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alpha-Synuclein pre-formed fibrils preparation and stereotactic injection into Mus Musculus brain

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We use this protocol and it's working



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Abstract

Preparation of seed-competent alpha-Synuclein pre-formed fibrils and subsequent stereotactic injection into Mus Musculus brain as a model of synucleinopathy.

Troubleshooting



Storage

- 1 Store alpha-Synuclein fibril preparations at  -80 °C with appropriate biohazard precautions and labels until use.

PFF preparation (to be done on the same day as experiment)

- 2 On the day of usage, briefly thaw fibrils on ice. 2m
- 3 Transfer required quantity of fibrils to the appropriate tube compatible with your sonicator. Dilute fibrils with normal saline (0.9% NaCl) if required. We used fibrils at a concentration of 5mg/mL. 2m
- 4 Program Covaris S220 water bath sonicator to:
18 W peak incident power;
20% duty factor;
50 cycles per burst;
150 s. 2m

Mouse preparation and administration

- 5 Anesthetize mouse with isoflurane in an enclosed chamber. 2m
- 6 Once deeply anesthetized, transfer the mouse to the stereotaxic frame. Insert mouse nose into isoflurane mask.
- 7 Using a 2µl Hamilton Company syringe, take up 1µl of sonicated PFFs or normal saline (0.9% NaCl) using a micro-syringe computerized pump. 2m
- 8 Program the micro-syringe computerized pump to deliver 1 µL mouse PFFs (5 mg/mL) or sterile saline (0.9% NaCl) at a rate of 0.1 µL/min (min).
- 9 Align needle to coordinates relative to bregma: -2 mm medial-lateral; +0.2mm antero-posterior.
- 10 Incise skin. Mark site on skull with a marking pen, and drill 1mm diameter burr hole with handheld drill.



- 11 Slowly position the needle to coordinates relative to bregma: -2 mm medial-lateral; +0.2mm antero-posterior and -2.6 mm dorso-ventral (right dorsal striatum).
- 12 Begin the administration sequence, following the micro-syringe computerized pump's instructions.
- 13 Leave the needle in place for at least 3 min before its slow withdrawal.
- 14 Close skin with topical skin adhesive.

Post-injection

- 15 Provide appropriate post-surgical care to the mice.
- 16 Wait 6 months for phenotype development, or however long your experiment requires.
- 17 Perform behavioural experiments then collect tissues for biochemical and histological analysis.