Effects of plasticizer di(2-ethylhexyl) phthalate and neuroprotectant rosmarinic acid on mammalian spinal locomotor activity

Meadow Jennings, 2026

Plastic pollution, specifically microplastic pollution, is a major environmental threat to many global ecosystems. One chemical additive to plastics that the Díaz-Ríos lab is especially interested in is di(2-ethylhexyl) phthalate (DEHP). Phthalates are a class of chemical plasticizers used in plastic products to give them flexibility and malleability (Rowdhwal & Chen, 2018). DEHP is the most widely produced plasticizer globally and it's commonly found in children's toys, cosmetics, medical equipment, and many other plastic products (Amara et al., 2020). This is concerning because DEHP can leach into water systems and cross the blood-brain barrier, directly affecting the health of the nervous system (Amara et al., 2020).

Plasticizers such as DEHP can affect the body on a cellular level. Acting as an oxidizer, these chemicals generate free radicals known as reactive oxygen species and prevent critical cell functions leading to oxidative stress, inflammation, and apoptosis (Kovacic, 2010). The cellular effects of DEHP create larger systemic problems, such as disruptions to the central pattern generator (CPG) which generates rhythmic activity such as breathing and walking (Acevedo et al., 2016). This project intended to further this research by investigating how locomotion is affected at a cellular level using electrophysiology. Furthermore, we investigated the neuroprotective properties of rosmarinic acid (RA), which is a key chemical in the cooking herb rosemary. Rosmarinic acid can combat oxidative stress as a reducing agent, stabilizing free radicals such as ROS (Noor et al., 2022).

To assess the effect of DEHP and rosmarinic acid on the CPG we first dissected and isolated the spinal cords of neonatal mice one to six days old (P0-P6) via ventral laminectomy. We obtained extracellular ventral root recordings from the lumbar L2 (flexor-related activity) and L5 (extensor-related activity) ventral roots. The preps were perfused with a control ringer solution (6uM NMDA and 9-12uM serotonin) and recorded for 20 minutes. Then we perfused with 100uM DEHP and ringer for an hour, followed by an hour wash in ringer. For tests with RA, cords were pre-incubated in 100uM RA and ringer for one hour, then 100uM DEHP, 100uM RA, and ringer, and then the wash (with 100uM RA). All conditions were analyzed for peak amplitude, burst duration, and cycle period using Spike 2. We hypothesized that DEHP would decrease peak amplitude, increase burst duration, and increase cycle period, indicating a reduction in excitability and disruptions to the CPG. Furthermore, preincubation with RA would reduce these effects.

Based on our preliminary data we found that the DEHP and wash conditions increased all three parameters for both the L2 and L5 in comparison to the control. There was a statistically significant increase in cycle period and burst duration from the control to the wash ($p=<0.001$). Additionally, DEHP and wash condition showed increased variability in the data in comparison to the control indicating that DEHP does have a disruptive effect on the prep. Preliminary data for 100uM RA and 100uM DEHP showed that RA had a visually stabilizing effect on the extracellular recordings in the DEHP condition but quantitatively showed no statistical significance in preventing the toxic effects seen in the DEHP and wash conditions. Furthermore, the RA preincubation notably increased all three parameters when compared to the control for both L2 and L5. This indicated that 100uM RA concentration may be too high, eliciting an excitotoxic effect. In the future, these problems could be addressed by increasing the length of the DEHP conditions both with and without RA, as well as decreasing the concentration of RA.

We began testing a 50uM concentration of RA in the last week and saw promising trends that indicated a less disruptive effect from RA while maintaining neuroprotective properties in the DEHP condition.

Faculty Mentor: Manolo Díaz-Ríos
Funded by the Kibbe Science Fellowship

References:

Acevedo, J., Santana-Almansa, A., Matos-Vergara, N., Marrero-Cordero, L. R., Cabezas-Bou, E., & Díaz-Ríos, M. (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

Amara, I., Timoumi, R., Annabi, E., Salem, I. B., & Abid-Essefi, S. (2020). Di(2-ethylhexyl) phthalate inhibits glutathione regeneration and dehydrogenases of the pentose phosphate pathway on human colon carcinoma cells. *Cell stress & chaperones*, *25*(1), 151–162. https://doi.org/10.1007/s12192-019-01060-5

Kovacic P. (2010). How dangerous are phthalate plasticizers? Integrated approach to toxicity based on metabolism, electron transfer, reactive oxygen species and cell signaling. *Medical hypotheses*, *74*(4), 626–628. https://doi.org/10.1016/j.mehy.2009.11.032

Noor, S., Mohammad, T., Rub, M. A., Raza, A., Azum, N., Yadav, D. K., Hassan, M. I., & Asiri, A. M. (2022). Biomedical features and therapeutic potential of rosmarinic acid. *Archives of pharmacal research*, *45*(4), 205–228. https://doi.org/10.1007/s12272-022-01378-2

Rowdhwal, S., & Chen, J. (2018). Toxic Effects of Di-2-ethylhexyl Phthalate: An Overview. *BioMed research international*, *2018*, 1750368. https://doi.org/10.1155/2018/1750368