Published in final edited form as:

J Neurosci Methods. 2016 April 1; 263: 7–14. doi:10.1016/j.jneumeth.2016.01.018.

Microelectrode Array Stimulation Combined with Intrinsic Optical Imaging: A Novel Tool for Functional Brain Mapping

Mykyta M. Chernov, Gang Chen, Luke A. Torre-Healy, Robert M. Friedman, and Anna W. Roe

Department of Psychology, Vanderbilt University, Nashville TN, 37240

Abstract

Background—Functional brain mapping via cortical microstimulation is a widely used clinical and experimental tool. However, data are traditionally collected point by point, making the technique very time consuming. Moreover, even in skilled hands, consistent penetration depths are difficult to achieve. Finally, the effects of microstimulation are assessed behaviorally, with no attempt to capture the activity of the local cortical circuits being stimulated.

New Method—We propose a novel method for functional brain mapping, which combines the use of a microelectrode array with intrinsic optical imaging. The precise spacing of electrodes allows for fast, accurate mapping of the area of interest in a regular grid. At the same time, the optical window allows for visualization of local neural connections when stimulation is combined with intrinsic optical imaging.

Results—We demonstrate the efficacy of our technique using the primate motor cortex as a sample application, using a combination of microstimulation, imaging and electrophysiological recordings during wakefulness and under anesthesia.

Comparison with Current Method—We find the data collected with our method is consistent with previous data published by others. We believe that our approach enables data to be collected faster and in a more consistent fashion and makes possible a number of studies that would be difficult to carry out with the traditional approach.

Conclusions—Our technique allows for simultaneous modulation and imaging of cortical sensorimotor networks in wakeful subjects over multiple sessions which is highly desirable for both the study of cortical organization and the design of brain machine interfaces.

Keywords

Utah array; optical chamber; microstimulation; cortical mapping; functional tract tracing

Conflicts of Interest: The authors have no conflict of interest to disclose.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

The cerebral cortex is functionally diverse, with specific regions being responsible for different sensory, motor, and higher cognitive functions. Its electrical excitability has lent itself well to mapping with electrical stimuli, something that led to the first proof of the so-called localization theory of brain function. Early studies included Hitzig's experiments on the victims of the Franco Prussian war (1870-1871) and, later, his and Fritsch's work in dogs (1). These experiments were carried out using DC currents and large surface electrodes, which provided only crude maps of cortical function. The next few decades saw a number of refinements in the technique and resulted in the publication of the first detailed homunculus by Penfield and Boldrey in 1937(2). Since then, microelectrode mapping of the cortical surface has remained a staple for localizing functional areas in the clinic as well as being an important experimental tool (3-5).

In addition to mapping, electrical microstimulation has proven useful for controlled modulation of sensory percepts and behaviors (6-10) and for brain-machine interface applications (11, 12). However, despite the exciting advances in brain stimulation technology, the understanding of circuits underlying these behavioral effects remains indirect and limited.

To associate stimulation induced behavioral effects with underlying neural circuitry, one approach is to develop an *in vivo* functional tract tracing method. Unlike traditional anatomical tract tracing, *in vivo* functional tract tracing opens new avenues for studying cortical connections without sacrifice of the animal or time-consuming anatomical reconstruction, and, furthermore, enables study of circuits activated by the stimulation sites which induced behavioral effects. Such methods have been developed in conjunction with intrinsic signal optical imaging (13-16), voltage sensitive dye imaging (17-19), and fMRI (20-22) following single site stimulation. These methods have revealed both local intra-areal and distant inter-areal connection patterns (15, 23).

Here, we further adapt this approach by using the Utah microelectrode array. Multielectrode arrays have introduced the possibility of mapping in a systematic grid with sufficient density to reveal local functional organization. The construction of multielectrode arrays in the early 1980's consisted of bundles of a few tens of wire electrodes. However, hand-building such a device was very time consuming and their utility was limited by the throughput of the computers used at that time to record the data at the necessary high sample rates. A few years later, a number of probes were developed using microlithographic materials processing, including the flat Michigan probe with contacts along its shank used to sample various cortical layers and the rectangular Utah probe, which is better suited for sampling millimeters of cortical area at a particular depth as well as flexible designs for surface stimulation.(24-26). Unlike probes designed solely for recording, these arrays are treated with a sputtered iridium oxide film (SIROF), making it possible to alternate between stimulation and recording at the same sites without degrading the electrode tip (27, 28).

Here, we demonstrate the feasibility of combining the use of the Utah multielectrode array with chronic optical imaging *in vivo* both in the anesthetized and the awake behaving

monkey. We hope this capability will open new avenues for investigation, including the ability to reveal in parallel: (1) the functional architecture of a local (several millimeter) cortical region, (2) the functional architecture of connection patterns arising from multiple points within this local cortical region, (3) the relationship of these connection patterns to specific sensorimotor behaviors and (4) the modulation of cortical activation patterns in response to electrically stimulated behavioral modulation.

Methods

Surgical Procedures

All procedures were performed in accordance with NIH guidelines and with the approval of the Vanderbilt Institutional Animal Care and Use Committee. Two rhesus macaque (*Macaca mulatta*) monkeys were sedated with ketamine (10 mg/kg), intubated and placed in a stereotaxic frame. The animals were ventilated with a 1-3% mixture of isoflurane in oxygen. Vital signs, such as expired CO₂, body temperature, heart rate and blood oxygen saturation were monitored continuously. A craniotomy and durotomy were performed to expose the brain for implantation of the array. A low-density functional map of the hand representation in premotor and primary motor cortical areas was obtained using a few (<10) microstimulation penetrations with a conventional parylene-coated tungsten microelectrode with an impedance of 1M Ω (World Precision Instruments, cf. (23)). The general location of the implanted array, chamber and major anatomical landmarks are shown in Fig. 1(A). When the appropriate area was located, the array (96 channels, 400 micron spacing, 1 mm shank length) was placed on the surface of the brain and its wire bundle was contoured with rubberized tweezers to conform to the curvature of the brain and minimize the torque on the array itself. A pneumatic injector (Blackrock Microsystems) was then lowered until it barely touched the array and ventilation was briefly stopped to minimize respiration-related brain pulsations during the injection process. The array was then pneumatically injected 1 mm into the brain (Fig. 1(B)). The wire bundle exiting the array was enclosed in rapid-curing biocompatible silicone (World Precision Instruments Kwik-Cast) *in situ* up to its point of termination in an implantable steel connector, which was secured to the head with bone screws. The silicone made it easier for the array to be removed *post mortem* from the surrounding cranioplastic cement. A custom-made rigid nylon chamber (20 mm outer diameter, 1 mm wall thickness, 6 mm wall height) was placed into the craniotomy and secured with bone screws and cranioplastic cement (Fig. 1(C) and (D)). Finally, a custom-made flexible hat-shaped silicone (Shin Etsu Chemical Co. KE-1300T) artificial dura was inserted inside the chamber with the edges tucked under the edges of the durotomy, allowing a clear view of cortex and the microelectrode array (Fig. 1(C) and (D)). The chamber was closed with a threaded cap and sealed with bone wax (29, 30).

Post-surgical care included analgesic (buprenorphine) and anti-inflammatory agents (dexamethasone) for 3 days. The chamber was opened and cleaned under aseptic conditions at least once per week and maintained with a prophylactic antibiotic (Amikacin Sulfate).

Experimental Procedure

We performed a series of experiments to demonstrate the utility of our technique. These included: (1) microstimulation with the Utah array under anesthesia to characterize the different movements evoked in each specific region of the brain covered by the array, (2) microstimulation with the Utah array combined with intrinsic signal imaging under anesthesia to visualize the cortical connections between the areas stimulated in nearby regions, and (3), multi-channel recordings of neuronal activity in an awake behaving animal.

Electrical microstimulation—Electrical microstimulation was performed under 0.5-1% isoflurane-oxygen/nitrous oxide mixture. To minimize contamination of imaged data by noise due to body movement, we conducted electrical stimulation evoked mapping and optical imaging acquisition at separate times, although it is possible to combine the two (16). Cortical sites were stimulated using a programmable multi-channel microstimulator (Blackrock Microsystems CereStim), connected to the Utah array through an implanted pedestal. Stimulation consisted of biphasic 300 Hz trains of 100 pulses with a 200 μ s pulse width and a 53 μ s interphase interval (16). For intrinsic imaging of cortical motor circuitry, the amplitude was set at 10 μ A, high enough to generate a signal but in most cases too low to evoke movements. For characterization of movements, which was done in a separate series of experiments, the stimulation current was stepped until a just noticeable motor movement was produced; the level was identified as the threshold current level (31). Thresholds were typically lower in primary motor cortex than premotor (31). The maximum current used was limited to 150 μ A in order to avoid the possibility of tissue damage or changes in electrode impedance.

Imaging—Imaging was performed using an Imager 3001 (Optical Imaging) system connected to a CCD camera with an 85/50 mm focal length tandem lens combination providing a 7 mm field of view. The cortex was illuminated using LED light sources placed at a slight angle with respect to the optical imaging path. Functional imaging was performed under red light (632 nm) while blood vessel maps were obtained using green light (578 nm) for maximal contrast. Images were recorded at a rate of 4 Hz and imaging was initiated 500 ms (2 frames) before the onset of electrical stimulation. The cortex was stabilized using 4% agarose in saline and a glass cover slip placed on top of the artificial dura within the optical window to reduce pulsation artifacts from respiration and heartbeat. Electrical stimulation trials were interleaved with blank imaging trials during which no stimulation was performed. The blanks were subtracted from the stimulated image to visualize the blood flow-related reflectance changes following electrical microstimulation. Custom MATLAB (MathWorks, Inc.) programs were used for image analysis. Recorded images were filtered using a Gaussian high pass filter (kernel size = 10 pixels) and a Ribot low pass filter (kernel size = 3 pixels) and pixel values 2 standard deviations or more from the mean were clipped to improve image contrast. A pixel-by-pixel single tailed t-test corrected for multiple comparisons was performed to examine the significance of the reflectance changes. The time course of the changes in intrinsic signal was visualized by taking pixels with significant activation and plotting the mean value as a function of time.

Awake behavior—A week after implantation of the Utah array under anesthesia, the animal was placed in a custom-designed chair with minimal restraint, allowing free movement of the head and arms. The animal was then trained to reach out and take small pieces of fruit (grape) with either the contralateral or the ipsilateral hand. The hand choice was determined by the animal and depended primarily on the direction from which the reward was provided. Electrophysiological data from the Utah array were acquired using a multichannel recording system (Cerebus, Blackrock Microsystems) and the animal's motion was recorded with a color CCD camera at 30 frames per second. Multi-unit activity recorded at each site was sorted off-line using the Cerebus system and further analysis was carried out with the aid of NeuroExplorer (NEX Technologies) software and Cerebus Central Suite was used to visualize the waveforms and spike trains at each individual electrode. Waveforms were sorted using the automatic histogram-sorting program included with Central Suite. The impedance of the electrodes was assessed using a built-in impedance testing function. Fewer than 5% of the electrodes demonstrated abnormally high ($>100\text{ k}\Omega$) impedance at any part of the data acquisition process.

Results

Microstimulation

Electrical microstimulation in primary motor cortex (M1) and dorsal premotor cortex (PMd) in the anesthetized subject allowed us to map movements evoked by activation of cortical motor areas (Fig. 2). The starting current was $40\text{ }\mu\text{A}$, which was gradually increased in $10\text{ }\mu\text{A}$ steps, up to $150\text{ }\mu\text{A}$. The stimulation threshold varied between 100 and $150\text{ }\mu\text{A}$. Of the 96 electrode sites where stimulation was attempted, 34 elicited movements, all of which were confined to the contralateral forelimb while stimulation of the rest did not elicit any observable movement. This ratio of responsive to unresponsive sites is reasonable for the current amplitudes used (32). Of the 34 movements evoked, 10 were confined to movement of the wrist, 7 were primarily movements of the elbow, 3 were digit movements and the rest involved a combination of joint-related movements, including some complex movements, such as grasping or reaching. There was some scatter in the topographic map but the overall somatotopy was consistent with published maps (33, 34).

Intrinsic Optical Signal Imaging

As PMd is known to have anatomical connections with topographically similar locations in other motor areas, we hypothesized that electrically stimulating PMd would activate functionally related areas in ventral premotor (PMv) and M1. The array was placed over both PMd and M1; we conducted optical imaging of electrical stimulation-evoked intrinsic signals for ten electrode sites from presumed PMd locations, selected from approximately the top two-thirds of the array as oriented on Figure 2 (10-15 trials for each site). We imaged from adjacent cortex located in M1 and PMv. Fig. 3(A) shows the relative arrangement of the imaging field (blue box) and the electrode array (green box), with the electrode being stimulated identified by a red dot; the field of view (blue box) was 7 by 7 mm . The array itself was out of the imaging field of view and slightly tilted with respect to the imaging field. As shown in Fig. 3(B), blank trials without electrical stimulation produced images with baseline levels of reflectance change (average of 10 trials). In contrast, stimulation at a

site which evoked movement at the elbow joint (circled in red in Fig. 2) produced two distinct regions of significant reflectance change (Fig 3(C), pixels with statistically significant reflectance change shown in 3(D)). These two regions were located in the PMv (spot 1) and in M1 (spot 2). The time course of the reflectance change at each of these two regions (Figure 3(E), M1: red line, PMv: blue line) peaked at about 0.1%, at about 16 frames (4 seconds) post stimulation (orange bar), values typical for reflectance changes induced by electrical stimulation in the anesthetized monkey (35). A time course for an area away from these two activated regions shows little activation (Fig 3(C), spot 3) and little reflectance change (Fig 3(E), green line). Stimulation of other electrodes in the Utah array also revealed activation in either M1 or PMd or both (See supplementary Fig. 1). We observed that different electrodes activated different regions; however, we did not collect enough data to show a definitive topography of activated sites.

Awake Recording

We trained two monkeys to sit in a primate chair and reach for grape reward. Monkeys reached with either the left or right hand, typically dependent on the direction from which grapes were presented. Subsequent to training, we mapped the motor and premotor cortex via microstimulation with the Utah array in these animals under anesthesia. This established that stimulation of many of these sites led to movement of the contralateral forelimb and revealed a rough somatotopy (Fig. 2). This suggested that activation of neurons at these sites lead to such movements. We therefore expected that voluntary movement of the contralateral forelimb in an awake animal would be associated with an increase in firing rate at the same electrode sites and that movements of other parts of the body would not be associated with increases in firing rate at those sites.

To examine this hypothesis, following microstimulation mapping, the monkeys were recovered and re-accustomed to performing the reach task. Approximately 20 trials of reaching behavior with either the ipsilateral or the contralateral (with respect to the chamber implantation site) forelimb were collected, (14 contralateral and 6 ipsilateral). Movement of the contralateral arm evoked robust increases in activity, particularly in the posterior region of the array (M1, towards bottom of Fig. 4, particularly in the wrist region). Plotted in Figure 4(A-C) are three maps of Utah array electrode firing rates obtained prior (left panel), during (middle panel), and following (right panel) elbow flexion during an arm movement. Prior to arm movement, the baseline spiking activity exhibited a relatively low level of spiking across all electrodes (left panel). However, once arm movement began, approximately 60% of the array electrodes showed significant increases in firing rate (middle panel, also see Fig. 4(M) for statistical significance of the changes). This elevated level of activity returned to baseline once the arm returned to rest (right panel). Spikes recorded from an electrode representing elbow flexion (red box in (A-C), red circle in Fig. 2) are shown in a spike raster plot (Fig 4(D)). Spiking activity at the electrode corresponding to elbow flexion revealed low activity levels prior to movement, and relatively elevated activity during arm movement (indicated by pink region of raster plot), and declining activity after the reaching movement; electrode firing rate maps (A), (B), and (C) are indicated at corresponding time points of the spike raster plot. A second trial is shown in the Fig 4(E-H) and demonstrates a similar result. In contrast, similar reach and grab movements

performed with the other hand, elicited low spiking activity and no increases in firing rate over baseline (Fig 4(I-L)). Other activity, such as chewing or head movements also did not elicit significant increases in firing rates (data not shown), suggesting that the recorded activity was correlated with motion of the forelimb contralateral to the site of array implantation. The increases in activity were highly statistically significant during limb movement as compared to rest (Fig 4(M)), even in an average of a few (8) trials.

Discussion

Limitations and Possible Future Advances

Summary—The development of a high spatial resolution functional tract tracing tool is imperative for interrogating the circuits underlying brain function and behavior. Our approach combines the use of a silicon micromachined electrode array (Utah array) and intrinsic signal imaging in a chronically implanted optical window preparation. The implantation of the Utah array provides the investigator with a dense (100 electrodes within 16 mm² in our study), evenly spaced grid of sample points for both stimulation and recording at a known depth (1 mm). Combined with intrinsic signal optical imaging through a chronically implanted optical window, cortical activation patterns can be mapped in response to electrical stimulation from any electrode in the array. These activation patterns can be correlated with optical maps obtained in response to sensorimotor activation in anesthetized or awake states. When applied to the awake behaving preparation, these methods provide a powerful approach to revealing the functional architecture of a cortical region, its cortical connections, and associated behavioral patterns. While in these experiments, we did not attempt to maintain this preparation longer than 2 months, given our previous experience with chronic optical chambers (36), we expect that this preparation can be maintained for extended periods needed for long-term behavioral studies in monkeys.

One of the potential benefits of combining the Utah array with imaging is the ability to study the interactions of multiple functional columns in the cerebral cortex. As these columns are small (~200 microns in diameter), visualizing and targeting them can be challenging. Many studies clearly show that cortical columns form networks that underlie functionally specific information processing in the brain.(37). By stimulating single or groups of single columns with the Utah array and observing activated cortical domains with optical imaging, our technique permits direct association of resulting effects on behavior with underlying cortical networks. Furthermore, simultaneous or sequential activation or presentation of different spatiotemporal stimulation patterns can be achieved by simply reprogramming the stimulation paradigm.

Limitations—We note that the method does have some limitations in its current form. While it has superior resolution to fMRI, optical imaging requires a direct view of the cortical surface. Therefore, it cannot be used for imaging of deep brain structures or within sulcal folds. Some of the limitations of the Utah array are: (1) it is invasive and its insertion can cause bleeding and tissue injury, (2) it is opaque, (3) it is rigid, (4) it does not allow for sampling of different cortical layers (although this could be addressed with a modified array design). However, the risks of a one-time implantation of an array should be weighed

against the combined risks of hundreds of manually-guided penetrations needed to map the brain with comparable density using conventional single electrode approach and the power and convenience of having simultaneous access to a hundred or more sites for stimulation or recording. These arrays have been successfully implanted in monkeys and humans and have remained operational for years (38, 39). The rigid and opaque nature of the array prevents imaging of cortex directly under it and makes it difficult to implant in areas of significant curvature, such as around sulci. Design of clear conductive materials has been a challenge and although some of them (such as indium tin oxide) have desirable optical properties, they tend to be brittle or have other drawbacks that prevent them from being readily used. Recently, a flexible transparent array has been created using a layered graphene parylene structure (40). However, this is a surface array and does not allow for access to deeper cortical layers. The array we used did allow for stimulation below the cortical surface. In general, fabrication of Utah arrays with shanks longer than 2 mm is challenging but longer arrays can be made using metal microwires inserted into a ceramic base (41). All of the shanks were of the same length but arrays with variable shank depths are commercially available (for example, from Blackrock Microsystems).

Another limitation of electrical stimulation is its lack of directional selectivity. Stimulation activates neurons both orthodromically and antidromically, as well as axons of passage. If all axons of passage were activated, then one might expect the distribution of activation to be relatively uniformly distributed. This is not the case. In an optical imaging study that examined the pattern of activation following focal electrical stimulation, local patchy activations were observed, similar in size and distribution to anatomically labeled patches following a focal tracer injection. These activation patterns were, moreover, relatively consistent following cortical stimulation at different laminar depths (15). In a 2-photon study that examined neuronal activation in response to electrical stimulation (typically less than 10 μ A), the authors reported that the activated subpopulation ($<300 \mu$ m zone) remained within the activation focus but that the subpopulation within the locus shifted, suggesting that the stimulated network was not randomly distributed.(42) Note, however, that these studies were conducted with relatively low current intensities. It is possible that different current stimulation amplitudes bias the activation towards local, feedforward, or feedback connectivity.(43) Concurrent electrical recordings from activation foci can provide additional clues about the underlying neuronal contributions to the elicited functional connectivity patterns.

Previous mapping studies using electrical stimulation—Previous uses of electrical stimulation for studying connectivity have included assessing the presence of a direct projection from an antidromically stimulated neuron to the target area; however, the purpose of such study was to identify projection neurons and not to study connection patterns per se (44, 45). Electrical stimulation has also been used in conjunction with optical imaging and fMRI mapping to reveal cortical connection patterns. We have shown that with relatively low electrical stimulation parameters (25 μ A, 250 Hz, 100 ms) in monkey somatosensory cortex, the imaged hemodynamic response to electrical stimulation mimics that of tactile stimulation; resulting in focal 1 mm activation sites consistent with a single digit representation (15). Importantly, such electrical stimulation elicited focal activations at sites

away from stimulated site; these included both intra-areal (15) and inter-areal (23) connections, similar to typical intra-areal and inter-areal columnar networks revealed by anatomical tracer studies (23). There have also been pioneering studies using electrical stimulation in conjunction with fMRI mapping. One of the first studies to employ this approach applied electrical stimulation in visual area V1 in the macaque while mapping with BOLD responses with fMRI (20). Perhaps due to high (up to 1800 μ A) stimulation currents, these revealed very large activation sites within V1 and other extrastriate areas. With somewhat lower electrical stimulation currents (100-300 μ A) applied to inferotemporal cortex, local patches of activation measuring roughly 1 mm- 1 cm in size were revealed, establishing the presence of face patch networks in inferotemporal cortex (21). Stimulation of closely spaced patches in the lateral bank of the interparietal sulcus in conjunction with fMRI revealed surprisingly varied activation patterns with the rest of the cerebral cortex, further strengthening the point that even relatively small cortical areas may be functionally heterogeneous (46).

Optical imaging and fMRI can therefore be used as complementary imaging modalities. While optical imaging offers higher spatial resolution and shows activation at lower stimulation amplitudes (10-150 μ A being usually sufficient) (15), allowing a more fine-grained view of functional connection patterns, fMRI may give a broader picture of the connections between one area and the rest of the brain, especially in areas inaccessible to optical imaging.

Conclusion

This methodology provides the ability to record from a local region of cortex and study the functional connectivity of different electrodes of the array, thereby potentially providing an understanding of cortical connectivity patterns and connectional functional architecture. These combinations of stimulation and mapping have great promise for mapping cortical connections at both the local and global scales. Moreover, the ability to conduct this in the awake behaving animal raises the possibility of relating such connectional architecture to specific sensorimotor behaviors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was funded by NIH grant NS 044375 to AW Roe.

References

1. Koehler PJ. Eduard Hitzig's experiences in the Franco-Prussian War (1870-1871): the case of Joseph Mousseau. *J Hist Neurosci.* 2010; 21(3):250–62. [PubMed: 22724487]
2. Penfield W, Boldrey E. Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. *Brain.* 1937; 60(4):389–443.
3. Dum RP, Strick PL. Motor areas in the frontal lobe of the primate. *Physiol Behav.* 2002; 77(4-5): 677–82. [PubMed: 12527018]

4. Bruce CJ, Goldberg ME, Bushnell MC, Stanton GB. Primate frontal eye fields. II. Physiological and anatomical correlates of electrically evoked eye movements. *J Neurophysiol.* 1985; 54(3):714–34. [PubMed: 4045546]
5. Bonini L, Maranesi M, Livi A, Fogassi L, Rizzolatti G. Ventral premotor neurons encoding representations of action during self and others' inaction. *Curr Biol.* 2014; 24(14):1611–4. [PubMed: 24998525]
6. Graziano MS, Taylor CS, Moore T. Probing cortical function with electrical stimulation. *Nat Neurosci.* 2002; 5(10):921. [PubMed: 12352975]
7. Murphey DK, Maunsell JH. Behavioral detection of electrical microstimulation in different cortical visual areas. *Curr Biol.* 2007; 17(10):862–7. [PubMed: 17462895]
8. Romo R, Hernandez A, Zainos A, Brody CD, Lemus L. Sensing without touching: psychophysical performance based on cortical microstimulation. *Neuron.* 2000; 26(1):273–8. [PubMed: 10798410]
9. Salzman CD, Britten KH, Newsome WT. Cortical microstimulation influences perceptual judgements of motion direction. *Nature.* 1990; 346(6280):174–7. [PubMed: 2366872]
10. Tehovnik EJ, Slocum WM. Depth-dependent detection of microampere currents delivered to monkey V1. *Eur J Neurosci.* 2009; 29(7):1477–89. [PubMed: 19519630]
11. Tehovnik EJ, Slocum WM, Smirnakis SM, Tolias AS. Microstimulation of visual cortex to restore vision. *Prog Brain Res.* 2009; 175:347–75. [PubMed: 19660667]
12. Chase SM, Kass RE, Schwartz AB. Behavioral and neural correlates of visuomotor adaptation observed through a brain-computer interface in primary motor cortex. *J Neurophysiol.* 2012; 108(2):624–44. [PubMed: 22496532]
13. Lieke EE, Frostig RD, Arieli A, Ts'o DY, Hildesheim R, Grinvald A. Optical imaging of cortical activity: real-time imaging using extrinsic dye-signals and high resolution imaging based on slow intrinsic-signals. *Annu Rev Physiol.* 1989; 51:543–59. [PubMed: 2653196]
14. Godde B, Leonhardt R, Cords SM, Dinse HR. Plasticity of orientation preference maps in the visual cortex of adult cats. *Proc Natl Acad Sci U S A.* 2002; 99(9):6352–7. [PubMed: 11959906]
15. Brock AA, Friedman RM, Fan RH, Roe AW. Optical imaging of cortical networks via intracortical microstimulation. *J Neurophysiol.* 2013; 110(11):2670–8. [PubMed: 24027103]
16. Stepniewska I, Friedman RM, Gharbawie OA, Cerkevich CM, Roe AW, Kaas JH. Optical imaging in galagos reveals parietal-frontal circuits underlying motor behavior. *Proc Natl Acad Sci U S A.* 2011; 108(37):E725–32. [PubMed: 21873212]
17. Sawaguchi T. Modular activation and suppression of neocortical activity in the monkey revealed by optical imaging. *Neuroreport.* 1994; 6(1):185–9. [PubMed: 7703411]
18. Kunori N, Kajiwarra R, Takashima I. Voltage-sensitive dye imaging of primary motor cortex activity produced by ventral tegmental area stimulation. *J Neurosci.* 2014; 34(26):8894–903. [PubMed: 24966388]
19. Suzurikawa J, Tani T, Nakao M, Tanaka S, Takahashi H. Voltage-sensitive-dye imaging of microstimulation-evoked neural activity through intracortical horizontal and callosal connections in cat visual cortex. *J Neural Eng.* 2009; 6:066002. [PubMed: 19794238]
20. Tolias AS, Sultan F, Augath M, Oeltermann A, Tehovnik EJ, Schiller PH, et al. Mapping cortical activity elicited with electrical microstimulation using fMRI in the macaque. *Neuron.* 2005; 48(6):901–11. [PubMed: 16364895]
21. Moeller S, Freiwald WA, Tsao DY. Patches with links: a unified system for processing faces in the macaque temporal lobe. *Science.* 2008; 320(5881):1355–9. [PubMed: 18535247]
22. Ohayon S, Grimaldi P, Schweers N, Tsao DY. Saccade modulation by optical and electrical stimulation in the macaque frontal eye field. *J Neurosci.* 2013; 33(42):16684–97. [PubMed: 24133271]
23. Kaas JH, Gharbawie OA, Stepniewska I. Cortical networks for ethologically relevant behaviors in primates. *Am J Primatol.* 2013; 75(5):407–14. [PubMed: 22865408]
24. Hambrecht FT. Visual prostheses based on direct interfaces with the visual system. *Baillieres Clin Neurol.* 1995; 4(1):147–65. [PubMed: 7633780]
25. Drake KL, Wise KD, Farrey J, Anderson DJ, BeMent SL. Performance of planar multisite microprobes in recording extracellular single-unit intracortical activity. *IEEE Trans Biomed Eng.* 1988; 35(9):719–32. [PubMed: 3169824]

26. Jones KE, Campbell PK, Normann RA. A glass/silicon composite intracortical electrode array. *Ann Biomed Eng.* 1992; 20(4):423–37. [PubMed: 1510294]
27. Davis TS, Parker RA, House PA, Bagley E, Wendelken S, Normann RA, et al. Spatial and temporal characteristics of V1 microstimulation during chronic implantation of a microelectrode array in a behaving macaque. *J Neural Eng.* 2012; 9(6):065003. [PubMed: 23186948]
28. Slavcheva E, Vitushinsky R, Mokwa W, Schnakenberg U. Sputtered iridium oxide films as charge injection material for functionalized electrostimulation. *J Electrochem Soc.* 2004; 151:E226–37.
29. Ruiz O, Lustig BR, Nassi JJ, Cetin A, Reynolds JH, Albright TD, et al. Optogenetics through windows on the brain in the nonhuman primate. *J Neurophysiol.* 2013; 110(6):1455–67. [PubMed: 23761700]
30. Chen LM, Heider B, Williams GV, Healy FL, Ramsden BM, Roe AW. A chamber and artificial dura method for long-term optical imaging in the monkey. *J Neurosci Methods.* 2002; 113(1):41–9. [PubMed: 11741720]
31. Burish MJ, Stepniewska I, Kaas JH. Microstimulation and architectonics of frontoparietal cortex in common marmosets (*Callithrix jacchus*). *J Comp Neurol.* 2008; 507(2):1151–68. [PubMed: 18175349]
32. Stepniewska I, Fang PC, Kaas JH. Organization of the posterior parietal cortex in galagos: I. Functional zones identified by microstimulation. *J Comp Neurol.* 2009; 517(6):765–82. [PubMed: 19852065]
33. Godschalk M, Mitz AR, van Duin B, van der Burg H. Somatotopy of monkey premotor cortex examined with microstimulation. *Neurosci Res.* 1995; 23(3):269–79. [PubMed: 8545075]
34. Preuss TM, Stepniewska I, Kaas JH. Movement representation in the dorsal and ventral premotor areas of owl monkeys: a microstimulation study. *J Comp Neurol.* 1996; 371(4):649–76. [PubMed: 8841916]
35. Grinvald, A.; Shoham, D.; Shmuel, A.; Glaser, D.; Vanzetta, I.; Shtoyerman, E., et al. In-vivo optical imaging of cortical architecture and dynamics. In: Windhorst, U.; Johansson, H., editors. *Modern techniques in neuroscience research.* Springer Verlag; 1999. p. 893-969.
36. Tanigawa H, Lu HD, Roe AW. Functional organization for color and orientation in macaque V4. *Nat Neurosci.* 2010; 12:1542–8. [PubMed: 21076422]
37. Sincich LC, Blasdel GG. Oriented axon projections in primary visual cortex of the monkey. *J Neurosci.* 2001; 21(12):4416–26. [PubMed: 11404428]
38. Normann RA. Technology insight: future neuroprosthetic therapies for disorders of the nervous system. *Nat Clin Pract Neurol.* 2007; 3(8):444–52. [PubMed: 17671522]
39. Hochberg LR, Serruya MD, Friehs GM, Mukand JA, Saleh M, Caplan AH, et al. Neuronal ensemble control of prosthetic devices by a human with tetraplegia. *Nature.* 2006; 442(7099):164–71. [PubMed: 16838014]
40. Park DW, Schendel AA, Mikael S, Brodnick SK, Richner TJ, Ness JP, et al. Graphene-based carbon-layered electrode array technology for neural imaging and optogenetic applications. *Nat Commun.* 2014; 5:5258. [PubMed: 25327513]
41. Musallam S, Bak MJ, Troyk PR, Andersen RA. A floating metal microelectrode array for chronic implantation. *J Neurosci Methods.* 2007; 160(1):122–7. [PubMed: 17067683]
42. Histed MH, Bonin V, Reid RC. Direct activation of sparse, distributed populations of cortical neurons by electrical microstimulation. *Neuron.* 2009; 63(4):508–22. [PubMed: 19709632]
43. Kudyba, KA.; Friedman, RM.; Gharbawie, OA.; Roe, AW., editors. *Optical imaging of electrical stimulation in somatosensory and motor cortices: A novel approach to tracing functional connectivity.* Society for Neuroscience Meeting; San Diego, CA: 2013.
44. Bishop PO, Burke W, Davis R. Single-unit recording from antidromically activated optic radiation neurones. *J Physiol.* 1962; 162:432–50. [PubMed: 13869505]
45. El-Shamayleh Y, Kumbhani RD, Dhruv NT, Movshon JA. Visual response properties of V1 neurons projecting to V2 in macaque. *J Neurosci.* 2013; 33(42):16594–605. [PubMed: 24133263]
46. Premereur E, Van Dromme IC, Romero MC, Vanduffel W, Janssen P. Effective connectivity of depth-structure-selective patches in the lateral bank of the macaque intraparietal sulcus. *PLoS Biol.* 2015; 13(2):e1002072. [PubMed: 25689048]

Highlights

- The method allows for visualization of cortical circuitry during evoked or spontaneous behavior.
- Circuit activity can be imaged or recorded electrophysiologically, or stimulated at a single or multiple sites, simultaneously or sequentially.

Fig 1. Utah array within a chronically implanted optical window

(A) Approximate location of array implantation (black square) in the motor region of the macaque monkey brain. Red circle indicates the extent of the chronically implanted optical window. (B) Image of the array after insertion into the motor cortex. The electrodes are numbered as shown (1 to 96). (C) View of the array beneath the silicone optical window, the walls of which are marked with blue arrows. (D) A schematic side view of the imaging chamber. Legend: A- anterior, L-lateral, AS- arcuate sulcus, CS-central sulcus, PS- principal sulcus, M1- primary motor cortex, PMd, dorsal premotor cortex, PMv, ventral premotor cortex.

Fig 2. Somatotopy within motor cortex obtained using Utah array microstimulation

The array was injected in the region outlined by the black square, over monkey motor cortex, covering both the primary (M1) and dorsal pre-motor (PMd) regions. The location of the array was determined by aligning photographs taking before and after the implantation through a surgical microscope, using blood vessel patterns as markers. Colored dots represent the Utah array electrode locations which evoked movements of the contralateral forelimb, with the specific arm regions labeled below. The electrodes are numbered as in Fig. 1. The electrode site circled in red was stimulated during the imaging session shown in Fig. 3 and awake recording session in Fig. 4. The electrode site circled in green was stimulated during imaging session shown in Supplementary Fig. 1. A-anterior, L-lateral.

Fig 3. Imaging of local projections during Utah array stimulation

(A) Blood vessel map of the cortical region containing portions of M1, PMd and PMv. The Utah array was located over M1 and PMd (orange square). The imaging field of view included parts of M1 and PMv (blue square). Images from this field of view are shown in (B), (C) and (D). The array orientation is approximately the same as in Fig.2 but tilted slightly anti-clockwise. Red dot indicates the electrode being stimulated in (C) and (D). (B) Intrinsic optical signal image without stimulation (first frame subtracted) of M1 and PMv located lateral to the Utah array. (C) Blank-subtracted map of activation following stimulation of electrode indicated by red dot (elbow) in (A). Two dark areas, labeled as 1 and 2, show activation following stimulation, while region 3 does not. (D) Same map as (C). Pixels with significant changes in intrinsic optical signal shown in color ($p < .05$, paired T-test). Each area was isolated and used as a mask for ROI analysis. (E) Time course of average reflectance changes from each of ROI's 1 (blue, PMv), 2 (red, M1), and 3 (green, ROI overlying an area of no activation). Orange bar indicates the period (300 ms) of stimulation.

Fig 4. Awake recording via Utah array during reach and grab task

(A-C) and (E-G). Two examples of color-coded spike rates recorded by the Utah array during an awake reach and grab experiment, with the animal using the arm contralateral to the array. (I-K). An example of spike rates recorded when the animal was using the arm ipsilateral to the array. Three panels with integration times of 0.46 second are shown for each of the three movements, including rest (left), reach (middle) and return to rest (right). (D), (H) and (L) represent raster plots of the electrode outlined by the red square. The distance between each tick mark is one second. Stimulation of this electrode evoked movement of the elbow joint under anesthesia (Fig 2). Lettering below the raster plots indicate the location at which activity maps (A-C), (E-G) and (I-K) were recorded and the period when forelimb movement was observed is highlighted in pink. (M) Electrode sites which demonstrated increased firing rate during the reach and grab task with the contralateral arm (N=8 trials), compared to rest, color coded by levels of significance using a paired T-test.