Volume Control for a Cortical Network

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ABSTRACT

Excitability is a fundamental property of cortical networks, shaping their responses to input. Here, we use ionic direct current (iDC) to modulate excitability with sub-10-ms temporal resolution and submillimeter spatial precision across the cortical surface, greatly surpassing the capabilities of pharmacological tools. In anesthetized rats, we recorded laminar neural responses in the S1HL cortex to spontaneous delta oscillations and to foot stimulation with and without iDC delivered to the cortical surface. Cathodic iDC suppressed, and anodic iDC enhanced, evoked responses across recording sites. iDC shifted the spatiotemporal excitability pattern in a graded manner, paralleling the effects of weaker or stronger foot stimuli. A computational model reproduced these effects and implicated dendritic summation at the axon initial segment (AIS) as a key mechanism for bidirectional modulation. This approach enables precise, causal manipulation of cortical responsiveness in vivo and offers a platform for dissecting functional circuits and developing targeted neurotherapeutic interventions.

INTRODUCTION

Cortical excitability defines how neural populations react to incoming synaptic input. Modulating network excitability, or gain, is essential for adaptive brain functions such as sensory filtering, attention, learning, and decision-making[1–6]. The gain of a neural population determines not only the magnitude of its response but also whether it contributes meaningfully to downstream processing. Dysregulation of excitability has been implicated in a wide range of neurological and behavioral disorders like epilepsy, schizophrenia and autism, motivating the development of tools to control excitability with high specificity[7–10].

Traditionally, excitability has been modulated using pharmacological agents targeting specific receptors. These approaches, such as agonists or antagonists of GABA, NMDA, or neuromodulatory receptors[11–13], can bias a network toward greater or lesser responsiveness to natural input. However, pharmacological methods are fundamentally limited in both spatial and temporal resolution: they act broadly across brain regions and operate on timescales of minutes to hours. These constraints limit their utility for dissecting fast, localized neural computations or for precisely controlling network-level dynamics in vivo.

A promising alternative involves applying weak, constant electric fields to modulate neuronal excitability. Early in vitro studies by Bikson and colleagues demonstrated that such fields can induce subtle membrane polarization in neurons depending on their orientation to the field, with

pyramidal cells being particularly sensitive due to their extended morphology[14–16]. Importantly, these effects are subthreshold and do not drive spiking directly. Instead, they shift the postsynaptic integration of excitatory and inhibitory inputs, modifying the amplitude and timing of excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) in a spatially specific and polarity-dependent manner. These findings suggest that extracellular electric fields can modulate network gain by biasing dendritic integration in targeted populations of neurons, especially within cortical layers.

Until recently, applying such fields in vivo lacked practical value, as existing technologies could not support chronic use with sufficient spatial and temporal precision. The development of the Freeform Stimulator (FS), combined with microcatheter-based delivery, now enables a viable platform for invasive ionic direct current (iDC) stimulation that is safe, spatially targeted, and suitable for long-term application [17–20].

Here, using FS emulation, we test the hypothesis that iDC can modulate the excitability of cortical networks in vivo in response to both endogenous cortical rhythms and exogenous sensory input. Using multielectrode arrays spanning cortical layers in anesthetized rats, we characterize how anodic and cathodic iDC affect spontaneous oscillatory activity and sensory-evoked responses. We then compare these effects to natural variations in stimulus intensity and employ computational models to identify the underlying mechanisms, specifically, how iDC alters dendritic summation at the axon initial segment of pyramidal neurons.

RESULTS

Modulation of Spontaneous Neural Activity

To investigate how ionic direct current (iDC) modulates spontaneous neural activity, we conducted a series of in vivo experiments in which a 32-channel single-shank microelectrode array was vertically inserted 1.65 mm into the S1HL cortex of a urethane-anesthetized rat, spanning all cortical layers over a 1.55 mm contact array. A 250 µm-ID iDC microcatheter filled with electrolytic gel was then positioned on top of the pia mater, with its center located at a fixed horizontal distance (0.2 mm) from the recording electrode (**Fig.1AB**).

Spontaneous multi-unit activity (MUA) was recorded in ~90-second recordings, each segmented into 30s pre-iDC, 30s during-iDC, and 30s post-iDC epochs (**Fig. 1C, top**). A representative 90s multiunit activity (MUA) recording from a single channel with +40 µA iDC stimulation is shown (**Fig. 1C, middle, bottom**). The magnified snippets illustrate that the transition in spontaneous neural activity occurs immediately after the onset and offset of iDC stimulation.

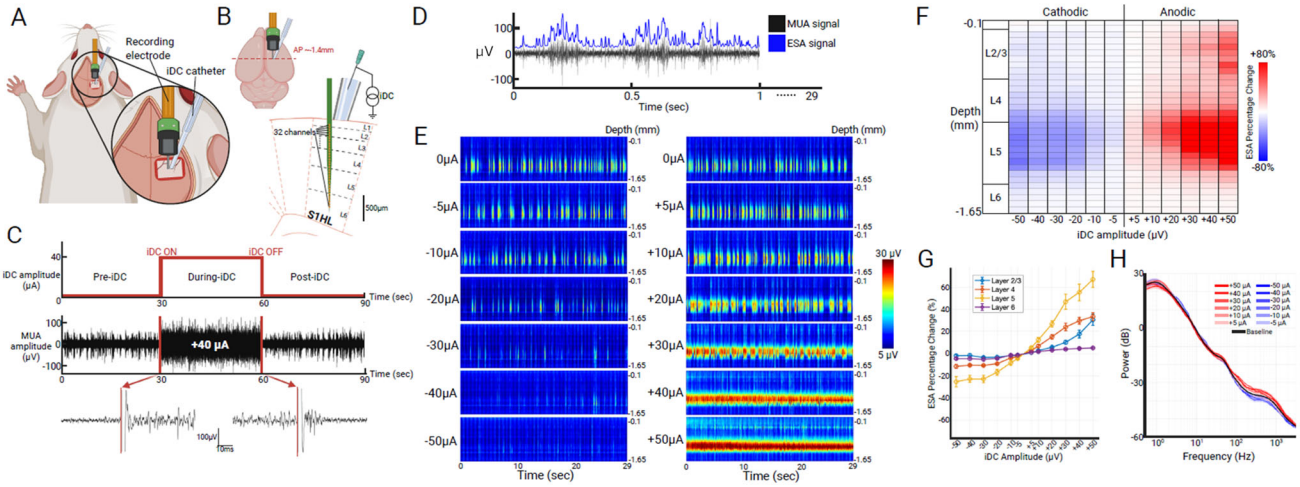

Figure 1. A) iDC stimulation catheter and recording electrode setup in rat S1HL. **B)** Scaled cartoon of the 32-channel recording electrode placed within the six layers of the S1HL cortex. **C)** *top:* iDC stimulation timeline when recording spontaneous neural activity. *middle:* Temporally-aligned representative MUA recording with +40µA anodic iDC stimulation from one electrode channel. *bottom*: Magnified MUA signal snippets during iDC onset and offset are shown. **D)** Representative MUA signal snippet (black) overlaid by processed Entire Spiking Activity (ESA) signal (blue). **E)** Time-resolved heat maps under various iDC stimulation amplitudes (±5 to ±50 µA) from one representative experiment. **F)** Relative percent change in average ESA across electrode channels over a range of iDC amplitudes. **G)** Cumulative ESA relative percent change grouped by layers (L2/3, L4, L5, and L6) (n=7 animals, mean ±SEM). **H)** PCA-derived column power spectral density (PSD) curve across various iDC amplitudes and pre-iDC baseline (n=3 animals, mean ±SEM).

To robustly quantify the spontaneous activity, the MUA signals were processed using the Entire Spiking Activity (ESA) method [21–23]. This processing method avoids threshold-based spike detection and yields a continuous measure of neural activity that is less sensitive to random changes in signal-to-noise ratio while retaining the spike contribution from smaller neurons (**Fig. 1D**) [21,22]. The 32 channels reported neural activity along cortical depth. We constructed heat maps from the processed ESA signals at various iDC stimulation amplitudes to examine its effects on neural activity across time and cortical depth (**Fig. 1E).** The heat maps show that before iDC stimulation is delivered (baseline, 0 µA iDC), the most prominent activity happens in deeper layers, revealing robust slow-wave oscillatory dynamics (~1 Hz) characteristic of the urethane-anesthetized state [24,25]. At lower iDC amplitudes (–30 to +30 µA), the slow wave oscillation remains intact, with only amplitude modulations in a graded fashion. In contrast, at higher iDC amplitudes (<-30µA or >+30µA), the heat maps reveal altered patterns: Cathodic iDC produces near-complete suppression of activity in deeper layers, while anodic iDC elicits a persistent, high-frequency firing pattern. These findings suggest a linear regime of graded modulation at lower iDC amplitudes (–30 to +30 µA), and a nonlinear transition at higher amplitudes (>±30 µA), where iDC begins to suppress or drive activity beyond endogenous oscillatory patterns.

To visualize the relative effects of iDC across the layers, we computed the percent change in averaged ESA between pre-iDC and during-iDC epochs (**Fig.1F**). The main effect that we notice

is in layer 5, with the intensity of the effect proportional to iDC amplitude. For anodic stimulation specifically, iDC also induced a gradual increase in layer 2/3 activity with higher amplitude.

We compiled the cumulative results across the seven animals that underwent these experiments. In **Figure 1G**, the ESA percent change values were averaged across channels within each layer, with the standard error of the mean (SEM) across multiple animals (n=7). The line plot shows that layer 5 is indeed the most affected layer across experiments, followed by layer 2/3 and layer 4. In contrast, layer 6 showed only minimal changes across the same range of iDC amplitudes. We also see the linear relationship in neural responses to amplitudes spanning -30μA to +30μA, suggesting the dynamic range of iDC-modulated network excitability.

To confirm that iDC modulates local neuronal gain without altering the overall oscillatory state, we compared the power spectral density (PSD) curves generated from unfiltered raw signals, rather than ESA to eliminate preprocessing effects. In **Figure 1H**, the PSD curves are shown for a range of iDC amplitudes relative to the baseline recording, with the shaded region indicating SEM across multiple animals (n=3). Due to reference-wire artifacts affecting raw signal quality in four animals, PSD analyses were restricted to the three datasets where this issue was absent. These artifacts did not affect ESA metrics (see **Supplemental Fig. S1**), supporting the reliability of the broader findings. All iDC stimulation amplitudes ranging from -50 to +50μA did not alter the ~1Hz slow wave oscillation observed in the pre-iDC baseline PSD curve that characterizes the urethane-anesthetized cortical state, indicating that the global oscillatory dynamics remained intact. Consistent with our observations, the higher-frequency components (~80–1000 Hz) of the PSD curves, which more directly reflect local spiking activity[26], exhibited consistent polarity- and amplitude-dependent spectral power changes at high amplitudes.

Computational Model and Experimental Assessment of iDC Modulation

Our computational model investigates how iDC stimulation modulates cortical activity in the rat S1HL by simulating electric field effects on the neuronal membranes. The model first establishes a two-dimensional grid representing a cortical cross-section and calculates the extracellular potential (V_e) generated by a 250 μm-diameter isopotential disk (mimicking the iDC catheter) placed on the pia mater. The output of the model establishes the extracellular voltage V_e (relative to distant reference) along the cortex and in-depth along the cortical layers (**Fig.2A**).

In our model, eight principal excitatory neuron types (layer 2/3 pyramidal (L2py, L3py), layer 4 spiny stellate (L4sp), layer 4 star pyramidal (L4ss), layer 5 slender-tufted (L5st), layer 5 thick-tufted (L5tt), layer 6 corticocortical (L6cc), and layer 6 corticothalamic (L6ct)) and two common inhibitory neuron types (basket cells (BC) and Martinotti cells (MC)) are parameterized by their top and bottom dendritic depths as well as their soma depths according to their biological morphologies [27–30] (**Fig.2B**). Neurons are modeled as simplified vertical one-dimensional “rods” (**Fig.2B**) whose lengths span to the extent of their dendritic arbors along the somatodendritic axis, and each rod includes a defined soma (indicated by black markers) that represents the location of the cell body. For each neuron, the “mirror estimate” is the measure of the membrane potential voltage change along the length of the neuron (rod) when it is exposed to the electric field. It is calculated as the difference between the mean extracellular voltage V_e along its vertical span and

the local V_e at each point [18,31,32]. The lower two rows of **Figure 2B** show each neuron as a single rod with the color indicating the membrane potential change along the length of the neuron. These are shown at incremental lateral positions across the cortical plane to illustrate how the neuron membrane voltages change across the horizontal distance under ±20 μA stimulation. Critical to the modeling explanation, the effect of the membrane change at the soma (and importantly, the colocalized axon initial segment (AIS)) is indicated with a black mark along each modeled neuron.

According to the model, L5 pyramidal cells with long somatodendritic axes (e.g., L5st, L5tt) display the most pronounced somatic depolarization under anodic (+20 μA) iDC currents: their superficial apical dendrites hyperpolarize while basal dendrites in deeper layers depolarize, lowering the action-potential threshold at the AIS and increasing firing probability. Conversely, cathodic (-20 μA) iDC currents invert this pattern, producing somatic hyperpolarization and suppressed somatic activity. By contrast, L2/3 pyramidal neurons (e.g., L2py, L3py), though vertically oriented, have shorter apical arbors whose medial soma positions lie near the biphasic zero-crossing of the field, resulting in minimal net polarization effects. L4 neurons (e.g., L4sp, L4ss) yield similarly attenuated effects. L6 pyramidal cells (e.g., L6cc, L6ct), despite long apical extensions and somas near their basal dendrites, experience reduced field strength because the end of their apical dendrites does not reach as superficially as L5 pyramidal cells.

The two most common types of inhibitory interneurons (basket cell and Martinotti cell) were also modeled accordingly (**Fig.2B, right**) [29]. Many cortical inhibitory interneurons— especially basket cells—exhibit compact, roughly symmetric dendritic arbors. Moreover, because inhibitory neurons make up a smaller fraction of the neuronal population (15-20%) and their synaptic outputs are more diffusely targeted, we expect the direct effects of iDC stimulation on these interneurons to be minimal (i.e. close to net zero) compared to the robust modulation seen in deep pyramidal cells [33].

To mimic the spatial variability found in the cortex, the rods, representing the dendrites of each cell type, are randomly scattered across the horizontal axis without overlapping, with the number of rods per type scaled according to their reported densities [28]. The model then extracts the membrane potential change at each soma and applies a Gaussian-weighted sum interpolation over a finer grid. This produces a continuous two-dimensional heatmap that reflects the cumulative effect of iDC stimulation on the local population of neuronal activity. Since the AP threshold is determined primarily at the axon initial segment (AIS) due to the high density of voltage-gated sodium channels (and since the recording electrode primarily picks up somatic spiking activity), this interpolated potential change heatmap serves as an approximation of the signal that might be detected by the recording electrode used in our in-vivo setup (**Fig. 2C**). The resulting figure indicates that the main depth of depolarization and hyperpolarization is around L5, with a decay of modulation seen in layers 2/3 and 4, which progressively decays as distance increases. Interestingly, the model also predicted a faint, reversed polarity band in L2/3 at distances >0.2 mm for these stimulation amplitudes.

Building on the model's predictions, we examined the spatial resolution of iDC modulation in-vivo using the same setup described in **Fig.1A**. To simulate spatial effects without having to move the recording electrode which is experimentally prohibitive, we shifted the iDC catheter horizontally along the pia at 0.20, 0.55, 0.90, and 1.25 mm away from the recording electrode (**Fig. 2D**). Spontaneous MUA was recorded in 90-second runs, each divided into 30 s pre-iDC, during-iDC, and post-iDC epochs. The relative percent change between the average ESA for pre-iDC and during-iDC epoch was computed for each channel from a representative experiment, which forms a table with rows representing individual channels and columns corresponding to different lateral distances of the iDC microcatheter (**Fig. 2D**). Anodic modulation (+20 μA) produced maximal ESA

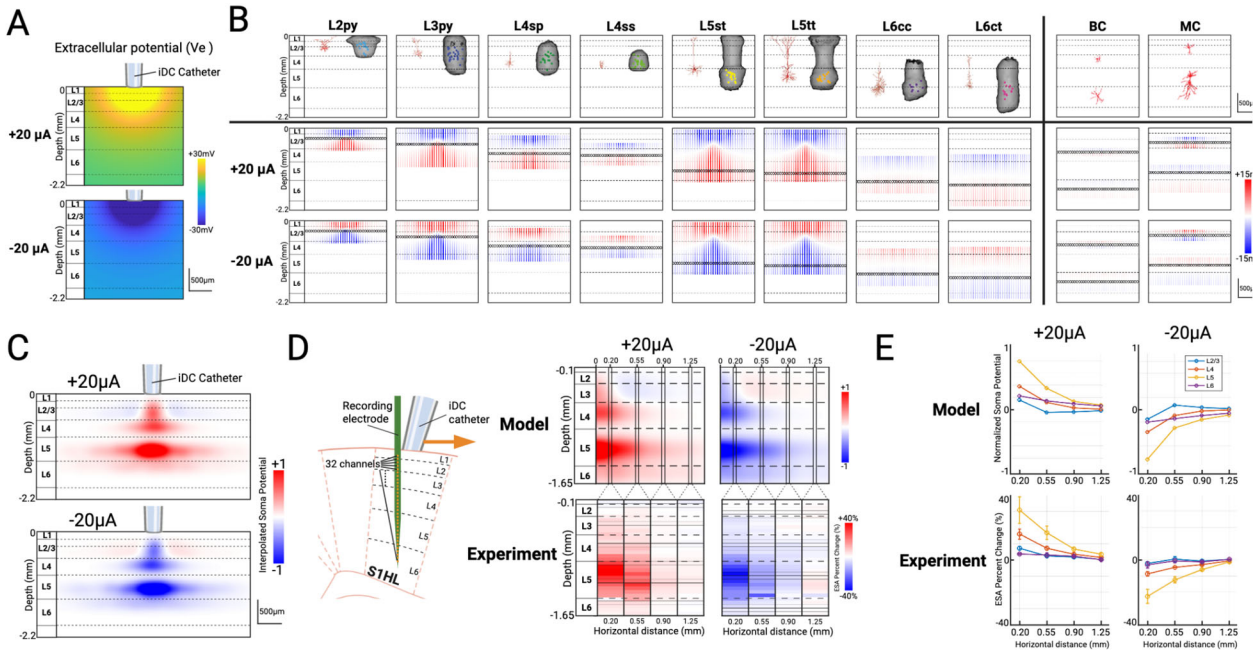

Figure 2. A) Extracellular potential within a two-dimensional cortical cross-section generated by the iDC microcatheter placed at the top of the pia mater. **B) First row:** representative morphologies and dendrite 3D volumes of the 8 most common types of excitatory neurons (L2py: L2 pyramidal cell; L3py: L3 pyramidal cell; L4sp: L4 star pyramidal cell; L4ss: L4 spiny stellate cell; L5st: L5 slender-tufted pyramidal cell; L5tt: L5 thick-tufted pyramidal cell; L6cc: L6 corticocortical pyramidal cell; L6ct: L6 corticothalamic pyramidal cell. Modified from Radnikow and Feldmeyer, 2018, Narayanan et al., 2017, and Oberlaender et al., 2012) and the 2 most common types of inhibitory interneurons (L4 and L6 basket cells (BC); L2/3 and L5 Martinotti cells (MC). Modified from Feldmeyer et al., 2018) within the rat somatosensory cortex. **Second and third row:** Each major neuron type is being modelled as vertical rods placed within the cortex cross-section, and their soma locations are shown as black circles. The color denotes the mirror estimate of the membrane potential change along the vertical length of each neuron in response to the extracellular electric field induced by anodic +20μA and cathodic -20μA iDC stimulation, respectively. **C)** The weighted sum of all membrane potential changes at excitatory neuron somas in proportion to their relative densities. **D)** Predicted iDC-induced somatic potential changes based on computational model versus representative experimental result by horizontally moving the iDC catheter to specific distances from the recording electrode (0.20, 0.55, 0.90, and 1.25mm). **E)** Cumulative model and ESA percent change versus horizontal distance plot from multiple animals (n=5, mean ± SEM).

increases in layer 5 that diminished with distance, effectively vanishing beyond ~1 mm, which confirms the sub-millimeter spatial resolution of iDC modulation. Cathodic stimulation (-20μA) yielded complementary decreases. These patterns in spatial decay observed in experiments closely align with our model's predictions. Interestingly, some channels in layer 2/3 also exhibited a weak, reversed polarity modulation when the horizontal distance is at 0.55, 0.90, and 1.25mm, though this pattern is inconsistent and could be due to random fluctuations in spontaneous activity. A cumulative ESA percent change versus horizontal distance plot from multiple animals (n=5, mean ± SEM) suggests that this pattern is consistent across repetitions (**Fig.2E**).

Modulation of Foot Stimulation-Evoked Responses

To assess how iDC modulates the gain of evoked responses in S1HL, we extended the experiment described in **Figure 1A** to deliver controlled foot shock stimulation while delivering iDC on top of the pia mater (**Fig.3A**). We first confirmed recording electrode placement by gently brushing the contralateral hindfoot, which elicited robust spiking responses across our 32-channel array. Foot stimulation was delivered through a surface electrocardiogram electrode using 200 μs cathodic pulses at 2-second intervals (10 repetitions per epoch) in a 90-second recording structure, comprising 30 s pre-iDC, 30 s during-iDC, and 30 s post-iDC epochs (**Fig.3B**). For control, the same stimulation protocol was applied to the ipsilateral foot, confirming that the observed responses were specific to contralateral stimulation.

Figure 3C shows a set of representative MUA responses recorded from a layer 5 electrode channel. Stimulation of the contralateral foot without iDC produced clear responses that emerged at approximately 20 ms post-stimulus, consistent with the delay associated with somatosensory signal propagation [34]. When a +20 μA anodic iDC was applied, the evoked responses were noticeably amplified, whereas –20μA cathodic iDC attenuated the response amplitude. As would be expected, ipsilateral stimulation elicited minimal activity with or without iDC stimulation. These observations indicate that iDC modulates the input/output gain of evoked responses in a polarity-dependent manner.

To investigate this gain modulation further, we generated time-resolved heat maps from foot stimulation ESA curves (averaged over 10 repetitions per epoch). In these heat maps, the x-axis represents time (10–50 ms window post-stimulus), and the y-axis represents electrode channels arranged by cortical depth (**Fig.3D)**. In this representative experiment, a range of foot stimulation intensities from 2.1 to 2.5 mA was empirically determined to be the effective range intensities eliciting distinct levels of evoked responses. Notably, the heat map for a 2.3 mA foot shock with anodic +30 µA iDC (2) closely resembled that for a 2.5 mA foot shock without iDC (1), while the heat map for 2.3 mA foot shock with cathodic -20 µA iDC (4) was similar to that for 2.1 mA foot shock without iDC (5). These comparisons suggest that iDC can potentially shift the effective input/output gain of the local network—anodic iDC amplifies the evoked response, making a lower-intensity stimulus behave like a higher-intensity one, whereas cathodic iDC attenuates it.

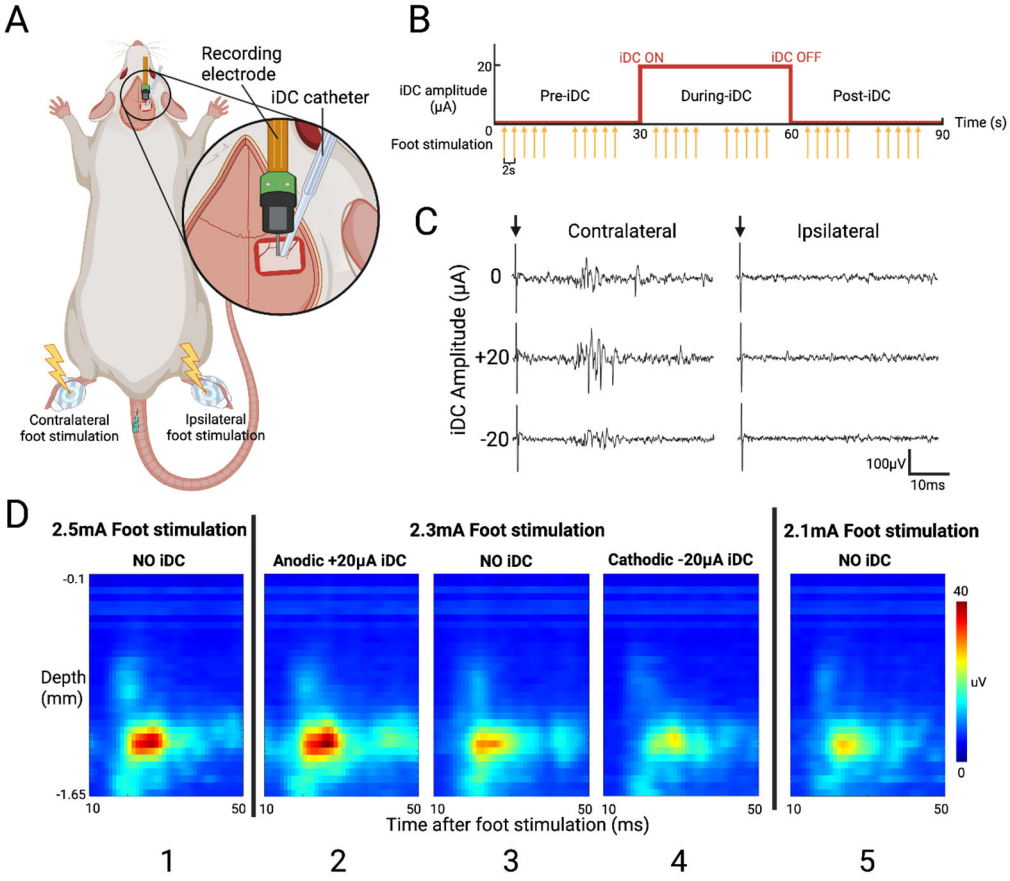

Figure 3. A) iDC stimulation catheter, foot stimulation surface electrode, and recording electrode setup used in the experiment. **B**) iDC (red) and foot shock (yellow) stimulation timeline in each 90s recording session. There are two cycles of 5 repeated foot stimulations per epoch, each 2s apart. **C**) Example MUA signal recorded from one L5 electrode channel. Evoked responses are mostly generated by the contralateral foot shock, with anodic iDC amplifying the response amplitude and cathodic iDC attenuating it. Arrows indicate the onset of foot shock. **D**) Averaged foot stimulation response heatmaps from one representative experiment. Three center panels (2,3,4) show the response to the same foot shock, but with anodic iDC gain amplification on the left (2) and cathodic gain attenuation on the right (4). Note the similarity of no-iDC responses to higher and lower amplitude foot stimulation on the corresponding far left (1 vs 2) and far right panels (4 vs 5).

To systematically assess the gain shifts induced by ±20 μA iDC, we constructed evoked response heat maps across a broader range of foot shock intensities (**Fig. 4A**). For each shock intensity there were two recordings, one for +20 μA iDC and one for -20 μA iDC, with the NO iDC condition included in the pre-iDC and post-iDC phases of the recording (**Fig. 3B**). These were ordered as follows: recording R1: +20 μA iDC and 0.8 mA foot shock, R2: -20 μA iDC and 0.8 mA foot shock, R3: +20 μA iDC and 1.0 mA foot shock, R4: -20 μA iDC and 1.0 mA foot shock, etc. Therefore, for each shock intensity there were four NO iDC conditions (two for odd and two for even recordings). In this analysis we expect the pre-iDC (as well as post-iDC) responses to be highly similar for any given foot shock amplitude. To verify this baseline consistency, we first compared the pre-iDC heat maps from odd and even cycles, expecting that there would be no difference between the odd and even response patterns. **Figure 4B** shows a mean squared error (MSE) matrix computed by comparing the even-numbered heat maps (along the columns) with the odd-numbered heat maps (along the rows). Notably, the lowest MSE values are situated along the diagonal, indicating that the baseline responses are reproducible across cycles.

We then compared these odd-numbered baseline heat maps with those recorded during iDC stimulation. Under the +20 μA (anodic) condition (**Fig.4C**), the matrix element with the lowest MSE values shifted below the diagonal toward the lower left, suggesting that the amplitudes and temporal patterns of the evoked responses under anodic iDC resemble those elicited by higher foot stimulation intensities with no iDC. Conversely, under -20 μA (cathodic) conditions (**Fig.4D**), the lowest MSE values shifted above the diagonal and to the right, corresponding to baseline responses evoked by lower stimulation intensities. These systematic shifts agree with the observation that anodic iDC amplifies evoked responses while cathodic iDC attenuates them in a predictable polarity-dependent manner, mimicking the natural response pattern elicited by higher or lower amplitude foot shocks.

To quantify this iDC-dependent shift in evoked responses, we calculated the mean MSE above and below the diagonal for all three matrices. The results computed from the matrices shown in **Figure 4B,C,D** indicate that iDC can reliably shift the network's input/output gain responding to foot shock stimulations (**Fig.4E**). Even though the absolute values of the mean ESA vary drastically from animal to animal due to individual differences, the trend of modulation remains consistent (**Fig. 4F**).

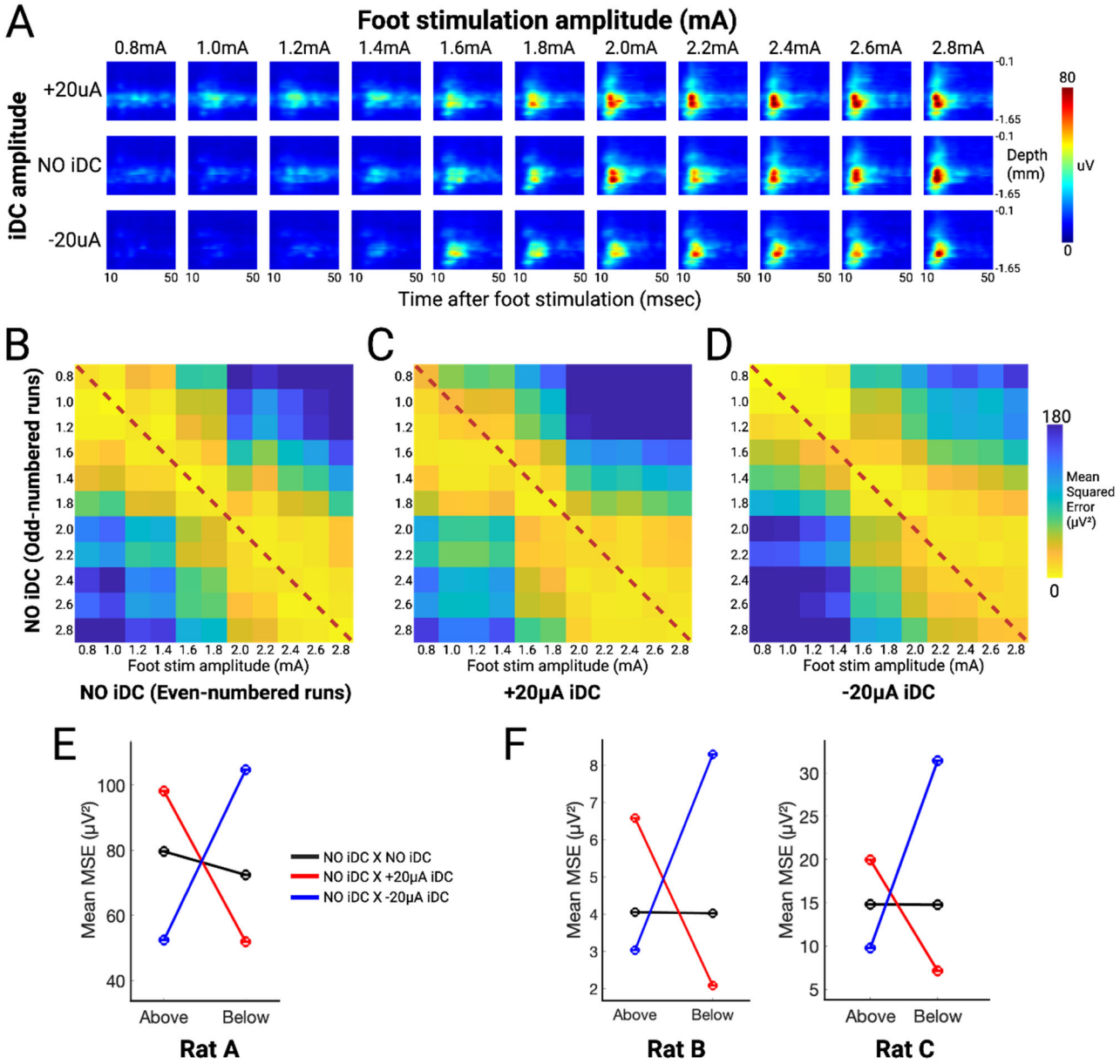

Figure 4. A) Representative experiment that delivers a broad range of foot shock stimulation amplitudes (0.8-2.8mA, increment in 0.2mA) while concurrently delivering 0µA (pre-iDC baseline), +20µA, and -20µA iDC. **B**) MSE matrix computed between even-numbered (x-axis) and odd-numbered (y-axis) pre-iDC baseline heat maps. Yellow matrix elements represent heatmap pairs with lower MSE and higher similarity, while blue elements represent higher MSE. **C**) MSE matrix computed between +20 µA during-iDC heat maps (x-axis) and odd-numbered pre-iDC baseline heat maps (y-axis). **D**) MSE matrix computed between -20 µA during-iDC heat maps (x-axis) and odd-numbered pre-iDC baseline heat maps (y-axis). **E**) Mean MSE above and below the diagonal for matrices 4B, 4C, and 4D. **F**) Similar plots generated from the data of two other animals (Rat B and C).

DISCUSSION

Mechanistic Insights of iDC and Cortical Gain Modulation

Our findings indicate that low to mild amplitude (±5 to 30 μA) ionic direct current (iDC) delivered via a cortical surface microcatheter can bidirectionally bias cortical responses in a controlled and graded manner, modulating local network excitability. Anodic iDC (positive current at the pia) increased both spontaneous firing and sensory-evoked response amplitudes, whereas cathodal iDC had the opposite, suppressive effect. Analysis of neural responses across multiple foot shock amplitudes with and without iDC indicates that iDC can modulate evoked activity in a way that mimics the natural response pattern elicited by higher or lower amplitude exogenous input.

The concordance between model predictions and experimental results indicates that field-induced modulation predominantly affects membrane potential near the axon initial segment (AIS), where action potentials are initiated. It suggests that whether that effect was suppressive or excitatory depended on where the cell body was positioned along the layers relative to the dendritic arbor. The vertical length of the dendritic arbor and its position within the electric field determined the membrane potential changes along its length [14,18,35,36] and at the soma. Long duration electric fields like those produced by iDC, are expected to have nearly the same effect on the cell body membrane voltage (or more accurately AIS) as the axons and dendrites [14,16,37]. The match between experiments and the model are then explained by the evidence that extracellular neural recordings are dominated by action potentials generated at the soma[38,39].

We propose that the mechanism by which exogenous electric fields modulate network sensitivity can be understood by recognizing that the axon initial segment (AIS), located near the soma, serves as the integration site for excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) originating from pyramidal dendritic synapses [40,41]. We can think of the change in the pyramidal neuron's AIS membrane potential (ΔV_{AIS}) as being the sum of the synaptic EPSPs ($\sum EPSP$) minus IPSPs ($\sum IPSP$) and the change in membrane potential imposed by the electric field (ΔV_{AIS}^{iDC}):

$$\Delta V_{AIS} = \sum EPSP - \sum IPSP + \Delta V_{AIS}^{iDC}$$

A slight depolarization of the AIS membrane potential (i.e. positive ΔV_{AIS}^{iDC}) biases the neuron toward excitation, lowering the threshold for excitatory postsynaptic potentials (EPSPs) to trigger an action potential. Conversely, slight hyperpolarization (negative ΔV_{AIS}^{iDC}) shifts the balance toward inhibition, raising the threshold and making it more difficult for EPSPs to elicit a spike.

Layer- and Cell-Type Specific Effects

The effects of iDC were not uniform across cortical layers or neuronal cell types. Our in-vivo and previous in-vitro studies [14,16] both found that under anodic iDC, layer 5 (L5) pyramidal neurons displayed significantly enhanced firing rates and larger sensory-evoked depolarizations, whereas superficial neurons in layers 2/3 (L2/3) and putative inhibitory interneurons exhibited comparatively modest responses.

The computational model offers a plausible explanation for this layer-specific modulation, demonstrating that somatodendritic orientation and the differential exposure of neuronal morphologies to the applied electric field govern the extent of voltage gradient "picked up" by neurons [14,16,42,43]. L5 pyramidal neurons are large projection neurons with long apical dendrites extending toward the cortical surface and somatic compartments positioned near the base of the dendritic volume, with their somatodendritic axis nearly parallel to the radial field emanating from the pia-mounted iDC source. Consequently, these neurons experience a significant voltage drop from dendrite to soma in the presence of iDC, resulting in a higher net membrane polarization at AIS. Additionally, previous studies [44–47] indicate that L5 neurons have the highest basal firing rates under urethane anesthesia, whereas L2-4 neurons exhibit sparser spontaneous activity, explaining why iDC modulation produces proportionally larger changes in L5 neuronal activity.

By contrast, smaller cells or those with more symmetric arborizations (such as some interneurons or small excitatory cells in upper layers) likely experience a more uniform electric potential across their structure, or that their AIS positions lie near the biphasic zero-crossing of the field, resulting in minimal net polarizations and thus modest modulation by iDC. These findings align with previous work highlighting neuronal morphology and orientation as critical determinants of responsiveness to electric fields [14,16,42,43,48–51].

Functionally, preferential modulation of L5 pyramidal neurons significantly impacts cortical circuit dynamics. L5 pyramidal neurons constitute major cortical outputs, projecting subcortically and providing excitatory drive to local microcircuits through translaminar synapses. From this perspective iDC neuromodulation can be thought of as “volume control” for the output of a local cortical circuit.

Comparison with Other Neuromodulation Techniques

The iDC approach provides neuromodulation distinct from—and complementary to—optogenetic and chemogenetic techniques. Optogenetics affords millisecond precision and cell-type specificity but necessitates genetic manipulation and implanted hardware, posing clinical translation challenges [52]. Chemogenetics (e.g., DREADD-based approaches) targets genetically defined neurons through engineered receptors activated by systemic ligands, achieving sustained cell-specific modulation. However, chemogenetic modulation incurs inherent delays (minutes) and relies on exogenous ligands, limiting real-time applications [52].

Transcranial direct current stimulation (tDCS) produces diffuse sub-millivolt membrane polarization due to the smoothing effect of the skull and scalp, functioning as a broad neuromodulatory influence rather than submillimeter focal stimulation[14,42,53]. In contrast, iDC provides superior spatial precision and efficacy due to direct placement on the pia mater rather than through the scalp, restricting current spread to targeted cortical columns. Neurons located approximately 1 mm from the iDC catheter displayed negligible modulation, highlighting iDC’s millimeter-scale focality.

Lastly, intracortical microstimulation (ICMS) with microelectrodes is a well-established technique for activating neural tissue in a localized manner (e.g., in brain–machine interfaces) [54]. ICMS

typically uses charge-balanced high-frequency pulses to evoke APs phase-locked to pulse presentations, but offers limited ability to produce sustained subthreshold changes or direct inhibition of neural activity [17].

By comparison, iDC occupies a middle ground: it uniquely combines spatial specificity and continuous modulation without requiring genetic tools, providing rapid, steady, and reversible neuromodulation suitable for causally probing cortical node functions in behavior and cognition, effectively allowing neuroscientists to “turn up or down the volume” of a cortical node and observe the consequences. Practically, iDC integrates seamlessly with electrophysiological recordings, avoiding electromagnetic interference associated with high-frequency stimulation [51,54]. This compatibility facilitates continuous monitoring of the network’s real-time response to the modulation.

Future Potential of iDC and Freeform Stimulator Technology

Invasive ionic direct current (iDC) offers precise, bidirectional neuromodulation without the electrochemical damage typical of conventional electrodes. Traditional stimulators rely on charge-balanced pulses to avoid tissue degradation but inherently favor excitation and limit sustained inhibition [55]. In contrast, the electrolyte-filled microcatheter, adapted from the Separated Interface Nerve Electrode (SINE)[56], safely delivers true DC by isolating metal components outside the brain. The Freeform Stimulator (FS) implant currently under development further advances this approach by using microfluidic rectification to generate arbitrary ionic waveforms, including direct current, without accumulating charge or contaminants, enabling repeated, long-duration use [17,18]. It is being developed for multichannel and wireless control, supporting complex current steering and spatial field shaping across cortical areas. FS provides a novel tool by enabling chronic application of iDC for long duration behavioral and clinical studies. Clinically, iDC’s gradual, stable modulation is promising for conditions involving abnormal excitability, including epilepsy, stroke, chronic pain, and depression. Together, iDC and FS represent a flexible platform for both research and therapy, enabling high-precision, low-side-effect neuromodulation in both acute and chronic settings.

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METHODS

In Vivo Experimental Procedures

Animals and Surgical Preparation

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Johns Hopkins University and conducted in compliance with NIH guidelines. Adult Sprague–Dawley rats (n=7; 4 males, 3 females; 450–600 g) were housed with a 12 h light/dark cycle and given food and water ad libitum. Animals were anesthetized with urethane (1.4 g/kg, intraperitoneal injection). Anesthetic depth was monitored regularly during surgery via heart rate, foot-pinch reflex, and blink response. Body temperature was maintained at 37 ± 0.5 °C using a feedback-controlled heating pad.

A craniotomy was performed to expose the hindlimb region of the primary somatosensory cortex (S1HL) in the right hemisphere (coordinates: posterior bregma −1.0 to −2.0 mm, lateral 2.0 to 3.5 mm). The dura mater was carefully removed, and a 32-channel single-shank microelectrode array (NeuroNexus A1x32-6mm-50-177-Z32) was vertically inserted ~1.65 mm into the cortex, spanning all layers with its 1.55 mm contact array (**Fig. 1AB)**. A stainless-steel reference electrode was positioned between the skin and muscle tissue on the contralateral side of the skull. S1HL localization was confirmed by gently brushing the contralateral hindfoot and verifying robust multi-unit activity (MUA) responses.

Stimulation and Recording

A 28-gauge MicroFil needle (250 µm inner diameter) was filled with agar-gelled artificial cerebrospinal fluid (aCSF). The microcatheter tip was gently placed on top of the pia mater within S1HL, adjacent to the inserted recording probe (~0.2 mm), and pointed vertically downwards (**Fig. 1AB)**. The microcatheter was connected to an external constant current stimulator (Keithley 6221) through a hypodermic needle on top. This setup safely delivers iDC currents and isolates metal electrodes outside the brain. A separate hypodermic needle served as the return electrode and was placed subcutaneously at the tail base. Prior testing using pH-sensitive dyes confirmed that electrochemical byproducts at the metallic portion of the circuit were contained away from the cortex for the duration of the experiments.

Neural signals were amplified (×1000) and digitized at 24.414 kHz using Tucker-Davis Technologies hardware (Subject Interface and processor) and Synapse software. For MUA extraction, recordings were bandpass filtered between 300 and 5000 Hz. Both spontaneous and foot-stimulation-evoked MUA recordings were conducted in approximately 90-second sessions, segmented into 30 s epochs for pre-iDC, during-iDC, and post-iDC intervals (**Fig. 1C**). iDC amplitudes varied randomly across sessions, ranging from -50 to +50 µA.

For spontaneous activity recordings, to minimize artifacts due to edge effects and stimulation onset/offset, a 500 ms interval at both the start and end of each 30-second epoch was excluded, yielding an effective analysis window of 29 seconds per condition (pre-iDC: 0.5–29.5 s; during-iDC: 30.5–59.5 s; post-iDC: 60.5–89.5 s).

Evoked responses were elicited by delivering cathodic foot stimulation pulses (200 μs, –0.5 to –2.5 mA; AM2100 stimulator) every 2 seconds, repeated 10 times per epoch (3 epochs × 10 stimulations per recording; **Fig. 3B**). Stimuli were applied via 3M Red Dot ECG electrodes (Model 2560), with the cathode on the plantar and the anode on the dorsal surface of the contralateral hindfoot (**Fig. 3A)**. A control stimulus was applied to a different dermatome area (e.g., ipsilateral foot) to confirm the specificity of iDC effects on contralateral S1HL. Evoked MUA responses were evaluated in the 10–50 ms post-stimulus window.

To evaluate spatial electric field effects, the iDC microcatheter was positioned at incremental horizontal offsets (0.2, 0.55, 0.90, and 1.25 mm) from the recording electrode (**Fig. 2DE**), and spontaneous activity was recorded at various iDC amplitudes for each offset.

Data Analysis

Having acquired spontaneous and evoked MUA under randomized iDC amplitudes, we next applied custom MATLAB (The Mathworks Inc) signal-processing pipelines to quantify gain changes due to iDC stimulation.

Entire Spiking Activity (ESA) Analysis

Both spontaneous and foot stimulation evoked MUA signals were processed using the Entire Spiking Activity (ESA) method over their relevant time window (e.g., 29 s for spontaneous activity or 10–50 ms for evoked responses) [21–23]. MUA signals were full-wave rectified and bidirectionally low-pass filtered with a Gaussian kernel (σ = 1ms, kernel length = 6σ) (**Fig. 1D)**. The ESA method preserves activity from smaller neurons by avoiding threshold-based spike detection, allowing more robust quantification under varying signal-to-noise conditions [21,22].

To visualize spontaneous activity across cortical depth, ESA signals from each channel were segmented into 50-ms non-overlapping bins across the 29 s analysis window, then vertically arranged by cortical depth to generate time-resolved heatmaps (**Fig. 1E, 3D, 4A**). For foot stimulation evoked activity, the MUA responses were first aligned to each stimulus onset, and a 5-ms post-stimulus “artifact” window was excluded. To ensure stable edge behavior, the retained 10–50 ms post-stimulus effective analysis window was padded by 5 ms on either side, yielding an extended 5–55 ms segment. This segment is then processed by the ESA method, cropped back to the 10–50 ms window, and divided into 1 ms bins. ESA segments from all 32 channels were depth-aligned and averaged over 10 repetitions to create one response heatmap per epoch (**Fig. 3D,4A**).

To quantify spontaneous activity changes, spontaneous ESA signals from each channel were also averaged over the 29 s during-iDC epoch and normalized to baseline (pre-iDC epoch) to yield a relative percent change value. These values were calculated across 12 iDC amplitudes (–50 to +50 μA) and arranged by channel depth to form the relative percent change table (**Fig.1F**). Recording channels were also assigned by depth into four cortical layers (2/3, 4, 5, and 6). Within each experimental cycle (one animal per cycle, 12 iDC amplitudes), we averaged the channel-

level relative percent changes for each layer and then plotted the mean ± SEM across multiple animals as line graphs with error bars (**Fig. 1G)**.

PCA-Derived Power Spectral Density Curves

To confirm that iDC does not disrupt intrinsic oscillatory dynamics, we analyzed raw (unfiltered) spontaneous activity from all 32 channels and 12 iDC amplitudes (–50 μA to +50 μA). Data segments (pre-iDC: 10.5–29.5 s; during-iDC: 40.5–59.5 s) were reduced via principal component analysis (PCA), with the first principal component (PC1) serving as a representative "column signal." Power spectral density (PSD) was calculated using a median short-time Fourier transform (STFT; 2 s Hamming windows, 0.5 s overlap), with spectral power derived from the median squared magnitude ($|STFT|^2$) over time. Spectra were interpolated onto a log-spaced frequency axis (0.5–3 kHz), smoothed with a 10-point moving average, and converted to decibels (dB). Mean ± SEM PSD curves across three animals (1 male, 2 females) were generated for the pre-iDC baseline (black shading and trace) and each iDC amplitude condition (colored shading and traces) (**Fig. 1H**).

Mean Squared Error (MSE) Analysis of Evoked-Response Heatmaps

To quantify changes in the gain of foot-stimulation evoked responses, we computed pairwise mean squared error (MSE) matrices comparing heat maps from odd- versus even-numbered baseline cycles and between baseline and ±20 μA iDC cycles (**Fig. 4BCD)**. For each pair of sessions (indexed along the matrix axes), the two response heatmaps were vectorized, and MSE was computed as the average squared difference (in μV^2). Diagonal elements of the matrices (self-comparisons) served as controls. To summarize polarity-dependent shifts, we computed the average MSE above the diagonal (indicating similarity to lower-intensity baselines) and below the diagonal (indicating similarity to higher-intensity baselines) for all matrices in each animal (**Fig. 4EF**).

Computational Modeling

iDC Electric Field Spread

The model first establishes a two-dimensional grid representing a 2.2 × 2.2 mm rat S1 cortex cross-section in MATLAB. The extracellular potential (V_e) at every point in the cross-section was computed by superposition of point-source contributions from a 0.25 mm diameter circular disk electrode (mimicking the iDC catheter) placed on the pia (**Fig. 2A)**. Each point source at radius r contributes:

$$V(r) = \frac{\rho I}{2\pi r}$$

where $\rho = 5 \times 10^3\ \Omega\cdot$mm is the in vivo measured tissue resistivity (see **Supplemental Fig. 2**), I is the applied current (i.e., ±20 μA), and $2\pi r$ is the hemispherical spread of voltage.

Neuron-Rod Representation and Mirror Estimate

We represent each cell as a vertical “rod” whose length matches its dendritic span, and assign a discrete soma depth (black markers) to approximate the electrotonic center (**Fig. 2B)**. Eight most common excitatory types (L2 pyramidal, L3 pyramidal, L4 star pyramidal, L4ss: L4 spiny stellate

cell, L5 slender-tufted pyramidal, L5 thick-tufted pyramidal, L6 corticocortical pyramidal, L6 corticothalamic pyramidal [27,28,30]) and two inhibitory types (basket and Martinotti [29]) were parameterized by empirically derived top and bottom dendritic extents and soma positions. Each rod type was evenly spanned across the cortex cross-section. For each neuron rod, we compute the "mirror estimate," defined as:

$$\Delta V_{\mathrm{m}}(z) = \overline{V_{e_{\mathrm{rod}}}} - V_e(z)$$

where $\overline{V_{e\,\mathrm{rod}}}$ is the mean extracellular potential across the rod's depth [18,31,32]. This metric serves as a DC analog of the extracellular activation function, predicting net transmembrane polarization.

Population-Level Heatmap via Weighted Sum Interpolation

To approximate population multiunit activity, rods from each excitatory neuron type were randomly scattered laterally across one cortex cross-section in proportion to their relative densities[28]. Soma potential changes ΔV_m were extracted and placed on a finer 200×200 grid, then summed with Gaussian weighting (σ=0.1 mm) to reflect local neuronal activity:

$$S(x,y) = \sum_i \exp\left[-\frac{(x-x_i)^2 + (y-y_i)^2}{2\sigma^2}\right] \Delta V_{\mathrm{m}}(i)$$

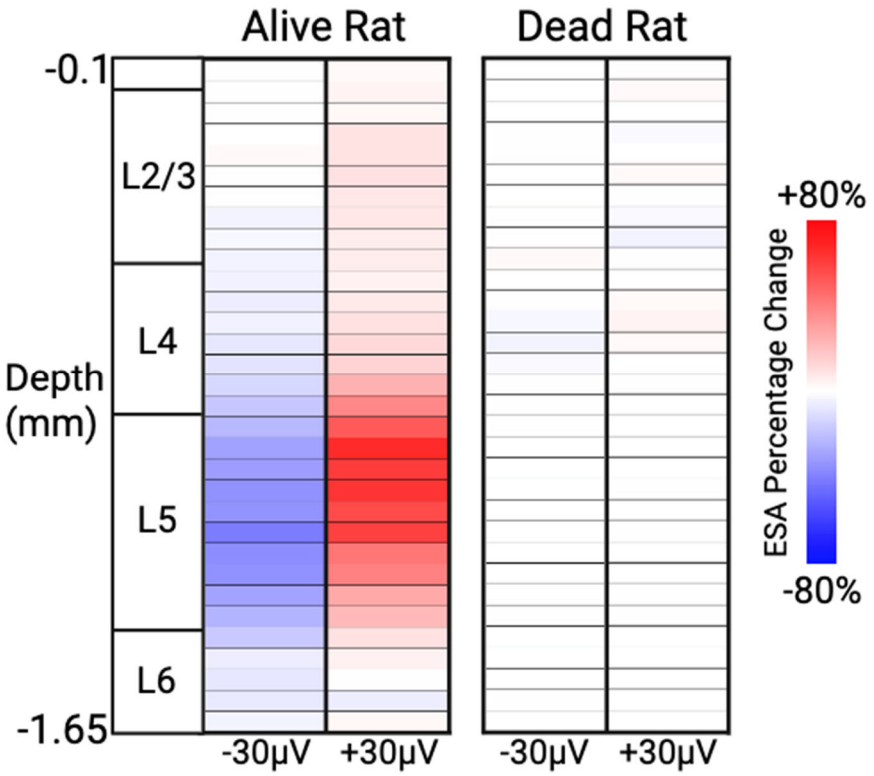
The resulting 2D heatmap displays the spatial distribution of net soma polarization under anodic or cathodic iDC, which serves as an approximation of the activity change that would be detected by the recording electrode used in our in vivo setup (**Fig. 2C)**.

The complete code can be found in https://github.com/RunmingTonyWang/iDC-cortex-model

Supplementary Material:

Control Experiment for Reference-Wire Artifacts in ESA Metrics

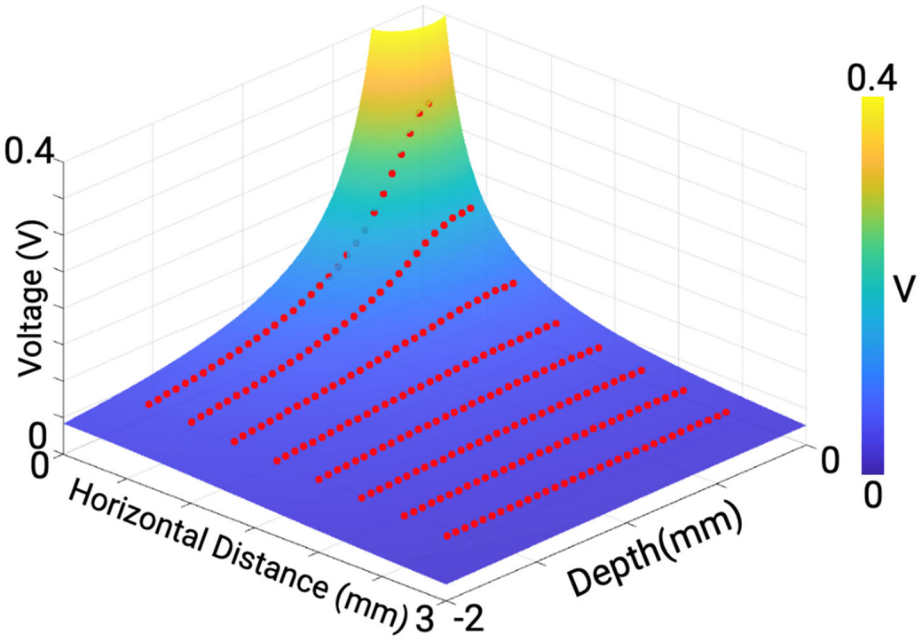
To confirm that reference-wire artifacts did not influence our ESA measurements, we performed control recordings in which anodic and cathodic iDC (±30 μA) were applied using identical stimulation and recording configurations in both living and euthanized rats. ESA relative percent changes during-vs.-before iDC were calculated for both conditions. Anodic and cathodic stimulation robustly altered ESA in layer 5 channels of the alive rat, whereas no change was observed across channels in the euthanized rat (**Supplemental Fig.1**). These results verify that ESA metrics reported here were unaffected by stimulation artifacts.



Supplemental Figure 1. Relative percent change in average ESA across recording channels for alive and euthanized rats.

Electric Field Mapping In Vivo

To validate the tissue resistivity parameter (ρ) used in our computational model, we delivered brief cathodic pulses (200 µs, -100 µA) through the iDC microcatheter placed at multiple lateral distances (0.25–2.6 mm) from the microelectrode array. Peak voltage amplitudes were recorded from all 32 channels spanning cortical depths (~0.10–1.65 mm) and lateral offsets (~0.25–2.55 mm), generating a two-dimensional voltage map of the induced electric field (**Supplemental Fig. S2**). We optimized the resistivity parameter by matching model-predicted voltages to experimentally recorded values, yielding a best-fit resistivity of approximately 5×10^3 Ω·mm, which was subsequently used in our computational model.



Supplemental Figure S2. Measured voltages (red dots) overlaid on model-predicted extracellular voltage spread.

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