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

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Plasticizers, additives that increase the malleability of plastic products, have become a ubiquitous form of environmental pollution due to their ability to leach off of objects such as children's toys, food wrappers, and cosmetics and cross the blood-brain barrier (1). Di(2-ethylhexyl) phthalate (DEHP), a member of the most common class of plasticizers, phthalate diesters, is a particularly prevalent and harmful pollutant. The carbonyl functional groups on DEHP withdraw electrons from water molecules, creating reactive oxygen species, which can trigger oxidative stress and large-scale, irreversible cell damage (2). A comprehensive body of research has linked DEHP to numerous health hazards, including impaired locomotion (3), damage to the endocrine, reproductive, cardiac, hepatic, and renal systems (4), emotional, cognitive, and neurodevelopmental decline (5), and an increased risk of certain cancers (6). While no large-scale solutions to the biological damage caused by DEHP have been implemented, natural neuroprotectants such as rosmarinic acid have been shown to work as effective antioxidants, suggesting they may have the ability to counter the oxidative stress generated by DEHP exposure (7).

Although the effects of DEHP on the nervous system at the organismal behavioral scale have been studied extensively, its effects at the cellular level are not fully understood. Particularly, there is a lack of clarity on how DEHP and rosmarinic acid affect the central pattern generator (CPG), a neural circuit that controls walking. In this study, we examined the effects of DEHP and rosmarinic acid exposure on the mammalian spinal CPG and its ability to control rhythmic locomotor activity.

We dissected and isolated the spinal cords of Swiss-Webster neonatal mice via a ventral laminectomy. Then, we utilized suction electrodes to record electrophysiological activity at the ventral roots L2 and L5, which control flexor and extensor movement respectively. We recorded activity in a control condition with serotonin (6-12μM) and NMDA (3-6μM) to induce fictive locomotor activity, followed by experimental conditions containing DEHP (100μM) with and without a 1-hour long preincubation in rosmarinic acid (50μM,100μM). We also recorded a wash in the control condition to assess the reversibility of DEHP-induced damage. We analyzed the data we collected for peak amplitude, burst duration, and cycle period in Spike 2.

In the presence of 100μM DEHP, we observed significant increases in burst duration and cycle period occurring in the wash condition, and a noticeable trend of increase in peak amplitude. Across all parameters, DEHP led to greater variability. While 100μM rosmarinic acid interfered with locomotor activity on its own, proving to be too high of a concentration, the 50μM condition appeared to be effective in decreasing variability and stabilizing burst duration and cycle period. No reversibility was observed during the wash of any of the conditions.

This study indicates that DEHP disrupts the CPG's ability to regulate locomotor activity and may induce excitotoxicity. Additionally, preliminary results suggest that rosmarinic acid at the 50μM concentration potentially mitigates these harmful effects. Future research should be conducted evaluating higher concentrations of DEHP and longer exposure times to build a more complete understanding of its risks.

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