Published in final edited form as:

J Neurosci Methods. 2009 February 15; 177(1): 60–66. doi:10.1016/j.jneumeth.2008.09.032.

A Method for Recording Single-cell Activity in the Frontal Pole Cortex of Macaque Monkeys

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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.

Keywords

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

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

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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 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.

Materials and Methods

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.

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 degrees 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 degrees is sufficient for this purpose. Special care was taken to insure 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 rostradorsal 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 to 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 mm and 20 mm from the top of the chamber, depending on the exact location within the chamber. The incision was closed with standard procedures. (Note: both monkeys had undergone an earlier procedure to implant a head-restraint device, which was attached to the caudal aspect of the calvarium.)

These procedures should be adapted to each individual monkey. The best approach would be to obtain an MRI scan on each monkey prior to surgery to examine the size and shape of the frontal air sinus. The specific angle of approach and estimated location of recordings can be decided on the basis of those data.

Chamber design and electrode-drive adaptor

Because of the unique trans-sinus implantation method, we had to modify our standard chamber and microelectrode drive to reach the frontal pole through the narrow, but deep access route. The modified recording chamber can be seen in Figure 2 (upper left). The chamber is machined from Ultem™, an autoclavable plastic, to provide a 10.65 mm diameter aperture to the frontal pole. The outside shape is adapted from more common 19 mm ports to fit the smaller craniotomy, which provides good stability against both rotational and radial forces. The top circumference of the recording chamber is grooved for a 14 mm (outer diameter) O-ring. The

O-ring provides an air- and water-tight seal for a lid (Fig. 2, lower right) that is installed between recording sessions. Outside threads of the lid match threads cut into the first 4.5 mm along the inner walls of the chamber. A small-caliber pressure-relief hole, which penetrates the lid, eases both insertion and removal. A #8 hex-head nylon set screw plugs the pressure-relief hold after lid insertion. We modified one lid to establish a dead center marker (Fig. 2, lower left) by pressing a 22 mm length of 18 gauge hypodermic tubing into an enlarged hole, with about half of the tubing above the top of the lid. This tube provides an accurate trajectory for a snug-fitting marker wire or electrode.

Three different snug-fitting positioning plugs can rotate inside the recording chamber aperture (see Fig. 2, upper right for an example). Each positioning plug has a 4.2 mm diameter radial hole and score marks every 15 degrees around the top circumference of the plug. The radial holes for the three plugs are drilled at 0.0, 1.4, and 2.8 mm from its center. Through the use of these offsets and 360 degrees of rotation, the positioning plugs cumulatively provide access to 75.43 mm² of the dura over the frontal pole. The 4.2 mm diameter radial plug holes accommodate the 4.0 mm outer cannula of a 16-electrode Thomas Recording (Giessen, Germany) microdrive, the microdrive used in our laboratory.

In our application of the method, a separate multi-jointed clamp (L. S. Starett Co. model 660 with magnetic base removed, see McMaster-Carr part number 85195A11) attaches from the preamplifier mount of the microdrive to a small (6.4 mm diameter) threaded anchoring post (Fig. 3, lower left). A 9.4 mm adapter threads onto the post to fit the clamp (Fig. 3, lower right, shown upside down), which squeezes the split walls of the adaptor and thus transfers force directly to the base of the post. The upper part of Fig. 3 shows how the clamp end of the multi-jointed clamp compresses the adapter on the threaded anchoring post. This rigid clamp near the chamber stabilizes the microdrive relative to the recording chamber without exerting force on the chamber itself.

Figure 4 shows the microelectrode drive head (Fig. 4A), the adaptors we used to mate the drive to the recording chamber (Fig. 4B), and the implantation angle of the recording chamber relative to the skull (including air sinus), skin, brain, acrylic and an electrode (Fig. 4C). To increase flexibility, we prefabricated outer cannulae for the microelectrode drive and cut it to size later (*e.g.*, the 36 mm tube in Fig. 4A). First, New England Small Tube Corporation (Litchfield, NH) provided custom 0.156" outer diameter $\times$ 0.16" wall (3.96 $\times$ 4.06 mm) 304 stainless steel tubing. Then a computerized (CNC) drill was used to make 16 holes into a 3 mm thick stainless steel disk cut from 1/8" (3.18 mm) bar stock. The pattern of 9 eccentric, 1 central, and 5 intermediate holes required a #80 solid carbide drill running at 5000 rpm. The peck feed rate was set at 4" (10.16 cm) per minute, and each hole was countersunk both before and after drilling. The disk was attached to one end of the tube using 9 welds (near each eccentric hole) with a Crafford-Laserstar (Riverside, RI) laser welder. Each inner guide cannula (cut to 85 mm in the example shown in Fig. 4A) was sculpted from standard 30-gauge regular-wall 316 stainless steel hypodermic tubing using a Dremel Cut-Off Wheel (#409). An outer tubing length of 36 mm and UltemTM spacers between 1 and 5 mm (3 mm in the example illustrated in Fig. 4B) worked best for our implants. Thomas electrodes of length 144/42 mm (wire/rubber) provided over 15 mm of penetration depth (Fig. 4B, C), although the bulk of our recordings were within a few millimeters of the first activity encountered in each penetration.

Results

Health outcome

We conducted daily recording sessions from the frontal pole cortex for 2-3 weeks in each monkey. Subsequently, a second recording chamber (18 mm inner diameter) was placed more laterally and caudally over the same cerebral hemisphere for access to other parts of the

prefrontal cortex. After 7-11 weeks of additional recording, data collection ended. The first monkey was taken off study for several months after the completion of data collection and had the frontal-pole chamber attached for 8 months. The second monkey had the chamber attached for 3 months. After implantation of the frontal pole chamber, the monkeys' health and well being were monitored carefully. Neither the craniotomy through the frontal air sinus nor any of the other procedures caused any obvious health problems in either monkey. We detected no signs of infection near the recording chambers, the monkeys maintained their weight within a typical range (8.6–9.1 kg for the first monkey; 10.6–11.3 kg for the second), and the monkeys continued to work vigorously for fluid reward.

Neither monkeys showed any abnormalities in their behavior or health status during the period between placement of the frontal pole chamber and their end-of-study euthanasia. Blood work that was done during routine tuberculosis testing was within normal limits and was consistent with blood work done prior to the surgery. This blood work consisted of a complete blood-cell count, which was made up of a total white-blood-cell count with a differential, red-blood-cell count, and a platelet count. The white-blood-cell count differential consisted of neutrophils, lymphocytes, monocytes, eosinophils, basophils, and nucleated red blood cells. All these measures were within normal limits, and thus there was no indication of any systemic infection in either monkey. Along with the red-blood-cell count, measurements of hemoglobin concentration, hematocrit, and reticulocyte numbers were also performed. These measures were also within normal limits, which rules out anemia. Platelet counts were also normal, indicating adequate blood clotting capacity in both monkeys.

Serum biochemical levels were also measured and all were found to be within normal limits. These measures consisted of blood urea nitrogen, creatinine, total protein, albumin, globulin, alkaline phosphatase, alanine transferase, and blood glucose, as well as the electrolytes sodium, chloride, and potassium.

During the time that the frontal pole chamber was implanted, one monkey developed a suspected superficial bacterial infection of the skin around the head-restraint device and was subsequently placed on appropriate antibiotics, but this development was unrelated to the frontal pole chamber.

Both monkeys maintained a good appetite and hydration status and maintained their weight near preoperative levels. This was a good indication that they remained healthy and exhibited no signs of pain or distress during the time that the frontal pole chamber was attached. There was no compromise of muscular control of the upper face or eye lid in either monkey.

Recording quality

Figure 5A shows a structural magnetic resonance image (MRI) illustrating the location of an electrode at the center of the recording chamber, from the second monkey. The electrode's location is indicated by the susceptibility artifact, the dark area in the rostral most cortex (left, arrow). Magnetic resonance imagery at the time of the recordings and later histological analysis confirmed that recordings were made in the frontal pole cortex for both monkeys. Examination of Nissl-stained sections in both monkeys revealed normal cytoarchitecture in the frontal pole, with no visible electrode tracks, which is typical for electrodes from Thomas Recording.

The activity of isolated neurons was collected while monkeys performed one of three tasks, two versions of a strategy task and one control task, here called tasks A, B, and C. At the start of each day, 4 to 13 electrodes were advanced independently until the activity of at least one neuron was well isolated by most of the electrodes. The signal from each electrode was monitored and recorded using a Multichannel Acquisition Processor (Plexon Inc., Dallas, TX), and every unit's isolation was later scrutinized offline using Off Line Sorter (Plexon Inc., see

Fig. 5B, C). For spike sorting, we accepted only individual spikes that had waveforms grouped tightly with other spikes in the time domain (Fig. 5C) and with waveform characteristics that clustered clearly and separately from other potentials in a three-dimensional (3D) parameter space (Fig. 5D). Most often, this 3D space consisted of the first three principal components, although other aspects of the waveform were also used as one of the sorting dimensions, such as the time of peak amplitude or the voltage range of the unit spike.

For monkey 1, frontal pole recordings were performed for 19 days, with 11.9 ± 4.3 (SD) sets of neurons recorded each day. Each set comprised a number of isolated neurons simultaneously recorded with multiple moveable microelectrodes. Over the 19 days, we monitored the discharge activity of 225 sets of neurons, with a mean of 5.0 ± 2.3 neurons per set (up to 12 neurons maximum). The frontal pole cortex of monkey 2 was tested for 13 days, which resulted in a total of 100 recording sets and a mean of 7.7 ± 2.5 sets per day. In this monkey, 5.1 ± 2.9 neurons were recorded per set (14 neurons maximum). Table 1 gives the total number of single-cells recorded in each of the three tasks, and all combinations of these tasks.

As shown in Table 2, each neuron was tested in more than 100 trials on average for task A. During the other tasks, each neuron was also tested for a considerable number of trials. In most cases, recording sessions ended because the neurons were tested sufficiently, not because of problems with recording stability. The number of neurons recorded in two of the three tasks or in all three tasks (Table 1) provides further evidence for the adequacy and stability of the recordings.

Discussion

Only modest changes from standard methods are necessary to obtain high-quality neurophysiological recordings from the frontal pole of awake, behaving monkeys. Recording from the frontal pole interfered with the optics for our overhead camera-based eye tracking system, which required the use of a front-mounted system. Likewise, certain head-holding methods and some microdrive systems will not work with the frontal position of the recording chamber. Otherwise, the mechanical changes were no more challenging than in typical recording arrangements. Our major result is that trans-sinus placement of a recording chamber can be stable and safe.

Appendix

Appendix

Contact information for suppliers

Bone screws

Synthes Cat. #400.006, 1690 Russell Rd., Paoli, PA 19301, 800-523-0322.

Trephine

Galt Skull Cat #98TRE3-3 Trephine, 3/4, Miltex 26-28c, AliMed, Inc., 297 High Street, Dedham, MA 02026, 800-223-1984.

Microdrive and electrodes

Thomas Recording GmbH, Winchester Strasse 8, D-35394 Giessen, Germany.

Data acquisition hardware and spike sorting software

Plexon, Inc., 6500 Greenville Ave., Dallas, TX 75206.

Stock materials and multi-joint clamp

McMaster-Carr, 600 N County Line Rd., Elmhurst, IL 60126

Custom tubing

New England Small Tube Corp., Litchfield Technology Park, 480 Charles Bancroft Hwy., Litchfield, NH 03052.

Laser welder

Crafford-LaserStar Tech., One Industrial Court, Riverside, RI 02915.

Acknowledgments

We thank Mr. James M. Fellows for performing the surgical procedures and additional aspects of animal care and use, Mr. George Dold for expert mechanical fabrication, and Dr. Asif Ghazanfar for comments on the manuscript. Contributions to this paper were as follows: SPW devised the surgical approach, ARM designed the recording chamber and electrode-drive adaptation, ST trained the monkeys and performed the recordings, and AJM evaluated the health of the monkeys. All authors contributed to the preparation of this report. This research was supported by the Intramural Program of the NIH, National Institute of Mental Health.

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Figure 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 indicated 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.

Figure 2.

Recording chamber and chamber parts made from Ultem™ plastic. **Upper left.** Recording chamber. This version of the chamber is 16 mm long and has a 10.65 mm bore. About 2.4 mm of the chamber is below the skirt. Threads are cut inside the chamber just below the O-ring for the caps. **Upper right.** One of three chamber positioning plugs. Each plug fills the chamber, adapts the microdrive to the chamber, and provides a rotational positioning mechanism. Score marks on the top of the plug are in 15 degree increments. The hole of this plug is drilled 1.4 mm off of center. When compressed against the chamber O-ring, the bottom of this plug rides about 1 mm above the bottom of the chamber. However, much shorter plugs can also be used. **Lower left.** Cap with dead-center cannula. When the cap is screwed onto the chamber, an electrode or wire lowered through the cannula will mark the center of the chamber for anatomical registration. **Lower right.** Sealing cap. This cap forms a seal that is both air-tight and water-tight when the chamber is not in use. A nylon screw in the cap can be removed to relieve pressure during insertion or removal of the cap.

Figure 3.

Threaded post assembly with adapter and clamp. **Top.** One end of the clamp that restricts relative movements between the multielectrode drive and the skull. This photograph shows how the clamp locks onto the threaded post assembly, which can be seen both within and below the clamp near the lower left of the image. **Lower left.** Threaded miniature post, which is implanted along with the recording chamber. **Lower right.** Bottom view of adapter, which screws onto the threaded post (to the left). The split part of the adaptor is compressed by the clamp, as shown above.

Figure 4.

Selected dimensions of microdrive and chamber components relative to the skull. **A.** The microdrive head with custom (36 mm) outer cannula and inner cannula (85 mm). 144/42 mm electrodes can reach up to 20 mm beyond the outer cannula. **B.** A spacer (3 mm in this case) is used to raise the microdrive above the positioning plug to match day-to-day changes in the depth of recording. Note the roughly 15 mm penetration range for the electrode. **C.** Location and angle of chamber after implantation. Acrylic bone cement is used to fix the chamber position and seal the frontal air sinus.

Figure 5.

A. MRI documentation of electrode placement. The monkey was scanned to obtain standard anatomical images, T1 and T2, with pulse sequences as follows: SPGR, TE6, TR25, flip angle 30, NEX4, 256-square matrix, FOV 100 mm, 1-mm slices. The figure shows a parasagittal slice from the right hemisphere, approximately 2 mm off the midsagittal plane. The arrow points to the location of the electrode, which was placed in the center of the chamber at the time of the scan. Abbreviations: PFp, frontal pole cortex; Cing, anterior cingulate cortex; Caud, caudate nucleus. **B–D.** Recording quality and stability. **B.** Continuous display of the voltage signal from an electrode. Data during first, middle, and last minute, out of a 15 minute recording session, is shown. Yellow ticks show spikes isolated as a single neuron, with white ticks showing the signals of smaller spikes and noise. **C.** Waveforms for two neurons. All the isolated spikes during first, middle, and last minute of the recordings are shown. Neuron 1 is the same cell as in B and D. Neuron 2 was recorded for 16 minutes from a different monkey. **D.** 3D principal-component (PC) space for spike sorting (same cell as in B and Neuron 1 of C). Each point shows a single spike in 3D-PC space (the first 3 principal components of a principal component analysis). Yellow dots indicate the spikes sorted and accepted as Neuron 1 in C; white dots were rejected.

Table 1
Number of frontal pole neurons recorded in each task. A, B, and C refer to the three tasks performed by the monkeys, with sequential recordings in multiple tasks indicated by combinations of these letters.

	A	B	C	AB	BC	AC	ABC	Total
Monkey 1	256	74	55	89	12	87	77	650
Monkey 2	165	31	13	54	6	43	33	345
Total	421	105	68	143	18	130	110	995

Table 2

Number of trials the monkeys performed during single-cell recordings from the frontal pole cortex, for each task (mean $\pm$ SD). A, B, and C refer to the three tasks performed by the monkeys.

	A	B	C
Monkey 1	105 $\pm$ 41	77 $\pm$ 32	69 $\pm$ 42
Monkey 2	112 $\pm$ 38	96 $\pm$ 21	58 $\pm$ 15