

# Neuroprotective Effects of Electrical Stimulation Following Ischemic Stroke in Non-Human Primates\*

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**Abstract**— Brain stimulation has emerged as a novel therapy for ischemic stroke, a major cause of brain injury that often results in lifelong disability. Although past works in rodents have demonstrated protective effects of stimulation following stroke, few of these results have been replicated in humans due to the anatomical differences between rodent and human brains and a limited understanding of stimulation-induced network changes. Therefore, we combined electrophysiology and histology to study the neuroprotective mechanisms of electrical stimulation following cortical ischemic stroke in non-human primates. To produce controlled focal lesions, we used the photothrombotic method to induce targeted vasculature damage in the sensorimotor cortices of two macaques while collecting electrocorticography (ECoG) signals bilaterally. In another two monkeys, we followed the same lesioning procedures and applied repeated electrical stimulation via an ECoG electrode adjacent to the lesion. We studied the protective effects of stimulation on neural dynamics using ECoG signal power and coherence. In addition, we performed histological analysis to evaluate the differences in lesion volume. In comparison to controls, the ECoG signals showed decreased gamma power across the sensorimotor cortices in stimulated animals. Meanwhile, Nissl staining revealed smaller lesion volumes for the stimulated group, suggesting that electrical stimulation may exert neuroprotection by suppressing post-ischemic neural activity. With the similarity between NHP and human brains, this study paves the path for developing effective stimulation-based therapy for acute stroke in clinical studies.

## I. INTRODUCTION

Ischemic stroke is a major cause of brain injury that results in serious long-term disability in adults [1], [2]. Among the few existing therapeutic options for stroke, most showed limited efficacy in restoring lost functions [3]. Meanwhile, novel neural modulation paradigms such as electrical brain stimulation have been proposed to exert neuroprotection and facilitate recovery in the post-ischemic brain [4]–[6]. Implementing such a technique for acute and chronic stroke treatments requires a comprehensive understanding of how electrical stimulation drives changes in the physiology of neuronal networks at a large scale, across multiple brain areas. As a result, two major gaps in knowledge need to be addressed before we can effectively use these techniques for therapy: 1) The protective effect of electrical stimulation need to be evaluated across large neural circuits, while most studies have mainly focused on the local or behavioral response to stimulation 2) We need to have a better understanding of the

mechanisms underlying stimulation-induced changes, from both electrophysiological and histological perspectives. However, non-invasive stimulation studies in human patients [7], [8] usually lacked sufficient resolution to investigate circuit- and cellular-level changes after stimulation, whereas invasive studies in rodents [5], [9] are often not clinically translatable due to the anatomical differences between rodent and human brains.

In this study, we used a novel set of approaches capable of addressing the two gaps above to investigate stimulation-induced neuroprotection. We combined a lesion-based toolbox [10], [11] with state-of-the-art neurophysiology techniques to study the neuroprotective effects of electrical stimulation following acute ischemic stroke in non-human primates (NHPs). We compared multiple aspects of stimulation-induced network changes from large areas ( $\sim 3 \text{ cm}^2$  per hemisphere) of the macaque sensorimotor cortex at 0–4 hours after stroke. With insights gained from these experiments, we hope to inform the development of next-generation electrical stimulation paradigms that can be used as an alternative treatment to minimize neuronal damage, promote functional recovery, and reduce severe disabilities for stroke patients.

## II. METHODS

### A. Animals and Surgical Procedures

All animal procedures were approved by the University of Washington Institutional Animal Care and Use Committee. Surgeries were conducted through the Tissue Distribution Program at the Washington National Primate Research Center (WaNPRC), which aims to conserve and fully utilize the NHPs no longer needed for other experiments. Using standard aseptic technique, 4 adult rhesus macaques (Control group: monkey D, female, 12.8 kg, 14 years; monkey E, female, 13.10 kg, 14 years; Stim group: monkey F, female, 13.8 kg, 14 years; monkey G, male, 14.6 kg, 7 years) were anesthetized with isoflurane and placed in a stereotaxic frame. The animal's temperature, oxygen saturation, heart rate, and electrocardiographic responses were monitored throughout the procedure. Bilateral craniotomies and durotomy were performed using stereotaxic coordinates that target the sensorimotor cortices. A semi-transparent multi-modal artificial dura containing 32 ECoG electrodes [12] was implanted bilaterally on top of the cortical surface in each

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cranial window for subsequent electrophysiology recording and electrical stimulation.

### B. Induction of Focal Ischemic Lesions

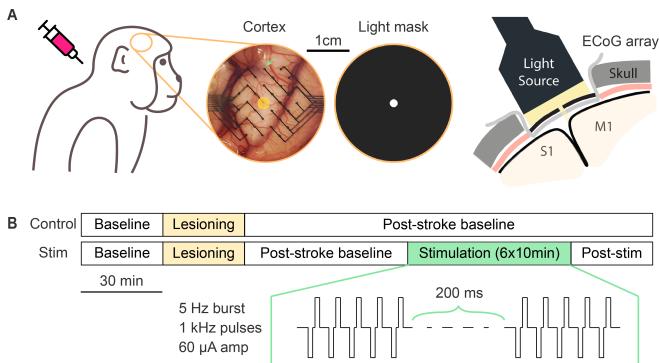
Our lab has previously developed a novel and practical method for creating ischemic lesions in NHPs based on the photothrombotic technique [10], [13], which produces focal infarct by photo-activation of a light-sensitive dye (Rose Bengal). Upon illumination, the intravenously administered dye produced singlet oxygen that damaged endothelial cell membranes, causing platelet aggregation and interrupting local blood flow. In this experiment, we first performed 30 minutes of baseline recording with the ECoG electrodes and then induced a unilateral infarct in the sensorimotor cortex of all 4 monkeys with consistent location. This was achieved by illuminating the ipsilesional cranial window for 30 minutes through an 1.5-mm diameter aperture using an uncollimated white light source after Rose Bengal injection (Fig. 1A). Simultaneous electrophysiology recording was performed to monitor the extent of neuronal damage and network dynamics around the site of injury as the lesion was forming and up to 4 hours post illumination.

### C. Electrophysiology Recording and Electrical Stimulation

All electrophysiology recording and electrical stimulation were performed with two Grapevine Nomad processors and four Nano front ends (Ripple Neuro, Salt Lake City, UT). In all monkeys, we collected ECoG data bilaterally for ~4 hours at 30 kHz sampling frequency (Fig. 1B). In monkeys F and G, we performed electrical stimulation delivered through a single ECoG electrode at ~8 mm medially from the lesion center on the ipsilesional (left) hemisphere (Fig. 1A, green arrow). We delivered the stimulation trains in 6 blocks lasting 10 minutes each, with 2 minutes of baseline recordings in between the blocks to track changes in neurophysiology as stimulation continues. The stimulation trains had a 5 Hz burst frequency and 5 biphasic charge-balanced pulses at 1 kHz within each burst. The stimulation amplitude was 60  $\mu$ A and pulse width was 200  $\mu$ s per phase with 50  $\mu$ s inter-phase interval. These stimulation parameters are chosen based on previous studies in animals and patients [14], [15].

### D. Electrophysiology Data Analysis

We down-sampled the raw 30 kHz signals to 1 kHz and filtered the down-sampled data into distinct frequency bands



**Figure 1.** Schematics of experimental procedures. **(A)** Methods to induce photothrombotic lesion in the NHP cortex. Cortical view includes the multi-model artificial dura used to record ECoG. Yellow circle indicates the area illuminated with light source. Green arrow points to the electrode that delivered electrical stimulation. **(B)** Experimental timeline the control (monkey D and E) and stimulated groups (monkey F and G).

including delta (1-4 Hz), theta (4-7 Hz) and gamma (30-59 Hz). We then calculated the signal power over multiple time windows, including pre-stroke baseline, post-stroke, during-stimulation, and post-stimulation, at each electrode for the frequency bands defined above.

Next, we analyzed the functional connectivity changes between electrodes within either the lesion region or the corresponding area in the contralesional cortex by their pairwise coherence. This magnitude-squared coherence ( $C_{xy}$ ) between signals at electrodes x and y was computed as a function of the respective power spectral densities of signals x and y ( $P_{xx}$  and  $P_{yy}$ ) and their cross-spectral density ( $P_{xy}$ ) using 10 s Hamming windows across every 2 minutes of data.

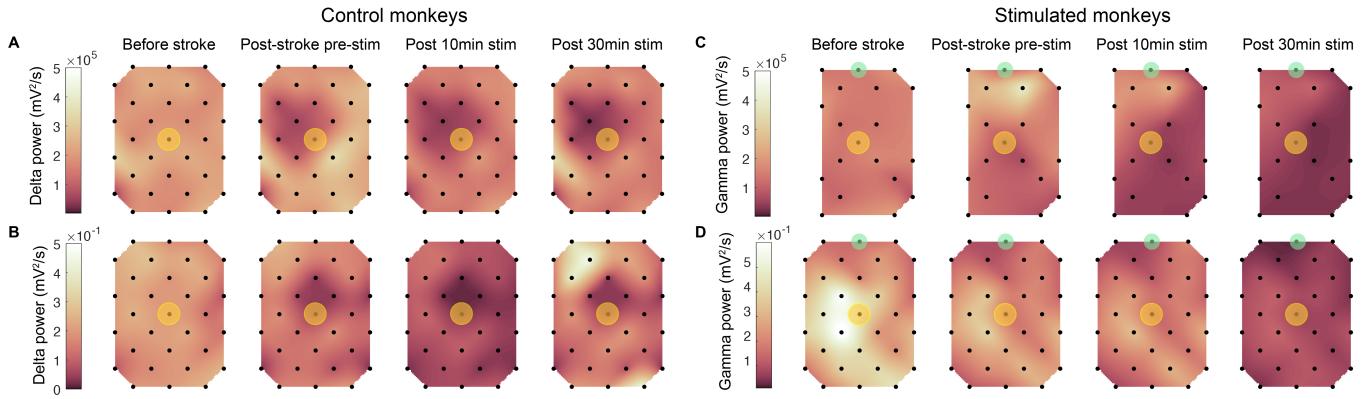
### E. Histological Analysis

At ~4 hours after the stroke is induced, animals were deeply sedated and transcardially perfused with phosphate buffered saline (PBS) and followed by 4% paraformaldehyde (PFA). The brains were harvested and post-fixed by immersion in 4% PFA for 24 to 48 hours. A coronal block containing the lesioned region was dissected using a custom matrix and then stored at 4 °C in 30% sucrose in PBS. For staining, the block was frozen and sectioned into 50  $\mu$ m thick coronal sections using a sliding microtome (Leica). Sliced sections were stored at 4 °C in PBS with 0.02% sodium azide. To evaluate the extent of ischemic damage and neuronal death, we performed Nissl staining on mounted coronal sections surrounding the lesion with ~0.45 mm separation between sections using Thionin acetate. Nissl-stained slices were then scanned and registered in MATLAB (2019b, MathWorks) for three-dimensional (3D) reconstruction and estimation of lesion volumes. The registered images were then smoothed, binarized, and went through edge detection so that infarct boundaries on each slice can be identified for 3D visualization. The widths and depths from representative coronal slices of each lesion were also calculated based on image resolution.

## III. RESULTS

First, we analyzed the large-scale ECoG recordings to monitor the sensorimotor neural activity and characterize the acute physiological changes driven by the ischemic lesion and electrical stimulation. In the gamma frequency band, we saw distinctively low power at electrodes closest to the previously illuminated region (center of the array) during all post-stroke periods in both control and stimulated monkeys (Fig. 2A-D). This observation confirmed the localized neuronal damage caused by photothrombosis. Interestingly, we also observed a gradual, large-scale downregulation of high frequency gamma activity across the entire ipsilesional sensorimotor region in response to post-stroke stimulation for monkeys in the stimulation group (Fig. 2C-D). This was distinctively different from what was observed in the control group where gamma power at some of the perilesional electrodes was elevated at 30 minutes post the stimulation start time (Fig. 2A-B). These results suggest that gamma power was selectively suppressed over large areas as stimulation continued, reflecting reduced neuronal activity level in response to post-stroke stimulation.

Next, we calculated the pairwise coherence between electrodes within the lesion region (Fig. 3A). Similarly, the coherence was also calculated between the corresponding electrodes in the contralesional cortex (Fig. 3B). We observed that within the delta band (1-4 Hz), the pairwise coherence



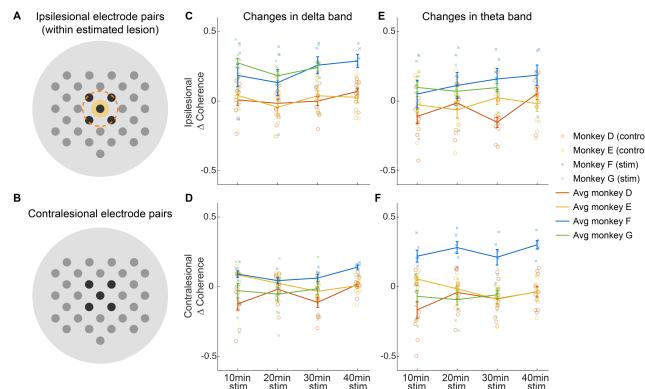
**Figure 2.** Heatmaps of ipsilesional gamma band power (30-59 Hz) at different time points. **(A-B)** Gamma power in monkeys D and E (control group). Yellow circles indicate the area illuminated with light source. **(C-D)** Gamma power in monkeys F and G (stim group). Green circles indicate the electrode that delivered electrical stimulation. Fewer electrodes are shown in C due to channel lost during recordings in monkey F.

after stimulation was significantly higher than pre-stroke baseline only in the stimulated monkeys, following 10 minutes (monkey G,  $p < 0.001$ , paired t-test), 20 minutes (monkey G,  $p < 0.001$ ), 30 minutes (monkey F,  $p < 0.01$  and monkey G,  $p < 0.001$ ), and 40 minutes (monkey F,  $p < 0.005$ ) of stimulation in the lesion area (Fig. 3C). Meanwhile, no significant changes in delta coherence was observed in the contralateral cortex, except for a significant increase in monkey F after 40 minutes of stimulation ( $p < 0.001$ , Fig. 3D). In comparison, the coherence changes in theta band (4-7 Hz) was less consistent across animals in both groups, on both hemispheres (Fig. 3E-F). These results suggest that electrical stimulation increased low-frequency local field potential (LFP) synchrony in the acute phase after stroke, especially in the delta band within the estimated lesion core and penumbra.

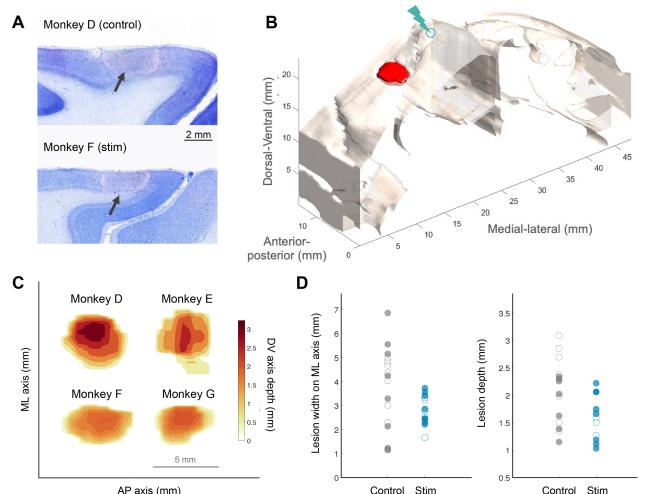
To confirm the extent of cell death and estimated lesion volumes in control and stimulation animals, we performed Nissl staining using fixed coronal sections. The loss of Nissl substance inside ischemic cores led to distinct pale areas and well-defined boundaries on the stained sections (Fig. 4A). Using this identified lesion boundary and linear interpolation, we reconstructed the lesions in 3D space (Fig. 4B) and estimated their volumes in each animal. We found that in control monkeys D and E, the estimated lesion volumes were 35.3 and 28.4  $\text{mm}^3$  respectively, while in the stimulated monkeys F and G, the lesion volumes were 20.3 and 15.9  $\text{mm}^3$  respectively, smaller than the controls on both the medial-lateral (ML) and dorsal-ventral (DV) axes (Fig. 4C-D). Note that the stimulation pulses were also delivered medially from the lesion center. Together, these results suggest that the downregulation of gamma activity observed in Fig. 2C-D was not caused by additional neuronal death in the stimulated region, and that monkeys receiving post-stroke electrical stimulation showed smaller infarction at around 4 hours after ischemic lesioning.

#### IV. DISCUSSION

In this study, we produced focal ischemic lesions using the phototherapeutic method [10]. In comparison to other surgical interventions for generating infarcts in NHPs [16], our method is less technically challenging and can reliably control the location and size of infarcts across animals by implementing the same aperture, intensity, and duration of light illumination. Meanwhile, a phototherapeutic infarct is largely limited to the cortex and does not cover other stroke pathways such as those



**Figure 3.** ECoG signal coherence in response to post-stroke stimulation. **(A)** Schematics of electrodes selected for coherence measurements in the ipsilesional hemisphere show in black. The shaded yellow region indicates the cortical area under illumination and the dashed circle indicates the estimated lesion area. Electrodes shown in black are used for coherence measurement. **(B)** Electrodes (shown in black) used for coherence measurements in the contralateral hemisphere. **(C, D)** Changes in pairwise delta (1-4 Hz) coherence from the pre-stroke baseline at different time points after the start of electrical stimulation. **(E, F)** Changes in theta (4-7 Hz) coherence from pre-stroke baseline at similar time points. In 4 animals, both individual data points and mean  $\pm$  standard error are shown.



**Figure 4.** Histological results. **(A)** Examples of Nissl-stained section from monkey D and F. **(B)** 3D reconstruction from coronal sections. Red volume indicates the interpolated lesion and green arrow points to the stimulation site. **(C)** Comparison of interpolated lesion volumes projected onto the dorsal-ventral (DV), anterior-posterior (AP), and medial-lateral (ML) axis. **(D)** Lesion widths and depths in control and stimulation animals.

dominated by subcortical and white matter damage. With this method, we generated unilateral lesions in the sensorimotor region while collecting ECoG data from both hemispheres in 4 animals. We then applied repeated electrical stimulation adjacent to the lesion on the ipsilesional cortex, 60 minutes after lesion induction in 2 monkeys. The stimulation train contains 5 Hz bursts of 5 biphasic pulses, which has been shown to promote greater Hebbian-like plasticity compared to single-pulse paradigms in rodents [15]. In the future, other stimulation design such as modulated pulses can be explored to optimize the parameters for enhancing neuroprotection and plasticity. Our ECoG data showed decreasing gamma power (30 – 59 Hz) over the course of stimulation but not in control animals. Since gamma LFP in ECoG has been shown to correlate with neuronal firing rate [17], it is possible that our electrical stimulation paradigm exerts a neuroprotective effect on cortical neurons in the acute phase after stroke by temporarily suppressing neuronal firing surrounding the damaged area and thus conserving critical metabolic energy. This protective mechanism may be similar to some of the pharmacological agents used for ischemic stroke which aims to restore the balance between oxygen supply and energy consumption by inhibiting neural activity [18]. This hypothesis is partially confirmed by the smaller infarct volume observed in the stimulation animals, although additional experiments are needed to validate this result statistically.

Additionally, we analyzed the pairwise coherence between ECoG signals recorded within the estimated ischemic core and penumbra, as well as the corresponding regions on the contralesional hemisphere, to investigate the change in functional connectivity in response to post-stroke stimulation. Coherence is a reliable and accessible measure of LFP synchrony that can be performed during spontaneous activity without the need of perturbing the network. We found that delta band coherence increased significantly from the pre-stroke baseline after multiple blocks of stimulation. Although higher delta coherence has been considered as a biomarker of acute ischemic injury in past electroencephalography and LFP studies [19], [20], it has also been shown that this low-frequency synchronous activity can also be correlated with axonal sprouting, a process critical to post-stroke plasticity and recovery [21]. Therefore, the higher delta coherence in the stimulated monkeys may suggest a neuroplastic effect of electrical stimulation, especially in the ischemic penumbra. We plan to test this hypothesis in the future by performing immunohistochemistry staining to quantify the expression of a biomarker of axonal sprouting, growth-associated protein 43, in the perilesional areas from all 4 animals.

In summary, this study revealed possible mechanisms of stimulation-induced neuroprotection after acute ischemic stroke by combining the latest technology in electrophysiology and histology. With ongoing work, we hope to investigate both protective and neuroplastic aspects of stimulation to gain a better understanding of the therapeutic benefits produced by electrical stimulation paradigms, before translating them into a viable treatment for stroke patients.

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