

Introduction

Designer stimulant drugs are an emerging public health problem that is confounded by the lack of rapid diagnostics and specific treatment regimens. Among the many types of designer drugs on the market, synthetic cathinones ("bath salts") have become increasingly popular. Typically bath salts are consumed by insufflation, ingestion or application to mucous membranes and are known to cause severe adverse effects including tachycardia, hypertension and respiratory distress. However, little is known regarding pharmacodynamic properties of bath salts in humans, specifically regarding their cardiogenic effects.

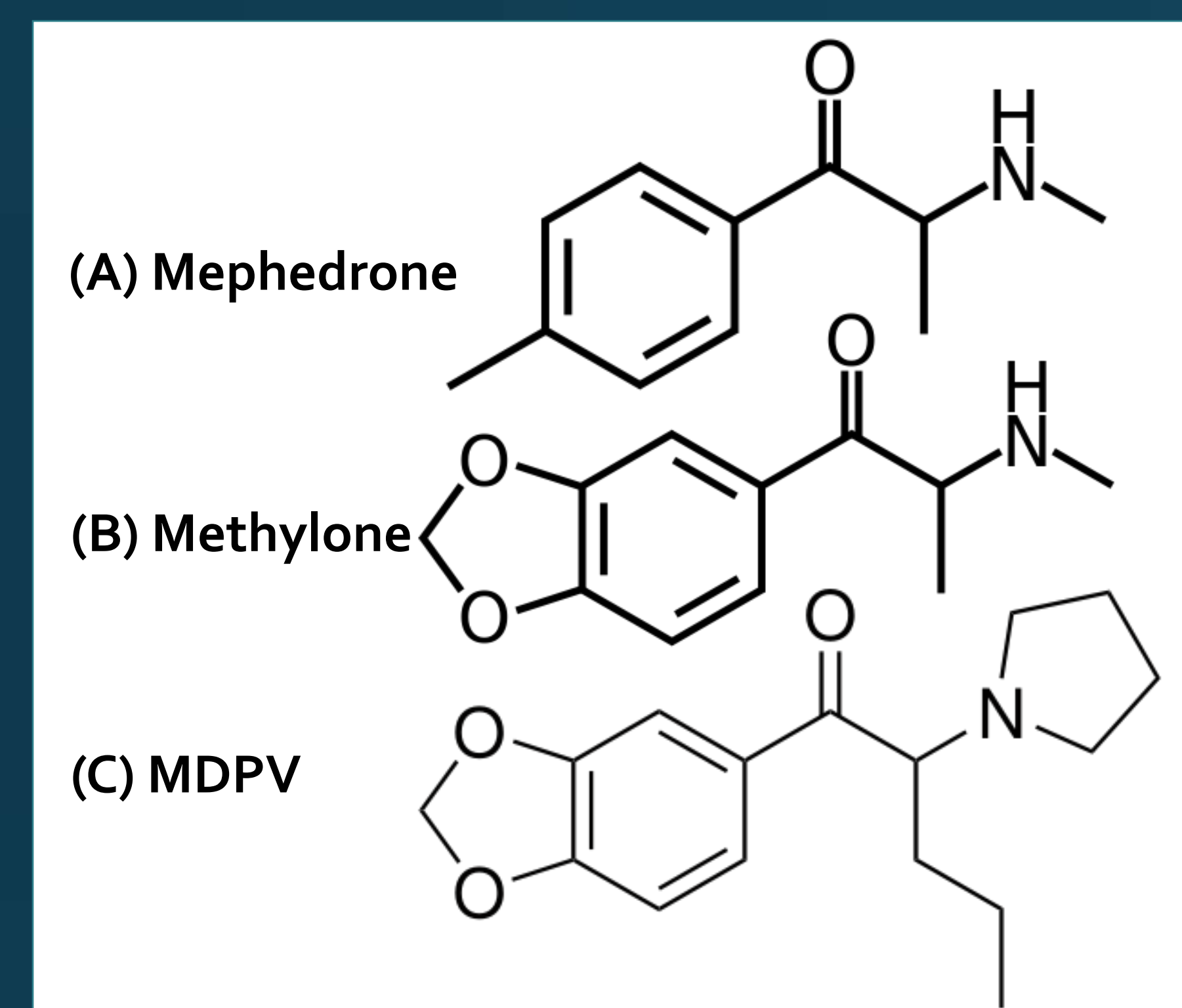


Figure 1: structure of the three synthetic cathinones used throughout the study in an equimolar mixture

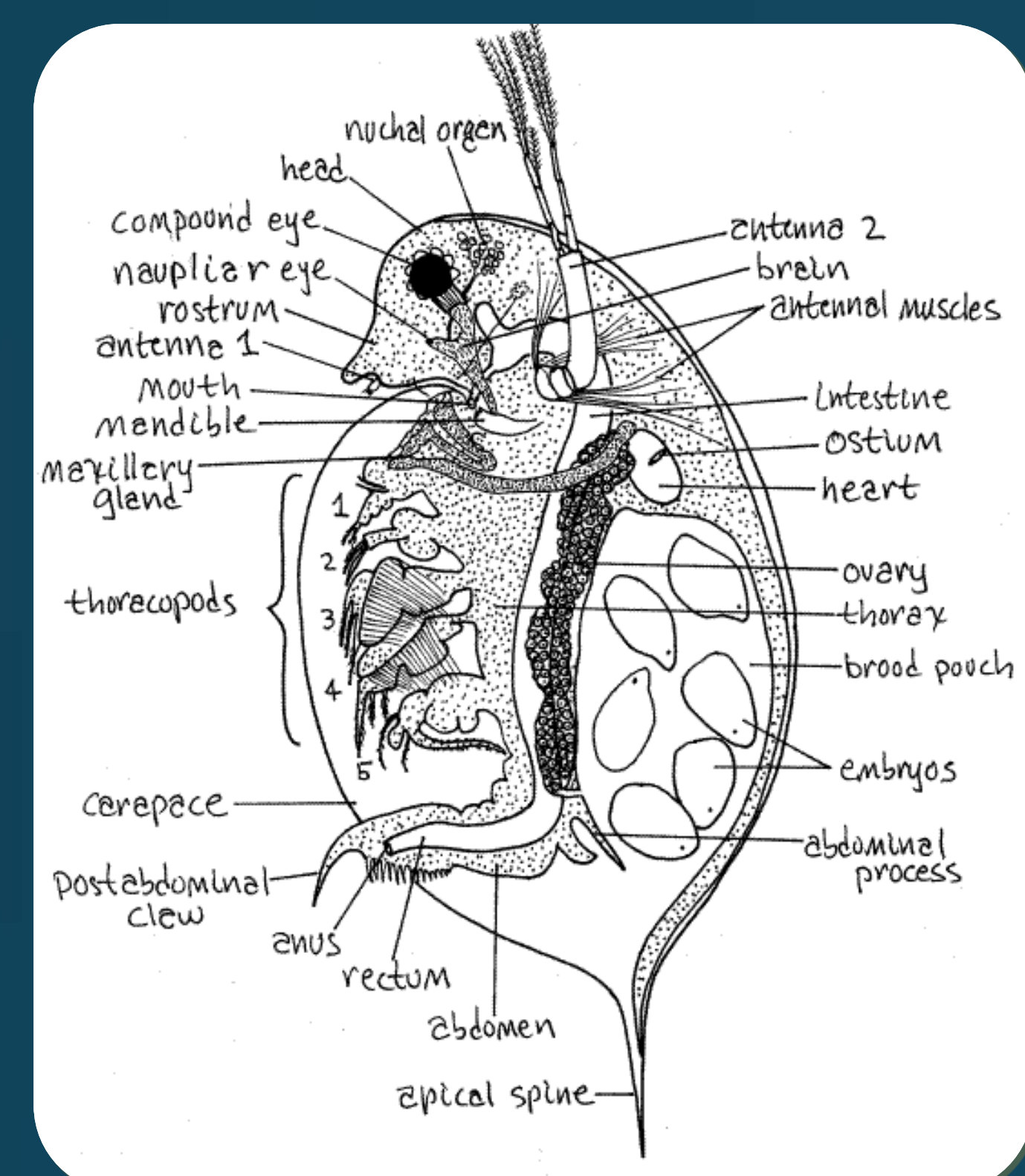


Figure 2: schematic of the anatomy of *Daphnia magna*.

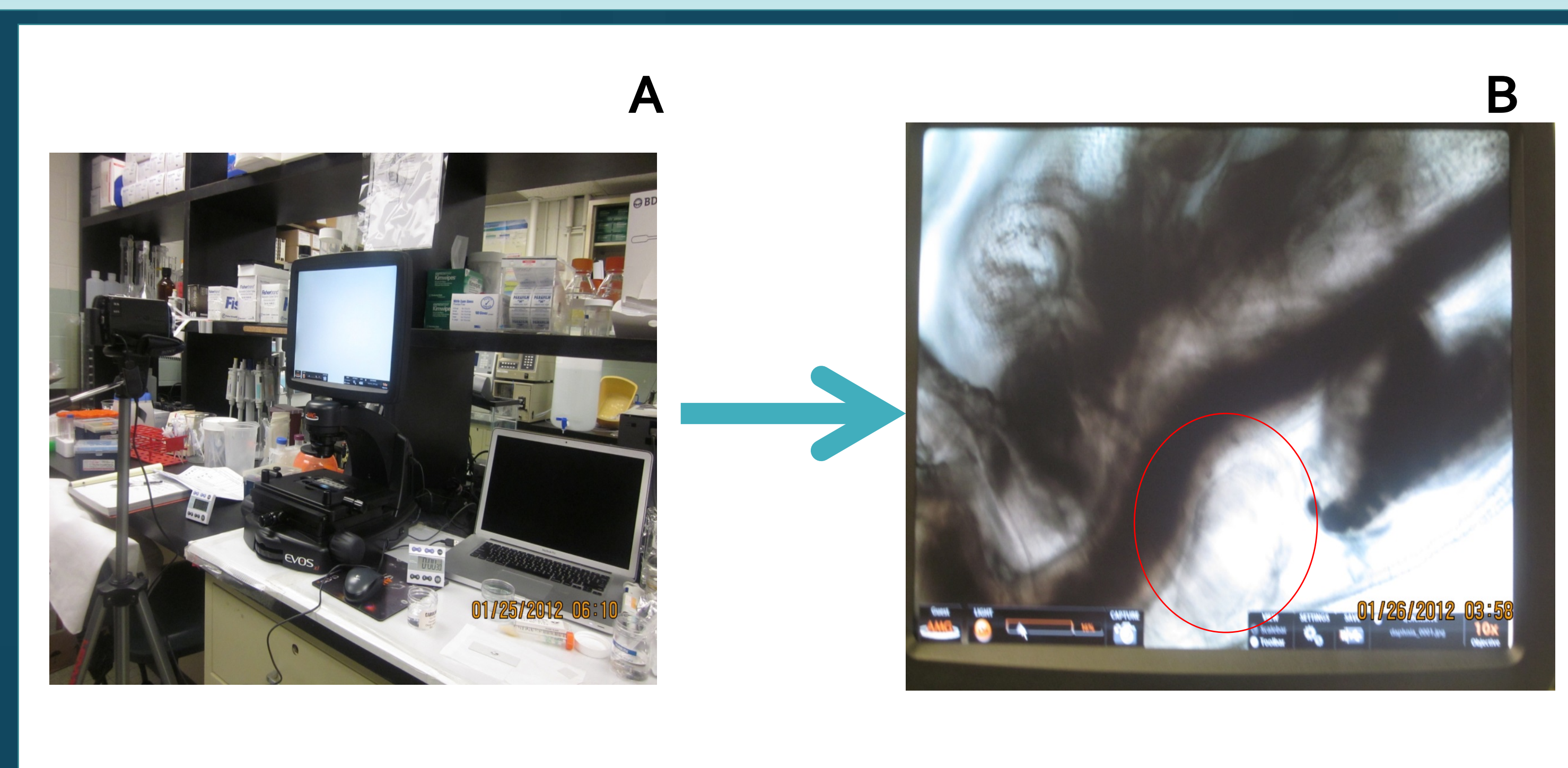


Figure 3: panel (A) shows the light microscope used for data acquisition. Briefly, the *D. magna* are placed onto slides and videos of their hearts are captured. Panel (B) shows the *daphnia* heart; following data acquisition the videos are analyzed by time delay analysis.

Results

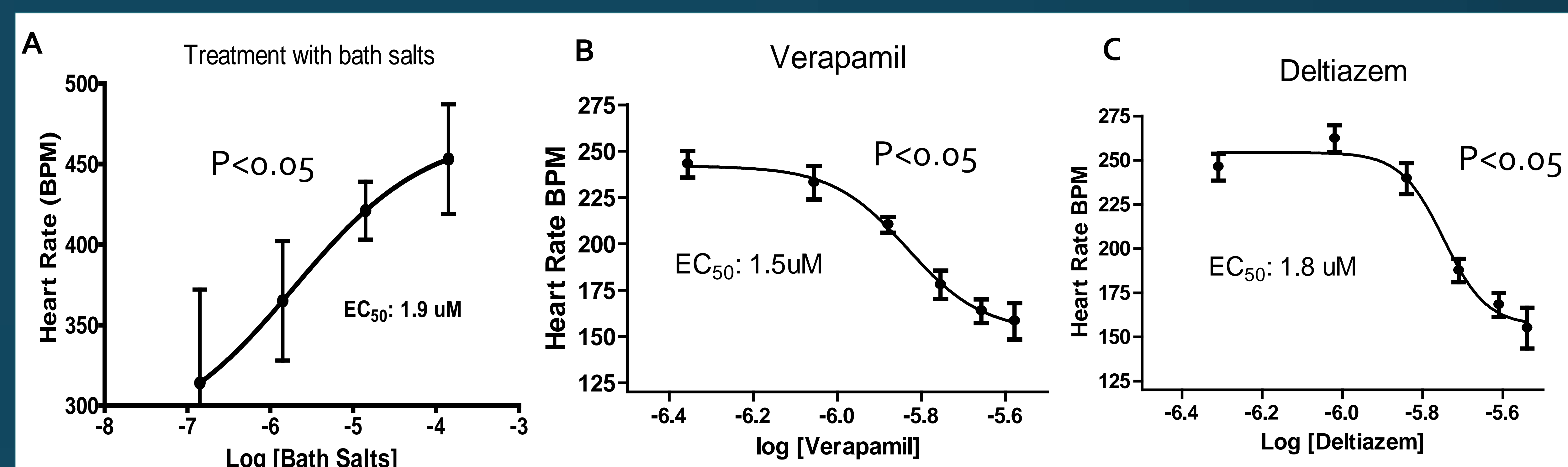


Figure 4: Panel (A) shows the effects of the mixtures of synthetic cathinones on the heart rate of *D. magna*. The heart of *D. magna* was significantly elevated following treatment with the highest concentrations of cathinones ($P < 0.05$, as determined by T-test). Treatment with the calcium channel blockers Verapamil (A) and Deltiazem (B) dose dependently depress the heart rate of *D. magna* following incubation. Verapamil (A) and Deltiazem (B) significantly decreased the heart rate of *D. magna* ($P < 0.05$).

Objective and Hypothesis

- To evaluate the effects of synthetic cathinones ("bath salts") on the heart rate of *Daphnia magna* and determine if elevations in heart rate could be reversed using antiarrhythmic drugs.
- We hypothesize that cathinones will significantly increase the heart rate of *D. magna*, and will be reversed by treatment with calcium channel blocking medication.

Materials and Methods:

- Daphnia magna* (Carolina; Burlington, NC), were maintained in fresh water at room temperature and feed once every other day. *D. magna* have myogenic heart similar to mammals and have been used extensively to study the effects of environmental toxins.
- Aqueous equimolar mixtures of mephedrone, 3,4 methylenedioxypropylvalerone (MDPV) and methylone were used to treat *D. magna*. The synthetic cathinones were provided by UTAK laboratories (Valencia, CA).
- Antiarrhythmic drugs (Diltiazem and Verapamil) were also characterized and used to determine if they could counteract the tachycardia caused by synthetic cathinones.
- Briefly, *D. magna* were incubated for 30 seconds in three different solutions: equimolar cathinone mixtures (concentration 0.14-14.1 μM), anti-arrhythmic drugs (conc. 0.4-2.9 μM), and combinations both classes of drugs. Negative control *D. magna* were treated identically, except they were placed in water.
- Following exposure, groups of five *D. magna* were transferred to slides and the heart rates were determined using a video microscopy and subsequent time-delayed video analysis. Significance was determined using the T-test.
- The effective concentration required to affect the heart rate 50% (EC_{50}) were determined and the t-test was used to demonstrate significant changes.

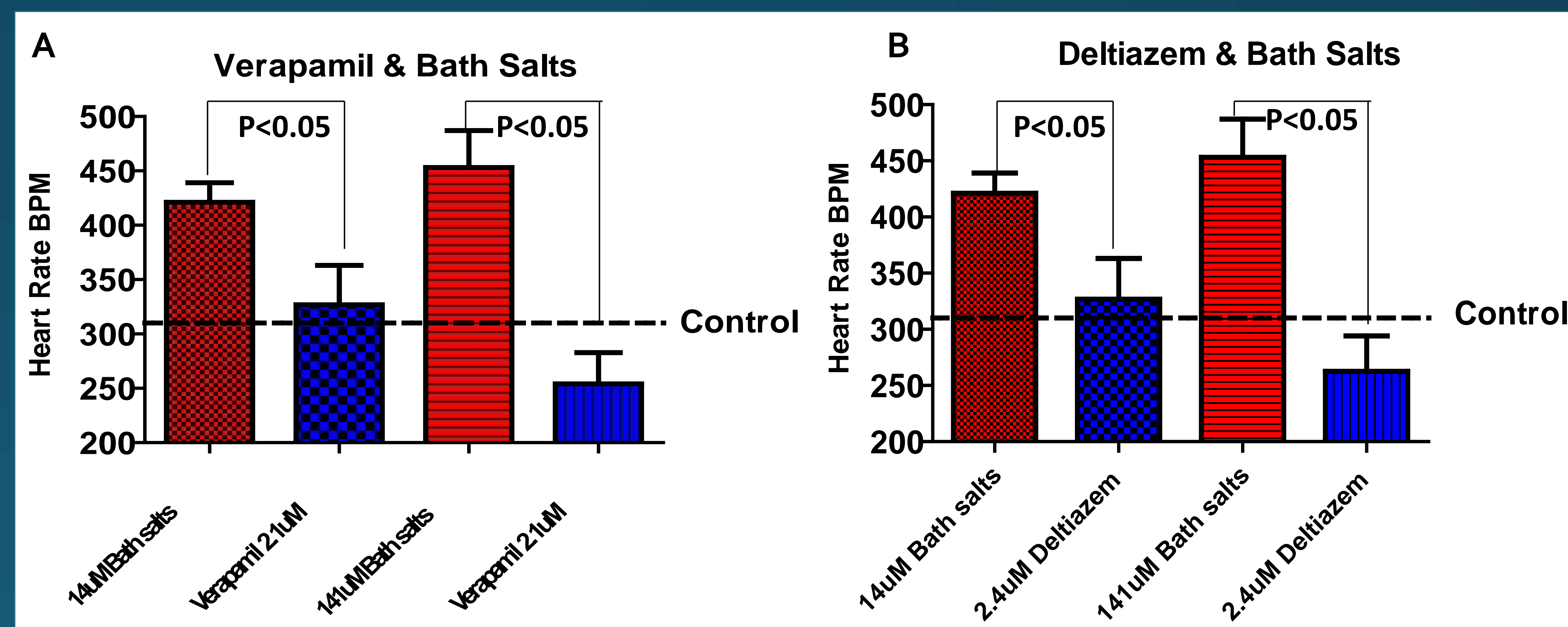


Figure 5: The tachycardia induced by the mixtures of cathinones were counteracted by incubation with the calcium channel blockers Verapamil (A) or Deltiazem (B)

Conclusions

- Mixtures of mephedrone, methylone and MDPV were able to significantly increase the heart rate of *D. magna* by 150% from 310 to 451bpm, with an EC_{50} of 1.9 μM .
- Heart rate did not significantly increase with cathinone concentrations higher than 14 μM .
- Antiarrhythmic drugs such as Verapamil and Deltiazem depress heart rate, decreasing the heart rate by 50% from 310 to 158bpm, with an EC_{50} of 1.5 μM .
- D. magna* exposed to the 14 μM of cathinones, followed by treatment with 2.1 μM Verapamil or Deltiazem, significantly reduced the heart rate from 451 to 326 bpm ($P < 0.05$), effectively counteracting the tachycardic effects of the cathinones.
- We have demonstrated the pharmacodynamic effects of synthetic cathinones on the heart, which lead to tachycardia. Our findings have important clinical implications.