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A technique for stereotaxic recordings of neuronal activity in awake, head-restrained mice

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ABSTRACT

Neurophysiological recordings of brain activity during behavior in awake animals have traditionally been performed in primates because of their evolutionary close relationship to humans and comparable behavioral skills. However, with properly designed behavioral tasks, many fundamental questions about how the brain controls behavior can also be addressed in small rodents. Today, the rapid progress in mouse neurogenetics, including the development of mouse models of human brain disorders, provides unique and unparalleled opportunities for the investigation of normal and pathological brain function. The development of experimental procedures for the recording of neuronal activity in awake and behaving mice is an important and necessary step towards neurophysiological investigation of normal and pathological mouse brain function. Here we describe a method for stereotaxic recordings of neuronal activity from head-restrained mice during fluid licking. Fluid licking is a natural and spontaneous behavior in rodents, which mice readily perform under head-restrained conditions. Using a head-restrained preparation allows recordings of well-isolated single units at multiple sites during repeated experimental sessions. Thus, a large number of neurons can be tested for their relationship with behavior and detailed spatial maps of behavior related neuronal activity can be generated as exemplified here with recordings from lick-related Purkinje cells in the cerebellum.

1. Introduction

A central goal of brain research is to understand how the brain controls behavior and how the brain's ability to generate normal behavior is affected by brain disorders. Studies of behavior related neuronal activity in awake and behaving animals yield the most promising method of accurately assessing the complex interactions of multiple cell types and networks during the generation of behavior. Although traditionally a domain of primate research, many fundamental questions about normal and pathological brain function can be successfully studied in small rodents. In fact, the development of genetic mouse models of human brain disorders now offers unparalleled opportunities to study the neuronal and behavioral defects associated with brain disorders. While the use of anesthesia alters the brain's mode of operation, sometimes producing results irrelevant for our understanding of the function of the conscious brain (Schonewille et al., 2006; Bengtsson and Jorntell, 2007), recordings in awake, behaving mice avoid this issue.

Recordings of neuronal activity in awake mice have been successfully performed with permanently implanted electrodes to study hippocampal long term potentiation (LTP) based on local field potentials (LFPs) and multi-unit signals (Errington et al., 1997; Davis et al., 1997; Jones et al., 2001; Koranda et al., 2008). Advantages of this established technique are that it allows users to make long term observations of neuronal activity at one location and animals can behave more naturally as they move around freely. Limitations include that implanted electrode recordings are not suitable for mapping studies and that stable single unit recordings are more reliably and easily obtained in a head-restrained paradigm because of reduced tissue movements and because micromanipulators can be used for micrometer precise electrode placement. Furthermore, not all brain areas are as amenable to recordings with implanted electrodes as the neocortex and particularly the hippocampus. We attempted to use implanted electrodes in the cerebellum and failed to get stable single unit recordings. Anatomical examination of the brains revealed large lesions around the implanted electrodes, which may have resulted from movement of the cerebellar tissue relative to the stationary electrodes (unpublished observations). Using the head-restrained preparation described here overcomes these problems and additionally allows the use of optical imaging methods and intracellular recording techniques in the awake mouse. Optical and physiological recordings from head-restrained mice have been performed by a small number of labs (Cheron et al.,

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2004; Goossens et al., 2004; Schonewille et al., 2006; Ferezou et al., 2007) and so far no comprehensive description of the experimental procedures involved is available in the literature. The experimental technique described here was initially developed to perform cerebellar recordings in awake behaving rats (Heck et al., 2002, 2007) and has been adapted for the performance of stereotaxic recordings from behaving mice during head fixation. The behavioral spectrum of head-restrained mice is limited, but the animals will perform spontaneous orofacial behaviors such as whisker movements (Ferezou et al., 2007) and rhythmic fluid licking (Hayar et al., 2006). These behaviors also yield a large number of repetitions, which is an important prerequisite for electrophysiological investigations of the underlying neuronal processes. Here, we investigated the neuronal representation of rhythmic fluid licking behavior in the simple and complex spike activity of Purkinje cells in the mouse cerebellar cortex. Fluid licking has been studied in a variety of contexts, including studies of taste preference (Lewis et al., 2005), chronic or acute drug treatment (Hsiao and Spencer, 1983; Genn et al., 2003), and to phenotype mouse models of autism spectrum disorders such as Angelman syndrome (Heck et al., 2008).

The rhythm or inter-lick-interval (ILI) of fluid licking in mice is strain-specific (Horowitz et al., 1977; Boughter et al., 2007). The experimental paradigm described here can therefore be useful for the investigation of strain differences in the neuronal mechanisms controlling the licking rhythm. This approach can be readily adapted for recordings from other brain areas of the mouse while monitoring either licking or other behaviors, like movements of the mystacial whiskers.

2. Materials and methods

2.1. Surgical procedures and head-fixation assembly

All experiments adhered to procedural guidelines approved by the University of Tennessee Health Science Center Animal Care and Use Committee. Principles of laboratory animal care (NIH publication No. 86–23, rev. 1996) were followed.

The following procedures were successfully performed with adult mice from four different strains (C57BL/6J, DBA/2J, Cbln1$^{-/-}$ and Lurcher mice). Here we present data examples obtained from C57BL/6J mice. Prior to surgery, mice were weighed and then anesthetized with isoflurane or tribromoethanol (Avertin). Tribromoethanol was used for anesthesia in Lurcher mice because this strain had a very low survival rate with isoflurane. With Tribromoethanol as anesthetic (250 mg/kg, IP), survival rates for Lurcher mice went up to around 90%. For all other strains, that tolerated it well, isoflurane was used because duration and depth of the anesthesia could be more accurately controlled with a vaporizer. Isoflurane anesthesia was induced in a chamber filled with a mixture of 3% isoflurane in oxygen (isoflurane from Baxter Pharmaceutical Products, Deerfield IL) and maintained during surgery (via nose-cone) at 1.0–2.5%. Isoflurane concentration was controlled with a vaporizer (Ohio Isoflurane vaporizer, Highland Medical Equipment, CA). Survival rates dropped markedly if the surgeries lasted significantly longer than 1 h. Thus, considerable effort was made to reduce the time in surgery through careful preparation and practice of all critical steps. Also, during the last 10–15 min of the surgery, i.e. application of the acrylic cement and injections of fluid supplement and analgesic, isoflurane concentration was always lowered to 1%. This measure reduced the time it took the mice to wake up from anesthesia.

The anesthetized mouse was mounted in a stereotaxic instrument with non-rupture ear bars (*Zygoma ear cups*, David Kopf Instruments, Tujunga, CA). Body temperature was monitored with an electronic rectal thermometer and maintained at 37–38.0 °C using a feedback controlled heating pad (FHC Inc., Bowdoinham, ME). The scalp hair at the surgical site was removed with a depilatory and the skin was treated with iodine solution (Xenodine, Veterinary Products Laboratory, Phoenix, AZ). A scalpel was used to make a 1–1.5 cm long midline incision in the scalp. The skin was retracted to expose the skull bone which was then cleaned of all tissue with a dental scraper and sterile cotton swaps. Three holes were drilled in the parietal and interparietal bones at the approximate positions indicated by the arrowheads in Fig. 1, using a 0.7 mm spherical stainless steel burr (SS White, Lakewood, NJ). Small machine screws (1/8″ dome head, 0.8 mm diameter, 2 mm long, part number: MX-000120-01B, Small Parts, Inc., Florida, USA) were carefully fastened about 1 mm deep into the holes. To provide access to the brain area of interest, a circular opening of 3–4 mm diameter was drilled into the lateral interparietal bone, exposing part of the cerebellar hemisphere. The same size drill bit was used to drill the screw holes and the skull opening. If bleeding occurred, it was immediately stopped with either an electric cauterizer (Aaron medical, St. Petersburg, FL) or Gelfoam (Pfizer Inc., NY, NY). Great care was taken to leave the dura intact. Immediately after removing the bone the dura was covered with Triple Antibiotic Ointment (Walgreens, US) to prevent drying and infections.

A cylindrical plastic recording chamber (0.45 cm diameter and 8 mm height) was fashioned from a drinking straw. Small curved scissors were used to shape the bottom of the chamber to fit the curvature of the bone. The chamber was treated with 100% ETOH, dried, and placed over the skull opening. A few drops of Vetbond tissue adhesive (3 M St. Paul, MN) were applied to where the bottom rim of the chamber touched the skull bone. This served to hold the chamber in place until it would eventually be embedded in acrylic cement. The chamber was then filled with Triple Antibiotic Ointment, which would seal the chamber, keep the dura moist and prevent infections. The Triple Antibiotic Ointment had to be removed for each recording session and was replaced with new ointment upon the end of each session.

A stereotaxic manipulator was used to place a custom-made aluminum headpost (Fig. 2D) in a stereotaxically defined position, relative to Bregma. The right caudal corner of the post was placed at Bregma as shown in Fig. 1. Thus, the corresponding corner on the upper end of the headpost served as a stereotaxic reference for electrode placements during awake recordings. Finally, the chamber and headpost were secured into place with dental acrylic (Teets methyl methacrylate denture material; CoOral-Lite Mfg. Co., Diamond Springs, CA, USA). At the end of surgery, mice were subcutaneously (SC) injected with 8 mg/g of the analgesic butorphanol tartrate (Torbugesic, Fort Dodge, USA) to alleviate pain. Body fluid was also supplemented towards the end of the surgery with subcutaneous injections of 0.5 ml of lactated Ringer's solution. For the first hour after surgery mice were kept underneath a 250 W infrared heating lamp (at a distance of 50 cm) to prevent a drop in body temperature during the recovery period. Butorphanol tartrate injections were repeated the next morning. Following surgery, mice were individually housed.

2.2. Training and experimental procedures

After a 3–4-day recovery period mice were adapted to the head-restrained experimental situation during 2 sessions of head fixation of 15 and 30 min duration performed on the same day at 8 a.m. (15 min session) and 4 p.m. (30 min session). During these sessions the head was held fixed and the body was covered with a loose fitting plastic half-tube (5 cm diameter, 10 cm long) to limit body movements (Fig. 1). Mice typically adapted to the head fixation within 2–3 sessions as judged from observations of markedly reduced walking and running movements during the third session compared to the first or second. After completing the adaptation