The Effects of the Plasticizer Dibutyl Phthalate (DBP) on Spinal Locomotor Activity

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Abstract

Plasticizers, such as phthalates, have shown to have numerous detrimental effects in various animal models, such as disrupting the body's oxidative balance, causing inflammation, altering the immune response, and even leading to excitotoxicity. The goal of this experiment was to understand and observe the nature and effects of phthalates, such as Dibutyl Phthalate (DBP) on the lumbar ventral roots of an isolated neonatal mouse spinal cord preparation, as relating to locomotor activity. The spinal cord was perfused with drugs that induced locomotion (5-HT, NMDA) and was then treated with DBP (10, 50, 100 µM). The ventral roots were recorded using suction microelectrodes, and the recordings were 1000x amplified. We observed and recorded rectified/smooth and raw traces on Spike 2 Software. The percent changes in burst amplitude, burst duration, and cycle period across the three conditions (control, DBP, wash) were graphed against each other for both the L2 and L5 roots. DBP led to an increase in burst amplitude, burst duration, and cycle period. DBP was seen to cause overstimulation by triggering excitotoxicity, affecting the neuronal network, and disrupting it potentially at the motor and interneuron levels. Further experiments including using intracellular recordings will help confirm these initial results.

The project objectives were to explore how rhythmic motor activity, specifically produced by the spinal cord central pattern generator network (CPG), can potentially be compromised by environmental factors, such as pollution with plasticizers. CPGs are circuits of neurons (i.e. interneurons and motor neurons) that work together to generate rhythmic movements such as walking. Although recent studies have started to assess the effects of plasticizers on bodily and brain function, research on the effect of phthalates or plasticizers on a neural system and motor control, such as the central pattern generator (CPG) for locomotion located in the mouse spinal cord, is lacking. Thus, my aim this summer was to explore the effects of DBP on spinal locomotor activity, using an isolated neonatal mouse spinal cord preparation, specifically at the lumbar spinal CPG network to examine hindlimb locomotion.

The experiments used P1-P6 (1-6 days old) Swiss Webster mice from Charles River Laboratories that were bred in the vivarium at Bowdoin College. They were rapidly decapitated (euthanized) before their spinal cords were extracted. The spinal cords were removed via a ventral laminectomy in ice-cold Low Calcium Mouse Ringer's Solution. Upon removal, we placed and pinned the spinal cord on the ventral side up in a perfusion chamber containing Normal Mouse Ringer's (NMR) solution, at room temperature. Serotonin (5-HT) (9-12 µM) and N-methyl-D-aspartate (NMDA) (6 µM) were perfused via a peristaltic pump to the Ringer's solution bath to induce locomotor-like activity as previously done (Acevedo et al. 2016). DBP (10, 50, 100 µM) was added (via perfusion) to this solution to observe its effects. The solutions changed from the control (NMR + 5-HT + NMDA) to the drug (NMR + 5-HT + NMDA + DBP) and then to the wash (NMR + 5-HT + NMDA). The spinal cord preparations were kept in these perfused solutions for 25-30 minutes, 75-90 minutes, and 45 minutes, respectively. Recordings were done extracellularly from the ventral roots using tightly sealed glass suction microelectrodes. The electrodes were placed on either the contralateral second lumbar (L2) or fifth lumbar (L5) roots, or on the ipsilateral L2 and L5 roots. The L2 and L5 roots were used because they act as hindlimb flexors and extensors, respectively, and thus alternate in activity. Recordings were 1000x amplified using an AC amplifier (Model 1700 from A-M system, Inc) with a low cut-off filter at 100 Hz and a high cut-off at 10 kHz. Data acquisition was done using a CED 1401 digitizer and Spike 2 Software. Microsoft Excel Software was used to organize and manage the data. Graphing and statistical analysis were done with GraphPad

Prism software. We used a script from Dr. Thomas Cleland, Cornell University to analyze three motor activity-related bursting parameters: burst duration (time from start and end of a burst), burst amplitude (height of the burst), and cycle period (time between the start of one burst to the beginning of the next burst).

Figure 1 illustrates the alternating phasing locomotor-like pattern that persisted in the presence of DBP (50 µM). It shows the smooth/rectified and raw traces for about 10 seconds from the right L2 and left L2 of neonatal mice. After the DBP application, we noted that the number of bursts decreased from the control condition, with not many changes between the DBP and the wash conditions. However, in these 10 seconds, the control traces experienced more bursts than the DBP traces, but the bursts seem higher in the DBP condition. Nevertheless, alternating bursting persisted in the spinal cord prep in the presence of DBP. Figure 2 shows three graphs illustrating the percentage change in burst amplitude, burst duration, and cycle period as recorded from the L2s of the neonatal mice. Each graph displays the effect of DBP at the different concentrations of 10, 50, and 100 µM. For all three parameters, the trend suggests an increase from the control for all three concentrations of DBP, with no reversibility seen after wash. However, when testing for statistical significance, only 50 µM and 100 µM of DBP have a significant increase in the cycle period as compared to the control recordings. Figure 3 is similar to figure 2, but illustrates the recordings from the L5 ventral roots of the neonatal mice instead. In this case, the only DBP concentration that had an increase in burst amplitude, as compared to the control, was that of 100 µM, which was also significant. The burst duration and cycle period have the same upwards trend as seen in figure 2. From these figures, it can be suggested that DBP led to an overall increase in burst amplitude, burst duration, and cycle period, with minimal changes in the alternating phasing locomotion pattern.

The overall increase in burst amplitude indicates DBP's role as an excitotoxin. It's triggering excitotoxicity in this preparation, which means it is overstimulating the glutamate receptors in the neurons, which leads to high influxes of cations such as calcium and potassium. This causes the excess recruitment of neurons, which can explain the increase in burst amplitude that is seen. The overstimulation of neurons due to excitotoxicity eventually can cause neuronal death, which would mean a decrease in neuron recruitment and a resulting decrease in burst amplitude. However, this has not been seen in the 75-90 minute DBP application period used in this study, suggesting a longer application might be necessary (2-3 hours) to assess this excitotoxicity hypothesis. The increase in the cycle period suggests that DBP is affecting the neuronal network. The increase in burst duration indicates that DBP disrupts the CPG network at the motor neuron and interneuron levels (Acevedo et al. 2016).

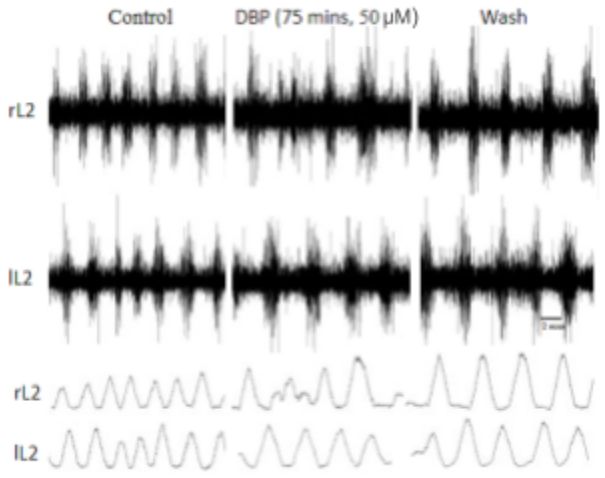

Figure 1. Raw (top) and rectified/smooth (bottom) traces over a time of 10 seconds show the effects of DBP (50 µM) on a drug-induced (9 µM of 5-HT, 6 µM of NMDA) locomotor-like activity in the lumbar (L2) ventral roots of a neonatal mouse spinal cord. Notice the persistence of the alternating rhythmic pattern throughout the three conditions (control, DBP, wash).

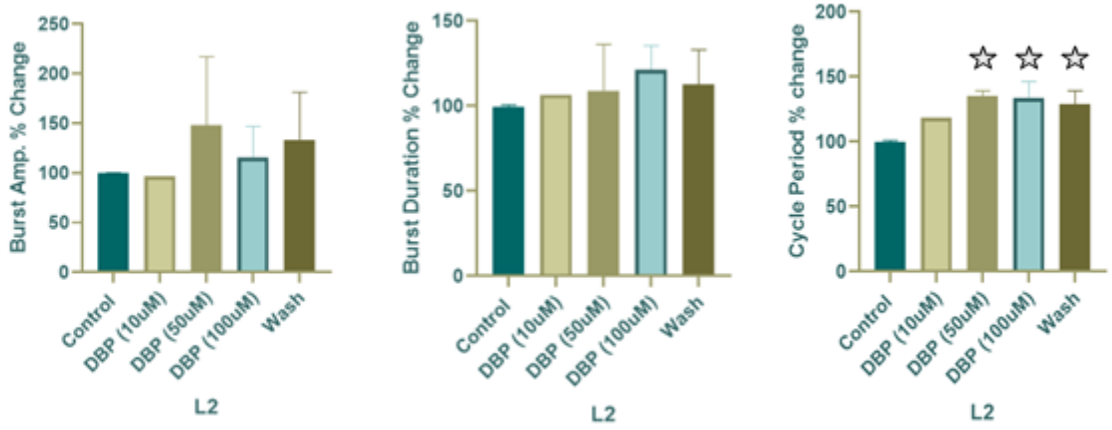

Figure 2. Data was taken from the last 5 minutes of the L2 recordings from the control and DBP conditions (75 - 90 minutes), and from the last 10 minutes for the wash condition. Notice an increase in the percent change (compared to control) in burst amplitude, burst duration, and cycle period following the addition of DBP to the bath (n = 5). The stars represent a significant difference as compared to the control ($p < 0.05$).

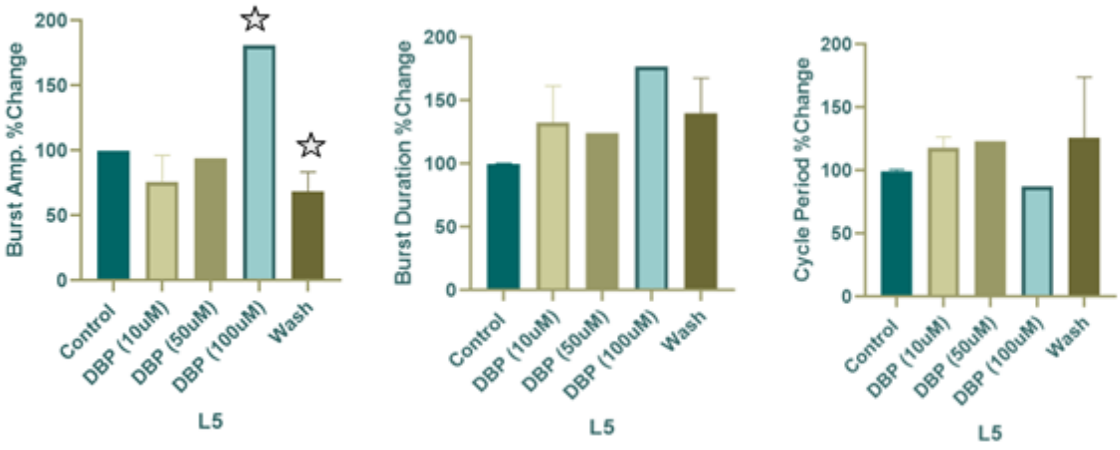

Figure 3. Data was taken from the last 5 minutes of the L5 recordings from the control and DBP conditions (75 - 90 minutes), and from the last 10 minutes for the wash condition. Notice a slight increase in the percent change (compared to control) burst duration, and cycle period following the addition of DBP to the bath (n = 4). However, the only DBP concentration that led to an increase in burst amplitude was that of 100 µM. The stars represent a significant difference as compared to the control ($p < 0.05$).

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