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Preparation of pharmacological agents V.2

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We use this protocol and it's working

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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).

Materials

To prepare these pharmacological agents, you will need
(1) a metal spatula
(2) a scale
(3) water and/or DMSO as a solvent
(4) pipettors
(5) 0.4 or 1.5 mL eppendorf tubes
(6) aluminum foil
(7) personal protective equipment, including coat, gloves
(8) a fume hood.

Safety warnings

Please check the Safety Sheet associated with each chemical before handling, as some are toxic upon inhalation and should be handled under a fume hood. Some chemicals also require logging as "particularly hazardous chemicals", depending on your local safety regulations.

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. 6-OHDA for intrastriatal depletions was prepared at 2.5 µg/µL in normal saline solution. These solutions are aliquoted and wrapped in tinfoil, stored at -20 C until use. Once defrosted, the aliquot can usually be used for 6-8 hours if kept on ice and wrapped in tinfoil. It turns pink when it begins to oxidize, which is a hint it should be discarded.

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. The solution is prepared fresh daily from powder and can be used for several hours from the time of preparation.

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 **Pramipexole** (PPX, Sigma Aldrich) was prepared in normal saline at a stock concentration of 0.1 mg/mL. It was administered at 0.5 mg/kg IP.

5 **A77636** (Tocris) was prepared in normal saline at a stock concentration of 0.1 mg/mL in a dark room, and the tube wrapped in aluminum foil to prevent light exposure. Aliquots can be frozen and used for several weeks. It was administered at 1 mg/kg IP.

6 **Clozapine-N-oxide (CNO, Tocris).** For *in vivo* experiments, CNO was prepared in normal saline at 0.1-0.3 mg/mL in a dark room, and the tube wrapped in aluminum foil to prevent light exposure. Aliquots can be frozen and used for several weeks. It was administered at 1-3 mg/kg IP. For local infusion by cannula, CNO was prepared in normal saline at 10 mM, and the tube wrapped in aluminum foil to prevent light exposure. Aliquots can be frozen and used for several weeks. CNO is then diluted in normal saline for a final concentration of 10 uM. For ex vivo slice experiments, CNO was prepared in normal saline at 1 mM in a

dark room, and the tube wrapped in aluminum foil to prevent light exposure. Aliquots can be frozen and used for several weeks. At the time of the experiment, CNO is diluted in Artificial Cerebrospinal Fluid (ACSF) for a final concentration of 1 uM.

7 **Ethanol** (Sigma Aldrich). For in vivo experiments, ethanol was prepared in a 20% solution in water (by volume), then administered at 1.5 g/kg IP.

8 **Picrotoxin** (Sigma Aldrich) was dissolved in warm water to prepare a 5 mM stock solution, which is kept a room temp and can be used for many months. Picrotoxin stock was subsequently diluted in ACSF for a final concentration of 50 µM in ex vivo (slice) electrophysiology experiments.

9 **Tetrodotoxin** (TTX, Abcam) was dissolved in water at a stock concentration of 1 mM, aliquoted and stored at -20 C. Aliquots were diluted in ACSF for a final concentration of 1 µM in ex vivo (slice) electrophysiology experiments.

10 **SKF 81297** (Tocris) was dissolved in water at a concentration of 1mM, aliquoted and stored at -20 C. Aliquots were diluted in ACSF for a final concentration of 5 µM in ex vivo (slice) electrophysiology experiments.

11 **Quinpirole** (Tocris) was dissolved in water at a concentration of 10 mM, aliquoted and stored at -20 C. Aliquots were diluted in ACSF for final concentration of 1 µM for ex vivo (slice) electrophysiology experiments.

12 **Sulpiride** (Tocris) was dissolved in water at 10 mM, aliquoted and stored at -20 C. Aliquots were diluted in ACSF for a final concentration of 1-10 µM for ex vivo (slice) electrophysiology experiments.

13 **SCH23390** (Tocris) was dissolved in water at 10 mM, aliquoted and stored at -20C. Aliquots were diluted in ACSF for a final concentration of 10 µM for ex vivo electrophysiology experiments.

14 **CNQX** (Tocris) was dissolved in water at 10 mM, aliquoted and stored at -20C. Aliquots were diluted in ACSF for a final concentration of 1-10 µM for ex vivo (slice) electrophysiology experiments.

15 **APV** (Tocris) was dissolved in water at 50 mM, aliquoted and stored at -20C. Aliquots were diluted in ACSF for a final concentration of 50 µM for ex vivo electrophysiology experiments.

16 **Scopolamine** (Sigma) was dissolved in water at 200 mM, aliquoted and stored at -20C. Aliquots were diluted in ACSF for a final concentration of 200 µM for ex vivo electrophysiology experiments.

17 **CGP55845** (Tocris) was dissolved in water at 300 mM, , aliquoted and stored at -20C. Aliquots were diluted in ACSF for a final concentration of 300 uM for ex vivo electrophysiology experiments.

18 **Dopamine** (Sigma) was dissolved in water with 0.1% ascorbic acid at 1-100 mM and added to ACSF for a final concentration of 1-100 uM for ex vivo electrophysiology experiments.