes (Ma, 2013; Lee et al., 2020; Tseng et al., 2013). For example, within the nervous system, phthalates disrupt neural plasticity, a crucial process that allows neurons to adapt and change after a stimuli input (Lee et al., 2020). However, the effects of phthalates on motor activity in the spinal cord remains elusive. In this study, we assess the effects of Dibutyl phthalate (DBP) on motor activity in the mammalian spinal cord. We hypothesize that DBP will induce neurotoxicity within the central pattern (CPG) network, a circuitry of interneurons and motors neurons capable generating rhythmic activity in the absence of a stimuli from the brain and the periphery. We expect to find a decrease in amplitude of electrical discharges termed bursts. Additionally, we expect to find an increase in burst duration and cycle period. Burst amplitude refers to motor recruitment while burst duration and cycle period denotes the overall excitability of the CPG network.

Methods

Using neonatal mice, we dissect the lumbar region of the spinal cord preserving the ventral roots L2 and L5. These ventral roots have been shown to control muscle flexion and extension, respectively (Acevedo et al., 2016). After, we isolate the spinal cord in a bath with a cocktail of serotonin (9 -12 μM) and NMDA (6 μM) to activate the CPG network, termed fictive locomotion (Acevedo et al., 2016). Electrophysiological techniques are used to record motor activity. When a stable rhythm is detected, we conduct our control recordings after which we introduce DBP into our recording bath at either 10, 50, 100 or 200 micromolar (μM) concentrations. After, we perform a washout of DBP by returning to our control solution containing serotonin and NMDA. For data analysis, we assess three parameters, burst amplitude, burst duration, and cycle period using Spike 2 software with a custom script (provided by Dr. Thomas Cleland, Cornell University) to observe changes in parameters over the course of the experiment.

Results

At 10 μM we observed a trend that DBP increased all three parameters with significant differences in the wash for L2's cycle period and L5's burst duration and cycle period. Distinctively, we observed a significant increase in burst amplitude at 50 μM for L2 with an increasing trend for L5 and no discernible effect on the other parameters. At 100 μM, burst amplitude increased for L2 but decreased for L5 while burst duration and cycle period generally increased with significant differences detected in the wash. For 200 μM, DBP decreased amplitude and burst duration for both L2 and L5; while cycle period increased for L5, we did not detect changes in L2.

Preliminary Conclusions and Discussions.

Our result from the summer indicates that DBP affects the CPG network by inducing neurotoxicity to generate motor recruitment and decreasing the excitability the CPG network by slowing down burst duration and cycle period. However, higher concentrations of DBP induced more drastic effects and reveals a potential difference in how L2 and L5 ventral roots respond the neurotoxicity. In the future, we hope to increase sample size and pre-incubate our spinal cord prep in Rosmarinic Acid, a neuroprotectant with antioxidative properties to assess its ability to mitigate DBP's effect. Additionally, we hope to conduct intracellular recordings, immunohistochemistry, western blots, and behavioral experiments.

Mentor: Professor Manuel Diaz-Rios
Funded by the Gibbons Summer Research 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 and PKA-dependent mechanisms. *Neuropharmacology.101.*490-505. https://doi.org/10.1016/j.neuropharm.2015.10.020

Lee, S. M., Jeon, S., Jeong, H. J., Kim, B.-N., & Kim, Y. (2020). Dibutyl phthalate exposure during gestation and lactation in C57BL/6 mice: Maternal behavior and neurodevelopment in pups. *Environmental Research*, *182*, 109025. https://doi.org/10.1016/j.envres.2019.109025

Ma, Q. (2013). Role of nrf2 in oxidative stress and toxicity. *Annual Review of Pharmacology and Toxicology*, *53*(1), 401–426. https://doi.org/10.1146/annurev-pharmtox-011112-140320

Tseng, I.-L., Yang, Y.-F., Yu, C.-W., Li, W.-H., & Liao, V. H.-C. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and afd neurons through oxidative stress in caenorhabditis elegans. *PLoS ONE*, *8*(12), e82657. https://doi.org/10.1371/journal.pone.0082657