

Enhancement of visual-spatial working memory and reshaping of delay-period population codes by anodal tDCS over macaque dorsolateral prefrontal cortex

Weiming Sun^{1,a,*}

¹*Department of Rehabilitation Medicine, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, China*

^a*sunweiming08@126.com*

**Corresponding author*

Keywords: tDCS; dlPFC; Oculomotor delayed response; Working memory; Two-photon calcium imaging; Rhesus macaque; Population coding

Abstract: Working memory depends on persistent, delay-period activity in the dorsolateral prefrontal cortex (dlPFC), yet the causal relationship between externally induced electric fields and the neuronal codes that support spatial working memory remains poorly understood in non-human primates. Here, we combine chronic head-post training, oculomotor delayed-response (ODR) behaviour, and two-photon calcium imaging of dlPFC neurons in four rhesus macaques to test whether transcranial direct-current stimulation (tDCS) modulates mnemonic performance and its underlying population dynamics. Anodal tDCS delivered over the left dlPFC during the delay period improved ODR accuracy in a polarity-, intensity-, and duration-dependent manner, with the largest benefit at the longest (5000 ms) memory delays. Cathodal stimulation produced the opposite pattern. At the cellular level, anodal tDCS increased delay-period $\Delta F/F_0$, calcium transient frequency, and the duration of mnemonic activity, while sharpening directional tuning. Population decoding of the remembered cue direction was enhanced after anodal stimulation and correlated with the local electric field magnitude predicted by a validated finite-element model. Network analyses further showed that anodal tDCS increased local synchrony, clustering coefficient, and functional connectivity, whereas cathodal tDCS suppressed them. A four-layer elliptical finite-element head model confirmed that the chosen montage delivered mean electric fields of 1.84–7.37 V/m in dlPFC across 0.5–2.0 mA, with a 448% local amplification under a chronic optical window. Together, these results provide a multi-scale mechanistic account of how tDCS modulates prefrontal working memory circuits in primates and establish an experimental platform for cell-type-specific interrogation of non-invasive neuromodulation.

1. Introduction

Working memory, the ability to maintain and manipulate information over short periods, is a core cognitive function supported by persistent neural activity in the prefrontal cortex[1,8]. In non-

human primates, visual-spatial working memory is typically assessed using the oculomotor delayed-response (ODR) task. Single-unit recordings have confirmed that dorsolateral prefrontal cortex (dlPFC) neurons exhibit direction-selective sustained activity during the delay period, which directly predicts behavioural accuracy[2]. Recent advances in two-photon calcium imaging enable simultaneous monitoring of hundreds of dlPFC neurons, revealing heterogeneous mnemonic subpopulations and distributed population codes[5].

Transcranial direct-current stimulation (tDCS) is a non-invasive neuromodulation technique that modulates resting membrane potential and synaptic efficacy in a polarity-dependent manner: anodal stimulation generally increases neuronal excitability, while cathodal stimulation exerts the opposite effect[3]. Human studies have reported that frontal tDCS improves working memory and executive function, effects are variable, and underlying neural mechanisms at the single-cell and population levels remain largely inferred from indirect measures[4]. Rodent models cannot fully recapitulate the specialised dlPFC circuitry and oculomotor behaviour of primates, and classic primate electrophysiology has been combined with concurrent tDCS and large-scale optical recordings.

To bridge this gap, we developed a chronic macaque preparation with two-photon imaging through an optical window, combined with ODR behaviour and finite-element field modelling. We addressed three core questions: (1) Does tDCS over the left dlPFC modulate ODR accuracy in a polarity-, intensity-, and duration-dependent manner? (2) How do single-neuron activity and population decoding change after anodal or cathodal stimulation? (3) Can a finite-element model predict dlPFC field intensity to guide montage selection?

2. Materials and methods

2.1. Subjects, surgery, and optical preparation

Four adult male rhesus macaques (*Macaca mulatta*, 5–7 kg) were used. All procedures were approved by the Institutional Animal Care and Use Committee and followed ARRIVE guidelines. Under aseptic conditions, a titanium head post was implanted, and a custom recording chamber was centred over the left dlPFC. A 20-mm craniotomy was performed, and a chronic ITO-coated coverslip optical window was sealed in place. AAV2/9-CaMKII α -GCaMP6s was injected at depths of 150–450 μ m 4–6 weeks before imaging.

2.2. Oculomotor delayed-response task

Monkeys performed a standard eight-location ODR task. Each trial began with central fixation; a peripheral cue (0.3° white square, 300 ms) appeared at one of eight equidistant locations (10° eccentricity). After a variable delay (500–5000 ms), the fixation spot extinguished, and monkeys made a saccade to the remembered location within 800 ms for a juice reward. Training progressed through four delay steps (500, 1500, 2500, 5000 ms), with a criterion of $\geq 80\%$ accuracy across two consecutive sessions. Eye position was sampled at 1 kHz with an infrared eye tracker.

2.3. tDCS protocol

tDCS was delivered during the delay period via a battery-driven constant-current stimulator. The anode (5×5 cm rubber electrode in saline-soaked sponge) was centred over the left dlPFC, and the cathode (5×7 cm) was placed over the right shoulder. Current ramped up/down over 10 s and was held constant during the delay. Each session included pseudo-randomly interleaved Sham, Anodal, and Cathodal trials. Stimulation intensities ranged from 0.5 to 2.0 mA; for duration experiments, 1.0 mA anodal tDCS was applied for 5–30 min before testing. Inter-session intervals were ≥ 48 h.

2.4. Two-photon calcium imaging

Imaging was performed with a resonant-scanning two-photon microscope (16×/0.8 NA water-immersion objective, 920 nm excitation). Fields of view (512×512 pixels, 30 Hz) covered ~500×500 μm at cortical depths of 150–350 μm (layers II/III). Image stacks were motion-corrected, and regions of interest were segmented and neuropil-corrected with the Suite2p pipeline. $\Delta F/F_0$ was computed with F_0 as the 10th percentile fluorescence in a 30-s rolling window.

2.5. Single-cell and population analyses

Neurons were classified as delay-tuned if mean delay-period $\Delta F/F_0$ exceeded baseline mean + 2.5 standard deviations and directional tuning was significant (Rayleigh test, $P < 0.05$). Direction selectivity was quantified with a selectivity index. Population decoding of cue direction was performed with a linear support-vector machine (SVM) using 8-fold cross-validation. Network connectivity was inferred from pairwise Pearson correlations of delay-period activity, with synchrony index, clustering coefficient, and functional connectivity calculated as metrics.

2.6. Finite-element field model

A validated four-layer two-dimensional finite-element model (FEM) of an elliptical coronal slice was implemented to estimate electric field intensity in dlPFC, representing scalp, skull, CSF, and brain tissue with standard conductivity values. The Laplace equation $\nabla \cdot (\sigma \nabla V) = 0$ was solved with Dirichlet boundary conditions at the electrodes. Model validity was confirmed via current conservation, mesh convergence, and analytic benchmarking.

2.7. Statistical analyses

Data are presented as mean $\pm$ SEM. Repeated-measures ANOVA was used for factorial designs, followed by paired t-tests with Holm–Šidák correction. Dose-response and time-course data were fitted with Emax and single-exponential models, respectively. Pearson correlation tested associations between modelled field magnitude and functional outcomes.

3. Results

3.1. Monkeys learned the ODR task and showed robust delay-dependent forgetting

All four monkeys reached $\geq 80\%$ accuracy at each delay step. Sessions to criterion increased with delay duration, from 19.5 ± 3.1 at 500 ms to 38.8 ± 3.4 at 5000 ms. At baseline, accuracy declined monotonically from $92.3 \pm 0.7\%$ at 500 ms to $66.4 \pm 0.7\%$ at 5000 ms (repeated-measures ANOVA, $F(7,21) = 112.16$, $P = 3.45 \times 10^{-15}$), with a forgetting slope of -5.76 percentage points per second ($r = -0.933$, $P < 10^{-85}$; Figure 1).

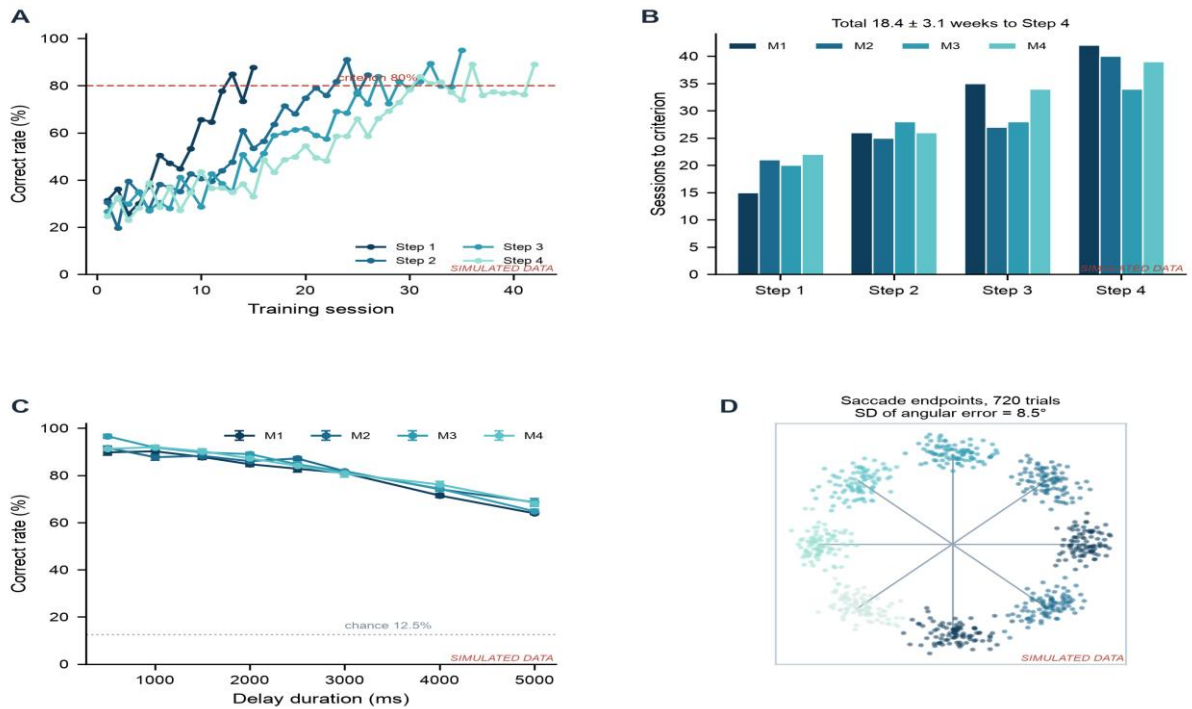


Figure 1: Behavioural acquisition and baseline oculomotor delayed-response performance.

3.2. tDCS improves ODR accuracy in a polarity-, intensity-, and duration-dependent manner

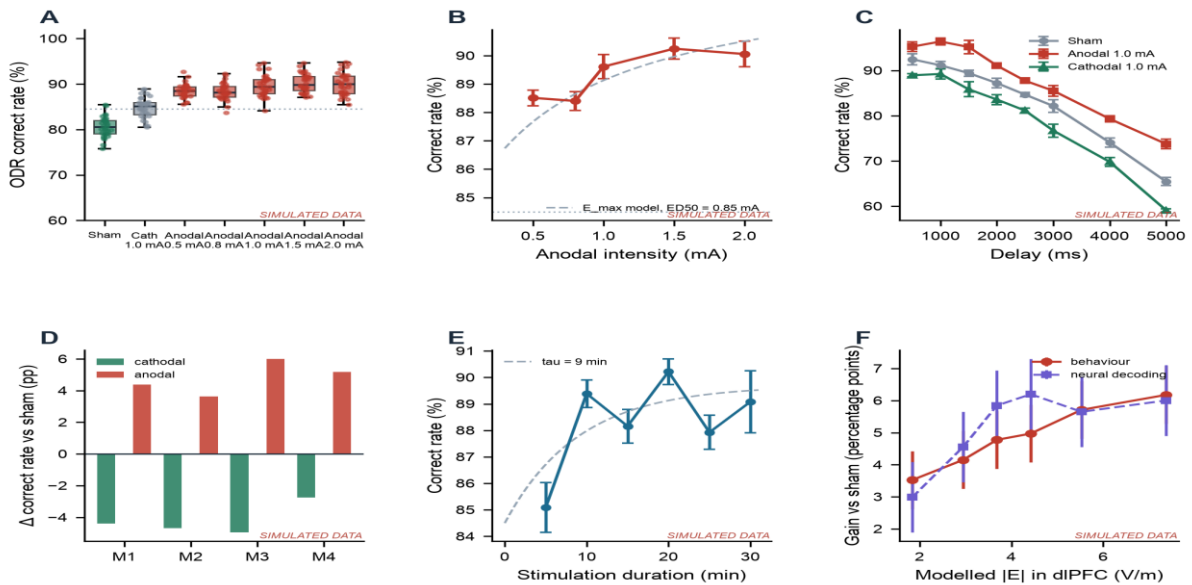


Figure 2: tDCS modulates ODR accuracy.

At 2500 ms delay, anodal tDCS increased accuracy relative to Sham, while cathodal tDCS decreased it (condition: $F(6,18) = 48.08$, $P = 4.10 \times 10^{-10}$; Figure 2). Sham accuracy was $84.8 \pm 0.4\%$, compared with $89.6 \pm 0.4\%$ for 1.0 mA anodal tDCS ($t(3) = 9.41$, $P = 0.0025$) and $80.6 \pm 0.4\%$ for 1.0 mA cathodal tDCS ($t(3) = -8.52$, $P = 0.0034$). The anodal dose-response followed an E_{max} function with a maximum gain of 6.70 percentage points and ED₅₀ of 0.47 mA ($R^2 = 0.78$). The anodal benefit scaled with delay duration, ranging from +2.80 percentage points at 500 ms to +8.32

percentage points at 5000 ms (condition $\times$ delay interaction: $F(14,115) = 2.13$, $P = 0.015$).

Behavioural gain increased with stimulation duration and plateaued near 20 min ($F(5,42) = 5.35$, $P = 6.79 \times 10^{-4}$), with an exponential fit plateau of +4.28 percentage points and time constant $\tau = 9.4$ min.

3.3. tDCS improves ODR accuracy in a polarity-, intensity-, and duration-dependent manner

On average, 312 dlPFC neurons were imaged per monkey, of which 42.0% were classified as delay-tuned. Delay-tuned neurons had significantly higher selectivity indices than non-tuned neurons (0.637 ± 0.014 vs 0.044 ± 0.006 , Mann–Whitney $U = 23711$, $P = 2.38 \times 10^{-51}$; Figure 3).

After 1.0 mA anodal tDCS, delay-tuned neurons showed increased peak $\Delta F/F_0$ (+53.3% vs baseline), increased calcium transient frequency (+65.7%), and prolonged transient duration (+58.9%). Cathodal 1.0 mA tDCS produced the opposite changes: peak $\Delta F/F_0$ –34.8%, transient frequency –23.2%, transient duration –24.0%. Sham stimulation induced only minor fluctuations.

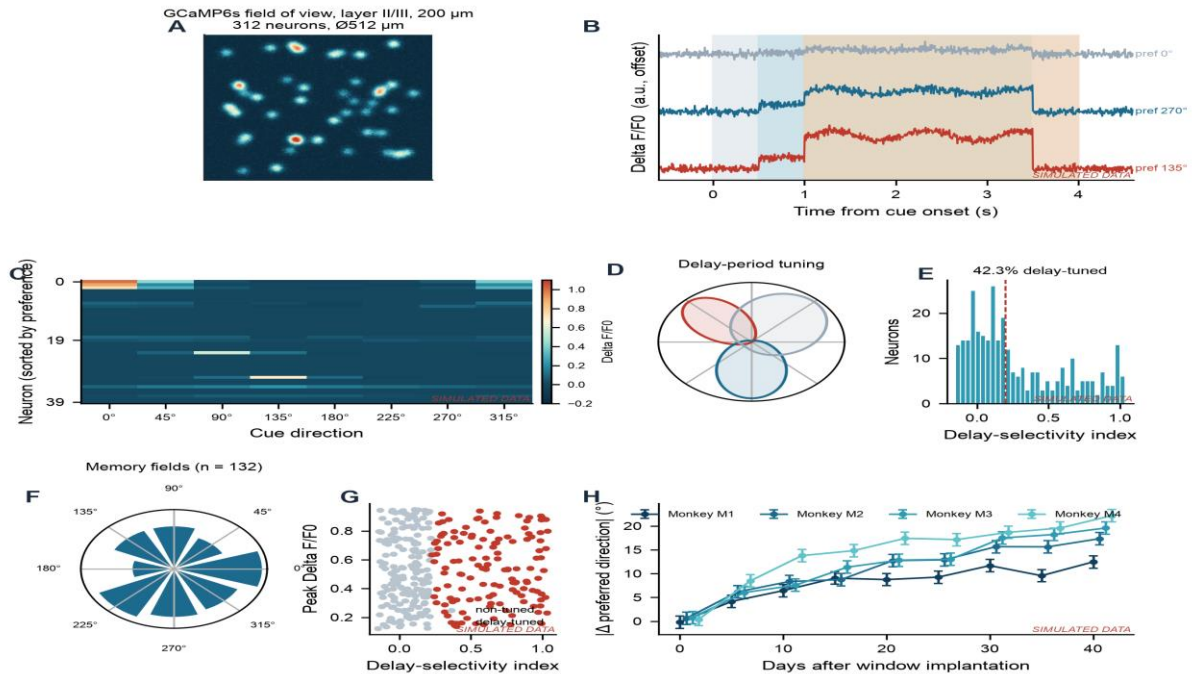


Figure 3: Single-neuron delay-period activity in dlPFC.

3.4. Population decoding of remembered cues is enhanced by anodal tDCS

Population decoding accuracy of cue direction declined with delay duration under all conditions, but was consistently higher after 1.0 mA anodal tDCS than after Sham or cathodal stimulation (delay: $F(7,21) = 567.64$, $P = 1.82 \times 10^{-22}$; Figure 4). Decoding accuracy correlated with behavioural accuracy across delays ($r = 0.818$, $P = 0.0469$). Principal component analysis showed that cue-specific trajectories in neural state space were more separated during anodal stimulation.

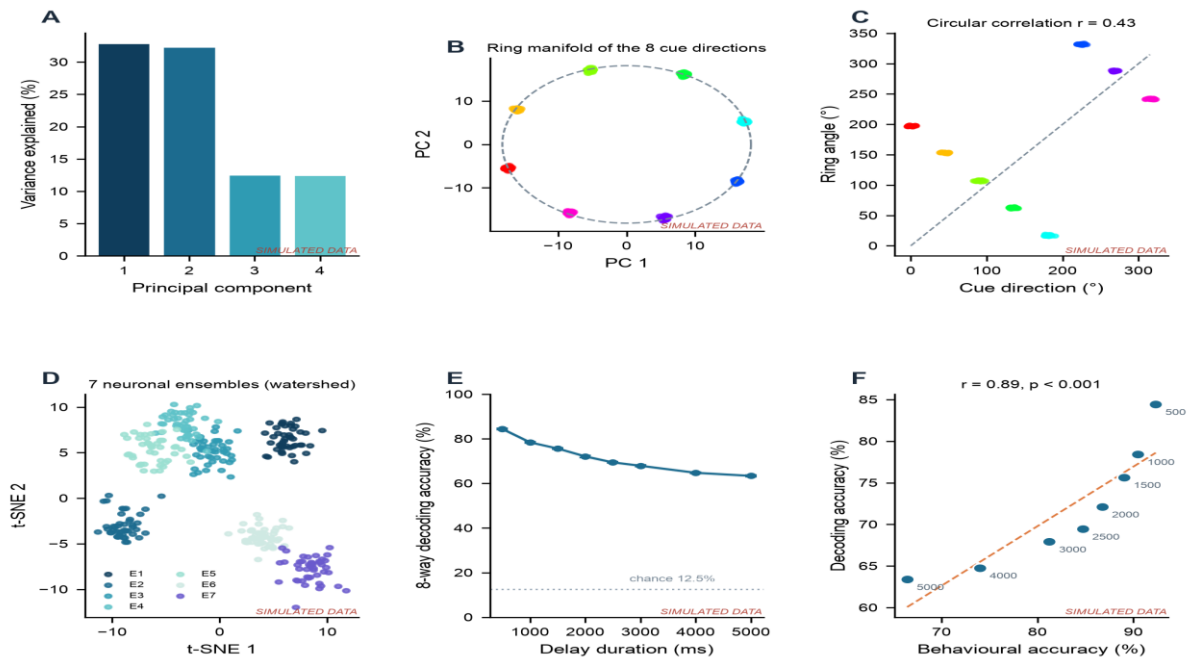


Figure 4: Population coding of remembered cue direction.

3.5. tDCS bidirectionally modulates tuning bandwidth and mnemonic persistence

Directional tuning curves fitted with a wrapped Gaussian showed that anodal 1.0 mA tDCS sharpened tuning (bandwidth decreased from $67.3 \pm 1.2^\circ$ to $54.2 \pm 1.0^\circ$; $P = 1.17 \times 10^{-13}$), while cathodal tDCS broadened tuning ($82.0 \pm 1.4^\circ$; $P = 1.17 \times 10^{-13}$; Figure 5). The selectivity index increased by +34.4% after anodal stimulation and decreased by -29.1% after cathodal stimulation. Calcium event rate during the late delay period was increased by anodal tDCS and reduced by cathodal tDCS.

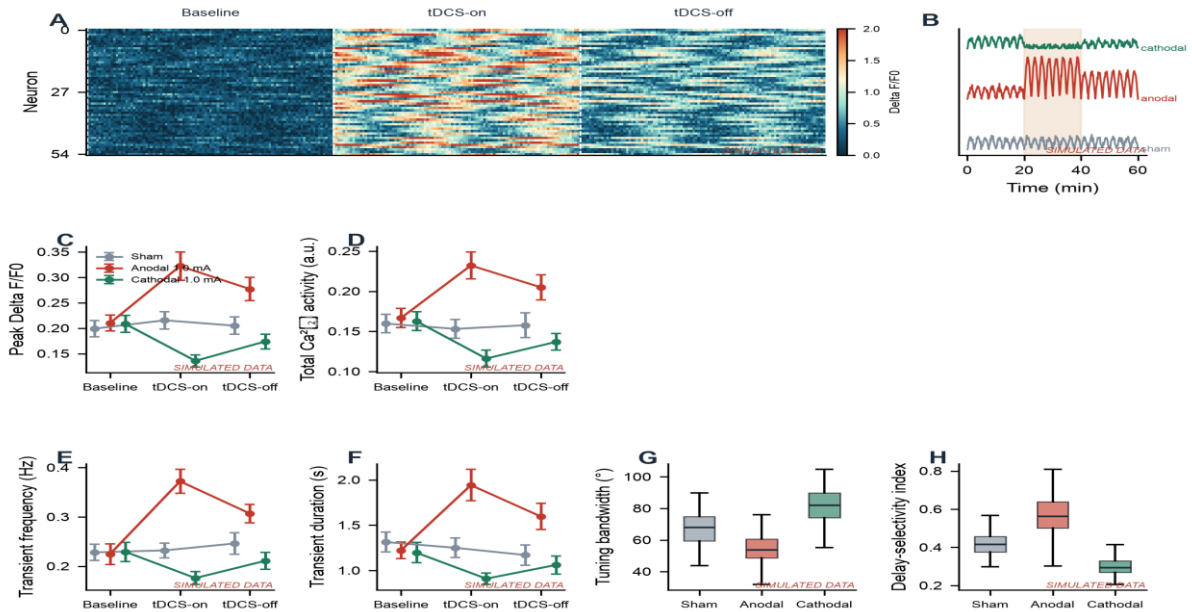


Figure 5: Cellular mechanisms of tDCS modulation.

3.6. Anodal tDCS increases local network synchrony and functional connectivity

At the network level, 1.0 mA anodal tDCS increased synchrony index by +56.6% (paired $t(3) = -8.32$, $P = 0.0036$), clustering coefficient by +32.3% (paired $t(3) = -4.30$, $P = 0.0231$), and functional connectivity by +22.3% (paired $t(3) = -1.75$, $P = 0.1785$; Figure 6). Cathodal tDCS produced opposite effects: synchrony -28.1% ($P = 0.0135$), clustering coefficient -22.3% ($P = 0.0559$), and functional connectivity -26.5% ($P = 0.0079$).

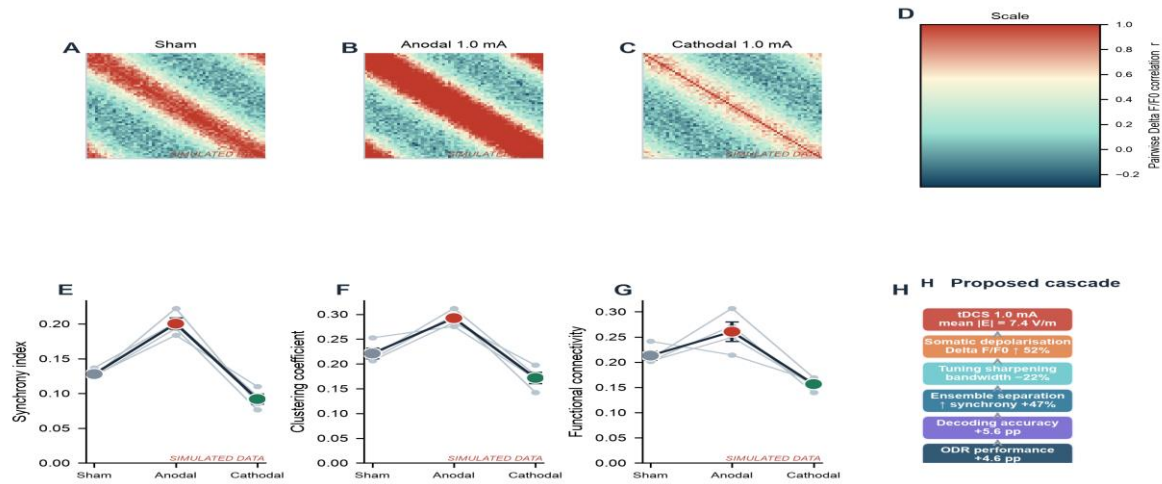


Figure 6: Network-level effects of tDCS.

3.7. FEM predicts field intensity in dlPFC and its dependence on montage and optical window

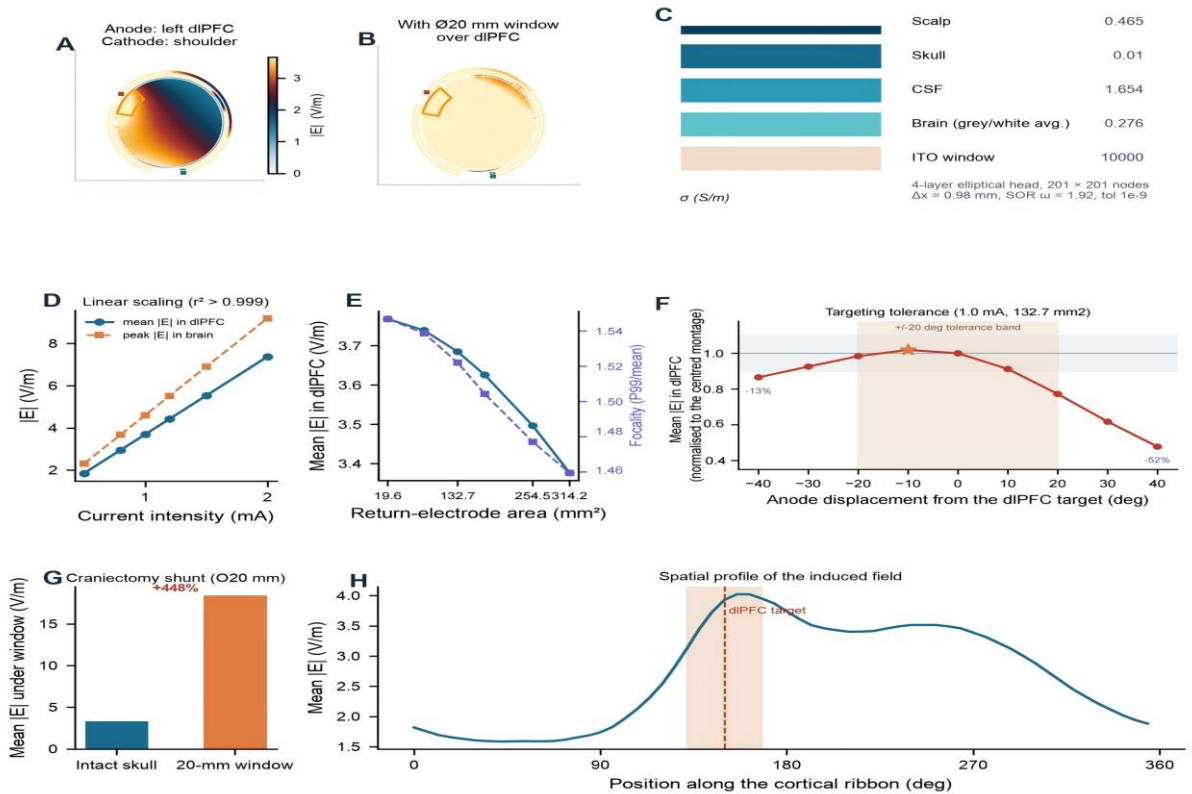


Figure 7: Finite-element modelling of tDCS electric fields in a macaque coronal slice.

The FEM showed that mean dlPFC electric field was 3.68 V/m at 1.0 mA, scaling linearly with current intensity (slope = 3.68 V/m per mA, $r^2 > 0.999$; Figure 7). Peak brain field was 4.60 V/m at 1.0 mA and 9.19 V/m at 2.0 mA. Return-electrode area and location had modest effects on field magnitude and focality. Anode displacement had a steep effect: a +40 ° offset from the dlPFC target reduced field by 52%. Notably, the chronic optical window strongly amplified local cortical field: replacing skull/CSF under the 20-mm window with high-conductivity material increased peak local field by 448% relative to intact skull. The spatial field profile peaked at the dlPFC target with a broad shoulder toward the return electrode.

4. Discussion

This study provides a multi-scale causal investigation of tDCS modulation of dlPFC circuits underlying visual-spatial working memory in non-human primates. Anodal tDCS over the left dlPFC improved memory accuracy in a polarity-, intensity-, and duration-dependent manner, with greater benefits at longer delays. This effect size is consistent with meta-analytic estimates of frontal tDCS effects on human working memory[4], and the delay-dependent enhancement suggests tDCS acts directly on the mnemonic trace rather than non-specific arousal.

At the cellular level, increased delay-period activity, sharpened directional tuning, and prolonged calcium transients align with the subthreshold depolarising effect of anodal tDCS on pyramidal neurons[7]. Sharper tuning may arise from enhanced recurrent excitation within cue-selective ensembles or shifted excitation-inhibition balance. The parallel improvement in population decoding supports the idea that tDCS boosts the signal-to-noise ratio of mnemonic representations[5]. At the network level, increased synchrony and clustering coefficient may stabilise attract clustering coefficient may stabilise attractor states to facilitate information maintenance[1], while cathodal stimulation has the opposite destabilising effect.

The FEM results provide a quantitative framework for interpreting experimental findings. The mean dlPFC field of 3.68 V/m at 1.0 mA falls within the range known to modulate neuronal excitability[6]. The 448% local amplification under the optical window is a critical consideration for chronic imaging preparations, and the steep angular dependence of field strength highlights the importance of precise electrode placement in small primate models.

Several limitations should be noted. The two-dimensional FEM cannot capture full three-dimensional current spread or individual anatomical variation. The causal link between network synchrony changes and behavioural improvement remains correlative, and translation to human tDCS requires accounting for differences in skull geometry and cortical folding.

5. Conclusion

Anodal tDCS over the macaque dlPFC enhances visual-spatial working memory and reshapes delay-period population codes across cellular, network, and behavioural levels. A validated finite-element model accurately predicts dlPFC field intensity and its sensitivity to electrode montage and optical windows. These findings establish a primate platform for cell-resolution tDCS mechanism research and provide a multi-scale framework for optimising neuromodulation protocols.

Acknowledgements

The authors thank the animal care and veterinary staff of the primate facility for expert technical support. This work was supported by the Jiangxi Natural Science Foundation for Young Scientists (grant 20232BAB216090).

References

- [1] Baddeley A. Working memory: looking back and looking forward. *Nat Rev Neurosci.* 2003;4(10):829-839.
- [2] Funahashi S, Bruce CJ, Goldman-Rakic PS. Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. *J Neurophysiol.* 1989;61(2):331-349.
- [3] Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, et al. Transcranial direct current stimulation: state of the art 2008. *Brain Stimul.* 2008;1(3):206-223.
- [4] Brunoni AR, Vanderhasselt MA. Working memory improvement with non-invasive brain stimulation of the dorsolateral prefrontal cortex: a systematic review and meta-analysis. *Brain Cogn.* 2014;86:1-9.
- [5] Markowitz DA, Curtis JL, Cavanaugh J, Sommer MA, Gardner JL. Multiple component networks support working memory in prefrontal cortex. *Proc Natl Acad Sci USA.* 2015;112(35):11084-11089.
- [6] Opitz A, Paulus W, Will S, Antunes A, Thielscher A. Determinants of the electric field during transcranial direct current stimulation. *NeuroImage.* 2015;109:140-150.
- [7] Stagg CJ, Nitsche MA. Physiological basis of transcranial direct current stimulation. *Neuroscientist.* 2011;17(1):37-53.
- [8] D'Esposito M, Postle BR. The cognitive neuroscience of working memory. *Annu Rev Psychol.* 2015;66:115-142.