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Preparation of pharmacological agents

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Abstract

This is a description of how we prepare different drugs for use in live animals (in vivo) or in brain slices (ex vivo).

This protocol provides a description of the preparation, dilution, application and administration of pharmacological agents.

1 **6-OHDA** (Sigma Aldrich) for MFB dopamine depletions was prepared at 5 µg/µL in normal saline solution.

2 **Levodopa** (Sigma Aldrich) was administered with benserazide (Sigma Aldrich) and prepared in normal saline solution. Levodopa (5–10 mg/kg) and benserazide (2.5-5 mg/kg) were given via IP
injection 5–7 days per week over the course of the experiment.

3 **4-hydroxytamoxifen** (4-OHT, 50 mg/kg in Chen oil, IP) was prepared as previously described (Guenthner et al, 2016; Girasole et al, 2018). To prepare a 20 mg/mL stock in ethanol of 4-OHT, 4-OHT was added to 200 proof ethanol, vortexed, and placed on a horizontal shaker at 37°C for 30 min or until the 4-OHT dissolved. The stock solution was kept covered in foil to minimize light exposure. Next, to prepare a 10 mg/mL working solution in oil, the 4-OHT/ethanol mixture was combined with Chen Oil (a mixture of 4 parts **sunflower seed oil** and 1 part castor oil) and placed into 1.5 mL Eppendorf tubes. The tubes were vigorously mixed, wrapped in foil, and left on a nutator for 45 min at room temperature, vortexed and shaken periodically. The tubes were then placed in a speed-vac for 2-3 h to evaporate the ethanol. If necessary, the final volume was adjusted with Chen Oil to 1 mL to reach a final concentration of 10 mg/mL.

4 **Picrotoxin** (Sigma) was dissolved in warm water to prepare a 5 mM stock solution. Picrotoxin stock which was subsequently diluted in ACSF for a final concentration of 50 µM in ex vivo (slice) electrophysiology experiments.

5 **Tetrodotoxin** (TTX, Abcam) was dissolved in water at a stock concentration of 1 mM and added to ACSF for a final concentration of 1 µM in ex vivo (slice) electrophysiology experiments.

6 **SKF 81297** (Tocris) was dissolved in water at a concentration of 1mM and added to ACSF for a final concentration of 5 µM in ex vivo (slice) electrophysiology experiments.