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Micro-coil design influences the spatial extent of responses to intracortical magnetic stimulation.

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Abstract

Objective: Electrical stimulation via cortically implanted electrodes has been proposed to treat a wide range of neurological disorders. Effectiveness has been limited, however, in part due to the inability of conventional electrodes to activate specific types of neurons while avoiding other types. Recent demonstrations that magnetic stimulation from a micro-coil can selectively activate pyramidal neurons (PNs) while avoiding passing axons suggest the possibility that such an approach can overcome some this limitation and here we use computer simulations to explore how the micro-coil design influences the selectivity with which neurons are activated.

Methods: A computational model was developed to compare the selectivity of magnetic stimulation induced by rectangular-, V- and W-shaped coil designs. The more promising designs (V- and W-shapes) were fabricated for use in electrophysiological experiments including in vitro patch-clamp recording & calcium imaging (GCaMP6f) of mouse brain slices.

Results: Both V- and W-shaped coils reliably activated layer 5 (L5) PNs but V-coils were more effective while W-coils were more selective. Activation thresholds with double-loop coils were approximately one-half those of single-loop coils. Calcium imaging revealed that both V- and W-coils better confine activation than electrodes.

Conclusion: Individual design features can influence both the strength as well as the selectivity of micro-coils and can be accurately predicted by computer simulations.

Significance: Our results show how coil design influences the response of cortical neurons to stimulation and are an important step towards the development of next-generation cortical prostheses.

Index Terms—

Micro-magnetic stimulation (μ MS); Intracortical micro-coil; Intracortical magnetic stimulation; Primary visual cortex (V1); Neural Prosthesis

I. INTRODUCTION

Intracortical electrical stimulation delivered via electrodes implanted into the neocortex has been proposed as a means to treat a wide range of neurological and psychological disorders [1–4]. The use of micro-electrodes allows for safe implantation into the cortex and much previous work with laboratory animals [5–8] and in clinical testing [2, 9, 10] has demonstrated the viability of intracortical electric stimulation for activation of cortex. However, efficacy remains limited, in part due to the inability to precisely target specific types of cortical neurons or even to confine activation to specific cortical regions. The electric fields arising from conventional, monopolar (distant return) intracortical micro-electrodes are spatially symmetric and therefore, the driving force for activation is similar for all nearby neurons and neuronal processes. As a result, both pyramidal neurons (PNs) in the local region around the electrode as well as horizontally-oriented passing axons that arise from neurons in distant cortical areas get activated [11–13]. This leads to a loss of precision with which the resulting neural activity can be spatially confined, and can lead to unpredictable and undesirable side effects during clinical use [2, 14, 15]. Several efforts to enhance spatial resolution utilizing two or more electrodes, e.g. with bipolar or hexagonal configurations, reported only limited improvement and also required a more complex electrode structure and driving circuitry [16, 17]. A second concern with the long-term implantation of cortical electrodes is that activation does not remain stable over time. Multiple factors contribute to the lack of stability but two principal contributors are the brain's response to the presence of foreign bodies [1, 18–21] and the complex series of chemical reactions [22–25] that can alter the electric properties of the electrode-tissue interface over time. The full extent of these factors are not completely known but the effectiveness of multi-channel stimulation has been greatly reduced in some studies [6, 20, 26] and concerns about stability have limited progress towards reliable long-term implantation of cortical prostheses.

Magnetic stimulation has several important advantages when compared to electric stimulation from conventional micro-electrodes including the ability to overcome some of the above-described limitations. For example, the electric fields induced by coils are spatially asymmetric and can therefore be aligned so as to create stronger activating forces for vertically-oriented PNs than for the horizontally-oriented passing axons that arise from distal neurons [27–30]. This helps to confine activation to a focal region around the coil, thereby enhancing the spatial resolution of cortical stimulation and reducing concerns about the undesirable side-effects that can arise when passing axons are activated. Another advantage of magnetic stimulation is that magnetic fields have high permeability to biological tissue and thus are not altered by glial scarring or other biological reactions that can lead to encapsulation of cortical implants. Thus, magnetic fields can continue to reliably 'carry' electric fields to the region beyond the encapsulation. Third, the stability of the

micro-coil array can be enhanced by hermetically sealing the implant with a dielectric coating [31–33]. In contrast to electrodes, which necessarily require some exposure of the metal surface to the extracellular space, complete sealing of coils greatly reduces the degradation of the device caused by water infiltration through the weak bonding between the exposed electrode and the dielectric coating and/or the delamination of the metal electrode from the substrate during chronic implantation [22, 25, 32–34]. Thus, a coil-based approach has the potential to provide a more precise and more stable activation process for cortical prostheses as well as for other applications that utilize cortical stimulation.

We have recently developed a micro-coil that is small enough to be safely implanted into cortex [35]. The micro-coil consists of a micro-wire with a single bend and is fabricated onto a silicon substrate that is similar in size ($50 \times 100 \mu\text{m}$) to some commercially-available microelectrodes that have been shown to be safe for chronic implantation [36]. Previous testing has shown that even this relatively simple coil design provides some selectivity for the activation of vertically-oriented PNs over that of horizontally-oriented passing axons during stimulation of cortex and, as a result, better confines activation to a focal region around the coil. Although this offers significant improvement over conventional micro-electrodes, the possibility exists that more sophisticated coil designs can further enhance the selectivity of activation. Several mouse models allow cortical activation to be directly visualized via calcium fluorescence imaging [37, 38] and so the selectivity of different coil designs can be directly compared. A second challenge that persists with coils is that power levels remain well above that of micro-electrodes. Although power usage has been reduced dramatically over the last few years [27–29, 35, 39], particularly as coil size has been reduced, the power requirements for coils are still high. Electromagnetic theory suggests that additional turns in the coil will increase the strength of the magnetic and electric fields but such an approach has not been evaluated. For multi-turn coils to be useful however, it will be important to determine whether additional loops will also increase the strength of the gradient of the electric field (the driving force for activation [40, 41]).

Here, we studied a series of increasingly complex micro-coil designs for their ability to enhance the strength and the selectivity of intracortical magnetic stimulation. We first built a computational model to compare the effectiveness of the different designs. We found that the use of a sharp bend at the coil tip (V-shaped) enhanced the selectivity of field gradients along the vertical orientation, and then, that the addition of a second bend at the tip (W-shaped) enhanced the selectivity for activation of vertically-oriented neurons even further. We fabricated the most promising designs and evaluated their performance via *in vitro* electrophysiological experiments using mouse brain slices. Both V and W-shaped coils could reliably activate L5 PNs but the V-coils were stronger than the W-coils. *In vitro* calcium fluorescence imaging revealed that the V and W-coils both produced spatially-confined activation in cortex relative to that of conventional electrodes, but the W-coil was more selective in a manner that was consistent with the model predictions. Double-loop coils enhanced the strength of stimulation over that of single-loop coils. Our results suggest that specific design features of micro-coils can greatly influence the strength and selectivity of neuronal activation in cortex.

II. METHODS

A. Modeling of micro-coils.

A finite element method (FEM) simulation was performed to calculate the spatial gradient of induced electric fields (E-field) arising from the flow of electric current through the micro-coils. Similar to previous studies [27–29, 35], we built 3D models of micro-coils using an ANSYS Maxwell 3D (ANSYS, Inc., Canonsburg, PA, USA). Single-loop gold micro-coils that have ‘rectangular’, ‘V’, ‘W’ shapes at the tips were modelled (Figs. 1C–E). A silicon substrate that supports the coils was incorporated into the model as well. The gold metal trace of the coils had a cross-sectional dimension of $2 \times 10 \mu\text{m}$ and the silicon substrate had a cross-sectional dimension of $50 \times 100 \mu\text{m}$. The length of the silicon substrate was $1000 \mu\text{m}$ (Fig. 1B) and tapered to a point over a length of $180 \mu\text{m}$. The outer dimension of the rectangular coil was $90 \times 820 \mu\text{m}$; the tips of the other coils extended $140 \mu\text{m}$ downward for V-coils (Fig. 1D) and upward for W-coils (Fig. 1E). Our previous study [35] showed that the region of the suprathreshold field gradient was located along the oblique segment of the coil loop and also that the vertical extent (y-axis) was not increased as the current amplitude increased. Therefore we fixed the height of the oblique segment of coils to $140 \mu\text{m}$ so that activation was confined to single cortical layers (thicknesses range from $100\text{--}200 \mu\text{m}$). Double-loop micro-coils with ‘V’ and ‘W’ shapes were also modelled (Figs. 1F and 1G) to explore whether they enhanced the strength and/or selectivity of stimulation.

The area surrounding the coils ($4 \times 4 \times 4 \text{ mm}$) was modelled as a homogeneous medium with properties of gray matter (electrical conductivity: $\sigma = 0.276 \text{ S/m}$; relative permittivity: $\epsilon_r = 12000$) [42]. The homogeneous gray-matter medium was chosen for two reasons. First, all *in vitro* testing (this study and previous ones) was performed with coils that were confined within the gray matter region of mouse brain slices. Second, the narrow spatial extent of elicited E-fields did not allow spread to the gray/white matter border. Thus, the model replicated the physiological experiments and eliminated the need for consideration of the border between gray and white matter as well as the border between gray matter and CSF (the height of the perfusion bath was maintained 6 mm above the tissue surface. The boundary conditions of the external surface of the medium were set to magnetic insulation (the magnetic field strength was set to zero). The boundary conditions of the coil loops and the silicon substrates were set to electric insulation.

The simulation cell was split up in a fine mesh in the ANSYS software, which was refined around the edges of the metal trace to resolve great changes of the E-field gradient. The current injection was realized by a port simulating the connection to a power supply with a current pulse generator. We used an eddy current solution mode of the ANSYS and the coil input was the sinusoidal current with a frequency of 5 kHz and amplitude of 1 mA . Based on the solutions from Maxwell’s equations which were described previously [27, 29, 35] the E-field induced by the coils was calculated by the software. Spatial gradient of the resulting E-field was then calculated with MATLAB software over the two-dimensional (2D) plane that was positioned $15 \mu\text{m}$ above the surface of the coil (Fig. 1B). The distance of $15 \mu\text{m}$ was based on the soma size of L5 PNs, and was chosen in consideration of the experimental situation in which targeted L5 PNs were positioned at the top and side surfaces of coils (Fig.

1B). There were also L5 PN's below the 50- μ m-thick silicon substrate but those were not considered in this study (modeling & experiment) due to the weak fields and corresponding low probability of activation.

All coils were modeled as a closed loop and therefore those had two right angle corners at the top edge of the silicon. We also built model coils using an open loop but there was no noticeable difference between the field distributions at the bottom; the long distance between the top and the bottom edges minimized the interaction between them. We therefore focused on the field gradients arising at the bottom of coils where the V- and W-shaped tips are located.

B. Fabrication and testing of micro-coils.

The micro-coil fabrication process was a modified version of the one used in the previous study [35]. Briefly, a 50- μ m-thick 4-inch silicon wafer was prepared and a 100–200 nm SiO₂ layer was deposited using plasma-enhanced chemical vapor deposition (PECVD). Then a 20 nm layer of Mo-Cr was sputtered after which a 2- μ m-thick gold (Au) layer was deposited using evaporation followed by a 3 nm Ti-W layer.

Next, the gold micro-coil patterns were created using a conventional photolithography and a wet etching. The Mo-Cr, Au and Ti-W layers were then etched using a combination of wet-etch and plasma etching. Next, a 500-nm-thick insulating SiON_x layer was deposited on top of the coil patterns using PECVD. After that, a second photolithography process was performed to create windows for the electrical contact pads. Following this step, the 50- μ m-thick silicon substrate was etched by a deep reactive ion etching (DRIE). The resulting micro-coil structures (Fig. 3A) were then separated from the 4-inch silicon wafer.

The fabricated coils were assembled with copper wire leads (34-AWG, polyurethane inner coat and nylon over coat) (Belden, Richmond, IN, USA). The electrical contacts of micro-coils were connected to the copper wire leads using a silver conductive epoxy (CircuitWorks Conductive Epoxy, ITW Chemtronics, Kennesaw, GA, USA). Assembled coils were mounted on a custom-made plastic holder with an instant adhesive and the distal ends of the copper wire leads were attached to the signal and ground leads of a BNC connector.

Each micro-coil assembly was tested both before and after each experiment to ensure that there was no leakage of electrical current from the coil into the brain tissue. Coils were submerged in physiological solution (0.9% NaCl) and the impedance between one of the coil terminals and an electrode immersed in the physiological solution was measured before and after each experiment. Impedances above 200 Ω at a frequency range of DC-10 kHz were considered indicative of adequate insulation. The high impedance ensures that direct electrical currents did not contribute to any of the elicited neural activity.

C. Micro-magnetic stimulation drive.

Similar to previous studies [27–29, 35], the output of a function generator (AFG3021B, Tektronix Inc., Beaverton, OR) was connected to a 1,000 W audio amplifier (PB717X, Pyramid Inc., Brooklyn, NY) with a gain of 1.43 V/V and a bandwidth of 70 kHz. The audio amplifier was powered by a battery (LC-R1233P, Panasonic Corp., Newark, NJ). The output

of the amplifier was monitored with an oscilloscope (TDS2014C, Tektronix Inc., Beaverton, OR). The stimulus waveform was one full period of a 5 kHz sinusoid waveform, the amplitude of which ranged from 0–2 V. The output of the amplifier ranged from 0 – 2.87 V. For threshold measurements, single stimuli were delivered at 1 Hz for 5 seconds. Burst stimulation for the calcium fluorescence imaging consisted of 20 presentations of the sinusoid delivered at 100 Hz (Fig. 4A).

The single period of sinusoidal current delivered to the coil generates an E-field with a tri-phasic waveform (Fig. 4A, inset, bottom) [27]. Note that the duration of the second (middle) phase was longer than the other phases and is thought to drive activation; longer durations reduce thresholds for activation [43,44].

D. In vitro brain slice experiments.

Electrophysiological recordings were performed using the similar methods to those of the previous studies [27–29, 35]. Briefly, brain slices were prepared from 17–30 days old mice (C57BL/6J; Jackson Laboratory, Bar Harbor, ME). The care and use of animals followed all federal and institutional guidelines, and the Institutional Animal Care and Use Committees (IACUC) of the Boston VA Healthcare System and the IACUC of the Massachusetts General Hospital. The mice were deeply anesthetized with isoflurane and decapitated. The brains were removed immediately after death and a section of the brain containing primary visual cortex (V1) (0.5–1 mm anterior from the lambdoid suture) was isolated on ice in a 0–5°C oxygenated solution containing (in mM) 1.25 NaH_2PO_4 , 2.5 KCl, 25 NaHCO_3 , 1 MgCl_2 , 25 glucoses, and 225 sucrose, equilibrated with 95% O_2 -5% CO_2 (pH 7.4). This cold solution, with a low sodium ion and without calcium ion content, improved tissue viability. In the same medium, 300–400 μm thick coronal slices were prepared using a vibrating blade microtome (Vibratome 3000 Plus, Ted Pella, Inc., Redding, CA) and were incubated at room temperature in an artificial cerebrospinal fluid (aCSF) solution containing (in mM) 125 NaCl, 1.25 NaH_2PO_4 , 2.5 KCl, 25 NaHCO_3 , 1 MgCl_2 , 2 CaCl_2 , and 25 glucose, equilibrated with 95% O_2 -5% CO_2 (pH 7.4). After a two-hour recovery period, slices that contained V1 were transferred and mounted, caudal side down, to a plastic recording chamber (RC-27L, Warner Instruments, LLC, Hamden, CT) with a plastic slice anchor (SHD-27LP/2, Warner Instruments). The chamber was maintained at $30 \pm 2^\circ\text{C}$, and continuously superfused (3.3 ml/min) with oxygenated aCSF solution.

V1 L5 PN were targeted under visual control. Spiking was recorded with a patch electrode (4–8 M Ω) that was filled with superfusate and positioned onto the surface of a targeted PN (a loose seal (15–20 M Ω) cell-attached mode). The patch-clamp amplifier was a MultiClamp 700B Amplifier (Molecular Devices, Sunnyvale, CA) operated in voltage-clamp with a holding potential (i.e. command potential) of 0 mV or similar levels at which the amplifier current I_{amp} is 0 pA. Two silver-chloride-coated wires served as the ground and were positioned at opposite edges of the recording chamber, each approximately 15 mm from the targeted cell. The micro-coil assembly was fixed in the micromanipulator such that the plane of the coil was held parallel to the top surface of the slice (Fig. 3B). The coil assembly was lowered into the bath until the coil was within the brain tissue and positioned close ($\sim 20 \mu\text{m}$) to the targeted PN (Fig. 3C).

In some experiments, antagonists for AMPA/Kainate channels (10 μ M 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)-quinoxaline (NBQX)) and NMDA channels (50 μ M D-2-amino-5-phosphonopentanoic acid (D-APV)) were added to the perfusion bath to block excitatory synaptic transmission. Both drugs were purchased from Sigma-Aldrich (Sigma-Aldrich Corp., St. Louis, MO). The GABA_A receptor antagonist (+)-bicuculline (Bicuculline; 10 μ M; Tocris Bioscience, Bristol, UK) was used to block inhibitory synaptic transmission. Tetrodotoxin (TTX; 1 μ M; EMD Millipore Corp., Billerica, MA) was used to block action potentials. Drugs were prepared daily from concentrated stock solutions; deionized water was added to dilute stock solutions to the appropriate concentration shortly before application.

E. Calcium fluorescence imaging and analysis.

Similar to previous work [35], calcium fluorescence imaging was performed using brain slices prepared from 17–30 days old transgenic mice (Thy1-GCaMP6f; Jackson Laboratory, Bar Harbor, ME). The slices were prepared and maintained using the same methods described above and were then incubated in a dark room at room temperature in the artificial cerebrospinal fluid (aCSF) solution. After a two-hour recovery period, slices that contained V1 were transferred and mounted, caudal side down, to the plastic recording chamber (RC-27L) with a plastic slice anchor (SHD-27LP/2).

Imaging was performed with a Nikon eclipse FN1 microscope (Nikon Instruments Inc, Melville, NY) through 4x, 10x, and 20x objectives (Nikon Fluor 4x and 10x air objectives; Nikon Fluor 20x/0.50 water immersion objective). The excitation light source (X-Cite 120Q; Excelitas Technologies Corp., Waltham, MA) was coupled to the epi-fluorescent port of the microscope. Calcium fluorescence changes were captured with a charge coupled device (CCD) camera (DFK 31BU03.H; USB 2.0 color industrial camera; 1024 $\times$ 768 pixels; 30 frames per second; The Imaging Source, LLC., Charlotte, NC). The actual imaging area was: 1335 $\times$ 1000 μ m with the 4x objective, 534 $\times$ 400 μ m with the 10x objective, and 267 $\times$ 200 μ m with the 20x objective.

Images were recorded using image capture software (IC Capture; The Imaging Source, LLC, Charlotte, NC) and processed using image analysis software (ImageJ; National Institute of Health (NIH)). Calcium fluorescent transients for individual L5 PNPs were extracted using methods described previously [35, 38, 45]. Briefly, outlines of individual PNPs were defined to create regions of interest (ROI) and the cellular calcium transients were calculated by averaging the pixels within each ROI. Calcium transients for neuropil within a 20 μ m annular-shaped region surrounding each neuron were also extracted for correction of neuropil contamination [45]. True fluorescence transients from a neuron (cell body) were estimated using the following equation:

$$F_{\text{true}}(t) = F_{\text{apparent}}(t) - r \times F_{\text{neuropil}}(t)$$

where t is time r is the contamination ratio ($r = 0.7$ was chosen for the 20x objective based on previous studies [38, 45]). After the neuropil correction, the calcium fluorescence transients for individual neurons were calculated as:

$$\Delta F/F_0 = (F - F_0)/F_0 \times 100\%$$

where F_0 was the baseline fluorescence level calculated by averaging over 2 seconds prior to the onset of stimulations. In order to compare the spatial extent of cortical activation for electric vs. magnetic stimulations, the calcium fluorescent transients for the entire imaging area ($F/F_{0,MAP}$) were calculated by dividing the imaging area into 256 grids and averaging the pixels within each grid. This $F/F_{0,MAP}$ shows stronger peak and baseline levels than those of the F/F_0 (individual neurons) because it includes the fluorescent signal from the surrounding neuropil.

Because it was difficult to keep the imaging plane perfectly level in our experimental setup, multiple cortical layers could not typically be brought into focus at the same time.

Therefore, when we imaged over large areas that include multiple layers, we adjusted focus to better show the activation in each layer individually. This limits the ability to compare (fluorescent) responses between layers from a single image.

F. Data Analysis

In all statistical analyses unpaired t-tests were used to assess whether the difference between the average values for different stimulation conditions was significant. Differences associated with P values <0.05 were regarded as statistically significant. Variances are reported as standard deviation, $\pm S.D.$, or standard error, $\pm S.E.$

III. RESULTS

A. Computational modeling of micro-coils.

We developed a computational model (Methods) that allowed us to study how different features of micro-coils influence the resulting fields as well as the field gradients that get induced from the flow of current through the coil. We were particularly interested in identifying designs that create strong field gradients in one spatial orientation without simultaneously creating strong gradients in orthogonal directions. Because the strength of the gradient along the length of a targeted nerve (or nerve fiber) is the driving force for activation, such a design could be used to strongly activate vertically-oriented PNs in the cortex (Fig. 1A, y-axis) without also activating horizontally-oriented passing axons and/or other horizontal processes (Fig. 1A, x-axis). The first design we evaluated was a simple rectangular-shaped coil on a silicon substrate (Fig. 1B and 1C). This approach resulted in designs for which the overall size was comparable to that of micro-fabricated electrodes that are routinely implanted into cortex without causing significant damage to the surrounding neural tissue [21, 36]. The coil length (1000 μm) was sufficient to target deep cortical layers in mouse or rat. The stimulus waveform was a single period of a 5-kHz sinusoid with an amplitude of 1 mA and was delivered with the initial phase in the counterclockwise direction (Fig. 1A, black arrows).

The resulting E-fields were evaluated over a 2D horizontal plane positioned 15 μm above the surface of the coil tip (Fig. 1B). Consistent with previous simulations, the E-fields were

strongest in the immediate vicinity of the wire with field strength generally uniform along the loop of wire (Fig. 1B, bottom). We plotted the spatial distributions of the field gradients (dE_x/dx and dE_y/dy) on the 2D plane of the coil (Fig. 1H top and middle panels) and found, as expected, that the peaks were strongest at the corners (sharp bends) of the coil. The bottom panel in Fig. 1H shows dE_x/dx vs. dE_y/dy along the horizontal component of the coil (see black horizontal lines with arrows in Fig. 1H top and middle panels). The positive peak of dE_y/dy arose at the left corner for a counterclockwise flow of current through the coil whereas dE_x/dx exhibited a negative peak at this same location. The polarity of the peaks was opposite at the bottom right corner of the coil. Also, the polarities of the gradients were inverted if the direction of the current flow through the coils was reversed. The peak value of dE_y/dy was 24 kV/m^2 , identical to the peak of dE_x/dx (24 kV/m^2). Both values are higher than the previously estimated threshold for activation of peripheral nerves with transcranial magnetic stimulation (TMS) coils (11 kV/m^2 , black horizontal dashed line in Figure 1H, bottom panel) [46]. Threshold levels here are dependent on the stimulus waveform frequency [44, 47]. The value of 11 kV/m^2 was derived from a stimulus pulse duration of $280 \mu\text{s}$ [46], similar to the duration of the 5-kHz sinusoid waveform ($200 \mu\text{s}$) used in this study. Actual threshold values for the cortical PNs have not yet determined but are likely to be equal to or less than the 11 kV/m^2 value given the higher sensitivity of the CNS [48–50], suggesting that both vertical PNs (y-direction) and horizontal passing axons (x-direction) in the region close to the coil would be activated by the configuration shown here. The activation of passing axons in the x-direction is undesirable however, as it spreads the effects of stimulation well beyond the local region of the coil and would therefore limit the spatial resolution that could be achieved.

We next considered a V-shaped coil (Fig. 1D), speculating that elimination of the horizontal segment might help to reduce the horizontal component of the induced field and gradient. This design was similar to that used in our earlier studies [35] and resulted in strong field gradients along the oblique component (the length of the ‘V’ portion of the coil) (Fig. 1I, top and middle). The field gradients were not uniform along the oblique components and so we plotted dE_y/dy vs. dE_x/dx at multiple locations along the coil (see black solid #1 and dashed #2 horizontal lines) in the bottom two panels of Fig. 1I. The peak of dE_y/dy was similar for the upper and lower parts whereas the peak of dE_x/dx was higher at the lower part. When we compared the peak field gradients over the 2D plane, we found that the V-design did indeed result in a slight increase in the ratio of vertical to horizontal gradients to $1.53\times$ (19 vs. 12.4 kV/m^2) (Figs. 2D and 2E). Although small, the increase in the ratio of the gradients was nevertheless encouraging because it suggested that additional changes to the coil geometry might lead to further enhancement of selectivity.

The effectiveness of the single bend in enhancing selectivity led us to question whether an additional bend might further reduce dE_x/dx . Therefore, we modeled a W-shaped coil (Fig. 1E) that was also $90 \mu\text{m}$ wide at the top but with two consecutive sharp bends at the bottom; the angle of each is 14° , $2\times$ smaller than the angle for the single bend of the V-coil (28°). Simulations revealed that the field gradients were also strong along the oblique component of the W-coil (Fig. 1J, top and middle panels) and that the ratio of gradients increased to $1.67\times$ (18.53 vs. 11.1 kV/m^2) (Figs. 2D and 2E).

While the V and W-coils generated a larger asymmetry in field gradients than the rectangular-coil, they also produced weaker peak gradients in the vertical direction (i.e. dE_y/dy was decreased by 20.8 % and 22.8 % for V and W-coils, respectively) (Fig. 2D). The reduction in strength of the field gradients led us to a question whether adding an additional loop might enhance the strength of field gradient. We simulated double-loop V- and W-coils (Fig. 1F and 1G) and found that they did enhance the strength of field gradients (dE_y/dy : 24.6 kV/m² for V-double and 23.64 kV/m² for W-double) (Figs. 1K–L and 2D). The ratio of the peak gradients was slightly reduced for the double-loop coils (1.4x for V-double and 1.53x for W-double) (Fig. 2E). Thus, the modeling results suggested that: (1) the use of one or more sharp bends enhances the selectivity of coil stimulation but reduces the effectiveness of stimulation; (2) the use of multiple loops enhances the strength of stimulation but slightly reduces selectivity.

To estimate the spatial extent of activation from the five different coil designs, we plotted contour lines indicating the area for which the field gradient exceeded the threshold of 11 kV/m² for each coil (Fig. 2A). Since estimated area could vary with current amplitude as well as with the threshold level for activation (not shown), we plotted contour lines when each coil generated the same peak level of dE_x/dx ; multiple levels were tested, e.g. 12–36 kV/m², in order to more completely assess sensitivity (Fig. 2A). While the rectangular-coil generated a region of activation that was similarly sized in both the x- and y-directions (0.9–1.03x) for all levels of peak dE_x/dx (Fig. 2A), the area for the V-coil was 2.5x larger in the y-direction at the 12 kV/m² level; the ratio decreased to 1.03–1.04x for the higher levels (24 and 36 kV/m²) (Fig. 2A). The area for the W-coil was even more asymmetric (11.7x larger in the y-direction) for the 12 kV/m² level while the ratio was similarly reduced (1.09–1.12x) at higher levels. Double-loop coils showed similar tendencies except maximum ratios were lower vs. single-loop coils (1.6x for V-double and 1.8x for W-double) (Figs. 2A). These results suggest that coil shape can influence the area of activation and that optimum stimulation strengths can produce highly preferential targeting of vertically-oriented neurons and thus highly asymmetric areas of activation. At higher stimulation levels however, the area ratios become more symmetric. The ratio of area was highly dependent on current amplitude and the estimate of threshold levels and therefore we did not use area ratios to further compare different coil designs and instead focused on the ratio of strengths of field gradients.

B. Fabrication of micro-coil probes and in vitro experiments.

V and W-coils were micro-fabricated for use in electrophysiological experiments so that the accuracy of model predictions could be evaluated and the performance of different coil designs compared (Figs. 3Aa and 3Ab). The coils consisted of a gold trace, 10 μ m wide x 2 μ m thick, on a silicon substrate that had a cross-sectional area of 50 $\times$ 100 μ m and a length of 4000 μ m (Fig. 3A). The gold coil patterns were insulated with a 500 nm-thick SiON_x layer to prevent the leakage of electric current into the tissue (Methods). V and W-coils with two loops (V-double and W-double, respectively) were also fabricated (Figs. 3Ac and 3Ad) to explore whether they enhanced the strength of stimulation. The single-loop coils had a DC resistance of $\sim$ 50 Ω and the double-loop coils had a DC resistance of $\sim$ 100 Ω . We used DC resistance for calculation of current amplitude for single and double-loop coils because

we used a low-frequency stimulus waveform (5 kHz) and the inductance of the coils was less than several tens of nH. The impedance of the coils at 5 kHz was therefore almost identical to the DC resistance.

The physiological experiments were performed using 300- μ m-thick coronal brain slices from mouse primary visual cortex (V1) (Fig. 3B); the preparation and positioning of brain slices (Methods) is identical to that used previously [27, 28, 35]. A cell-attached patch-clamp recording electrode was positioned on the soma of individual L5 PNs to record action potentials elicited by magnetic stimulation. A fabricated micro-coil was mounted on a micro-manipulator and its tip was positioned over the brain slice such that the long-axis of the probe was parallel to the long-axis of the targeted PN (i.e. aligned with the vertical orientation of the cortical column). The tip of the probe was further aligned so that the location at which the field gradient was strongest was situated directly over the proximal axon of the targeted L5 PN (Fig. 3B, red circle, ~ 50 μ m below the soma), the portion of the neuron known to have the highest sensitivity to stimulation [48–51]. This typically placed the tip of the coil over Layer 6 (L6) although for W-coils the tip was 140 μ m deeper vs. the tip for V-coils (Fig. 3C, compare left and right panels). With this alignment, the region of suprathreshold field gradient (yellow shaded area in Fig. 3C) largely overlapped L5. Once aligned, the height of the coil was lowered until the coil tip was fully inserted into the slice. This resulted in confinement of elicited fields to within gray matter and greatly reduced the boundary effects such as those at the border between gray and white matter, or between gray matter and aCSF, and thus best replicated the configuration that arises *in vivo*. Responses were recorded from a total of 25 L5 PNs (10 slices); somas were always located within 20 μ m of the edge of the silicon probe (Fig. 3C).

To determine whether magnetic stimulation from the fabricated micro-coils activated L5 PNs directly, initial experiments were performed in the presence of synaptic blockers for AMPA receptors (10 μ M 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX)), NMDA receptors (50 μ M D-2-amino-5-phosphono-pentanoic acid (D-APV)), and GABA_A receptors (10 μ M bicuculline). The stimulus waveform delivered to the coil was the same one used in the simulations: a single period of a 5-kHz sinusoid delivered repetitively at 1 Hz. At amplitude levels of ~ 15 mA, a multiphasic waveform was recorded from the cell (Fig. 3D, thick gray curve) that had a duration of ~ 2 ms, considerably longer than that of the electrical artifact (~ 0.4 ms). To determine whether the prolonged waveforms contained one or more action potentials, we added 1 μ M tetrodotoxin (TTX) to the bath to block all voltage gated sodium channel activity and found the prolonged waveform was eliminated (Fig. 2D, red curve). We used the TTX waveform as a template of the stimulus artifact and subtracted it from the raw (gray) waveform. The resulting waveform (Fig. 3D, blue curve) had an identical shape to that of action potentials arising spontaneously (not shown), confirming the ability of micro-coils to elicit action potentials (APs) in L5 PNs.

Similar to electric stimulation, the likelihood of eliciting action potentials was dependent upon the amplitude of stimulation. Typical response curves are shown in Fig. 3E (n=5 cells); because the curves overlap, we used small arbitrary offsets to better visualize the individual responses. Threshold levels were calculated (Methods) by fitting a sigmoid to the response curves and determining the stimulus amplitude levels that produced spikes in 50% of trials.

The mean threshold for activation with the V-single coil was $13.07 \text{ mA} \pm 0.61$ (S.D.). We repeated the same measurements for the other coil designs of Fig. 3A and calculated the average number of spikes as a function of current amplitude for each (Fig. 3F). Mean threshold levels are plotted in Fig. 3G (red crosses). Consistent with the modeling predictions (Figs. 1 and 2), the mean thresholds of V-double coils ($6.54 \text{ mA} \pm 0.44$, $n=5$ cells) were approximately one-half that of V-single coils (13.07 mA). Similarly, thresholds for the W-double coils ($8.14 \text{ mA} \pm 0.49$, $n=8$ cells) were about one-half that of W-single coils ($15.08 \text{ mA} \pm 0.83$, $n=7$ cells).

Interestingly, the mean threshold for W-single coils ($15.08 \text{ mA} \pm 0.83$, $n=7$ cells) was $\sim 1.15\times$ higher than that for V-single coil (13.07 mA) (two tailed t-test, $p=0.001$) and similarly, the mean threshold for the W-double coils was $\sim 1.24\times$ higher than that for the V-double coils (two tailed t-test, $p=0.0001$). The differences in the thresholds between the V and W-coils were consistent with the simulation results in which the strength of field gradient (dE_y/dy) at the target cell location (Fig. 3C) was $1.16\times$ and $1.39\times$ higher for the V-single and V-double coils, respectively (not shown). The consistency between the simulations and the physiological results is encouraging because it suggests that the model provides accurate predictions for different coils designs and thus can be used to effectively screen different design features in future evaluations. The physiological testing also supports the modeling finding that V-shaped coils produce stronger field gradients for a given level of current through the coil than W-shaped coils.

C. Coil design influences the spatial extent of activation.

The modeling results of Figures 2E indicated that W-coils result in a larger ratio of vertical to horizontal field gradients than V-coils and therefore suggest that W-coils will be more selective for activating vertically-oriented PNs without simultaneously activating horizontal processes. The reduced likelihood of activating horizontal processes with W-coils suggests that the resulting region of activation is likely to be more spatially confined than that from V-coils. To explore this, we conducted a series of calcium fluorescence imaging experiments using brain slices from the GCaMP6f mouse (Methods). PNs from these animals express a fast calcium indicator that increases its level of fluorescence when the concentration of intracellular calcium increases, e.g. as the result of spiking [37, 38]. A $500\text{-}\mu\text{m}$ -thick coronal brain slice that contained V1 was mounted in a recording chamber under an epi-fluorescence microscope with a CCD camera (Methods) and the fluorescent response across the slice was evaluated for different coils and different stimulating conditions.

Prior to evaluating the responses to magnetic stimulation, we first measured the responses to electric stimulation delivered via a microelectrode ($1 \text{ M}\Omega$, platinum-iridium electrode, PI2PT31.0A10, Microprobes for Life Science, MD, USA); the tip of the electrode was positioned over the proximal axon of the L5 PN (Fig. 4B). The stimulus train consisted of 20 pulses delivered at a rate of 100 Hz . Individual pulses were biphasic rectangular waveforms that were $200 \mu\text{s}$ in duration and $20 \mu\text{A}$ in amplitude; pulses were cathodic-first without an inter-phase-interval (Fig. 4A, inset, top). This pattern of stimulation is similar to those previously shown to elicit behavioral responses *in vivo* in non-human primates (NHPs) [8,52]. The amplitude was set to a level $1.3\times$ higher than the activation thresholds measured

in the earlier electrophysiological experiments (referred to as 1.3T). Initially, the region over which the fluorescence change was measured was limited to the soma of a single L5 PN (yellow circle in Fig. 4B). Delivery of the stimulus resulted in a rapid increase in the level of fluorescence (F/F_0 of 98%) during the period of repetitive stimulation (blue vertical bar) and a more gradual decrease after termination of the stimulus; this response is consistent with previous studies of fluorescent responses during moderate to strong levels of spiking [38]. To examine the spatial extent of the fluorescence change (Methods), the region of interest (ROI) was expanded to the full field of the microscope ($267 \times 200 \mu\text{m}$) and a color map was used to represent the level of fluorescence change over the entire imaging area (F/F_0 , MAP, Methods, Fig. 4D); a blue color indicates no change in the baseline level of observed fluorescence at that location and red indicates a 100% increase over the average baseline level. In this manner, separate images could be used to depict the time-lapse progression of the response and images corresponding to 0, 200, 400 and 600 ms after stimulus onset are shown. Similar to the responses in individual cells, the population fluorescence response ($F/F_{0, \text{MAP}}$) peaked ~ 200 ms after the onset of stimulation with a strength $> 140\%$ ($F/F_{0, \text{MAP}}$, Fig. 4D). Note that the peak response level across the larger field was slightly stronger than the peak for the single cell responses (140% $F/F_{0, \text{MAP}}$ vs. 110% in F/F_0), probably because responses within the neuropil (Methods) were included in $F/F_{0, \text{MAP}}$. Strong fluorescent responses were observed across the entire ROI ($267 \times 200 \mu\text{m}$) and the broad spatial spread of activation is consistent with previous reports [35, 53–57].

We replaced the electrode with a V-single coil (Fig. 4E) so that the analogous responses to magnetic stimulation could be measured; a train of 20 ‘pulses’ was delivered at a rate of 100 Hz although similar to the earlier experiments with coils, each pulse was actually a single period of a 5 kHz sinusoid (Fig. 4A). The stimulus amplitude again corresponded to a 30% increase over the activation thresholds measured in the single cell electrophysiology experiments (1.3T, corresponding to 17 mA).

Similar to the responses to electric stimulation, there was a strong increase in fluorescence ($F/F_0 > 70\%$, Fig. 4E and 3F) although the magnitude of the response was somewhat less than that of the electrical response. When responses over the larger region were evaluated, the region strongly activated by stimulation (i.e. $F/F_{0, \text{MAP}} \approx 100\%$) was confined to only the left side of the coil (Fig. 4G, red colored area) and matched well to the region predicted by computational modeling (Fig. 2A). The location of activation shifted from the left side of the coil to the right (not shown) by reversing the direction of current flow through the coil (Fig. 4E, yellow upward arrow); this switch was consistent with model predictions. The spatially confined region of activation found here supports earlier results and suggest that activation from a magnetic coil can be limited to focal regions around the coil, and further that such regions can be accurately predicted from theoretical models. The narrow region of activation from the coil is in sharp contrast to the spatially broad activation from the electrode. It is possible that non-linear scaling differences arise when the two thresholds are scaled, e.g. multiplying the threshold for electric stimulation by a factor of 1.3 is somehow not equivalent to multiplying the threshold for magnetic stimulation by the same factor. If so, these differences could contribute to the observed differences in the extent of spatial

activation. We did not attempt to explore this further however in part because such differences are inherent to their use.

The V-coil was replaced by a W-coil (Fig. 4H) and a 100 Hz train of twenty 5-kHz sinusoidal waveforms at 1.3T (20 mA) was delivered. The direction of current was reversed for this experiment (Fig. 4H, yellow upward arrow) and so the CCD camera was focused on the right side of the W-coil (e.g., Fig. 1J and Fig. 2A). Consistent with the modeling predictions, there was a strong increase ($\sim 50\%$) in fluorescence (Fig. 4I) in the single cell (Fig. 4H, yellow circle) as well as for the population response with the region of strong activation ($F/F_{0,MAP} \approx 100\%$) confined to the right side as predicted (Fig. 4J).

D. W-shaped coils improve spatial resolution in stimulation of L5.

Because the size of the imaging area ($267 \times 200 \mu\text{m}$) in the earlier experiments was not large enough to compare the responses from both the left and the right sides of the coil, we expanded the imaging area by a factor of 4 ($534 \times 400 \mu\text{m}$) by replacing the 20x water immersion objective of the microscope with a 10x air objective (Methods). This allowed neurons from both sides of the coil to be visualized and also allowed neurons in Layers 4–6 to be observed (Fig. 5); L4 neurons do not express fluorescence in the *Thyl-GCaMP6f* transgenic mice used here however and so observations of the spread of activation were limited to L5 and L6. Responses were measured at 200 ms after the onset of stimulation, the latency for which they are strongest (Fig. 4D). Electric stimulation at high amplitude (2T) produced strong responses ($F/F_{0,MAP} > 100\%$) that extended in all directions over L5 and L6 (red zone in Fig. 5B). In contrast, the same level of magnetic stimulation (26 mA, 2T) from the V-coil produced only a moderate increase ($\sim 50\%$) in $F/F_{0,MAP}$ that was confined to a much smaller spatial region (white zone in Fig. 5D). The relatively weak responses observed here to a strong (2T) stimulus are in contrast to the stronger responses ($\sim 100\%$ in $F/F_{0,MAP}$) observed with the smaller imaging area and weaker (1.3T) stimulus (Fig. 4G). The difference likely arises because fluorescence ($F/F_{0,MAP}$) from the V-coil was localized to a relatively small region and therefore had more of a limited effect with the larger imaging area, especially because the signal-to-noise ratio was lower than that of the smaller imaging area.

The zone of activation for the V-coil was found mostly on the left side of the coil although some weak activation ($\sim 30\%$ in $F/F_{0,MAP}$) was also observed on the right side as well (Fig. 5D, yellow arrow). This ‘extra’ activation was confined mainly to L6 PNs. While we did not investigate the underlying mechanism in detail, it seems likely that axons in the L5/L6 region were mildly activated by stimulation. A sub-population of axons in this area are known to course from the upper left to the lower right [58] and thus could underlie the responses observed below and to the right side of the coil. While the driving force for activation with V-coils is stronger in the vertical vs. horizontal directions, the ratio of 1.53 suggests only moderate selectivity, and would be even less for diagonal orientations. Magnetic stimulation with the W-single coil (Fig. 5E) (30 mA, 2T) also produced only a moderate increase (40–50%) in $F/F_{0,MAP}$ but the spatial extent of activation was now narrowly confined to the left side of the coil only (light blue and whitish zone in Fig. 5F).

We did not observe a lateral spread of activation, as occurred with the V-coil (Fig. 5F, yellow arrow), likely due to the increased selectivity for activation of vertical neurons with W-coils.

E. Spread of excitation to other cortical layers.

Electric stimulation delivered to deeper cortical layers (e.g. L5/6 and the white matter (WM) below) can lead to strong excitation in the superficial layers (e.g. L1-L2/3) [53, 56, 57, 59]. This activation can spread widely in the horizontal direction as well, up to 2–3 mm, via the horizontal axonal projections that connect neighboring cortical columns [56, 60]. We hypothesized that by confining the extent of activation at the site of stimulation (with coils), it might help to reduce the resulting spread in the superficial layers. To explore this, we increased the imaging area to $1335 \times 1000 \mu\text{m}$ (Fig. 6) which allowed us to view responses in both L2/3 and L5/6 PN. Similar to our earlier approach, electrical stimulation (30 μA , 2T) from an electrode positioned at the L5/6 border (Fig. 6A) produced a strong increase in $F/F_{0,\text{MAP}}$ in both L5/6 (~100%) as well as L2/3 (~100%) (Fig. 6B). Similar to L5, the cortical activation in L2/3 spread extensively in the horizontal direction (Fig. 6B, yellow arrows).

To quantitatively analyze the extent of the spread in L2/3, we measured the F/F_0 for several different regions of interest (ROIs), each corresponding to a different horizontal location within L2/3 (Fig. 6A, boxes labeled 1–7). Each ROI was $50 \times 50 \mu\text{m}$ square and the 7 ROIs (#1 – 7) were tiled along L2/3, extending a distance of 350 μm from the electrode (Fig. 6A, yellow square regions). Figure 6C shows the F/F_0 in response to repetitive electrical stimulation (30 μA , 2T) as a function of horizontal distance for all 7 ROIs; the level of fluorescence (F/F_0) increased in all ROIs in response to stimulation (Fig. 6C). The peak F/F_0 was strongest (~100%) in ROIs #1 and #2 and gradually decreased with increasing distance from the electrode. Peak levels remained substantial (>25%), even at the farthest ROI (350 μm , #7).

When the electrode was replaced with a V-single coil positioned at L5/6 (Fig. 6D), stimulation at 2T resulted in a moderate increase (50–70%) in $F/F_{0,\text{MAP}}$ in L2/3 on the left side of the coil (Fig. 6E, left arrow). The lateral spread of activation in L2/3 was restricted to ~200 μm on the left side (Fig. 6E, left arrow) and to ~50 μm on the right side (Fig. 6E, right arrow). It should be noted that Figure 6E showed the fluorescence transients from a very large area so that there was a relatively high level of went above this level of response, e.g. a green color in Fig. 6E representing activity levels of 50–70%. It is also worth noting that Figure 6E shows the fluorescence transients in L2/3 when stimulation was delivered to L5/6. Note that the focus in these images was adjusted to better show activation in L2/3. We were unable to keep the imaging plane perfectly flat and so multiple layers could not be brought into focus at the same time (Methods). As a result, the strong fluorescence response seen previously in Layers 5 & 6 (Figs. 4G & 5D) are not visible here.

Figure 6F summarizes the fluorescence changes produced by the V-coil as a function of horizontal distance and confirms that fluorescence changes (5–22% in F/F_0) were confined to only the 4 closest ROIs (distance $\leq 200 \mu\text{m}$, Fig. 6D and 6F). There were no noticeable fluorescence changes for distances of 250 μm or greater (#5–7 ROIs).

Similar to earlier experiments, magnetic stimulation at 2T from the W-single coil resulted in a lateral spread of activation that was confined to only one side of the W-coil (see the green area in Fig. 6H, right arrow). The changes in fluorescence level were modest (5–28%) and confined to the 4 closest ROIs (distances $\approx$ 200 μ m, Fig. 6G). The peak fluorescence levels decreased more rapidly with increasing distance for the W-coils than with the electrode or the V-coil (Fig. 6I, compare the peaks of #1–3 traces).

To quantitatively compare the spatial extent of activation in L2/3, we repeated these measurements in 5 different brain slices and plotted the average peak fluorescence changes in L2/3 as a function of distance for the electrode and the two coils (Fig. 6J). Consistent with the results above, electrical stimulation produced strong fluorescence changes in L2/3 with activation that extended beyond the 350 μ m measured region. The response to magnetic stimulation with both the V and W-single coils was weaker and more narrowly confined (Fig. 6J). The region of activation with the W-single coil was slightly narrower than that from the V-single coil (Fig. 6J), suggesting that the W-shaped coil design better confines the lateral spread of activation in L2/3. Figure 6K shows the fluorescence changes over distance normalized to the peak value from each trace. Dashed vertical lines reveal the half-maximum level (50 %) for all three methods of stimulation tested here; the half-maximum extent for all brain slices was plotted in Figure 6L. The mean half-maximum extent for the W-single coil was $132.6 \mu\text{m} \pm 17.99$ (S.D.), whereas the levels for the V-single coil and the electrode were $192 \mu\text{m} \pm 20.68$ (S.D.) and $259.2 \mu\text{m} \pm 23.13$ (S.D.), respectively (Fig. 6L). This further supports the notion that W-shaped coils better confine activation (two tailed t-test: $p=0.0001$ for W-coil vs. Electrode; $p=0.0013$ for W-coil vs. V-coil; $p=0.0013$ for V-coil vs. the electrode) and that the effects extend not just to the region immediately surrounding the coil but to synaptically connected regions as well. This feature may be useful for visual prostheses as well as other cortical implants that require enhanced spatial resolution.

In our final experiment, we explored whether the activation in L2/3 was mediated by the direct activation of passing axons from L2/3 PNs. Figure 7 shows the effect of the use of the synaptic blockers (10 μ M NBQX and 50 μ M APV). Overall calcium fluorescence responses along with the baseline activity became weaker with synaptic blockade but the spread of activation in L2/3 remained for the electrode and coil stimulations ($n=3$ different brain slices). Under synaptic blockade, the spatial extent of activation of L2/3 was confined to smaller areas during the stimulations with the coils than with electrodes. The W coils could also better confine the activation of L2/3 than the V coils. These results suggest that coil-based stimulation reduces the direct activation of passing axons from L2/3 PNs during stimulation of L5. However, our results did not show activation of vertically-oriented thalamic projection axons therefore much additional testing is needed before we understand the full extent of the response differences for electric vs. magnetic stimulation.

IV. DISCUSSION

A. Different design features influence the strength and selectivity of stimulation.

The goal of this study was to test whether changes to the micro-coil design improved the strength of stimulation and/or the selectivity for activation of vertically- vs. horizontally-oriented neurons in cortex. Initially, simulations were used to calculate the strength of the

gradient of the induced electric field as a measure of the driving force for activation [27, 35, 41] and we compared strengths in the vertical (dE_y/dy) and horizontal (dE_x/dx) directions arising from rectangular, V-shaped and W-shaped coils (Fig. 1H–1J). The models indicated that the rectangular coil produced the strongest field gradient in both directions (dE_y/dy : 24 kV/m^2 vs. dE_x/dx : 24 kV/m^2); this suggested effective activation of PNs but the similarity of gradients (ratio ~ 1) suggested horizontally-oriented neurons would be strongly activated as well. When V- and W-shaped coils were modeled, the peak strength of the gradients in the vertical direction was found to decrease by 20.8% and 22.8%, respectively, suggesting higher-amplitude currents would be required for activation. However, the even-larger increases needed for activation in the horizontal direction resulted in an increase in the ratio of gradients to 1.53x and 1.67x for the V and W-coils respectively. This suggested less activation of horizontal processes and therefore better spatial confinement. Therefore, our modeling results suggest that the use of a sharper bend enhances the selectivity of intracortical stimulation but also reduces the strength of stimulation and thus there is a trade-off between the strength of stimulation and selectivity.

Physiological recordings in mouse brain slices confirmed that the different coil designs do indeed influence the thresholds for activation of L5 PNs. Consistent with the model prediction that the V-single coil produces a stronger field gradient vs. the W-single coil for a given amplitude of current flow through the coil, the thresholds for V-coils were ~ 1.15 x lower than those for W-coils (Fig. 3G, V-coil: 13.07 mA; W-coil: 15.08 mA). The close correlation between the modeling predictions (factor of 1.15) and the physiological thresholds supports the validity of these types of models, especially for evaluating the relative effectiveness (i.e. strength and selectivity) of various coil designs. It is important to note however that the model predicted a threshold level of 0.58 mA for the V-coil (Fig. 2D) while the actual thresholds were closer to 14 mA (Fig. 3G). This suggests that although the model could produce qualitatively accurate predictions, the absolute value of some of the predictions could be off by more than an order of magnitude. This is not entirely surprising given the need for estimations and the use of simplifying assumptions (in the model). It will be interesting in future studies to explore whether more accurate estimations can improve the quantitative accuracy of the simulations. Future improvements to the model will also be useful for assessing the relative contributions of field strength vs. gradient strength in mediating activation. The model used here predicted field strengths of ~ 2 V/m with a 1 mA stimulus. The actual (physiological) thresholds were often over 10 mA and would therefore correspond to predicted field strengths > 20 V/m. In a few experiments, thresholds were over 20 mA and the resulting fields would therefore exceed 40 V/m. While still below the thresholds thought necessary for activation by TMS (60–100 V/m) [61], the fields are strong enough that some activation might have occurred. The large field strengths from TMS have relatively weak gradients and therefore are thought to initiate spiking in regions where the axon bends or at locations where neuronal processes terminate [46]. However, in much preliminary testing, both here and earlier [27] we could not induce activation from stimulation off of the central axis of the neuron, e.g. over dendritic processes as might be expected from strong fields [62]. The strong qualitative agreement between model predictions, e.g. Fig. 2A, and the physiologically-measured spatial extent of activation,

supports the notion that gradient indeed underlies activation and is consistent with previous work [27].

B. Coil design shapes the spatial extent of cortical activation.

Our *in vitro* calcium fluorescence experiments showed that the spatial extent of activation arising from coils was confined to a region much smaller than that arising from conventional electrodes. Consistent with the model prediction, the zone of activation for coils was confined to only a single side of the coil (where field gradient was strongest) (cf. Fig. 1I, 3C and 4G); this was in marked contrast to the uniformly distributed zone of activation around the electrodes (Fig. 4D). Also, consistent with the model prediction, the V-coil produced a change in fluorescence that was stronger and spatially broader than that of W-coils (cf. Fig. 5D vs. 5F), supporting the notion that coil design influences the selectivity of activation and further that computational modeling can be used to accurately predict the spatial extent of cortical activation around the coils. One additional finding from the present study is that the zone of activation can be shifted from one side of the coil to the other side by simply reversing the direction of current flow (cf. Fig. 4J and 5F, yellow upward arrows). This is most likely due to the tri-phasic waveform of current through the coil [27] (Fig. 4A, inset, bottom); the direction of the induced fields are opposite on the two ends of the coil and thus neurons on one side are depolarized while those on the other side are hyperpolarized. The prolonged duration of the second phase may be the dominant factor for activation [43, 44] but it is also possible that the first phase is supra-threshold and further, complex interactions may arise from the different phases. Regardless of the actual mechanism, because a single coil can be used to induce activation in two discrete regions in cortex, it is possible that each coil will be able to provide two distinct ‘channels’ of stimulation. This may be useful in visual prostheses or other devices for which high-acuity stimulation is desirable. Note that the use of two or more electrodes, e.g. bipolar configurations may improve the selectivity of activation but given the predominate use of the monopolar configuration with micro-electrodes, we felt it appropriate to focus on this configuration. It is worth noting however, that previous testing with bipolar electrodes still found it difficult to avoid the spread of activation [54–56] and were not able to confine activation to within a small volume of cortex.

C. Multi-loop coils enhance the strength of stimulation but not the selectivity.

Our simulation and experimental data support the notion that double-loop coils can increase the strength of the resulting E-field gradient and thus can enhance the strength of stimulation, e.g. reduce the thresholds required for activation (Figs. 2D and 3G). Our simulation data showed that the strengths of induced fields with the double coils were 1.65x stronger than that with the single coils (Fig. 2B) and that the field gradients with the double coils were 1.4x stronger than that with the single coils (Fig. 2D). The higher strengths with the double coils may be useful for lowering the thresholds for activation, i.e. in future psychophysical testing.

In physiological experiments, the thresholds for double V-coils were 6.54 mA and for double W-coils were 8.14 mA, a 2.0x and 1.9x reduction in threshold vs. that for the corresponding single coils. The peak power level for the double V-coil was 2.1 mW, a level that is ~4.2x

higher than that for intracortical electric stimulation (e.g. 0.5 mW) [63, 64], but is comparable to that for existing clinical devices, e.g. the power levels for deep brain stimulation range from 2–24.5 mW [65]. The use of materials that enhance magnetic field strength (e.g. ferrite, Mu-metal, and permalloy) may also help to reduce power levels even further although concerns about the biocompatibility of such materials will need to be explored.

Increasing the number of loops will also increase the physical size of coil-based implants and therefore, raises concerns about safe implantation into cortex. Figure 1F shows an example of the flat (2D) concentric double-loop configuration in which each loop is positioned on the surface of a silicon substrate. The dimension of the double-loop coil itself (the metal trace) is $90 \times 2 \mu\text{m}$ and therefore does not significantly increase the overall size of the substrate ($100 \times 50 \mu\text{m}$), e.g. the increase is only to $100 \times 52 \mu\text{m}$. Adding an 8-loop design would increase the coil dimension from $90 \times 2 \mu\text{m}$ to $280 \times 2 \mu\text{m}$, and the resulting device dimension would increase to $280 \times 52 \mu\text{m}$. This more than doubles the width of the implant over that of the existing electrode-based devices currently used for chronic implantation [6, 36, 66, 67], and therefore would introduce concerns about increased damage to the surrounding brain tissue. There is the possibility that a more complex configuration, such as a 3D multi-layered structure (Fig. 8), implemented by alternately stacking a small single loop ($60 \times 2 \mu\text{m}$) and a dielectric layer ($60 \times 0.5 \mu\text{m}$), would enable the 8 loop coil to be confined to a smaller physical size ($60 \times 20 \mu\text{m}$). This multi-layered structure may still be able to endure some of the mechanical stress that arises during implantation, even without the bulky substrate, but the design and fabrication of the 3D structure is more challenging than that of the 2D flat structure and will require further developmental efforts.

D. Coil designs influence the spread of activation to other cortical layers.

Previous studies [53, 56, 57, 59] have shown that electric stimulation of L5 with monopolar and/or bipolar electrodes results in a spread of activation in the vertical direction to L2/3 and also a spread in the horizontal direction within L2/3 by as much as 2–3 mm. Consistent with these earlier studies, our results showed that electric stimulation of L5 in V1 with the monopolar electrode produced strong cortical activation (i.e. $F/F_{0,MAP} > 80 \%$) in not only L5 (see Fig. 4D and Fig. 5B) but also L2/3 (see Fig. 6B and 6C). The activation in L2/3 spread extensively in the horizontal direction and remained strong ($F/F_{0,MAP} > 20 \%$) for distances up to $325 \mu\text{m}$. In contrast, magnetic stimulation of L5 using the V- and W-coils produced strong cortical activation (i.e. $F/F_{0,MAP} > 80 \%$) within a smaller area in L5 ($\sim 100 \mu\text{m}$; see Fig. 4G and 4J) but activation levels in L2/3 were weak (i.e. $F/F_0 < 30 \%$, Fig. 6F and 6I).

The weak activation in L2/3 did not spread in the horizontal direction beyond a distance of $175 \mu\text{m}$. This suggests that magnetic stimulation with the V- and W-coils can strongly activate L5 PNPs in the region immediately surrounding the coils and that this confinement of activation minimizes the subsequent spread of activation in other layers. It is worth noting again that our imaging plane was not perfectly level and therefore layers 2/3 and 5 could not be brought into focus at the same time. We adjusted focus to better show the activation in

L2/3 in Figs 6E and 6F and even though there did not appear to be a strong response in layers 5 & 6, the response could actually be quite strong, e.g. Figs. 4G & 5D.

Thus, these results suggest that W-coils can selectively confine activation to single cortical layers, i.e. the coils may be useful for activating L2/3 PNs without activating L5/6 PNs, and vice versa. This selective targeting of L2/3 PNs but not L5/6 PNs may be desirable for the application of visual prosthetics because the L2/3 PNs and L5 PNs are thought to have different roles in visual processing. The axons of L2/3 PNs form the principal projection to secondary visual cortex (V2) and they are therefore thought to process much of conscious visual perception [68] while the axons of L5 PNs form a strong projection to the superior colliculus [69, 70] and have been shown to mediate saccades. Implants that can selectively target individual layers may allow the functional role of the outputs from each layer to be studied in isolation.

V. CONCLUSION

In the present study, we explore how different design features of micro-coils influence the strength and the spatial resolution of intracortical magnetic stimulation. Our principal findings are: (1) V-shaped coils enhance selective activation of vertical PNs over horizontal axons, probably because the sharp bend at the end of the coil helps to minimize the driving force for activation of horizontally-oriented passing axons; as a result, the spatial extent of activation in cortex is better confined than that of conventional electrodes; (2) the addition of a second bend (W-shape) enhances selectivity even further, but slightly reduces the strength of stimulation; (3) the addition of multiple loops to the coil increases the strength of stimulation over that of single-loop coils, thereby enabling the reduction of activation thresholds.

The enhanced selectivity of coils is highly attractive for a variety of cortical prostheses currently under development that strive aim to restore vision [2, 3, 5], hearing [71, 72], and tactile sensations [1, 9]. Given that the spatial extent of cortical activation by the W-coil (50~100 μm) is smaller than the size of a single cortical column (~500 μm), or, the thickness of a single cortical layer (~200 μm), a micro-coil array could be developed whereby neural activity at different locations in single cortical columns could be independently and precisely controlled. This level of activation with micro-coils would also be potentially advantageous for studies with small laboratory animals as it allows for very precise targeting of specific neurons within a focal region. Further optimization of coil design may be possible via the combination of modeling & physiological experiments used here. It will be interesting to learn whether the benefits of new designs translate into enhanced performance over that of conventional electrode implants.

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Fig. 1.

Micro-coil shape influences selectivity. (A) Conceptual diagram of intracortical magnetic stimulation using a bent micro-wire. (B) Simulation of electric field arising from a model coil. Electric field arising from the coil was evaluated in a $4 \times 4 \times 4$ mm volume surrounding the coil (Methods). The spatial gradient of the electric field was calculated on the plane that was positioned 15 μm above the surface of the coil. (C) Single loop rectangular shape coil. (D) Single loop V shape coil. (E) Single loop W shape coil. (F) Double loop V shape coil. (G) Double loop W shape coil. (H-L) Spatial gradients of the E-fields for the different coil designs in C-G. Top panels show the distributions of the field gradients along the x-axis (dE_x/dx) and middle panels show the distribution along the y-axis (dE_y/dy) for the different coil designs. Bottom panels show the traces of dE_x/dx (blue curve) and dE_y/dy (red curve) along horizontal lines depicted (with arrowheads) in the top and the middle panels. Note that the field gradient traces for the coils in I-L were plotted along two horizontal arrow lines (solid and dashed).

Fig. 2.

Micro-coil shape influences the spatial extent of activation. (A) Red and blue contour lines indicate the areas for which dEy/dy and dEx/dx were respectively suprathreshold ($> 11 \text{ kV/m}^2$). Different line thicknesses correspond to different peak dEx/dx values: 12, 24, and 36 kV/m^2 (see scale below). (B) Peak electric fields in x- and y-directions for the coil designs with a current amplitude of 1 mA. (C) Ratios of E_y to E_x in B. (D) Peak field gradients in x- and y-directions for the coils for the 1 mA. (E) Ratios of dEy/dy to dEx/dx in D.

Fig. 3.

Coil design influences activation threshold. (A) Schematics & photographs of the four different micro-coils designs. (B) Schematic representation of the *in vitro* test configuration. Micro-coils were positioned in V1 of mouse brain slice and a patch electrode was used to record neural responses of targeted PNs to magnetic stimulation. Red dotted circle indicates the approximate region of activation from the micro-coil. (C) Illustrative diagram of targeted PNs in the region around micro-coils. The long axis of coil probes was aligned with the principal axis of the PNs. Cell bodies of the PNs were located 15 μm above the surface of micro-coils, 20 μm away from the side of the coils and 50 μm above the sharp bend of the coils. Yellow shaded area indicates the estimated region for which spatial field gradients along the y-axis (dE_y/dy) exceed 11 kV/m^2 . (D) Responses of PNs to stimulation from micro-coils under pharmacological blockade of synaptic input (thick gray curve, normal aCSF + 10 μM NBQX + 10 μM Bicuculline + 50 μM D-APV) and after the addition of 1 μM

TTX (red curve). The blue curves were computed by subtracting the TTX traces (red curve) from the raw recording (thick gray curve). Arrow indicates the evoked action potential. (E) Number of evoked action potentials as a function of stimulation strength (Each trace was from a separate L5 PN, 5 presentations at a repetition rate of 1 Hz, $n = 5$ cells). Bars indicate the standard deviation (S.D.). (F) Averaged number of evoked action potentials as a function of stimulus amplitude for four different coil designs (V single: $n=5$ cells; W single: $n=7$; V double: $n=5$; W double: $n=8$). Bars indicate the standard error (S.E.). (G) Threshold current for eliciting action potentials for the four coil designs. (two tailed t-test: * $p=0.001$; ** $p=0.0001$) Red crosses indicate mean threshold currents.

Fig. 4.

Coil design shapes the spatial extent of activation. (A) Stimulus waveforms consisted of 20 presentations delivered at 100 Hz. Each stimulus consisted of one full period of a 5-kHz sinusoid (left). The tri-phasic waveform of the field induced by the stimulus is depicted in the inset (bottom, red trace). For comparison, the bi-phasic electric stimulus waveform is shown above (inset, top blue trace). (B) Epi-fluorescence microscope photograph of a V1 coronal slice from a Thy1-GCaMP6f transgenic mouse; a microelectrode (1-MΩ 3Iridium-Iridium) is positioned directly on the slice and a schematic representation is overlaid to more clearly show its location. (C) The change in fluorescence of the L5PN soma (yellow circle in B) in response to electrical stimulation. The stimulus was a train of 20 pulses at 100 Hz with current amplitude of 20 μ A. (D) Time-lapse images of the fluorescence change in response to the stimulus. (E) Similar to B, except a V-shaped micro-coil is implanted in the V1 slice. (F) Similar to C, in response to magnetic stimulation from the V-shaped coil in E. (G) Similar to D, but with a V-shaped coil. (H) Similar to B and D, but with a W-shaped micro-coil. (I) Similar to F, showing the fluorescence level change of the L5 PN (yellow circle) in response to magnetic stimulation from the W-shaped coil in H. (J) Similar to G, but for the W-shaped coil. Note that the direction of the coil current (yellow upward arrow) was inverted so that the zone of excitation (red) occurs on the right side of W coil; this is consistent with the model prediction (Fig. 1J).

Fig. 5.

Coil design influences spatial resolution. (A) Epi-fluorescence microscope photograph of an electrode in a V1 slice. Dashed horizontal lines indicate approximate border between L4 and L5. (B) The peak fluorescence change (at 200 ms) in response to electrical stimulation (30 μA , 200 μs , 20 pulses at 100 Hz) from the electrode in A. (C) Similar to A, showing a V coil in a V1 slice. (D) Similar to B, showing the peak fluorescence change in response to magnetic stimulation (26 mA, a full period of 5 kHz sinusoid, 20 pulses at 100 Hz). Yellow downward arrow points to a region of activation on the right side off the V coil. Dark blue region in the bottom left represents a saturated level of brightness in the CCD camera image. (E) Similar to A and C, showing a W micro-coil in a V1 slice. (F) Similar to D, but for the W coil (30 mA). Yellow downward arrow indicates lack of spread of activation on the right side of W coil.

Fig. 6.

Coil design influences the spread of activation in L2/3 in response to repetitive stimulation of L5. (A) Epi-fluorescence microscope photograph of an electrode in L5/6 of a V1 slice. Regions of interest ($50 \times 50 \mu m$ squares, labeled 1–7) were used to compare fluorescence changes in L2/3 as a function of horizontal distance from the implant. (B) The peak fluorescence change in response to electrical stimulation (30 μA , 200 μs , 20 pulses at 100 Hz) from the electrode in A. Dark blue region in the bottom represents a saturated level of brightness in the CCD camera image. Arrows indicate the lateral spread of activation in L2/3. (C) The fluorescence change over time for the regions of interest (#1–7). (D) Similar to A, showing a V coil in L5/6 of a V1 slice. (E) Similar to B, showing the peak fluorescence change in response to magnetic stimulation (26 mA, 5kHz sinusoid, 20 pulses at 100 Hz) from the V coil in D. (F) Similar to C, but for magnetic stimulation from the V coil. (G) Similar to A and D, showing a W coil in L5/6 of a V1 slice. (H) Similar to B and E, but for the W coil (30 mA) in G. Note that the lateral spread of cortical excitation in L2/3 was smallest with the W coil (yellow downward arrows in H). (I) Similar to C and F, but for the W coil. (J) Plot of averaged peak fluorescence changes in L2/3 as a function of horizontal distance for stimulation from electrode, V coil, and W coil ($n=5$ slices). Error bars indicate Standard Error (S.E.). (K) Similar to J, showing normalized peak fluorescence changes and approximate lateral distances at half-maximum level of fluorescence change. (L) Plot of half-maximum distance for stimulation from electrode, V coil, and W coil. ** indicates statistical significance.

(L) Half-maximum distance for electrode, V coil, and W coil. (two tailed t-test: * $p=0.0013$; ** $p=0.0001$) Red crosses indicate mean half-maximum distances.

Fig. 7.

Activation of passing axons of L2/3 PNs influences the response to repetitive stimulation of L5. (A) The peak fluorescence changes in response to electrical stimulation (30 μ A, 200 μ s, 20 pulses at 100 Hz) from the electrode in L5/6 of a V1 slice. The fluorescence changes were measured under control conditions (left panel, normal aCSF) and the presence of the synaptic blockers (right panel, aCSF + 10 μ M NBQX + 50 μ M D-APV). Note that the wide spread of activation in L2/3 remained even after the blockade of synaptic transmission. (B) Similar to A, showing the peak fluorescence changes in response to magnetic stimulation (26 mA, 5 kHz sinusoid, 20 pulses at 100 Hz) from the V coil. (C) Similar to A and B, but for the W coil (30 mA).

Fig. 8.

3D Multi-loop coils enhance the strength of the field gradient. (A) A model of a single loop structure with a cross-sectional dimension of $60 \times 2 \mu\text{m}$. The electric field gradient in the y-axis was evaluated along a horizontal line running $15 \mu\text{m}$ above the surface of the coil (blue line with arrow). (B) A model of a 3D multi-layered structure with a cross-sectional dimension of $60 \times 20 \mu\text{m}$. The 3D structure was implemented by alternately stacking the single loop coil in A and a dielectric layer ($60 \times 0.5 \mu\text{m}$). Similar to A, the field gradient in the y-axis was evaluated along a horizontal line running $15 \mu\text{m}$ above the 3D coil (red line with an arrow). (C) Plots of the field gradients for the single and the 3D coils. The horizontal axis represents the horizontal distance from the center of the coil loop. Note that the 3D 8-loop structure produces a 8–10x stronger field gradient than that of the single loop structure.