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Dendritic spine analysis

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We use this protocol and it's working



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Abstract

Haloperidol is reported to induce a series of homeostatic synaptic and intrinsic adaptations primarily in the indirect pathway circuits. These adaptations include dendritic spine loss in iSPNs, which occurs after 5 days and persists for up to 14 days of daily treatment. We examined the haloperidol-induced dendritic spine density patterns in iSPNs after 7 days of treatment

Stereotaxic Surgeries

- 1 P4-5-day-old LRRK2GS/WT-Adora2aCre pups were cryoanesthetized and received ketoprofen for analgesia
- 2 Pups were placed on a cooling pad on a stereotaxic frame
- 3 200 nl of the AAV-DIO-EGFP virus (7×10^{12} vg/mL) were delivered into the dorsal striatum at a rate of 100 nl min⁻¹ using Micro-4 controller (WPI).
- 3.1 In order to ensure the dorsal striatum was targeted, the needle was placed 1 mm anterior to the midpoint between the ear and eye, 1.5 mm from the midline, and 1.8 mm ventral to the brain surface
- 4 After the injection, the pups were warmed on a heating pad before returning to their home cages.

Confocal microscopy

- 5 Confocal images of fixed 80-um-thick brain sections of P30 pups injected with the AAV-DIO-EGFP were obtained with the Nikon A1R microscope
- 6 Fluorescence projection images of dendrites and the corresponding dendritic spines were acquired with a 60x oil immersion objective (NA = 1.4) at 0.1 um intervals with 1,024 x 1,024 pixel resolution

Dendritic spine analysis

- 7 Segments from secondary and tertiary dendrites without overlap with other neurons or discontinuities were chosen for analysis
- 7.1 Dendritic spine density was assessed using Imaris 10.1 software
- 8 Dendritic segments were traced using autopath mode of the filament tracer at default settings
- 9 Settings used for spine detection



- 9.1 Thinnest diameter of seed points at 0.45 μm ; maximum length at 2.5 μm ; no branched spines allowed; and seed point threshold at auto