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.

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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 wit 2m normal saline (0.9% NaCl) if required. We used fibrils at a concentration of 5mg/mL.

4 Program Covaris S220 water bath sonicator to: 2m
18 W peak incident power;
20% duty factor;
50 cycles per burst;
150 s.

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.