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Juxtacellular Recording and Single-Cell Labeling of VTA Neurons

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We use this protocol and it's working

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Abstract

This protocol describes juxtacellular recording and labeling of single VTA neurons in urethane-anesthetized adult mice. Following baseline and sensory stimulation recording, individual neurons were labeled juxtacellularly and brains were processed for histological identification and reconstruction.

Materials

Animals
- Adult mice, 25-30 g.
- Anesthesia: urethane, 1.25 g kg^-1, intraperitoneal injection.

Electrode Preparation
- Glass micropipettes with tip diameter 3c 1 µm.
- Resistance: 6-15 MOhm.

Pipette internal solution (in mM):
- 250 K-gluconate
- 5 KCl
- 1 MgCl2
- 2 EGTA
- 5 HEPES
- 2 MgATP

Tracer added to internal solution:
- Tetramethyl-rhodamine-biocytin 2 percent w/v, or
- Neurobiotin 1.7 percent

pH adjusted to 7.2.

Recording Setup
- Dual-channel intracellular amplifier: NeuroData IR-283 (Cygnus).
- Signals digitized using micro1401 A/D converter.
- Data acquired in Spike2 (Cambridge Electronic Design).

Procedure

1 Induce anesthesia with urethane (1.25 g kg^-1, i.p.).
Confirm surgical plane of anesthesia.
Secure animal in stereotaxic frame.
Expose skull and perform craniotomy above VTA.

2 Slowly lower themicropipette into VTA (depth -4.0 to-5.0.)
Obtain stable extracellular juxtacellular recording.
Record 200 s baseline (no stimulation).
Deliver ipsilateral hindlimb foot-pinch series: 3 trials; 15 s pinch per trial; inter-trial interval ≥ 60 s.
Pinch delivered with blunt forceps to plantar surface of hindlimb.

3 After physiological recording, label the neuron juxtacellularly using the standard procedure (Pinault, 1996). Confirm successful entrainment during labeling.

4 Perform transcardial perfusion with 0.01 M PBS followed by 4 percent paraformaldehyde in PBS.
Post-fix brains for 12 h in 4 percent paraformaldehyde.
Cryoprotect in 30 percent sucrose until sunk.
Section at 40 µm using freezing microtome.

Outputs

5 Single-neuron spike recordings aligned to sensory stimulation.
Histologically labeled single neurons suitable for immunofluorescence and 3D reconstruction.

Critical Steps

6 Maintain stable urethane anesthesia throughout recording.
Use clean, high-resistance pipettes for stable juxtacellular contact.
Avoid excessive current during labeling to prevent tissue damage.
Ensure complete perfusion and cryoprotection to preserve morphology.

Expected Outcome

7 Successful recordings yield stable baseline activity and clear responses to hindlimb pinch stimulation. Juxtacellular labeling results in complete filling of somatodendritic domains and proximal axons, enabling post hoc anatomical and neurochemical identification.

Protocol references

Pinault, D. A novel single-cell staining procedure performed in vivo under electrophysiological control: juxtacellular labeling. J. Neurosci. Methods 65, 113–136 (1996).