Studying task-related activity of individual neurons in the human brain

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Single-neuronal studies remain the gold standard for studying brain function. Here we describe a protocol for studying task-related single-neuronal activity in human subjects during neurosurgical procedures involving microelectrode recordings. This protocol has two phases: a preoperative phase and an intraoperative phase. During the preoperative phase, we discuss informed consent, equipment setup and behavioral testing. During the intraoperative phase, we discuss the procedure for microelectrode recordings. Because patients are often awake during these procedures, this protocol can be performed in conjunction with behavioral tasks for studying a variety of cognitive functions. We describe the protocol in detail and provide two examples of expected results. In addition, we discuss the potential difficulties and pitfalls related to intraoperative studies. This protocol takes ~1.5 h to complete.

INTRODUCTION

The development and refinement of single-neuronal recording techniques from the 1920s through the 1950s, first with intracellular glass microelectrodes[1,2] and subsequently with extracellular metallic microelectrodes[3–5], revolutionized the field of neurophysiology. Pioneering efforts in the 1960s heralded the era of single-neuronal recordings in humans[6–9]. By applying extracellular microelectrode recording techniques to the developing field of stereotactic neurosurgery, these investigators were able to precisely target and study the physiology of deep brain nuclei. Initial efforts were focused on mapping individual thalamic nuclei during ablative procedures (thalamotomy) for patients with Parkinson disease (PD). Subsequently, these techniques have been applied to targeting a variety of other subcortical nuclei, including the subthalamic nucleus (STN) and pallidum, as well as the cingulate cortex and ventral striatum.

Currently, microelectrode recordings have widespread use in stereotactic neurosurgery, for both ablative procedures and deep brain stimulation (DBS). They provide physiological information that helps to confirm and to refine the trajectory of the lesioning probe or DBS macroelectrode. Relying on anatomical targeting with stereotactic coordinates alone can lead to inaccurate positioning because of brain shift resulting from cerebrospinal fluid (CSF) loss, imaging distortion caused by magnetic field inhomogeneities and other sources of error[10,11]. Physiological mapping with microelectrode recordings allows the neurosurgeon and neurophysiologist to measure the discharge patterns of structures along the expected trajectory, as well as of the target itself[12]. For example, the trajectory to the ventral intermediate (Vim) nucleus of the thalamus, the preferred target for patients with essential tremor, courses through the striatum, with its characteristic low-frequency phasic activity. The trajectory may terminate just posterior to the Vim in the ventral caudal (Vc) nucleus, the principal somatosensory nucleus, containing neurons with sensory receptive fields. The trajectory to the STN, a common target for DBS in PD patients, usually passes through the caudate and thalamus, with their characteristic neuronal signatures, and terminate in the dorsolateral STN, a structure with many active neurons and responses to active or passive joint movements. Current applied through the microelectrode permits microstimulation, which can be used to test for unwanted motor pathway activation due to inadvertent proximity to the internal capsule, which lies just lateral to Vim and STN. The globus pallidus internus (GPi) may be distinguished by the predominance of tonic, high-firing rate neurons, and the presence of visually responsive neurons just inferiorly, in the optic tract.

The benefits of physiological targeting using microelectrode recordings have made them a mainstay in many functional neurosurgery programs. In addition to their clinical importance, they also provide a unique opportunity for studying brain function with a resolution previously unattainable. The ability to record from individual neurons in the human brain represents a great leap forward from noninvasive methods such as scalp electroencephalography and magnetoencephalography. The identification of Vim neurons whose spontaneous discharge is synchronous with the patients' tremors helped in establishing the nucleus as the target rather than more anterior thalamic nuclei[7,13]. As another example, studies comparing spontaneous discharge patterns of the Parkinsonian human STN and the normal and Parkinsonian nonhuman primate brain (i.e., the l-methyl-4-phenyl-l,2,3,6-tetrahydropyridine model) have shed light on the role of the pathophysiological oscillations observed in PD[14,15].

In addition to spontaneous firing patterns, task-related evoked neuronal activity has provided a wealth of information about human brain function. By engaging subjects in an appropriately designed behavioral task, the specific function of individual neurons in a particular brain region can be interrogated. Although there are important limitations inherent to task-related intraoperative recordings,

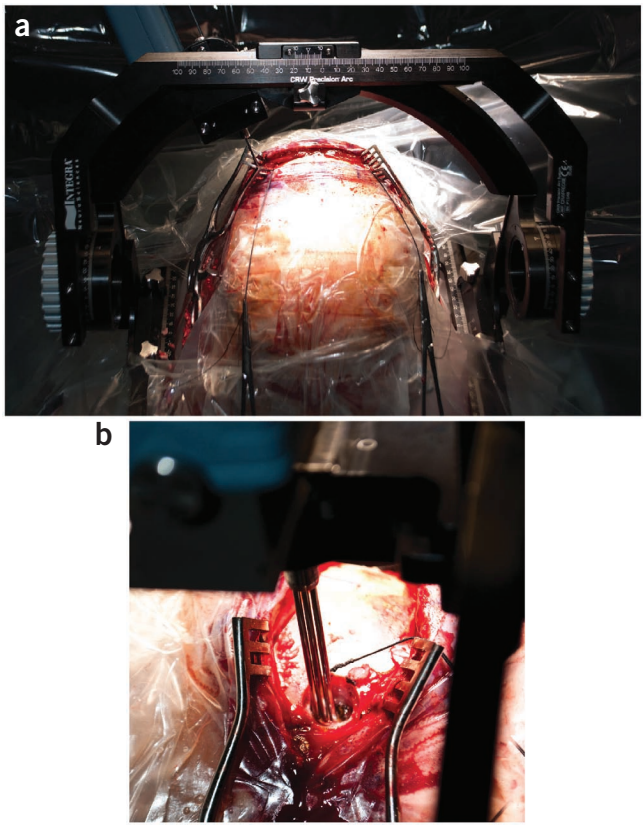

Figure 1 | Photographs of intraoperative patient orientation and cannula placement. (**a**) Photograph of stereotactic frame positioned on the patient cranium. (**b**) Close-up image of three linearly placed cannulae within the burr hole. Three microelectrodes will be advanced in the predetermined trajectory toward the target brain region.

Figure 2 | Forward view of intraoperative physiology rig. Individual components are numbered. (1) Storage space for presentation monitor, mounting arm and input devices. (2) Acquisition system monitor. (3) Behavioral system monitor. (4) Research acquisition system (Cambridge Electronic Design (CED) PowerLinc 1401, Plexon Omniplex). (5) Breakout box for behavioral system (National Instruments BNC-2090a). (6) Computer running acquisition software. (7) Computer running behavioral software (MonkeyLogic).

including limited time, neuronal drift and subject fatigue, they do permit the investigation of uniquely human cognitive processes with a precision unmatched by functional MRI or other methodologies. Previous studies have investigated the role of the basal ganglia in motor planning[16] and of the substantia nigra in reward expectation[17]. We recently demonstrated the function of the cingulate cortex in processing cognitive interference[18], of the nucleus accumbens in financial decision making[19] and of the dorsolateral prefrontal cortex in abstract rule encoding[20].

Experimental design

As the field of functional neurosurgery continues to grow and the clinical indications and surgical targets continue to expand, opportunities for research will rapidly increase. The purpose of this protocol is to describe the methodology for performing acute, intraoperative, task-related single neuronal recordings in consenting human subjects. We assume that this protocol will be performed by an experienced clinical team consisting of a functional/stereotactic neurosurgeon, neurophysiologist, anesthesiologist and operating room staff. We also assume a thorough (at least postdoctoral) understanding of the principles of behavioral and cognitive neuroscience, as well as of single-neuronal recording and analysis. We pay particular attention to describing the unique challenges and opportunities of the operating room environment and human subject that differentiate these types of studies from those in the laboratory on animal subjects. While designing and performing these studies, it is of paramount importance to prioritize patient safety and comfort over research goals, as subjects who consent to participate do so out of altruism rather than expectation of direct benefit.

Subjects. Each individual patient should be evaluated and considered for surgery by a multidisciplinary clinical team. An independent member of the research team should approach each patient to describe the possibility of study inclusion. We typically ensure that the consenting physician is not directly involved in the patient's clinical care to avoid potential conflicts of interest during the informed consent process. At this time, risks and benefits must be clearly explained to each subject. All study subjects must enroll voluntarily and must provide informed consent under the guidelines approved by the Institutional Review Board (IRB). All subjects

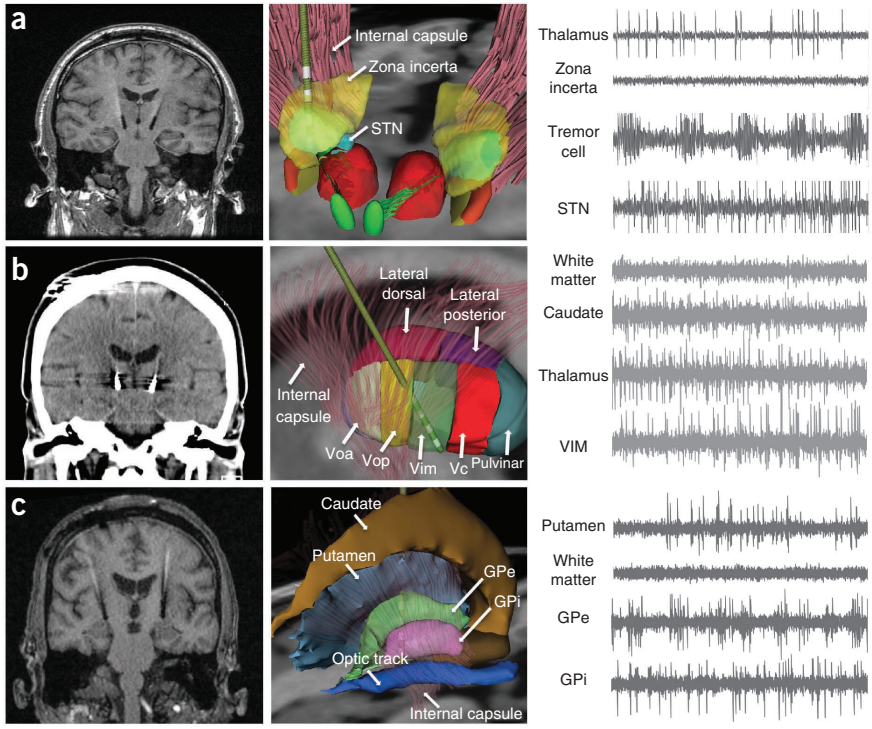

Figure 3 | Composite image demonstrating electrode trajectory. (**a**–**c**) MRIs of STN and globus pallidus internus (GPi) targets, respectively (**a** and **c**, left); computed tomography (CT) of VIM target (**b**, left); schematic 3D reconstruction of DBS implants with surrounding white and gray matter (not exact reconstruction of trajectory, **a**–**c**, middle) and example physiological recordings sampled along the electrode trajectory (**a**–**c**, right). Gpe, globus pallidus externus; Vc, ventral caudal nucleus; Voa, ventralis oralis anterior; Vop, ventralis oralis posterior. 3D reconstructions are reprinted with the permission of Medtronic.

should be free to withdraw from the study at any time, including during surgery, without consequence to operative approach or clinical care.

Surgical planning and procedure. Before surgery, acquire a high-resolution 1.5- or 3.0-Tesla MRI (volumetric Tl- and T2-weighted images). In addition, obtain post-contrast Tl-weighted images in order to identify and avoid vascular structures. On the day of surgery, a stereotactic frame (Cosman-Roberts-Wells or Leksell) is affixed under local anesthesia (xylocaine with epinephrine) to the subject's cranium. A reference frame containing fiducial markers is then attached to the stereotactic frame, and a computed tomography scan is acquired.

A neuronavigation system (e.g., Brainlab or Medtronic Stealth Station) is used for surgical planning. The MRI and computed tomography images are merged into a common 3D space. Standard anatomic landmarks, such as the anterior and posterior commissures (which define the intercommissural plane), are then chosen from the MRI, as is the target point (and optionally the entry point). The fixed location of the fiducials allows the target point to be converted into stereotactic coordinates. These coordinates can then be programmed into the stereotactic frame.

After planning is complete, the patient is positioned on the operating table (**Fig. 1a**). The operative area is shaved, sterilely prepped and draped in the standard manner. The surgical procedure, including skin incision, placement of skull burr holes and opening of dura, is performed according to the neurosurgeon's standard practice[12,21]. The microdrive (Alpha-Omega Engineering or similar) is attached to the frame and positioned above the burr hole (**Fig. 1b**). One or more high-impedance (500–1,500 kΩ) tungsten electrodes are positioned and advanced in 0.05–0.1-mm increments for microelectrode recordings.

Data acquisition. A portable rack system should be used to house all of the equipment required to run the behavioral task and record the neuronal data intraoperatively (**Fig. 2**). The components of the rack consist of: computers, monitors, an acquisition system, input devices (button box and joystick) and the monitor-mounting equipment. Two computers are required: one to run the behavioral software and a second to run the acquisition software. It is important to meet the recommended specifications to minimize latency and ensure high-quality data. In all described tasks, a monitor and button box are fixed to the surgical bed such that they are within comfortable viewing and reaching distance. Subjects are in a comfortable semi-reclined position. The behavioral task is presented using custom-written software (MonkeyLogic[22,23]). The choice of the behavioral task depends upon the investigator's question and the area being recorded. To maximize success, design the task with several issues in mind: (i) subjects will have specific limitations, such as motor limitations in patients with movement disorders or excessive anxiety in patients with anxiety disorders such as obsessive-compulsive disorder (OCD); (ii) subjects will fatigue with time, causing drift in performance; (iii) recording time is limited and must be managed efficiently to avoid insufficient trial repetition; and (iv) recordings will drift because of CSF and brain relaxation over time. For these reasons, we recommend simple, well-balanced tasks with sufficient repetition. There are many available references for examples of behavioral tasks[16,18–20,24]. Placing fibrin glue (Tisseel, or other similar biological material) within the burr hole helps to reduce CSF loss and to dampen brain pulsations, thereby improving recording stability, especially for cortical recordings.

Electrophysiology recordings. The microelectrode recordings are amplified, high-pass–filtered at 300 Hz and displayed on a clinical acquisition system (Alpha-Omega or similar). The electrical signal is passed through a speaker and/or oscilloscope, allowing the neurosurgeon and team to hear the activity of the sampled neurons. Some clinical acquisition systems do not have the capacity to store neural data in any relevant form for experimental use. In order to store the data for *post hoc* analysis, the neuronal signal must be split off from the clinical acquisition system and sent into the research acquisition system (Cambridge Electronic Design PowerLinc 1401, Plexon Omniplex or similar) for storage. Visualize the data and store it at 20 kHz or more. Spurious electrical noise must be

reduced during microelectrode recordings by powering down or unplugging overhead lights, infusion pumps or other sources of electrical noise in the operating room.

As the microelectrode is gradually lowered along the planned trajectory, the audible neuronal activity indicates the physiological properties of the surrounding gray and white matter. Through this procedure, the neurosurgeon is able to map the electrode trajectory by correlating the hallmark firing rates and patterns, density of active units, silent regions (white matter), and sensory and/or motor responses (e.g., to tactile stimulation and movements) along the microelectrode trajectory in real time, thereby providing physiological confirmation of the targeted brain region before insertion of the lesioning probe or DBS macroelectrode (**Fig. 3**). Although it is not our typical practice, some groups use fluoroscopy (intermittent or continuous X-rays) to confirm electrode placement[25,26].

MATERIALS

REAGENTS

Human subjects

- Study participants should be able to provide informed consent. In certain circumstances, the investigator's IRB may permit consent acquisition from parents or legal guardians of minors or health care proxies of patients unable to provide informed consent **! CAUTION** Before enrolling subjects, the protocol and consent form must be approved by the appropriate IRB and human subjects research committees.

EQUIPMENT

Computer, × 2

- Two computers are required: one for the behavioral system and one for the acquisition system **▲ CRITICAL** It is important to verify compatibility of the input/output data acquisition cards with the behavioral computer. **▲ CRITICAL** The behavioral computer requires a video card capable of dual-monitor output: one to run the control system for the behavioral software and the other to present the behavioral task to the subject.

Monitor, × 3

- Three monitors are required: a behavioral system, an acquisition system and a presentation monitor to the subject

Adjustable arm mount

- A two-arm adjustable LCD monitor (3M or similar) mount is required to mount the presentation monitor to the operating bed

Data acquisition card, × 2

- Two acquisition cards (National Instruments, PCI-6229 or similar) need to be installed in the behavioral computer; this will allow analog input from the button box/joystick. Duplicating the data acquisition cards substantially improves data acquisition speed and fidelity[23]

Breakout box

- A breakout box (National Instruments, BNC-2090a or similar) attached to the portable rack system is required to attach the analog input signal from the button box/joystick

Input device

- Button box, joystick, eye position tracker or other input device(s)

Programming environment

- Scientific computing language (MATLAB, MathWorks) or similar software

Behavioral software

- Behavioral software, MonkeyLogic (http://www.monkeylogic.org) or similar software

Clinical acquisition system

- An Alpha-Omega Micro-Guide Pro or similar clinical acquisition system is required to control the motorized microdrive, amplify and digitize neural signals, as well as output neural signals to the research acquisition system for offline analysis

Research acquisition system

- A Cambridge Electronic Design PowerLinc 1401, Plexon Omniplex, or similar system is required to sample and store neural signals from the clinical acquisition system for offline analysis

Data analysis software

- Plexon Offline sorter or other similar software is required to sort spikes for data analysis

EQUIPMENT SETUP

Behavioral task Please refer to the MonkeyLogic documentation (http://www.monkeylogic.org) for information on coding a behavioral task.

Intraoperative rig It is important to have your institutional Biomedical Engineering group approve any research equipment brought into and used in the operating room.

PROCEDURE

Subject consent ● TIMING ~20 min

1| On the day before surgery, discuss with the patient the opportunity to participate in the research. Highlight the steps of the research protocol, potential risks and benefits, and the ability to withdraw at any time without any effect on operative/clinical care. On the day of surgery, remind the patient about the research component, and reinforce the issues addressed during the consent process.

! CAUTION The informed consent discussion must occur before any anesthetics are given and must be performed by an IRB-approved study staff member.

2| Describe the behavioral task.

▲ CRITICAL STEP It is important to note the subject's understanding and ability to perform the task. Rehearsing the task with the subject (using a different set of cues from the actual task cues if necessary) usually improves subject performance.

Intraoperative recording setup ● TIMING ~45 min

3| Position equipment in the operating room before the start of the surgical procedure (**Fig. 4**).

! CAUTION It is important to ensure that all cables are of sufficient length to reach from the research rig to the operating bed.

▲ CRITICAL STEP It will take an hour for the neurosurgery and anesthesiology teams to prepare the patient for surgery. This hour is an opportune time for the research team to set up and troubleshoot the research setup and equipment before

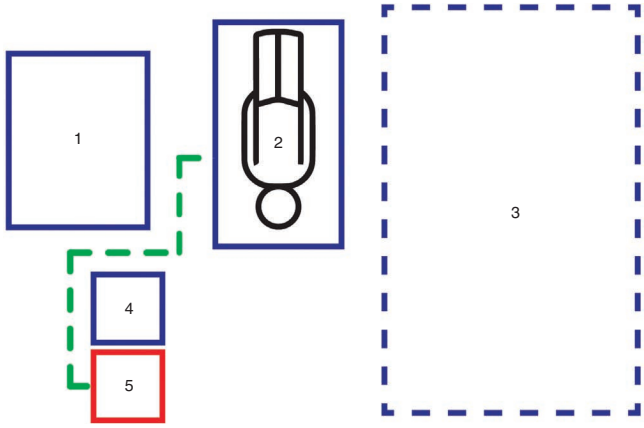

Figure 4 | Top-down perspective of the operating room. Clinical equipment is depicted in solid blue and clinical space in dashed blue. Research equipment is depicted in solid red. Cables from the research rig to the operating bed are depicted in dashed green. (1) Anesthesiology equipment. (2) Operating table. (3) Sterile area for surgical equipment and tools. (4) Clinical acquisition system. (5) Research rig containing computers, monitors, an acquisition system and a button box/joystick.

surgery. Because time in the operating room is limited, it is important to avoid wasting any unnecessary time during the microelectrode recording portion of the surgery.

4| Attach the computer monitor and input device to the operating bed and place them in a comfortable viewing and reaching distance.
! CAUTION The use of a two-arm monitor mount with an articulating arm will provide the most flexibility and viewing comfort when mounted to the surgical bed. It is important to take into account other clinical equipment attached to the bed and the positioning of the patient for surgery.

5| Load the behavioral and acquisition software.
▲ CRITICAL STEP Perform a quick test of all the equipment, including acquisition software, to ensure that all systems are in working order. This checkpoint is important as there are typically many cables and wires between the research, anesthesia and surgical equipments.

6| Connect the outputs of the clinical acquisition system to the inputs of the research acquisition system.
! CAUTION The number of inputs into the research acquisition system will depend on the number of electrodes used during the microelectrode recording portion of the surgery. Organize them carefully, and make a note of their arrangement.

Intraoperative recording ● TIMING ~30–60 min
7| The surgeon should perform the opening and prepare for microelectrode recordings. Advance the electrodes via a computer-controlled motorized microdrive system in increments of 0.05–1 mm. At the start of the microelectrode recording procedure, begin sampling neuronal data on the research acquisition system.

8| Once the electrode is within the target, ensure stable neuronal isolation before continuing with the protocol (**Fig. 5**).
▲ CRITICAL STEP Isolating a neuron requires a delicate balance between the time expenditure and the signal quality. In the operating room, it is important to consider the time limits imposed by the study protocol. Subject patience and performance dwindle as anxiety and fatigue increase. Respect for the subject is critical, as the environment is extremely challenging, and the subject's motivation for participation in research is usually altruism rather than direct benefit.
? TROUBLESHOOTING

9| Start recording on the research acquisition system.
! CAUTION We advise recording at least 60 s of baseline firing activity to ensure the stability of the neuron before initiating the task.

10| Start the behavioral task.
▲ CRITICAL STEP Research time in the operating room is limited by the study protocol, and thus it is crucial that the design of the task allows for enough trials to obtain statistically significant results within the allotted study time. In addition, it is difficult to hold stable neuronal recordings for tens of minutes in the operating room. We design a single session to take ~10 min, thus allowing for the potential to record multiple sessions and multiple neurons during one study. This step can be performed if, at the end of the first session, there is remaining research time left for the study and the subject is agreeable to performing another session. The total amount of time allocated to research-related recordings is usually prescribed in the IRB protocol. We typically restrict recording time to ~30 min.
? TROUBLESHOOTING

11| Adjust the electrode position, reisolate the neurons and repeat the behavioral task.

Remaining surgical procedure

● TIMING 90–120 min

12| After the research portion of the recordings, the neurosurgery team should continue with the rest of the procedure including microelectrode mapping, creation of the lesion or implantation of the stimulating electrode and closing. We advise allowing the neurosurgery team to finish before removing the research equipment from the operating room in order to minimize noise and disturbance.

? TROUBLESHOOTING

Step 8

If there is poor or no neural activity on the microelectrode traces, be sure to double-check whether the power is supplied to the headstage, whether the connections between the headstage and the microelectrode connectors are intact, whether the grounding clip to the headstage/frame is firmly attached and whether the impedance of the electrodes is appropriate.

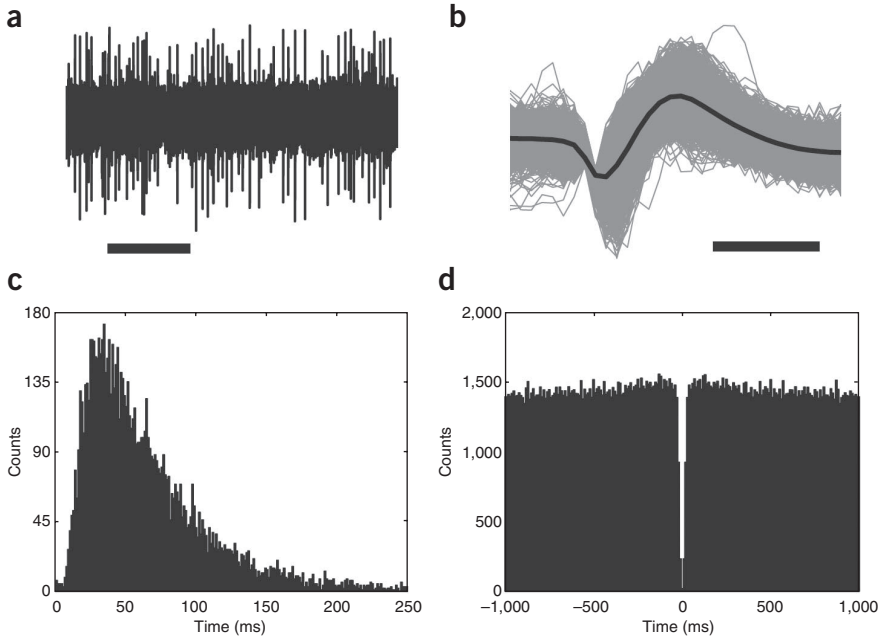

Figure 5 | Representative neuronal data. (**a**) Raw voltage trace from one microelectrode in the dorsal anterior cingulate cortex. Horizontal bar depicts 500 ms. (**b**) Each individual waveform from one recording session (gray); average waveform from entire session (black). Horizontal bar depicts 0.5 ms. (**c**) Interspike interval histogram from example anterior cingulate neuron. (**d**) Autocorrelation histogram from example anterior cingulate neuron.

Owing to the high gains and electrode impedances, electrical noise poses a frequent problem in the operating room environment. If possible, it is best to perform microelectrode recordings in an electrically shielded operating room. The best approach to dealing with excess electrical noise is to unplug equipment (simply turning it off will not suffice) one at a time in an attempt to identify the source of the noise. Potential sources of electrical noise include cautery equipment, anesthesia pumps and florescent overhead lights. If none of these maneuvers eliminates the noise, moving recording equipment away from main power outlets might reduce noise. It is also possible that individual wall outlets may not be effectively grounded; changing wall plugs to a well-grounded outlet may help to reduce excess noise. It is always a possibility that the electrical noise is coming from outside the operating room, perhaps from a neighboring operating room. In revision or multiple DBS implant surgeries, unwanted electrical noise might arise from an active contralateral pulse generator, which can be turned off during the recording portion of the surgery. Enlisting the assistance of the operating room's biomedical engineering team or a third-party neurophysiological monitoring company may also be useful for troubleshooting electrical interference.

Another source of noise during microelectrode procedures in humans is the presence of cardioballistic effects in the neural recordings. This contamination occurs when the electrode is sufficiently close to an artery or arteriole and results in the introduction of a rhythmic mechanical disturbance to the electrode at the frequency of the expansion or contraction of the artery. These effects can be detected in the neural recording as an underlying low frequency change in the amplitude of the noise. This contaminant should be considered during spike sorting and appropriate methods for waveform detection should be applied, as this underlying oscillation will change the amplitude of recorded action potentials[27,28]. In addition, this type of recording session generally results in poor signal quality and stability. If such effects are noticed during the recording procedure, the best solution is to advance the electrode slightly and acquire a new unit isolation.

Step 10

The most common bottleneck in human single-neuronal studies of this type is obtaining enough stable, high-quality neuronal recordings, given the constraints of the operating room. A manual microdrive, instead of a motor-controlled positioner, may provide better unit stability while reducing noise artifacts at the cost of remote control and computer interface. Task design is critical, as demanding or complicated tasks result in performance drift and insufficient trial repetitions. Even experienced animal physiologists will have to modify their approach to neuronal isolation, as the desire for perfect isolation has to be balanced with the necessity to obtain data within a finite time limit. Working with a high-volume neurosurgeon is extremely helpful, as most projects will require recording from a dozen or more subjects in order to obtain sufficient high-quality data.

A fundamental limitation of this type of research design is the issue of experimental controls. This issue relates both to limitations of the environment and to the question of the applicability of data from patients to the general population. Regarding the former, it is important to consider the extreme conditions of the operating room environment that contribute to patient anxiety, fatigue and discomfort. Specific limitations arise as a result of the disorder for which the procedure is performed, and these limitations should be taken into consideration in the experimental design (e.g., reaction time data are likely to be distorted in patients with movement disorders). Certain controls for alertness may be used, such as a 'start trial' button that requires the subject to press a button to begin the trial. This requirement helps to ensure that the subject is attending to the ensuing trial. Tasks should also be designed by considering whether the measured behavior (e.g., joystick movement) should be contralateral or ipsilateral to the recording.

A second key issue is that of the generalizability of the data. By definition, the subjects are patients with a diagnosed disorder, probably involving the brain structure under investigation. There are a number of possible ways to address this issue. First, common behavioral or functional imaging metrics may be observed between the study population and a matched healthy cohort, such as similarities in reaction times or error rates, or similarities in functional MRI activation patterns[18]. These commonalities may increase confidence in the applicability of the data to the general population. Second, the opportunity may arise to record from the same brain region in orthogonal populations. For example, the cingulate gyrus is a target for cingulotomy (for OCD and major depressive disorder) and for depth electrodes (for seizure localization in epileptic patients), and the ventral striatum is a target for both OCD and major depressive disorder. Similar findings from subjects with dissimilar disorders also increase confidence in the data.

● TIMING

Steps 1 and 2, subject consent: 20 min
Steps 3–6, intraoperative recording setup: 45 min
Steps 7–11, intraoperative recording: 30–60 min
Step 12, remaining neurosurgical procedure: 90–120 min

ANTICIPATED RESULTS

In contrast to similar neuronal recordings performed in nonhuman primates, human recordings will be much shorter in duration for any given neuron, and human subjects are likely to provide more variable behavioral data. This occurs because intraoperative recording time is more limited than that in an animal laboratory, and humans are typically much less practiced at the behavioral tasks than monkeys, who may have been performing a given task daily for weeks or months. The selection of an appropriate behavioral-task design that works within these limitations is thus critical. Therefore, these types of studies in humans complement, rather than supplant, similar experiments performed in nonhuman primates.

We provide two examples from previous studies conducted in our laboratory to demonstrate potential results. Perhaps the most substantial benefit from a single-neuronal study is to study how individual neuronal activity modulates in a task-dependent manner.

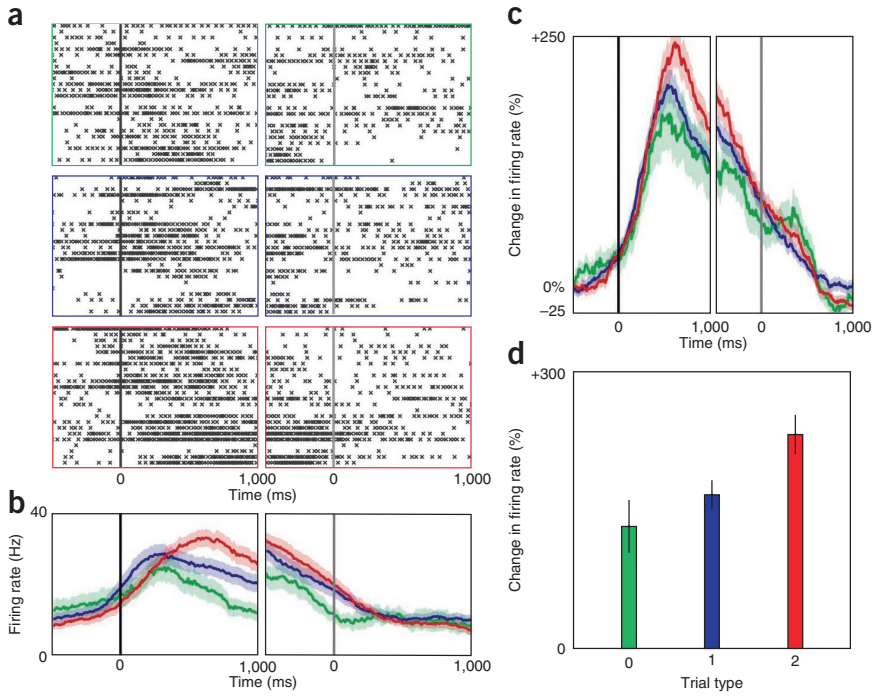

Figure 6 | Individual and population neuronal responses of dorsal anterior cingulate cortex neurons during a cognitive interference task. (**a**) Example neuron showing modulation of firing based on cue-related interference. Rasters for Type 0 (low interference; green), 1 (medium interference; blue) and 2 (high interference; red) trials are shown aligned to the cue (black line) and choice (gray line). (**b**) Average firing rates of the same neuron, demonstrating increasing firing with increasing interference. Error bars (s.e.m.) are depicted by shading. (**c**) Average firing of all cue-related neurons. (**d**) Same as in **c**, but showing activity averaged within a 200-ms-wide window centered 500 ms after the cue. Neuronal firing increased with cognitive interference (n = 1625, P = 0.02, ANOVA), correlating with ref. 18. Error bars show s.e.m.

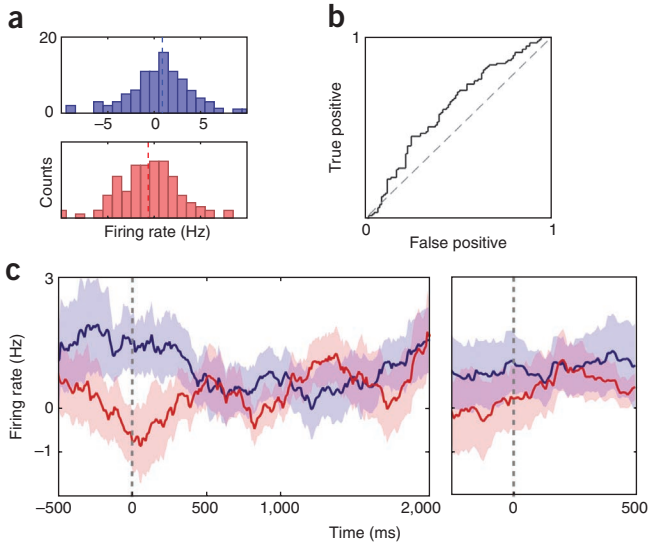

Figure 7 | Population response of nucleus accumbens neurons predicting behavioral choice during a financial decision-making task. (**a**) Histogram of average nucleus accumbens activity during the first 500 ms after the go-cue for trials during which the subject placed a high (blue) or low (red) wager. The mean activities for high- and low-wager trials are represented by the blue-dashed and red-dashed lines, respectively. (**b**) Receiver operating characteristic (ROC) curve for high- and low-wager trials (ROC, area under the curve = 0.62; randomization test, $P = 0.003$). (**c**) Continuous time firing rate histograms for high (blue) and low (red) wager trials centered on the go-cue (left) and choice (right). Reproduced with permission from ref. 19.

The most common method for exploring this activity is to compute a peristimulus time histogram (PSTH) of a neuron's spiking activity relative to a particular behavioral event. As an example, we present a neuron recorded from the dorsal anterior cingulate cortex during performance of a cognitive interference task[18]. By performing a PSTH analysis, we demonstrated that this neuron responds in a dose-response fashion to trials with increasing levels of cognitive interference. Furthermore, because of the high spatial resolution of this approach, we were able to identify a subgroup of cells with this important functional feature that might not have been detectable with another modality (**Fig. 6**). In a different study exploring financial decision making, we were able to perform trial-by-trial analyses correlating neurophysiological signals with upcoming behavior[19]. By using a receiver operating characteristic (ROC) analysis, we showed on a trial-by-trial basis that neurons in the nucleus accumbens encoded upcoming decisions well in advance of the physical manifestation of that behavioral choice (**Fig. 7**). In general, single-neuronal studies provide high spatial and temporal resolution and the unique ability to study an individual neuron's activity in *in vivo* system cognitive processes.

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AUTHOR CONTRIBUTIONS S.R.P., S.A.S., C.M.-R., M.K.M., W.F.A., J.L.G., C.-S.K., J.T.G., Z.M.W. and E.N.E. designed, conducted or analyzed the data. D.D.D., A.W.F., B.D.G., Z.M.W. and E.N.E. evaluated the patients and provided clinical care. S.R.P. and S.A.S. wrote the manuscript. All the authors edited the manuscript.

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