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Protocol

In vivo preparation and identification of mitral cells in the main olfactory bulb of the mouse

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Abstract

The mouse main olfactory bulb (MOB) is commonly used as a mammalian model to study olfactory processing. The genetic techniques available with the mouse make its MOB a powerful model for analysis of neuronal circuitry. The mouse has been used as a mammalian model for all types of MOB neurons, but especially to study the activity of mitral cells. However, mouse mitral cell activity is most commonly studied in vitro. Therefore, we aimed to develop a protocol to record the activity of antidromically identified mitral cells in mouse in vivo. Currently, such a protocol does not exist. Using extracellular techniques, we report a protocol that is able to record neurons from all mouse MOB layers. Specifically, mitral cell single-units were identified by antidromic activation from the posterior piriform cortex, and their spontaneous activity was recorded for more than 30 min. This protocol is stable enough to record from single-units while buprenorphine was applied both topically to the surface of the MOB and injected systemically.

Theme: Sensory systems
Topic: Olfactory senses

Keywords: Mouse; In vivo; Main olfactory bulb; Mitral; Spontaneous activity; Chloral hydrate

1. Type of research

The mammalian frontal cortex receives information about the chemical environment through connections from the main olfactory bulb (MOB). The electrophysiology of the principal output neurons of the MOB, mitral cells, has primarily been studied in vivo in rat [4,26,34,36,43,44] and rabbit [11,18,30]. Detailed analysis of MOB circuitry and pharmacological manipulations of mitral cell membrane physiology have primarily occurred in mouse and neonatal rat in vitro models [1,9,21,26]. In vitro brain slice preparations are able to maintain relatively stable physiology, including spontaneous activity [5,27]; however, this activity may be altered by a loss of input from several classes of neurons. In rat, in vitro recordings of mitral cell spontaneous activity average about 3 Hz [5], while in vivo recordings average about 30 Hz [16]. The mouse is an attractive model for physiology because of the many specific genetic alterations available [3,4,23,32]. An in vivo mouse model would facilitate analysis of MOB circuitry and mitral cell membrane physiology in an intact and genetically alterable system.

Mitral cells can be identified in vivo by antidromic activation from the posterior piriform cortex (pPC), since only mitral cells project from the MOB to this region [12,34,36,37]. However, the invasive surgery and the small size of the mouse have presumably deterred the development of a protocol for recording from mitral cells in vivo in mouse. Although mouse in vivo techniques are becoming more common [3,7,8,17,21,22,32], a protocol to record antidromically identified mitral cells in mouse does not exist. This protocol describes the anesthetic and surgical

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procedures necessary for studying mouse mitral cell physiology in vivo. We tested the usefulness of the protocol by characterizing 6 mitral cells and by observing the effect of the pharmacological agent buprenorphine.

2. Time required

Depending on the specific goal, and including the inherent variability of in vivo experiments, each experiment will last 4–12 h.

2.1. Surgical preparation: 30–65 min from induction of anesthesia to ground electrode insertion

(a) Induction of anesthesia, shave surgery site, subcutaneous injections of lidocaine: ~10 min. In some animals, induction is problematic and requires additional injections of anesthesia: extra 10–30 min.
(b) Insertion of intraperitoneal tube, insertion of rectal thermometer, and incision of scalp: <10 min.
(c) Insertion of ground electrode: <10 min.

2.2. Surgery: 75–150 min

(a) Placement of animal in stereotaxic: <5 min.
(b) Insertion of EEG electrode: 15–25 min.
(c) Exposure of dorsal MOB: 15–40 min.
(d) Exposure of posterior piriform cortex: 40–90 min.

2.3. Recording set-up: 15 min

(a) Electrode set-up: 12 min.
(b) Clean airline: <3 min.

2.4. Recording: most variable, dependent on experimental design: 2–10 hrs

(a) Location and recording of single-units based on extracellular techniques: minutes to hours (based on experimental design).

3. Materials

3.1. Animals

All procedures in this study are in accordance with federal animal care guidelines and were approved by the University of Cincinnati Institutional Animal Care and Use Committee. Animals were housed in filtered, clear plastic cages on a 12-h light/dark cycle. Food and water were provided ad libitum. Generally, mice were ICR females or C57BL6J (B6) males weighing between 19 and 40 g (Harlan; Indianapolis, IN). However, both sexes in both strains have been used successfully with this protocol.

3.2. Special equipment

(a) Micropipette puller: Sutter Instrument Co. (Model p-80; San Francisco, CA).
(b) Stereotaxic frame and microelectrode micropositioner: David Kopf Instruments (Model 1640, Model 650; Tujunga, CA).
(c) Pre-amplifier and window discriminator: Dagan (Model 2400a and Model WD-2; Minneapolis, MN).
(d) Oscilloscopes and amplifiers: Tektronix (Model 5111A, Model 5A22N; Gaithersburg, MD).
(e) Stimulation and isolation units: Grass (Model S44, Model SIU5; West Warwick, RI).
(f) Data acquisition hardware and software: Cambridge Electronic Design (Model μ1401, Spike2 ver4.0; Cambridge, UK).
(g) Analog-to-digital data storage: Medical Systems (Model PCM-8; Greenvale, NY).
(h) Stimulation and EEG electrode wire: California Fine Wire (0.125 mm stainless steel insulated wire; Grover Beach, CA).
(i) Micropipette glass: World Precision Instruments (Item 1B15OF-4; Sarasota, FL); pulled to 5–10 MΩ resistance.
(j) Teflon and glass tubing for delivery of clean air.
(k) Dental drill: Fordom electronic company (Model 73; Bethel, CT).
(l) Dental burs: SS White (Model HP-35; Lakewood, NJ).
(m) Polyethylene tubing: Becton Dickinson (Intramedic PE10: I.D. 0.28 mm, O.D. 0.61 mm; Sparks, MD).
(n) Water heater and pump: Gaymar Industries, (Model TP-500; Orchard Park, NY).

3.3. Chemicals

(a) Chloral hydrate and lidocaine: Sigma (St. Louis, MO).
(b) Buprenorphine: Reckitt Benckiser (Buprenex; Richmond, VA).
(c) Dental acrylic: Lang Dental (Jet; Wheeling, IL).

4. Detailed procedure

4.1. Anesthesia

Anesthesia was induced by an i.p. injection of 4% chloral hydrate solution at a dose of 400 mg/kg. Additional doses of anesthesia at 40 mg/kg were administered via a polyethylene (PE) tube inserted into the abdominal cavity. The tubing was inserted using an 18-gauge needle to pierce the abdominal wall; the tubing was fitted inside the needle. After the needle was retracted, the tubing was sutured to the skin. The tube was connected, via a blunted 30-gauge needle inserted into the PE tubing, to a syringe filled with chloral hydrate.