A new method for piercing the tentorium cerebelli for implanting fragile electrodes into the brain stem in the rhesus monkey (*Macaca mulatta*)

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Wu J, Wang W, Rizak JD, Wang Z, Wang J, Feng X, Dong J, Li L, Liu L, Xu L, Yang S, Hu X. A new method for piercing the tentorium cerebelli for implanting fragile electrodes into the brain stem in the rhesus monkey (*Macaca mulatta*). *J Neurophysiol* 111: 1027–1032, 2014. First published December 11, 2013; doi:10.1152/jn.00781.2013.—Recent developments in neuron recording techniques include the invention of some fragile electrodes. The fragility of these electrodes impedes their successful use in deep brain recordings because it is difficult to penetrate the electrodes through the dura mater, especially the tentorium cerebelli (TC) enclosing the cerebellum and brain stem. This paper reports a new method to pierce the TC for inserting fragile electrodes into the inferior colliculus of rhesus monkeys. Briefly, a unique tool kit, consisting of needles with sharp tips, a guide tube and an “impactor,” was used in a multistep protocol to pierce the TC. The impactor provided a brief force that quickly thrusts the needles through the meninges without causing significant damage to the brain tissue under the TC. Using this novel approach, tetrodes were successfully implanted into the inferior colliculus of a rhesus monkey and neuronal discharge signals were recorded. This method, which is simple, convenient and economical, allows neurophysiologists to study the electrophysiological characteristics of deep brain structures under the TC with advanced, albeit fragile, electrodes.

The dura mater is the thickest and outermost layer of the meninges that enclose the central nervous system. The meninges act to support the brain and spinal cord, as well as separate the cerebrospinal fluid from the rest of the body (Greenstein and Greenstein 2000). Consequently, the dura mater is a rigid and tough membrane that poses challenges to electrophysiological studies in live animals.

To insert electrodes in brain structures, the electrodes must first penetrate the dura mater. Often, the electrodes used are fragile and cannot directly penetrate the dura mater, requiring electrophysiologists to either make incisions in the meninges, or use guide tubes (Malpeli et al. 1992), needles (Chen et al. 2001) or cannulas (Matsuzaka et al. 2009) to penetrate the dura and then implant the electrode. However, there are various challenges associated with these conventional methods. First, making incisions has a risk of infection following the surgery (Vällfors et al. 1981). Second, the use of guide tubes, needles or cannulas requires a certain amount of force to breach the dura mater, and this force may cause the dura to deform and compress underlying brain structures before the needle or cannula actually penetrate the dura (Abolhassani et al. 2006; Wittek et al. 2008). Furthermore, when the guide tube or cannula does break through the dura mater, it may be embedded in the brain tissue (Wittek et al. 2008), which risks unnecessary damage to the tissue of interest (Mahvash and Dupont 2009).

These difficulties are only exacerbated when attempting to record neuronal discharges from deep brain structures, such as the brain stem or cerebellum. The meninges, and dura mater, closely outline the brain and separate adjacent brain structures by invaginating between the structures. The two most notable folds or reflections in the dural structure are the falx cerebri and the tentorium cerebelli (TC). The falx cerebri folds into the longitudinal cerebral fissure separating the two hemispheres of the brain, although it poses less of a problem to electrophysiological studies because it can be easily avoided when implanting electrodes through the cranium, whereas the TC is much more problematic because it lies directly upon the deep brain structures of the brain stem and cerebellum and separates them from the occipital lobes of the cerebrum (Greenstein and Greenstein 2000).

Here, a new method is reported that is designed especially to insert fragile electrodes in deep brain structures while passing through the TC. The principle behind the method is straightforward. In brief, a novel apparatus was developed, in which needles of various sharpness were accelerated quickly to a high speed, and the corresponding momentum was used to puncture the TC without causing significant damage to the brain tissue. To accelerate the needles, a novel tool was created from a remodeled blood sampling device. The device was subsequently termed the “impactor,” because its quick recoil would impact the needles and drive them through the dura mater. This approach and the basic apparatus can be used to implant fragile electrodes needed for electrophysiological characterizations into different subjects and/or different deep brain structures or nuclei within the cerebellum or brain stem. A preliminary MRI scan can be used in conjunction with this method to determine the location of the target area and to ensure accurate tetrode implantation and prevent unnecessary tissue damage (Wu et al. 2010).
Materials

Dura mater piercing tool kit. The dimensions of the tool kit were based on the average brain size of the rhesus monkey (Paxinos et al. 1999). The kit can be used in surgeries on different monkeys with varying brain dimensions as the following protocol allows for an individual variation of ±10 mm in piercing depth. The kit consisted of needles, a guide tube, an impactor, and a fragile wire to confirm that the piercing of the dura mater was successful.

Needles. The needles were made of 304 stainless steel. The kit contained five specialized needles (Fig. 1). The names and dimensions of the needles were as follows: 1) TC localization needle [length (l) = 50 mm, diameter (d) = 0.55 mm, with a round blunted tip]; 2) impactor localization needle (l = 10 mm, d = 0.55 mm, with a round blunted tip); 3) piercing needle no. 1 (l = 33 mm, d = 0.55 mm, with a sharp pointed tip: where the length to apex (L) = 4.0 mm); 4) piercing needle no. 2 (l = 32.5 mm, d = 0.55 mm, with a pointed tip: L = 0.8 mm); and 5) piercing needle no. 3 (l = 32.5 mm, d = 0.55 mm, with a pointed tip: L = 0.2 mm). Each needle had a steel cap fixed opposite to the apex. The dimensions of the cap were l = 4 mm and d = 0.8 mm.

The TC localization needle was used to locate the TC and measure the distance between the skull and the TC. The impactor localization needle was used to adjust the precise position of the impactor without damaging the TC or IC. The piercing needles (nos. 1, 2, 3) were used to pierce the dura mater. The tips of the three piercing needles are shown in Fig. 2. The caps prevented the needles from falling in the guide tube and acted as a target for the impactor.

Guide tube. The guide tube was made of 304 stainless steel. Its external dimensions were l = 45 mm and d = 0.64 mm. The guide tube was hollow with an inner diameter of 0.58 mm (Fig. 3). The tip was sharpened into an angle of 45° to lessen the damage to the cortex when implanting it. A circular incision or notch was made by filing around the guide tube at a distance 30 mm from the tip of the tube. The guide tube was anchored to the skull after the TC localization and functioned as a tunnel to guide the needles to the TC. The incision was used as a marker to precisely adjust the guide tube’s length to 30 mm after anchoring it to the skull. The notch could then be easily fractured to provide an accurate depth for piercing the dura mater.

Impactor. The impactor was modified from a blood sampling device (Fig. 4). The tip of the blood sampling device was replaced with a copper cap with a flat bottom. The copper cap could be compressed into the device and could be easily released with the press of a button. The resulting recoil was quick and provided an adequate force, about 3 N, to hit the cap on the needles and to pierce the dura mater with a significant velocity. The complete dura mater piercing

Animals

Three rhesus monkeys (Macaca mulatta, females, 5, 6 and 15 yr old, weighing 5.8, 6.3 and 5.4 kg, respectively) were used in this study for electrode implantation into the IC. The monkeys were obtained from the breeding colonies of the primate center at the Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, China).

Monkeys were housed singly under standard conditions (a 12:12-h light-dark cycle with light on from 0700 to 1900, humidity at 60%, temperature at 21 ± 2°C) in the animal house. Monkeys were fed four times daily a regimen of fruits, nuts and monkey chow.

The monkeys’ feeding schedules were stopped 1 day prior to performing the surgeries (as described in Preliminary MRI Localization Scan, Post-MRI Surgery and Dura Mater Penetration, and Auditory Stimulation sections below). Prior to the surgeries, monkeys were anesthetized with ketamine (10 mg/kg im) and maintained with pentobarbital sodium (20 mg/kg im).

All experimental procedures were approved by the Kunming Institute of Zoology Animal Safety Committee (authorization no. SYDW-2012012) and were conducted in accordance with the guidelines for the National Care and Use of Animals of the National Animal Research Authority of China.
process is outlined in Post-MRI Surgery and Dura Mater Penetration below.

Confirmation wires. Two Teflon-coated platinum iridium wires (A-M Systems) were twisted together. The wires were relatively fragile and were used to determine if the dura mater piercing was successful. Generally, any kind of material used in a laboratory to make an electrode, which is fragile compared with the dura mater, but tougher than the brain tissue, can be used to confirm dura mater piercing. A fragile confirmation wire will bend or break when it is forced in contact with the more rigid dura mater. The Teflon-coated platinum iridium wires, as well as the tips of three piercing needles are shown in Fig. 2.

Preliminary MRI Localization Scan

Magnetic resonance imaging was performed at the Kunming General Hospital of the People’s Liberation Army (Kunming, China) on a 1.5-T scanner (GE). Prior to the MRI scan, the monkeys underwent surgery while mounted on a stereotaxics (RWD Life Science) to anchor three thin glass tubes filled with a paramagnetic solution, Vitamin AD (Qingdao Double Whale Pharmaceutical). The glass tubes were anchored to the monkey’s skull with dental cement (Shanghai Medical Instrument). The three tubes acted as external markers. Two of them were placed along the calvarial sagittal suture, 10 mm in front of and 20 mm behind the bregma, respectively. They were used to adjust the MRI scanning angle by ensuring the sagittal image was scanned along both of them, such that only one particular plane passed through the two parallel lines. The third marker was placed near the estimated target area: the IC of the left hemisphere. The location of the third marker was determined from the stereotaxic coordinates of the rhesus monkey anatomical atlas (Paxinos et al. 1999).

Once the markers were anchored, an MRI scan of the monkey brain was taken with the following parameters: slice thickness = 1 mm; spacing between slices = 1.3 mm; repetition time = 4,000 ms; echo time = 99.96 ms; inversion time = 0; number of averages = 4; and acquisition matrix = 0, 320, 256, 0. The sagittal and coronal MRIs displaying both the IC and its estimated external marker were used to calculate the position and angle needed for implantation of the electrode. This MRI localization method was previously reported (Wu et al. 2010) and offers a localization error of less than 1 mm.

Post-MRI Surgery and Dura Mater Penetration

The monkeys were anesthetized with ketamine (10 mg/kg im) and maintained with pentobarbital sodium (20 mg/kg im) while performing the surgery. Each monkey’s head was mounted on a stereotaxic frame. The scalp and muscles overlying the skull were incised and retracted to give access to the skull bone. The surface of the skull was cleaned with 2% hydrogen peroxide and dried thoroughly before continuing. The coordinates of the left IC determined from the MRI localization were used to accurately locate the region on the skull that provided the best approach to implant the electrode in the IC. An implantation hole (1.2 mm) was drilled through the skull, exposing the dura mater that covers the cortex. A needle was used to pierce through this first layer of dura mater.

Step 1 (Fig. 5A). Then the guide tube was positioned in the hole on top of the pierced dura mater layer. The guide tube was held in place by a standard electrode carrier (RWD Life Science) that clapsed the guide tube above the incision notch located 30 mm from its tip. The TC localization needle was inserted into guide tube and was pushed down until the needle’s cap was flush with the end of the tube. This allowed the extra 5 mm of length of the TC localization needle to protrude into the hole in the dura mater. Once the TC localization needle was positioned in the implantation hole, the dorsoventral coordinate of the guide tube and TC location needle were adjusted and recorded by the electrode carrier such that they were perpendicularly aligned with the IC.

Step 2 (Fig. 5B). Then the guide tube and the TC localization needle were driven down into the cortex by the electrode carrier. The TC localization needle was free inside the guide tube and was used to penetrate the brain tissue. When they reached the second layer of dura mater at the base of the cortex and on top of the IC, the blunt end of the TC localization needle prevented it from puncturing the TC. As the electrode carrier continued to embed the guide tube into cortex, the TC localization needle was impeded, and a small space (<3 mm) between the needles cap and the end of the guide tube appeared. This movement allowed the apparatus to detect precisely when it reached the surface of the TC. Once the TC had been identified, the guide tube’s progress was stopped.

Step 3 (Fig. 5C). The guide tube was then retracted (~2 mm) such that it was again flush with the cap of the TC localization needle, and the TC localization needle tip was still in contact with the TC. The dorsoventral coordinate was recorded again by the electrode carrier, and the distance between the TC and the skull surface was calculated by subtracting the two recorded coordinates (distance = TC coordinate − skull coordinate). This measurement was used to determine the implantation depth of the confirmation wire and electrodes.

Step 4 (Fig. 5D). The TC localization needle was removed, and the guide tube was then driven down another 3 mm. This adjustment left the space between the TC and the guide tube tip to be ~2 mm because the TC localization needle was 5 mm longer than the guide tube. Then the guide tube was anchored on the skull with dental acrylic, maintaining the distance between the guide tube tip and the TC at ~2 mm. The end of the tube was broken away at the incision notch, such that the length of the remaining tube was 30 mm, and the distance from the end of the tube to the TC was 32 mm.

Step 5 (Fig. 5E). The impactor localization needle was put into the guide tube until its cap was flush with the end of the tube. The impactor was held by the electrode carrier, and its location was adjusted until the copper tip of the impactor squarely faced the bottom image of the guide tube's progress. The impactor was held by the electrode carrier, and its location was adjusted until the copper tip of the impactor squarely faced the bottom image of the guide tube's progress.
accelerate the needle to pierce the TC. The tip of piercing needle no. 1 had a very sharp point \((l_1 = 4.0 \text{ mm})\) which would create a very fine hole in the TC. To increase the size of the puncture hole, piercing needle no. 1 was replaced with piercing needle no. 2. The length of piercing needle no. 2 \((32.5 \text{ mm})\) allowed it to rest on the TC while having its cap \(~0.5 \text{ mm above the guide tube. The length to apex of the needle’s tip was } 0.8 \text{ mm, such that its broader aspect would widen the puncture hole when the impactor struck piercing needle no. 2, without damaging the IC below.} \) This process was repeated with piercing needle no. 3 \((l = 32.5 \text{ mm, } l_a = 0.2 \text{ mm})\) to further increase the size of the puncture hole in the TC. In total, the impacting process was performed one time only with piercing needle no. 1 to prevent the dura mater from being pierced in different places by the sharp tip. However, the piercing process with needles nos. 2 and 3 was repeated a few times to ensure a wider hole had been made in the dura.

To confirm that the puncture hole was successfully created by the piercing procedure, the Teflon plated platinum-iridium confirmation wire was carefully inserted by the electrode carrier into the brain through the guide tube until it reached the depth of the TC. The wire was driven down to and gently pressed against the TC and then removed from the tube. The tip was visually inspected under a microscope to determine whether it was straight or bent. A straight tip indicated that a puncture hole had been successfully made in the TC as the wire had passed through it, whereas a curled or bent tip indicated the dura mater was still intact and had impeded the wire by bending the tip. In such cases, the piercing apparatus was adjusted, and the process was repeated until a successful puncture was found. After the TC was successfully pierced, a tetrode made of Teflon-coated platinum iridium wires \((A-M \text{ Systems})\) was passed down the guide tube through the puncture hole in the TC and then implanted into the IC.

**Auditory Stimulation**

Monkeys were anesthetized with ketamine \((10 \text{ mg/kg im})\) and maintained with pentobarbital sodium \((20 \text{ mg/kg im})\) while being restrained in a primate chair. Electrodes were implanted in the IC as described above. A sound stimulation, \(1,000 \text{ Hz in frequency, 50 ms in duration, and 95 dB in intensity, was generated by the series Function Generator AFG3000 (Tektronix) and presented by a D3004 loudspeaker (Scan Speak, Videbaek). The auditory stimuli were presented to the animals at 1- to 2-s random intervals.**

**Neuronal Discharge Recording**

A tetrode made of Teflon-coated platinum iridium wires \((A-M \text{ Systems})\) was implanted into the IC. Neuronal discharge signals in the IC were recorded with a PXI-4498 Dynamic Signal Acquisition Module \((\text{National Instruments})\). Signals were improved by reducing background noise with an amplifier and analyzed with LabVIEW \((\text{National Instruments})\). Signals were used to demonstrate successful implantation and recording from an electrode after surgery.

**Histology**

Following the neuronal recording, one monkey \((\text{female, 15 yr old, weighing 5.4 kg})\) was euthanized with an overdose of pentobarbital, and then the animal was perfused with 4% paraformaldehyde. The animal was decapitated, and the skull was sawed along the calvarial sagittal suture to expose the left hemisphere. Then it was dissected with a scalpel to reveal the guide tube, the tetrode, the TC and IC. The dissection was used to assess the path of the guide tube and electrode through the TC and to evaluate the condition of the IC after electrode implantation.
METHOD TO PIERCE THE TENTORIUM CEREBELLI IN RHESUS MONKEYS

A portion of the neuronal discharge signal recorded is displayed in Fig. 6. Discharge signals of approximately 100 μV were recorded by the tetrode. Multiple electrical discharges were found which corresponded to the administration of the sound stimuli with neuronal discharges. The vertical axis unit of \( A \), \( B \), and \( C \) are in Volts. Horizontal axis (\( A \), \( B \) and \( C \)) is time (seconds). The vertical axis of \( A \) does not reflect the intensity of the sound stimuli directly. The intensity was measured by a Digital Sound Level Meter AR814 (Smart Sensor, Hongkong, China). Neuronal discharges displayed in \( B \) are synchronous to the time of the sound stimulation displayed in \( A \). Black arrows indicate the same neuronal discharges in \( B \) and \( C \).

**DISCUSSION**

Recent developments in neuron recording techniques have led to the use of a number of diverse types of electrodes. Some examples of these electrodes include silicon-based multichannel electrodes (Brozoski et al. 2006; Miller et al. 2004; Nordhausen et al. 1994), glass pipettes (Thiele et al. 2006), carbon fiber electrodes (Millar and Pelling 2001), wire-bundle electrodes such as tetrodes (Aur et al. 2005; Liao et al. 2011), etc. It is hopeful that the use of these advanced electrodes will bring important new findings to the neurophysiological and neuropharmacological fields that are currently limited by conventional metal electrodes. However, application of these “next generation” electrodes has not been widespread because the electrodes are fragile and are easily broken when inserting them through the dura mater. This problem is exacerbated when probing deep brain structures, especially when the TC needs to be breached. The TC is generally a tough and rigid membrane whose location varies between subjects and is invisible during the implantation surgery.

In this paper, a new method that is convenient, economical, and universally available to most laboratories for the implan-
tation of electrodes in deep brain structures is reported. Using this method, tetrodes were inserted into the IC by successfully passing through the TC, and neuronal discharges were subsequently recorded.

To accurately insert the electrodes, the precise location of the IC was identified using an MRI localization method which was previously developed in our laboratory (Wu et al. 2010). Then a precise surgery was performed to pierce the TC using a novel tool kit, containing 5 needles, a guide tube and an impactor. The five needles included the TC localization needle, an impactor localization needle and three sharp needles with different tips named piercing needle nos. 1, 2 and 3. The length to apex of each tip was 4.0 mm (no. 1), 0.8 mm (no. 2) and 0.2 mm (no. 3). Briefly, the method consisted of first using the TC localization needle to orient the piercing needles above the TC. Then piercing needle no. 1 was used to pierce the TC by quickly accelerating it with a quick impact force provided by the impactor. The slightly broader tips of piercing needles nos. 2 and 3 were subsequently used to enlarge the puncture hole created by piercing needle no. 1.

The impactor was modified from a blood sampling device to provide the quick acceleration burst (about 3 N) needed to drive the needles into the dura mater and quickly pierce it without damaging the brain tissue (IC) beneath it. To adjust the impactor over the guide tube, a short (10 mm) impactor localization needle was used. Its short length allowed the impactor to be tested on a needle cap, without driving a needle into the TC or IC. Furthermore, piercing needles nos. 2 and 3 were also designed 0.5 mm shorter than piercing needle no. 1 to prevent their broader tips from causing further damage to the IC.

This new method proved effective and reliable in implanting fragile electrodes into deep brain structures, like the IC, while passing through the TC. Going forward, it will enable researchers to address the characteristics of neural structures which lie in the brain stem or cerebellum with advanced and modern electrodes.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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