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Postnatal astrocyte labeling by electroporation (PALE)

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Abstract

Postnatal astrocyte labeling by electroporation (PALE)

1. Late P0/early P1 mice were sedated by hypothermia until anesthetized, and 1 µl of plasmid DNA mixed with Fast Green Dye was injected into the lateral ventricle of one hemisphere using a pulled glass pipette (Drummond).

2. For shRNA knockdown experiments in wild-type CD1 mice, the 1 µl of DNA contained 1 µg of pGLAST-PBase and 1 µg of pPB-shRNA-mCherryCAAX was injected.

3. To label astrocytes in WT and LRRK2 G2019S$^{ki/ki}$mice, the 1 µl of DNA contained 1 µg of pGLAST-PBase and 1 µg of pPB-mCherry-CAAX was injected per mouse.

4. For PALE-mediated overexpression of phospho-mimetic EZRIN in shRNA knockdown experiments, 0.5 µg pGLAST-PBase, 0.5 µg pPB-shRNA-mCherryCAAX, and 1 µg pZac2.1-GfaABC1D-Ezrin T567D-BioID2-HA were injected in a total volume of 1 µl.

5. For phospho-dead EZRIN overexpression in WT and LRRK2 G2019S$^{ki/ki}$mice, 0.5 µg pGLAST-PBase, 0.5 µg of pZac2.1-gfaABC1D-mCherry-CAAX and 1 µg of pZac2.1-GfaABC1D-Ezrin T567A-BioID2-HA were injected in a total volume of 1 µl.

6. Following DNA injection, electrodes were oriented with the positive terminal above the frontal cortex and the negative terminal below the chin, and 5 discrete 50 ms pulses of 100 V spaced 950 ms apart were applied.

7. Pups were recovered on a heating pad, returned to their home cage, and monitored until collection at P21.

8. The number of mice used for each experiment is indicated in the figure legends.

9. All animals appeared healthy at the time of collection. Brain sections were examined for the presence of electroporated cells before staining.