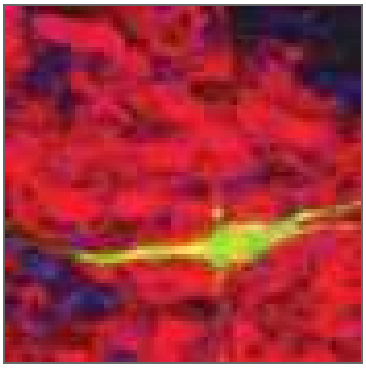

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Protocol status: Working
We use this protocol and it's working

Juxtacellular Recordings in Ventral tegmental area and Locus coeruleus

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ABSTRACT

This protocol describes juxtacellular recordings in the VTA and LC of adult mice, involving anesthesia, precise brain targeting, and neuron labeling. Using specialized electrodes and a dual-channel amplifier, neuronal activity is recorded and modulated with current injections. Following recordings, brains are perfused, fixed, and sectioned for detailed morphological analysis.

SAFETY WARNINGS

- Ensure all solutions and equipment are prepared and calibrated according to your lab standards.
- Follow all ethical guidelines and protocols for animal care and use.
- Maintain a sterile environment throughout the procedure to prevent contamination.

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Preparation of Adult Mice for juxtacellular recordings

1

- Use adult mice weighing 25-30 g.
- Anesthetize the animal with urethane at a dosage of 1.25 g/kg.
- Place the mouse in the stereotaxic frame and align the head angle according to Bregma and Lambda, with a tolerance of a maximum of 50 microns between them.
- Expose the skull by shaving the mouse's head and making a sagittal incision on the skin.
- Find the coordinates and drill the bone. Once the dura is exposed, carefully remove it with the forceps (n°).

  VTA: A/P 3.25, L/M 0.5
  LC: A/P 5.34, L/M 1.1

- Drill a small hole around Lambda and install the reference screw.

Juxtacellular set-up

2

- Employ an intracellular recording amplifier (NeuroData Dual channel IR-283), an analog-to-digital converter (CED Micro-1401-3), and the Spike2 software..
- Use 5-16 MΩ (40-45 MΩ for intracellular) glass electrodes with a tip diameter of less than 1 µm.
- Fill the electrodes with a solution containing:
- 250 mM k-gluconate, 5 mM KCl, 1 mM MgCl2, 2 mM EGTA, 5 mM HEPES, 2 mM MgATP, and Tetramethyl-Rhodamin Biocytin tracer or Neurobiotin (2% w/v); pH 7.2.

Juxtacellular/Intracellular Recordings and Labelling

3

- After penetrating the brain, ensure the electrode impedance is correct by injecting 0.5 nA pulses and adjusting the bridge/balance module.
- Move down the electrode at a constant low speed until reaching the dorsal VTA (z=-4.0).
- Start the exploration by moving down not faster than a micron per second.
- Isolate spontaneously active neurons until the extracellular spike amplitude is approximately 0.7 to 1.0 mV (for intracellular penetration about 2.0-2.5 mV.)
- After baseline recordings, label neurons using the juxtacellular (Pinault, 1996) or intracellular methods.

4
- For juxtacellular labeling:
- Label neurons by injecting a small current (1-4 nA for 5-10 minutes, 250 ms ON-250 ms OFF). Make sure that the neuron is being modulated by the current injection.

5
- For intracellular labeling:
- Apply AC pulses to gain intracellular access. If unsuccessful, adjust by 2 micrometers and reapply AC pulses.
- Once intracellular access is achieved, stabilize neurons with negative current.
- Label neurons by injecting a small current (1-2 nA for 10-15 minutes, 250 ms ON-250 ms OFF).

6 Wait for at least 2 hours before perfusion.

7

Post-Recording and Labeling Process:

8
- Perfuse the animals via the ascending aorta with 0.01 M PBS followed by 4% paraformaldehyde in PBS.
- Post-fix brains between 6-12 hours in 4% PFA.
- Cryoprotect the brain in 30% sucrose until it sinks.
- Section the brains at 40 µm using a freezing microtome.