Journal of Neuroscience Methods 177 (2009) 60–66

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

A method for recording single-cell activity in the frontal-pole cortex of macaque monkeys

Andrew R. Mitz[a,*], Satoshi Tsujimoto[a], Arthur J. MacLarty[b], Steven P. Wise[a]

[a] SN/Laboratory of Systems Neuroscience, National Institute of Mental Health, MSC 4401, 49 Convent Drive, Building 49, Room B1EE17, Bethesda, MD 20892-4401, USA
[b] Veterinary Medicine and Resources Branch, National Institute of Mental Health, MSC 4415, 49 Convent Drive, Building 49, Room 1B80, Bethesda, MD 20892-4415, USA

ARTICLE INFO

Article history:
Received 4 August 2008
Received in revised form
23 September 2008
Accepted 23 September 2008

Keywords:
Neurophysiology
Prefrontal cortex
Primate
Single-unit recording
Frontal air sinus
Behavioral neurophysiology

ABSTRACT

Neurophysiological research has explored most of the prefrontal cortex of macaque monkeys, but the relatively inaccessible frontal-pole cortex remains unexamined. Here we describe a method for gaining access to the frontal-pole cortex with moveable microelectrodes. The key innovation is a direct approach through the frontal air sinus. In addition, the small size of the frontal-pole cortex in macaques led to the design of a smaller recording chamber than typically used in behavioral neurophysiology. The method has proven successful in two subjects, with no adverse health consequences.

Published by Elsevier B.V.

1. Introduction

After the advent of single-cell recordings from awake, operantly conditioned monkeys (Evarts, 1965), the prefrontal cortex became one of the first brain regions studied with this method, often called behavioral neurophysiology (Kubota and Niki, 1971; Fuster and Alexander, 1971; Fuster, 1973). Over the subsequent decades, most of the areas composing the prefrontal cortex (Fig. 1A) have been explored by behavioral neurophysiologists, with the notable exception of the frontal-pole cortex. The frontal-pole cortex, called area 10 by Walker (1940) (Fig. 1A), is the region of frontal cortex that expands most dramatically during primate evolution (Semendeferi et al., 2001). It is, therefore, of considerable interest from a comparative perspective, alone.

In addition, evidence from neuroimaging and clinical neuropsychology in humans suggests that the frontal-pole cortex contributes to several aspects of high-order cognition, including the establishment of task sets, control of hierarchically complex behaviors, prospective coding and deferral of goals, mediation of internal versus external influences on cognition, integration of independent neural computations, detection of behavioral outcomes, generation of unconscious decisions, and evaluation of self-generated knowledge (Ramnani and Owen, 2004; Burgess et al., 2007; Badre, 2008). In monkeys, this area has long been known to have reciprocal connections with much of the remainder of the prefrontal cortex, as well as with the temporal pole and a limited number of additional cortical areas (Jones and Powell, 1970), a finding that has been confirmed recently (Rempel-Clower and Barbas, 2000; Petrides and Pandya, 2007).

Despite this knowledge from anatomy, brain imaging, and neuropsychology, much remains unknown about the functions and mechanisms of the frontal-pole cortex, and no neurophysiological studies have been devoted to it. One reason for this omission is that this area is relatively inaccessible in macaque monkeys because it lies beneath the frontal air sinus (Fig. 1B), a reticulated, boney air space embedded within the brain case at its rostral margin. This structure is typically 15 mm or more in thickness, and it covers the frontal-pole cortex from dorsal, lateral and medial angles of approach. The function of these and other postnasal air sinuses remains uncertain, but regardless of their function a compromise of the barrier between the air sinus and the meninges could have adverse health consequences for the monkeys, notably in the form of microbial infections. The most direct surgical approach for providing microelectrode access to the frontal-pole cortex, a complete penetration through the frontal air sinus, involves this potential risk. Accordingly, we evaluated the safety and efficacy of

* Corresponding author. Tel.: +1 301 402 5573; fax: +1 301 402 5441.
E-mail address: arm@nih.gov (A.R. Mitz).

0165-0270/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.jneumeth.2008.09.032

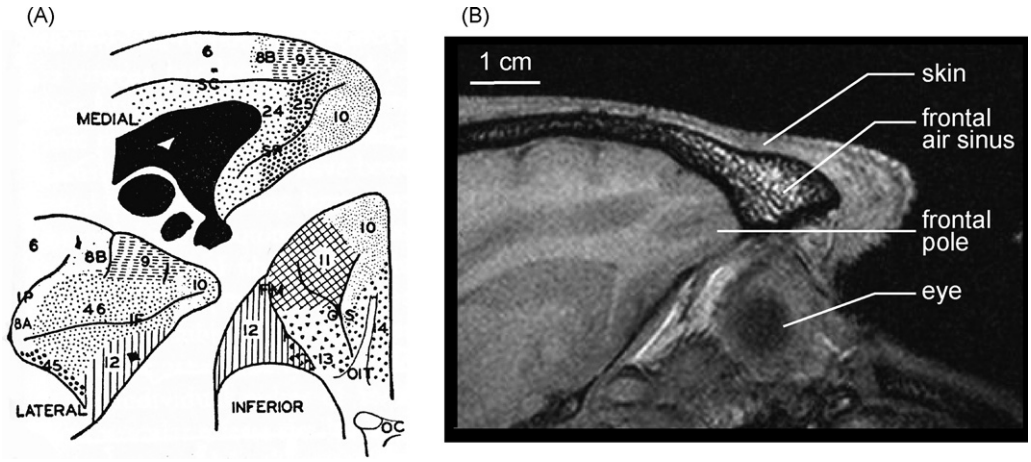

Fig. 1. The frontal-pole cortex in macaque monkeys and its coverage by the frontal air sinus. (A) Cytoarchitectonic map of Walker (1940) showing a medial view (top), a lateral view (bottom left) and a ventral view (bottom right) of the frontal lobe. Areas are designated by number and their extent indicted by the various fill patterns. (B) Magnetic resonance image in the parasagittal plane, showing the frontal air sinus of a rhesus monkey in relation to the frontal pole.

this direct surgical approach. In addition, commercially available recording chambers are either too large or shallow for frontal-pole recordings. We therefore devised an appropriate recording chamber, which required modification of our microelectrode manipulator.

2. Materials and methods

2.1. Subjects

Two male rhesus monkeys (*Macaca mulatta*) served as subjects in this project. The first monkey weighed approximately 10 kg and was 10 years old at the time of the recordings. The second monkey weighed approximately 11 kg and was 9 years old. The research program and procedures used here were approved in advance by the Animal Care and Use Committee of the National Institute of Mental Health. In this initial neurophysiological investigation of the frontal-pole cortex (Tsujimoto et al., 2008), we used a direct surgical approach, making a defect though the entire frontal air sinus, exposing the dura mater covering the frontal pole. A modified recording chamber was then cemented into place within that craniotomy and, subsequently, we obtained successful single-cell recordings by inserting moveable microelectrodes into the cortex of the frontal pole. Both monkeys maintained good health throughout the daily recording sessions, which combined with additional recordings from the other parts of the prefrontal cortex, lasted 2–3 months in each monkey.

In our study, the monkeys were operantly conditioned preoperatively to perform two variations of the strategy task used by Genovesio et al. (2005) and a control task. Accordingly, they were placed on a fluid-controlled diet and worked for fluid reinforcement. All monkeys maintained their weight at greater than 90% of their preoperative baseline, which provides motivation to perform the tasks without appreciable physiological stress. The monkeys began each trial by fixating a small white spot on a video monitor. After a period of steady fixation, two unfilled white squares appeared, one to the left and one to the right of the fixation point. After the fixation point disappeared as a "go" signal, the monkey's task was to make a saccadic eye movement to one of the two squares. If, according to the rules in place for any given task, the monkey chose the correct square and maintained fixation on it for 0.5 s, a 0.1 ml fluid reward was delivered. The monkeys worked for hundreds of such trials each day.

2.2. Surgery

We describe here the surgical methods unique to the present method of accessing the frontal-pole cortex. In all other respects, standard surgical procedures were employed. Each monkey was prepared for surgery with standard methods. The monkey was then placed in a stereotaxic frame, the head oriented in the Horsely–Clark planes, surgically cleaned and draped. An incision was then made along the midline from 0.5 cm rostral to the frontal air sinus for 6–7 cm caudally. The temporal muscle on the right side was displaced mechanically, but no muscle was removed.

After removal of soft tissue and cleaning the cranial surface with a periosteal elevator, an 18 mm (outer diameter) trephine was used to make a craniotomy, displaced 2–3 mm from the midline laterally and oriented at an angle of 63° from the horizontal. The precise angle is not critical, but it must be shallow enough to permit entry into the frontal pole. A range of ±2° is sufficient for this purpose. Special care was taken to ensure that the angle remained reasonably constant and that the trephine had not exposed dura mater or compromised the underlying orbital bone. In a practice surgical procedure, the surgeon made a small, unintentional defect in the orbit. The reason that the orbit can be damaged without injury to the cerebral cortex is that the rostrodorsal aspect of the orbit at its medial extent is overlain by the air sinus and not by the brain. Otherwise, there was nothing remarkable about the progress of the craniotomy as the trephine cut through the porous, reticulated mass of the frontal air sinus, except its depth. After the trephine had cut through the sinus, bone shelves and a small amount of additional bone were removed with a rongeur.

When the craniotomy was complete, a 10.65 mm (inner diameter) recording chamber (described below) was placed within it. Next, 7–10 small holes were drilled in the skull (#2 stainless steel burr), and titanium bone screws were inserted along the caudal and medial edges of the chamber (1.5 TI cortex screw, 6 mm). In addition, a small threaded anchoring post (described below) was positioned 2.5 cm from the chamber, mainly laterally but also slightly caudally, to stabilize the microelectrode-drive assembly. Bone screws were also placed near this anchoring post.

Acrylic bone cement was then placed around the recording chamber, taking care to seal the entire circumference of the craniotomy and to mechanically couple the bone screws, chamber, and anchoring post. Measurements were taken to show that the dura mater was between 17 and 20 mm from the top of the chamber,