Caffeine Pre-exposure Effects on Caffeine Preference in *Caenorhabditis elegans*

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Caffeine is the most commonly used psychoactive drug and is found in many popular soft drinks and coffee-based beverages. Caffeine is consumed for its mild stimulant properties, and like other stimulant drugs, produces tolerance with continued use and withdrawal symptoms with deprivation. Animal models allow us to investigate neurological effects and the behavioral changes associated with exposure to a drug. The nematode, *Caenorhabditis elegans* (*C. elegans*) is one of the simplest animals to study with only 302 neurons making 5000 synapses, and a short, three-day generation time, yet, its molecular systems are similar to numerous animals, including humans. Animals modify their behavior based on previous experience, including alterations due to drug exposure where chronic drug use can increase future drug use. Behavioral alterations in response to previous exposure to drug stimuli can manifest as sensitization or tolerance to that same stimuli. Furthermore, pre-exposure to the same or a different drug can alter subsequent responses. For example, previous work in our lab has shown that previous exposure to caffeine (50 µM) sensitized worms in avoidance behavior towards low concentrations of caffeine in future preference assays. The purpose of this study is to determine if *C. elegans* alter their preference of caffeine after being pre-exposed to a range of concentrations of caffeine (.5µM, 5µM, 50µM, 100µM, 250µM, and lifetime exposure). Preference for the drug will be measured through a simple choice test where drug will be placed on one side of the assay plate and worms will have free access to move to either side for 30 minutes. Caffeine will be tested in a range of 0 to 50 mM to examine concentration-dependent effects. Worms will be synchronized and placed into a buffer solution during the hatching/L1 phase. For the pre-exposure experiments, caffeine (or control vehicle) will be added to the buffer solution in a range of concentrations. An additional analysis will be done to determine how life-time exposure to caffeine changes caffeine preference. Approximately 60 hours post-hatching, adult worms will be washed from their maintenance plates and around 100 worms will be placed at the center of a test plate. Worms will be counted at 30 minutes to determine how many animals are on the drug-paired side in comparison to the total number of worms on the plate. Similar to previous findings in our lab, it is expected that *C. elegans* will show a greater sensitization to the aversive effects of caffeine with greater caffeine pre-exposure concentrations and with life-time caffeine exposure.