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Protocols for stereotaxic injections into mouse brain and ex-vivo electrophysiology

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We use this protocol and it's working

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ETHICS DISCLAIMER

The **protocols.io** team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board

Abstract

This protocol describes the method for injection of α-Synuclin PFF and monomer into the mouse brain. The second part of the protocol describes preparation of acute slices from these mice and whole-cell patch clamp recordings.

Materials

Oxygenated (95% O_2/5% CO_2) ice-cold choline artificial cerebrospinal fluid (Choline ACSF) containing (in mM):

	A	B
	choline	110
	NaHCO3	25
	NaH2PO4	1.25
	KCl	2.5
	MgCl2	7
	CaCl2	0.5
	glucose	20
	sodium ascorbate	11.6
	sodium pyruvate	3.1

ACSF (32 °C) containing (in mM):

	A	B
	NaCl	127
	NaHCO3	25
	NaH2PO4	1.25
	KCl	2.5
	MgCl2	1
	CaCl2	2
	glucose bubbled with 95% O2/5% CO2	20

Backfill glass electrodes (3-3.5 MΩ) with an internal solution containing (in mM):

	A	B
	cesium gluconate	126

	A	B
	HEPES	10
	sodium phosphocreatine	10
	magnesium chloride	4
	Na2ATP	4
	Na2GTP	0.4
	EGTA (pH 7.3 with cesium hydroxide)	1

Protocol for stereotaxic injections

1 Anesthetize the mouse with isoflurane by placing in the anesthesia chamber.

2 Place the anesthetized mouse in the stereotaxic frame and continually maintain isoflurane anesthesia using a vaporizer (Harvard Apparatus).

3 Locate the injection sites relative to bregma and make holes into the skull using a drill.

4 Lower the injection pipette into the brain at the predetermined coordinates. Perform the injection at a rate of 20 nL/s using a Nanoject III microinjector (Drummond Scientific).

5 Following the injection, leave the pipette in place for 10 minutes to prevent backflow while withdrawing.

6 Seal the incisions with sutures and allow the mice to recover on a heating pad until conscious.

7 Continue to monitor the mice and administer the post-operative analgesia.

Protocol for acute slice preparation and electrophysiology

30m

8 Anesthetize the mouse with isoflurane.

9 Transcardially perfuse the anesthetized mouse with oxygenated (95% O_2/5% CO_2) ice-cold choline artificial cerebrospinal fluid (Choline ACSF) containing (in mM):

	A	B
	choline	110
	NaHCO3	25
	NaH2PO4	1.25
	KCl	2.5

	A	B
	MgCl2	7
	CaCl2	0.5
	glucose	20
	sodium ascorbate	11.6
	sodium pyruvate	3.1

10 Decapitated the mouse and quickly remove the brain.

11 Mount the brain in the vibratome and cut 300 µm thick slices in the coronal plane.

12 Transfer the slices to warm ACSF (32 °C) containing (in mM):

	A	B
	NaCl	127
	NaHCO3	25
	NaH2PO4	1.25
	KCl	2.5
	MgCl2	1
	CaCl2	2
	glucose bubbled with 95% O2/5% CO2	20

13 After incubating at 32 °C for 00:30:00 , transfer the slices to Room temperature (RT). 30m

14 Transfer the slices to the slice chamber that is perfused with oxygenated ACSF.

15 Identify layer 2/3 pyramidal cells in the primary somatosensory cortex.

16 Backfill glass electrodes (3-3.5 MΩ) with an internal solution containing (in mM):

	A	B
	cesium gluconate	126
	HEPES	10
	sodium phosphocreatine	10
	magnesium chloride	4
	Na2ATP	4
	Na2GTP	0.4
	EGTA (pH 7.3 with cesium hydroxide)	1

17 Add [M] 1 micromolar (µM) tetrodotoxin to the bath to record miniature postsynaptic currents.

18 Voltage-clamp the cells at -70mV to record miniature excitatory postsynaptic currents (mEPSCs) and at 0mV to record miniature inhibitory postsynaptic currents (mIPSCs).

Note

All protocols were carried out in accordance with Yale IACUC guidelines.