Workstream 2a – in-vitro assays for neurotoxicity using neurons

Preliminary in vitro results with **methedrone** for **neurotoxicity** using mice hippocampal primary cultures of neurons indicate that in concentrations above **10 µg/mL** it causes decrease of neuronal viability (MTS, assay) and lead to the release of alarmins, such as high mobility group box protein 1 (HMGB1) at **50 µg/mL** (Figure 1). Levels above 150 µg/mL lead to a reduction on neurite length, compromising neuronal network (Figure 2).

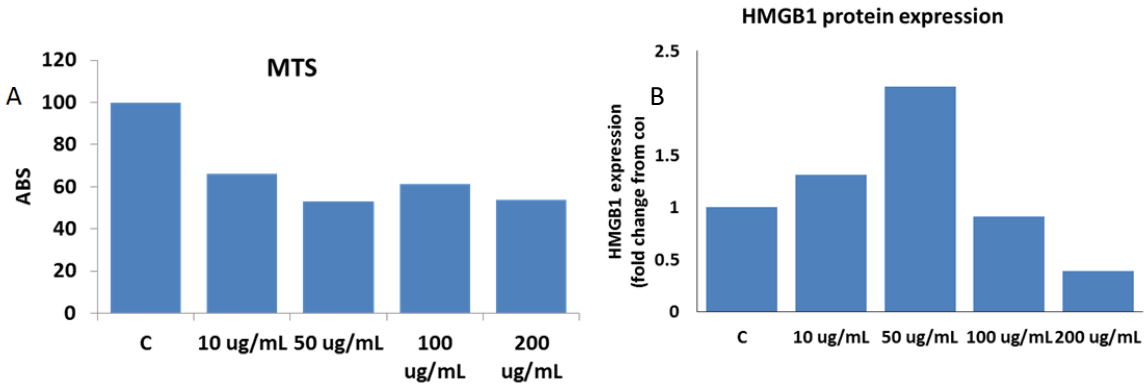


Figure 1. First assays in mice hippocampal neurons cultured for 8 days in vitro incubated with methedrone for 24 h show a decrease in neuronal viability (A) and the release of the alarmin high mobility group box protein 1 (HMGB1) at 50 µg/mL (B), indicating a marked neurodegeneration at such concentration. Hippocampus was isolated from mice embryos at E18.

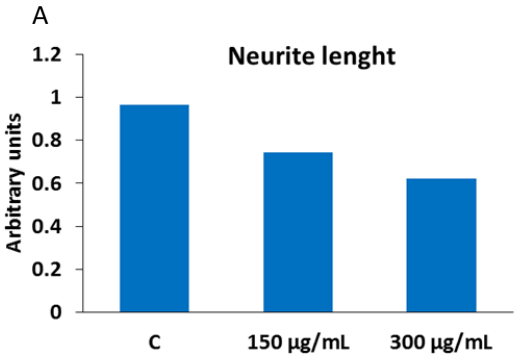
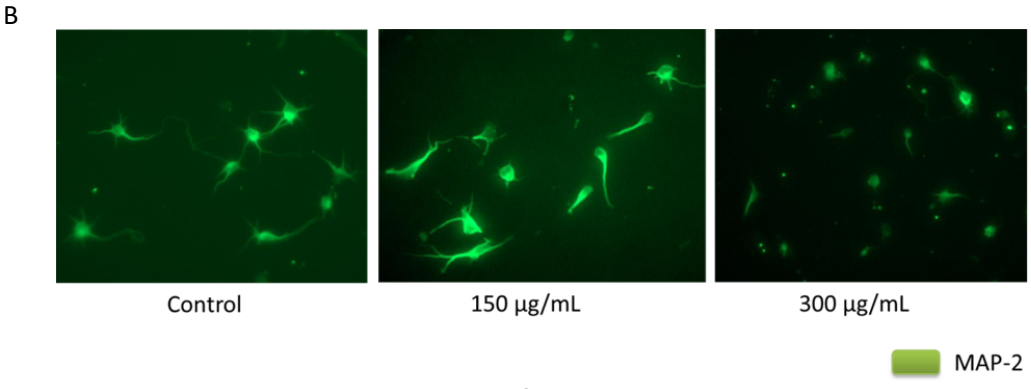


Figure 2 – Preliminary data on the effects of methedrone on neuronal plasticity, evidencing a reduction of neurite length from concentrations of **150 µg/mL** (A) and MAP-2 detection by immunocytochemistry and representative images (B). Hippocampus was isolated from mice embryos at E18.



Workstream 2a – in-vitro assays for neuroinflammation using microglia

Preliminary in vitro results with **methedrone** and **buphedrone** for **neuroinflammation** using cortical primary cultures of microglia from 2-day old mice pups indicate that buphedrone was the main trigger of reduced microglia viability mediated by increased apoptosis in concentrations of **10 µg/mL** or above. Methedrone that also induced a reduction in viability for the same **10 µg/mL** did not induce significant apoptosis and produced late apoptosis/necrosis from **50 µg/mL** on (Figure 3). Interestingly, while buphedrone induced apoptosis for the lower levels it causes decrease of neuronal viability (MTS, assay) and lead to the release of alarmins, such as high mobility group box protein 1 (HMGB1) at **50 µg/mL** (Figure 1). Levels above

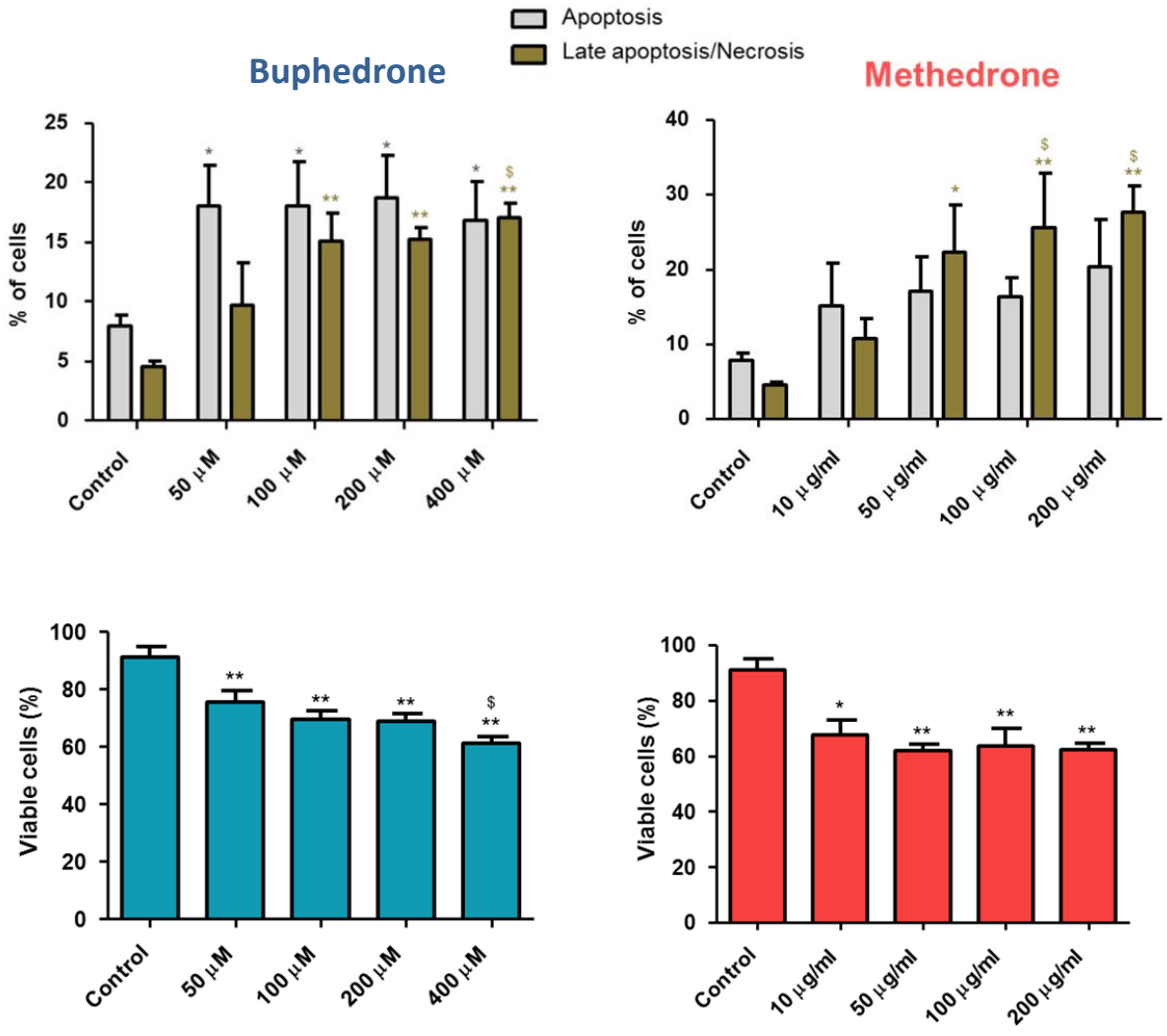


Figure 3. Evaluation of microglia viability loss using the Nexis assay by FACS after treatment with buphedrone and methedrone. Primary microglial cultures were obtained from the cortex of mice at 2 day-old.