Computational methods for precise and effective neuromodulation

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1. <u>English abstract</u>

Human brain activity in different brain regions is highly specialized, and in most regions requires very finely tuned oscillatory activity to properly function. When this rhythmicity is disturbed, it can have significant consequences, as is the case in many neurological diseases. On the other hand, if we can modify these oscillations in a desirable way from baseline, we may be able to enhance certain cognitive functions. By targeting specific brain areas with stimulation of a specific frequency, researchers and clinicians may be able to enhance cognitive functions, or correct deficits in brain function in patients with certain neurological conditions. To this end, we are interested in developing transcranial alternating current stimulation (tACS) to be an effective and precise non-invasive method for stimulating brain areas rhythmically. This technique involves delivering low strength alternating current through two electrodes placed on the scalp. This creates an electric field in the brain which influences the electrical properties of neurons. In order to target the stimulation precisely, we must simulate what the resulting electric field in the patient's brain will be. To do that we take MRI scans and turn those into 3D computer models. These models are then run through a simulator software which gives us a very good estimate of the electric field in the brain during stimulation. This allows us to maximize the field targeting a specific brain region. While the electric field strength is easy to interpret, the effect of the field direction on how neurons are affected is less understood. In a vacuum, certainly a polarizable membrane's orientation relative to an electric field would matter, and indeed this is the case as found in *in vitro* studies, where a single cell's morphology and orientation change how a field polarizes it. However, the effects of cell morphology and type seem to disappear *in vivo,* likely because all neurons are highly interconnected. However, orientation may still matter *in vivo,* since smaller cell effects may be cause by downstream effects of larger pyramidal cells. We set out to interpret the results of single

neuron *in vivo* recordings during stimulation in monkeys and compare them with the simulations in order to determine the effect of the electric field direction on the ability of tACS to modify the neurons' rhythmic spiking activity. We hypothesized that the electric field direction would be a significant factor in the efficacy of tACS. From the data we have, we found that the electric field direction indeed was a significant factor in the ability of tACS to cause changes in oscillatory behavior *in vivo*. Additionally, we sought to model the relationship between all the factors at play, namely the electric field's angle and strength, and the baseline rhythmic activity in the frequency of the stimulation. However, we were not able to draw any conclusions about this complex relationship with the data we have. More work will need to be done to fully understand the role of the electric field angle in the targeting of tACS. These results provide some insight for tACS researchers, namely the use of *in vivo* recordings to confirm that electric field angle should align with a neuron in order to maximize efficacy. Thus, tACS researchers should consider field orientation when planning studies.

2. French abstract

L’activité cérébrale humaine est hautement spécialisée en fonction des régions cérébrales, et, dans la plupart d’entre elles, son fonctionnement correct nécessite une activité oscillatoire très finement régulée. La perturbation de cette rythmicité par rapport à son état de base peut avoir des conséquences importantes, comme dans le cas de nombreuses maladies neurologiques. Néanmoins, modifier ces oscillations de manière souhaitable pourrait améliorer certaines fonctions cognitives. En effet, les chercheurs et cliniciens pourraient améliorer des fonctions cognitives ou corriger des déficits de fonction cérébrale chez les patients souffrant de certaines maladies neurologiques en stimulant des zones cérébrales spécifiques d'une fréquence particulière. À cette fin, nous souhaitons développer la stimulation transcrânienne à courant alternatif (tACS), à titre de méthode non invasive efficace et précise pour stimuler rythmiquement les zones cérébrales. Cette technique consiste à délivrer un courant alternatif à faible niveau au moyen de deux électrodes placées sur le cuir chevelu. Ceci crée un champ électrique qui influence les propriétés électriques des neurones dans le cerveau. Afin de cibler la stimulation avec précision, il nous est essentiel de simuler le champ électrique résultant dans le cerveau du patient-même. Pour ce faire, nous créons d’abord des modèles informatiques 3D à partir de scans IRM du patient. Ensuite, nous exécutons ces modèles à travers un logiciel de simulation pour obtenir très bonne estimation du champ électrique dans le cerveau durant la stimulation. Tout ceci permet de maximiser le champ électrique ciblant une région spécifique du cerveau. Bien que l’intensité du champ soit facile à comprendre et interpréter, ceci n'est pas le cas pour l’effet de la direction de ce champ a sur le fonctionnement des neurones. Dans le vide, il est certain que l’orientation d’une membrane polarisable par rapport à un champ électrique soit importante, et ceci en est effectivement le cas. En effet, dans les études *in vitro*, la morphologie et l’orientation d’une seule cellule modifient la façon dont un champ la polarise. Néanmoins, ces effets de morphologie et type cellulaire semblent disparaître *in vivo*, probablement à travers la forte interconnexion des neurones. Mais l'orientation pourrait toujours avoir de l'importance *in vivo*, puisque les effets des plus petites cellules peuvent être conséquence des plus grandes cellules pyramidales. Nous avons donc entrepris d'interpréter les résultats d'enregistrements de neurones unitaires *in vivo* lors d'une stimulation chez le singe et de les comparer avec nos simulations pour déterminer l'effet spécifique de la direction du champ électrique sur la capacité de la tACS à modifier l'activité rythmique de décharge neuronale. Nous avons émis l'hypothèse que la direction du champ électrique serait un facteur important dans l'efficacité de la tACS. À partir des données à notre disposition, nous avons constaté que cette direction était effectivement un facteur important dans la capacité de la tACS à provoquer des changements dans le comportement oscillatoire *in vivo*. De plus, nous avons cherché à modéliser la relation entre tous les facteurs en jeu, à savoir l'angle et la force du champ électrique, et l'activité rythmique de base de la fréquence de stimulation. Cependant, nous n'avons pas pu tirer des conclusions concernant cette relation complexe à travers les données dont nous disposons. Ainsi, des recherches supplémentaires devront être effectuées pour élucider le rôle de l'angle du champ électrique dans le ciblage de la tACS. Cependant, nos résultats fournissent un aperçu aux chercheurs en tACS, notamment l'utilisation d'enregistrements *in vivo* pour confirmer que l'efficacité de l'angle du champ électrique est maximale quand il s'aligne avec le neurone stimulé. Ainsi, l'orientation du champ devrait être prise en compte lors de la planification des études portant sur la tACS.

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4. <u>Contribution of authors</u>

This thesis was written entirely by Tudor Sintu.

Dr. Matthew Krause and Dr. Pedro Vieira collected the data used in this project analysis and performed some primary data analysis.

5. List of Figures

6. List of Abbreviations

- CSF: Cerebrospinal fluid
- CT: Computed tomography
- DBS: Deep brain stimulation
- FEM: Finite element modelling
- MRI: Magnetic resonance imaging
- PLV: Phase locking value
- PPC: Pairwise phase consistency
- tACS: Transcranial alternating current stimulation
- tDCS: Transcranial direct current stimulation
- tES: Transcranial electrical stimulation
- TMS: Transcranial magnetic stimulation
- rTMS: Repetitive transcranial magnetic stimulation

7. Introduction and Objective of research

Introduction

In many subfields of neuroscience, the general principle of research is simple: what makes neural circuits act the way they do, and how can we modify them to suit our purposes? This can be enhancement of cognitive traits or treatment of neurological conditions, but either way they require modifying the brain's activity. To this end, researchers and clinicians are looking at using brain stimulation: application of electric currents to disrupt abnormal patterns of neurological activity or enhance desirable ones. Different brain stimulation techniques are already being used for various conditions: deep brain stimulating pacemakers are used in many Parkinson's disease patients to alleviate motor dysfunction (Lozano et al., 2019), and transcranial magnetic stimulation is being used as a treatment for drug-resistant major depressive disorder (Perera et al., 2016). One such technique, transcranial alternating current stimulation (tACS), is a focus of new development. It passes alternating current through electrodes on the scalp in order to target brain activity at specific frequencies in the brain area being targeted. It is a useful technique because it can alter baseline rhythmicity in certain brain areas, which can be important when dealing with specific brain functions that rely on that rhythmic activity, especially when those rhythms are disturbed by some condition (Krause et al., 2019). Additionally, since the only required equipment is surface electrodes, it requires no surgery to perform and can be done with a relatively simple and inexpensive setup when compared with other brain stimulation techniques.

In order to target specific brain regions for stimulation, simulations of the electric field must be performed. These are done by taking an MRI of the patient, from which a 3D computerized model is produced. Then, software is used to optimize electrode position to maximize the electric field at the target location (Datta et al., 2016). The main obstacle in the way of being able to use this technique to target whatever brain area we like effectively is that the process of optimizing the position of electrodes to target the desired areas while minimizing off target effects is not very well understood. There are certain factors, namely the direction of the electric field at the target region, which are known to affect the outcome, but are not fully understood and are therefore not properly accounted for in simulations (Saturnino et al., 2021).

Objective of research

The principal issue I wanted to explore with this research is the lack of understanding of the effect of field direction on entrainment of neurons to tACS. While it is generally assumed that the field direction matters, there is no mathematical description of it backed by real neural data. Furthermore, there is a lack of *in vivo* data used to verify the assumptions regarding this subject, which I believe would lend more credibility to the field in general.

8. Literature review

Brain stimulation

Transcranial electrical stimulation (tES) is a family of brain stimulation techniques that involve passing weak electric currents through electrodes on the scalp in service of modulating brain activity. This is in contrast with other electrical stimulation techniques such as deep brain stimulation (DBS) in that it is non-invasive, requiring no surgical intervention to perform. The electrodes on the scalp create electric fields within the brain that modify neurons' electrical activity. The parameters of the stimulation, including the electrode positions, the duration of stimulation, and the strength of the current all play a role in determining the effect the stimulation has on the subject (Krause et al., 2019).

The simplest of these methods is transcranial direct current stimulation (tDCS), in which a constant current is passed from one electrode to another. This technique elicits subthreshold effects, meaning it does not directly induce firing in neurons, but rather modifies their excitability. When neurons are more or less likely to fire, their patterns of firing are altered in the targeted brain regions. It can also use neurons' mechanisms of long-term potentiation and depression to impart longer lasting changes. Through these mechanisms, it has been shown to modulate various cognitive and behavioral metrics, and there is potential for research and clinical use in patients with neurological conditions. (Stagg et al., 2018). However, despite the potential, there is a significant amount of debate regarding the reliability of tDCS to produce consistent neurophysiological changes, though much of the disagreement arises from differences in methodology. (Antal et al., 2015; Horvath et al., 2015).

The other major tES method, transcranial alternating current stimulation (tACS), involves passing alternating current of a specific frequency through the pair of electrodes. By using tACS, researchers can attempt to modulate brain functions that rely on neural oscillations of some particular frequency. More specifically, the goal is to entrain the neurons in a targeted brain region to fire in synchrony with the frequency of stimulation. This technique has been shown to produce neuromodulation in single neurons in the primate brain (Krause et al., 2019), however producing reliable behavioral modifications in human and animal subjects has proven to be difficult. Inconsistencies arise between and within subjects, due to differences in neuroanatomy and ongoing brain activity. Even in the same brain region in the same subject, different neurons are affected differently. A major cause of this variability is that different brain regions have ongoing oscillatory activity at different frequencies and at different strengths. This causes tACS to sometimes decrease entrainment to the frequency of stimulation rather than increase it, when the entrainment at baseline in the region is already high. (Krause et al., 2022). If altered brain oscillations could be corrected through the employment of tACS, then it could be possible to use tACS to treat the symptoms of conditions that involve altered brain oscillations, including schizophrenia, depression, obsessive compulsive disorder, bipolar disorder, and others. No clinical applications have yet been found to be fully effective, though many clinical trials are ongoing to assess the efficacy and safety of its use (Elyamany et al., 2021).

Another method of brain stimulation distinct from tES, though also non-invasive, is transcranial magnetic stimulation (TMS), in which a wire coil is placed on the scalp where it generates a magnetic field. The field induces electric currents in the brain and affects the electrical properties of neurons. Repetitive TMS (rTMS) involves stimulating with many pulses over a period of time with the intention of producing longer-lasting effects in the brain via long-term plasticity, whereas single-pulse TMS only modulates activity during stimulation. Like tES methods, TMS also suffers from a great amount of variability, largely owing to the different kinds of coils and stimulation protocols used for experiments and clinical trials (Klomjai et al., 2015). TMS does have potential for clinical use, as it has already been demonstrated to be effective and safe in treating major depressive disorder in adults who have resisted prior treatment options (Perera et al., 2016).

Deep brain stimulation (DBS) is the most invasive of the brain stimulation techniques, requiring surgical intervention to implant a stimulating pacemaker into a deep brain structure. From that point, the stimulator delivers an adjustable electrical stimulation. However, the mechanisms of action are not well understood. It is known that the frequency and strength of stimulation can change the effects greatly, and it is mostly accepted that the current alters the local field potential in the area to make neurons more or less likely to fire. There are other hypothesized mechanisms of action, though evidence exists both for and against each popular theory. DBS has therapeutic applications in many cases. In patients with movement disorders such as Parkinson's disease and dystonia, DBS is very effective in alleviating motor symptoms such as tremors, rigidity, and dystonic repetitive movements. These are the only approved uses in general clinical practice, though there are other conditions in which it has shown some benefit. It can improve some epileptic patients' conditions, though for the majority of them it does not eliminate seizures completely. It is being explored as a treatment option for other conditions, such as depression and Alzheimer's disease, though with limited positive results. There are issues with using DBS for these conditions, even the ones it is approved for. Since it requires surgery, there is risk of surgical complications. DBS also requires a lot of resources, requiring multiple professionals to make sure the patient is receiving proper treatment. Finally, it commits the patient to an implant that will last them the rest of their life, requiring battery replacements every few years, which involves additional surgical operations (Lozano et al., 2019).

Many neurological conditions could be made easier to treat with the addition of tACS as an accurate, potent non-invasive oscillatory brain stimulation to the roster of potential therapeutic options. One example of such a condition is Parkinson's disease. It is a neurodegenerative disorder, mainly involving significant loss of dopaminergic neurons in the substantia nigra. Features of Parkinson's include major motor dysfunction in the form of rigidity and tremors, and other significant non-motor symptoms, such as olfactory processing issues, disordered sleeping, and impaired cognition. The standard symptom treatment for motor symptoms is L-DOPA, the precursor to dopamine, or other dopamine agonists. Both can have significant side effects, and other dopamine agonists are typically avoided for their association with hallucinations. Long-term use of L-DOPA is also associated with other motor complications, making symptom management even more difficult. Deep brain stimulation in various areas is known to effectively treat motor symptoms in some patients, but due to the requirement of surgical installation of the stimulator, it is usually ignored as a treatment option until later in the course of the disease, when the patient develops complications with L-DOPA. Some trials have shown that earlier treatment with DBS improves outcomes significantly over the current standard of treatment (Kalia & Lang, 2015). It has been shown that DBS acts by altering the oscillatory behavior of brain regions that it stimulates (Kringelbach et al., 2007), the same mechanism by which tACS attempts to alter brain function. Little research has been done to examine the potential of tACS as therapeutic treatment in Parkinson's Disease, limited to reducing the severity of some motor deficits in Parkinson's patients (Teo et al., 2017). However, if it is possible to target the relevant structures that DBS targets through accurate, specific application of tACS, then it should be possible to produce similar results without the need for surgical intervention. While these would likely be lesser effects, due to the distance from the electrodes to the targets, it could supplement early treatment before DBS becomes an option.

<u>Optimizing tES</u>

When delivering tES, it is important to place the electrodes in the most optimal position to stimulate the target areas to maximize the effect, and to minimize off-target stimulation to minimize any undesired effects. This is generally done using computational finite-element model (FEM) simulations of multiple possible sets of electrode positions, known as montages. The FEM model is an individualized model of the subject's head, based on MRI scans. It is comprised of many small tetrahedra, each assigned their own conductivity based on the type of tissue they belong to, determined by properties of the scans. These models are well accepted as producing relatively accurate models of current flow within the head, such that the electric field that the model predicts would occur at some position is accurate to what that electric field actually is during stimulation (Datta et al., 2016).

One possible explanation for some of the variability in how neurons are affected by tES is simple biophysics: for a long time, it has been known that *in vitro* neurons can be stimulated by electric fields. In this preparation, they are preferentially stimulated by electric fields in the direction of the longest neural process. A small deviation in angle of the electric field away from optimal has little effect, but when perpendicular to the preferred direction of stimulation, the electric field has a significantly attenuated effect (Gluckman et al., 1996; Rushton, 1927; Terzuolo & Bullock, 1956). This effect is dependent on cell type and morphology, as cells with long projections in a single direction tend to experience much greater effects of electrical stimulation than neurons with short projections in all directions. This could be a long projecting axon, or a dendrite in the case of excitatory pyramidal cortical neurons (Gluckman et al., 1996; Kabakov et al., 2012; Radman et al., 2009).

It is generally assumed that tES in all its forms causes significant changes in brain activity principally by affecting cortical pyramidal cells, whose apical dendrites are typically aligned upwards toward the local cortical surface. This is because in the cortex, most other neuron types do not have long processes and are believed to experience relatively little effects of tES (Radman et al., 2009; Tran et al., 2022). Thus, most often it is assumed that tES has the strongest effect when the generated electric field in the target area is perpendicular to the local cortical surface (Stagg & Nitsche, 2011). Some methods of optimization of electrode position account for this, but others argue that in certain cases, particularly that of multiple stimulation targets in different brain regions, it is better to optimize purely for field strength in the target areas while ignoring any preferred direction, especially since it is not well defined how the neural effects actually depend on the field direction (Saturnino et al., 2021).

In vivo and *in vitro* contributions

Animal models provide an important framework for allowing us to test methodologies without human experimentation. Most importantly, they allow us to perform neural recordings without disturbing the overall network of connections that makes individual neurons work as they do. There have been many important contributions of *in vitro* studies to the knowledge base for brain stimulation. Brain slice preparations were the first to indicate the significance of the orientation of neurons to the efficacy of brain stimulation (Gluckman et al., 1996; Kabakov et al., 2012; Radman et al., 2009). *In vivo* studies are necessary to measure true outcomes of stimulation, including both real neuronal effects and behavioral outcomes. Animal studies have allowed researchers to assess the entrainment of neurons by tACS, the variability in responses, the behavioral effects, and the responses of other cells (Huang et al., 2021; Krause et al., 2019; Krause et al., 2017; Mishima et al., 2019). Using computational modelling to help interpret *in vivo* data, it should be possible to properly test the conclusions drawn by *in vitro* studies in live animals.

9. Rationale, hypothesis, aims

Rationale for the study

The reason for using tACS over other methods of neuromodulation is that it provides researchers and clinicians with the possibility of modulating rhythmic activity in order to reduce harmful rhythms or enhance helpful ones, something that tDCS is incapable of doing. Though rhythmic TMS is also capable of modulating neural rhythms (Thut et al., 2011), TMS is significantly more expensive and requires a more difficult to use setup, only lending its use to clinical environments, whereas tACS is a cheaper option which could be used by the patient at home. TMS has gained traction recently for its use in treating major depressive disorder, though this requires frequent application in a clinical setting, which may not be feasible for many patients due to scheduling issues or access to a TMS clinic. If tACS could be used instead, it could be administered by the patient at home like any other medication. In some early assessments, there is the possibility of the use of tACS in major depression disorder patients to ameliorate depressive symptoms (Alexander et al., 2019; Haller et al., 2020; Riddle et al., 2020)

The reason we want to use tACS instead of DBS, which has been proven to be effective, is that DBS requires surgical intervention. Avoiding this would make it easier for people to set up delivery of electrical stimulation quickly, and at lower risk to the patient. However, a significant obstacle in this case is that tACS is generally less able to achieve sufficiently powerful electric fields in deep brain structures, making it harder to elucidate the same changes as is possible in surface brain structures.

The ultimate goal for our research in improving methodology of tACS is to eliminate the need for other, more expensive and invasive methods of rhythmic brain stimulation. If it could be used in these conditions such as depression instead of rTMS and Parkinson's instead of DBS, it could be used earlier, at greater convenience to the patient, and at lower cost, which would improve access to therapeutic treatment.

In order to get tACS therapy to the same or a similar level of effectiveness as DBS, it is important to properly optimize the dosage delivered to the target area(s) while minimizing off-target effects. This allows us to reduce possible side effects of neurological stimulation in unrelated areas, as well as minimize discomfort for the patient caused by the electric current. To optimize this delivered dosage of electric field, we must fully understand the variables that cause neurons to be affected differently. In this study, I sought to elucidate the manner in which field direction affects how strongly neurons are entrained by tACS. This would help researchers and clinicians better optimize their electrode positions in computational simulations for effect on neurons rather than just maximum electric field. If we know exactly how the magnitude of effect depends on the electric field direction, this can be better accounted for during montage optimization. Another important goal of mine is to provide a comparison for the simulations using *in vivo* data, something very rarely done to support researchers' hypotheses in this field. It is important to note that these findings would be specific for tACS, and are not generalizable to tDCS or TMS, since the entrainment effect measured is specific to tACS.

Hypothesis

I hypothesized that the electric field direction will have a sizeable effect on how strongly neurons are entrained by tACS *in vivo,* such that for the effect of an electric field to be maximized in a neuron, the electric field should be aligned within a small range of angles around the neuron's preferred direction.

Aims

My goal is to provide a mathematical description or model for the effect of field direction for researchers and clinicians to use to optimize their electrode montages for use in tACS, and to provide *in vivo* data analysis to verify assumptions about field direction not previously properly assessed.

10. Methods, problems encountered and solutions, analysis and interpretation of data

Experimental procedures for animal subject data acquisition

Animal subjects: The acquired data for these experiments were collected from two adult male rhesus macaques (*Macaca mulatta).*

MRI scan acquisition: T1- and T2-weighted MRI scans were acquired of each monkey's head. See the lab's previous work for full details and parameters of MRI scan acquisition (Krause et al., 2019).

Animal preparation: Surgery was performed on each animal in order to attach a titanium head post, which is used to keep the animal's head in a fixed position to look at a screen while experiments are performed. Animals were given time to recover, and then familiarized with the laboratory environment and visual fixation task. A second surgical procedure was done to attach a ceramic chamber with a precise grid of recording electrode positions to the area of the skull through which recording electrodes would be passed. In monkey N, the hippocampus was targeted from above, using deep piercing electrodes. In monkey S_1, visual area V4 was targeted from above and behind, with shallower electrode placement required. Trajectories for these electrodes were planned using neuronavigation software. These recording sites were verified using post-operational CT scans, during which a guide tube was inserted into the recording chamber, which visualizes the trajectory of the guide tube on the scan.

Behavioral Task: An animal's arousal, motivational state, and oculomotor activity are known to strongly affect rhythmic brain activity. In order to maintain a relatively constant state of arousal and motivation, and to minimize eye movements, a simple visual fixation task was employed. The animals were placed in front of a computer screen, and their eyes were tracked with an infrared eye tracker. The monkeys were trained to fixate on a small black target on a grey background. Juice rewards were given whenever they fixated on the target for a brief period of time, roughly 1-2 seconds. Reward distribution delays were randomized from an exponential distribution to avoid entrainment to rewards. Full details on the behavioral task protocol can be found in the lab's previous work (Krause et al., 2019).

Transcranial Electrical Stimulation: Individualized finite-element models were used to optimize stimulation electrode montages in order to maximize electric field strength at the target areas, while avoiding placement near the surgical components. Stimulation strength was limited within ±2 mA in order for the data to be comparable to human experiments and reduce discomfort for the animals, which would also increase the difficulty of data collection. Optimal electrode locations for monkey N were FP1 and O1, and for monkey S_1 were FP1 and O1, and in some cases FP1 and OZ. tACS was applied through the stimulating electrodes using a StarStim8 system. The electrodes were round, and 2 cm in diameter. While animals performed the behavioral tasks, active and sham tACS were applied in 5-minute blocks. Frequencies of tACS applied include 5, 10, 20, and 40 Hz.

Neural Data Collection: Single-neuron data was recorded from the animals. During each recording session, the dura was penetrated with a stainless steel guide tube, through which a 32-channel V-Probe recording electrode was then inserted. Target depth and position on the chamber's grid was measured using the previously described MRI and CT scans. At each channel along the length of the electrode, the raw data is recorded along with the exact depth of each unit.

Finite Element Modelling: Individualized head models were created for each monkey subject. First, aligned T1- and T2-weighted MRI scans were segmented using a combination of automatic and manual techniques into six tissue types: grey matter, white matter, cerebrospinal fluid, skull, skin, and eyes. This process creates an overlay on the MRI scans called a segmentation, which assigns each pixel of the scan a tissue type. This segmentation is then exported as its own image which is used to create the head model.

For monkey N, a finite-element 3D head model was then generated from the segmentation in SimNIBS 3.2.6 using the headreco meshing pipeline (Nielsen et al., 2018). For monkey S_1, a finite-element 3D head model was generated from the segmentation in SimNIBS 4.0 using the charm meshing pipeline (Puonti et al., 2020). These pipelines take the segmentation image and turns the representation from a series of 2D slices into 3D models of each segment, combined to form a single head model. The result for each one was a 3D model comprised of many small tetrahedra, also referred to herein as a mesh, with each tetrahedron labelled as a specific tissue type. **Figure 1** shows the segmentation separated only by gray and white matter, and an approximation of the resulting 3D model. It also shows a full head model for monkey S_2, clipped to see the elements of all the different segments.

<u>Simulation of Transcranial Electrical Stimulation:</u> All simulations were performed using SimNIBS 4.0, which allows the user to place electrodes on the scalp of the finite-element model and simulate the electrical stimulation paradigm used in the monkey experiments, including electrode placement, electrode size and shape, and current strength. This was done for both monkeys using their respective electrode positions during stimulation experiments. The software then employs a finite element solver which, using the electrode parameters and the model's elements, numerically solves the equations governing the current flow through the head model; the solver constructs and solves a linear system involving the electric potentials at the electrodes to determine the electric potential at every element of the model. Then, the electric field is calculated from this result at every tetrahedron in the mesh. This simulation is done for the peak

input current in the tACS paradigm, and the resulting electric field simulation is what tDCS at this current would be. Because the frequency used in tACS is not high, where the electric properties of the tissues would change significantly, this is a reasonable approximation of peak electric field in tACS, and the field scales approximately linearly with the variation in current throughout the tACS paradigm (Saturnino et al., 2019; Thielscher et al., 2015). **Figure 2** shows an example of the result of a simulation of peak electrode stimulation during tACS for monkey N. This is a 3D model representation, and the field direction and magnitude at each point are determined by inputting coordinates to get the result.

Analysis and Interpretation of Data

Neural Data Analysis: The raw data from the electrodes was filtered to extract spiking activity of putative single neurons, which were then verified. The pairwise phase consistency (PPC) is then calculated, which is a measure of the synchronization of the single neuron's spiking specific and the tACS signal during recording. For details on how PPC is calculated, refer to Vinck et al. (2010). This metric has the advantage of being significantly less biased than other measures of neuronal synchronization like the commonly used phase locking value (PLV). In fact, the PPC gives an unbiased estimation of the squared value of the PLV. As such this could be used to compare to other works by taking the square root of the calculated PPC values (Vinck et al., 2010). This metric was calculated during sham and active tACS periods, in order to provide a measure of entrainment to the frequency of stimulation during baseline activity and when tACS is applied. The PPC serves as our primary outcome measurement for tACS specifically, as our desired outcome is either an increase in entrainment to the frequency of tACS or a decrease in such, which is what the PPC measures.

Localization of Neurons on Scans: In order to determine exact neuron positions on the scans, first the post-operative scans were aligned to the pre-operative scans, and fiducial markers were placed along the position of the guide tubes in the scan viewer software 3D Slicer. Then, a straight line was extrapolated downwards into the brain on the scan from the fiducials to draw the trajectory of the recording electrodes as they are pushed through the guide tubes during recording sessions. To locate individual neurons, a distance measuring line was drawn from the gray matter surface inward to the brain, and a point was placed at the end of the line corresponding to the recorded depth of each neuron. **Figure 3** shows an example of this process, where a neuron is found through a guide tube at a depth of 26.35mm.

Determination of Preferred Direction: In order to determine the most likely preferred direction of individual neurons found in the gray matter, a manual approach was taken where a vector was created using 3D Slicer from the point of the neuron to the nearest white matter surface, an approach based on the known histology of the cortex, though an estimation since we do not know the exact type of every cell we record from (Rampersad et al., 2019). The difference in angle between the preferred direction and the electric field was then calculated in 3D Slicer.

Analysis of Angle Data: The first avenue of analysis was to determine the correlation between the difference in angle between the preferred direction of the neuron and the direction of the electric field, between 0 and 90 degrees, on the change in PPC from the sham period to the tACS period. Another important factor, however, is the value of the baseline PPC itself, since high baseline PPC neurons are known to detrain from the frequency of stimulation when tACS is applied, so their PPC is reduced, whereas low baseline PPC neurons are more likely to entrain to the frequency of stimulation (Krause et al., 2022). To incorporate this into analysis, a polynomial surface was fit in 3 dimensions to the data, with the other independent variable being the baseline PPC.

It is more likely that the strength of tACS is dependent on the cosine of the angle between the field and the neuron's preferred direction, and on the electric field strength itself. As such, the angle difference was replaced in the analysis by the electric field strength times the cosine of the angle, as a measure of what can be changed via moving electrodes and changing current size. This metric measures the strength of the component of the electric field along the neuron's preferred direction. The other independent variable analysed was baseline PPC, and the measured outcome was change in PPC.

11. Results

Field angle analysis: Data was collected from monkeys N and S_1. From monkey N, 121 isolated neurons were recorded, of which 48 were analyzed in this experiment. From monkey S_1, 157 isolated neurons were recorded, of which 61 were analyzed in this experiment. Neurons that were located in the basal ganglia of monkey N were not included due to the inability to determine their preferred direction from the known histology of this area. From monkey S, neurons whose stimulation parameters involved multiple blocks of different stimulating frequencies were not included due to possible effects of varying frequency within a neuron. Some neurons were excluded due to being located in the white matter on the scan. Some other neurons were excluded based on their location on the MRI scans not matching up with the corresponding location in the head model. These two facets of the exclusion criteria are elaborated further in the discussion.

Figure 4 shows the graph of the effect of angle difference between the electric field and the neurons' preferred direction on the change in PPC during tACS. When comparing the angle difference against the change in PPC, there does appear to be an effect of angle on the effectiveness of tACS. It seems that neurons at a low angle experience a wider range of entrainment and detrainment effects. A test of circular-linear correlation between the difference in angle and change in PPC revealed a correlation coefficient of 0.3984 ($p = 3.0543\text{x}10^{-8}$). Thus, the difference in angle on its own has a significant correlation with the ability of tACS to entrain or detrain neurons. However, this data, due to the combination of different sets of data and some baseline properties of the neurons, is difficult to draw conclusions from. Firstly, it is worthwhile to analyze not just the angle difference, but to change that variable to the strength of the electric field in the direction of the neurons' preferred direction, calculated by the strength of the electric field at that point times the cosine of the angle difference. It is easier to work with due to being a linear variable rather than angular, and it represents something more likely to be a direct outcome measure, since this is what we are attempting to maximize when we minimize angle difference. **Figure 5** shows a polynomial regression for how the electric field in the neurons' preferred direction affects the change in PPC. The fit equation is $\Delta PPC = -0.4187x^2 + 0.2635x - 0.0068$, where x is the field strength in the preferred direction. Though the r squared is 0.47 and the fit is significant (p = $1.998\text{x}10^{-15}$, F-test), this model is misleading due to the base entrainment of the neurons affecting whether neurons experience a positive or negative change in entrainment. Neurons at higher baseline entrainment are usually detrained by tACS, and those at low baseline entrainment are usually entrained. Since in the data set all the high base PPC neurons are at high values of electric field in the preferred direction, mostly due to angle, then the model is skewed downward at that high value despite this not necessarily reflecting reality if the data set had been evenly distributed across the range of base PPC values. **Figure 6** shows the influence of baseline entrainment on the change in entrainment caused by tACS, confirming that this data set follows the same pattern as previous findings, namely that low base entrainment neurons are entrained by tACS and high base entrainment neurons are detrained by tACS ($R^2 = 0.7593$, p = 0, F-test). Note that there are a number of neurons lying along the line y = -x, these are neurons whose PPC during stimulation was 0, but whose baseline PPC was greater than 0. The fit equation is $\Delta PPC = -0.9755x + 0.053$ where x is the baseline entrainment of the neuron. Based on these results, I will instead analyze the effect of the field strength in the preferred direction on the magnitude of the effect, to avoid biasing model results based on negative and positive changes. **Figure 7** shows this relationship. The fit question is $|\Delta PPC| = 0.2457x^3 - 0.1831x^2 + 0.0656x + 0.0412$ where x is the field strength in the preferred direction. This model fits significantly with an R^2 of 0.3265 (p = $4.65\text{x}10^{-9}$, F-test). It is reasonable to expect that the field strength in the preferred direction accounts for roughly a third of the variance in effect size, due to the significance of the other potential factors like baseline entrainment, and the relatively random nature of the brain's activity. Additionally, this model fits the assumption of a factor with which the magnitude of the effect of tACS increases monotonically. **Figure 8** shows the relationships between the E field magnitude and the cosine of the angle difference each on their own with the magnitude of the effect of tACS. Both factors show a decrease in effectiveness in the middle, which, based on the assumption of factors that increase the effectiveness of tACS monotonically, does not make sense. Since these models break the assumption, it can be said that these factors on their own are not predictive of the effectiveness of tACS. It is important now to see how the baseline entrainment and electric field strength in the preferred direction interact with each other. The fact that the base entrainment restricts the analysis for the electric field strength and direction to only the magnitude of the effect of tACS highlights the necessity of using 3D analysis, as the baseline entrainment cannot be disentangled from the data.

A model was then fit to the data in 3 dimensions by polynomial regression, with independent variables being the base PPC and the electric field strength in the preferred direction. This model's equation is $\Delta PPC = 0.054 - 0.0037x - 0.9684y$ where x is the field strength in the preferred direction and y is the baseline entrainment. When fitting a model to the 3 dimensional data as shown in **Figure 9**, we generally see the same result at high field strengths in the preferred direction that has been previously described in our lab's results; the higher base entrainment neurons are more likely to be detrained and the low base entrainment neurons either experience no change or are entrained. At low field strength in the preferred direction and low baseline entrainment, neurons are also entrained. With an r-squared value of 0.7593, the model is able to significantly account for a large amount of the variation in the ability of tACS to affect neurons

($p=0$, F-test). However, the model does not significantly outperform the 2 dimensional polynomial model using only the base ppc as an independent variable (F-test).

12. Discussion

Interpretation of results

Previous studies have looked at the relationship between the angle of an electric field and its ability to depolarize a neuron *in vitro*, and these principles have been applied to some modelling papers for optimization of tACS montages, but there is little *in vivo* data used to verify this assumption. As a result, studies that use FEM for optimization of tACS often overlook the significance of electric field angle. In this study, I have demonstrated that the angle does have a significant correlation with the ability of tACS to affect the synchronicity of neurons *in vivo*, and with a correlation coefficient of nearly 0.4, should almost certainly not be ignored in the future for optimization of tACS. The nature of the relationship between angle and the efficacy of tACS has not been elucidated here, and as such more data will have to be collected in order to find exactly how optimization should be conducted, and how much emphasis should be placed on electric field angle. However, this result is significant in that it suggests that the effect of angle is not completely extinguished *in vivo* due to network effects. If this were the case, the connections between various neurons in an area would distribute the effect of tACS over a wide area comprised of neurons at many different orientations, and the effect of tACS would be very similar across the entire local network of neurons, and thus the angle of the neurons themselves would be mostly irrelevant. It follows from this that it is important for tACS researchers to consider field orientation when planning studies and interpreting results.

It is worth noting here that the range of effect of tACS represented in the data is significant enough to drive behavioral changes. Effects of tACS on the PPC range from about 0.15 increase, with one outlier at 0.3, down to 0.4 decrease, representing a complete extinction of the baseline entrainment of the neuron. Changes in PPC of less than ~0.1 have been associated with changes in sensory representations in working memory (Bahmani et al., 2018), behavioral states (Vinck et al., 2015), reward expectancy (van Wingerden et al., 2010), and memory retrieval (Foster et al., 2013). These small but observable changes in spike synchronicity are correlated with significant modulations in behavior in the brain. Because the neurons we recorded from exhibit a similar range or greater of changes in synchronicity, we can thus manipulate endogenous rhythmic activity to produce behavioral changes.

The modelling section of my project did not yield results, yet it is clear that if more data was collected and more work could be done, an accurate model over the whole range of the variables of angle, field strength, and baseline entrainment, could have a significant impact on how tACS is delivered in both research and clinical settings. Such a model could inform how researchers should plan their delivery of tACS. The way tACS electrode montages are optimized currently relies on the electric field strength only, due to the lack of understanding of how field direction affects the outcomes of tACS (Saturnino et al., 2021). While this does have a demonstrated relationship with tACS, that is, a stronger electric field will generally have a stronger entrainment effect, it does not capture the whole picture of how neurons are affected by tACS. A multiple-factor model involving angle as well as field strength could inform the creation of an algorithm for optimizing electrode position to generate the electric field which will most strongly influence neurons in the desired area in a manner specific to tACS.

<u>Problems, solutions, and limitations</u>

<u>Segmentation Difficulties:</u> Automatic segmentation tools are used for human brain scans in order to create 3D head models for the purposes of non-invasive brain stimulation. However, these tools are lacking for non-human primates, as they are based on human brain atlases. Rhesus macaque brain atlases do exist, though the tools have not been expanded in their function

appropriately to accommodate these. Thus, the automatic portion of the segmentation was limited to determining the grey matter surface on the scan, and even this had to be manually edited extensively. The rest of the segmentation was performed manually. Any project which seeks to use a much larger set of non-human primate subjects would require an effective automatic segmentation and meshing software package built to work for non-human primates. As such, my work was limited to data from two monkeys, though in this case the sample size is not an issue since the measured effect is on individual neurons and not any behavioral outcome of the animals themselves.

<u>Neurons Located in White Matter:</u> Some recorded neurons, when placed on the scan, were revealed to be located in the white matter. Since the histology in this area is much more variant than in the grey matter of the cortex, the direction of axons cannot be reasonably estimated, and this data had to be excluded. Some other neurons had to be excluded for similar reasons, namely being too close to, or even located inside, cerebrospinal fluid on the 3D model, due to being close to the grey matter surface and slight inaccuracies in the model. This results in wild inaccuracies in the electric field strength and most likely its direction as well, and these neurons thus had to be excluded from the analysis.

<u>Data set limitations:</u> There is a problem with the data set: one part of the data, from monkey N, has a range of angle differences between 47.2 degrees and 82.5 degrees, and neurons in this set are all at very low baseline PPC, with a max baseline PPC of 0.0535, as they were stimulated at a frequency which the hippocampus does not have high baseline activity in under these conditions (Krause et al., 2022). The other set of data, from monkey S_1, mostly has neurons within the range of angle differences between 14 and 37 degrees, with a few being much higher. The neurons of this set are of a much wider range of baseline entrainments, ranging from 0 to 0.42. This leaves an

empty space in the range of independent variables, namely there are no neurons with high baseline entrainment and a high angle between the field and preferred direction. As such, it is impossible with this current data set to get an accurate model over the whole theoretical range of data.

Future directions

If this work is expanded upon in the future, improvements to the methodology could be made to improve the quality of the results. Namely, more data should be collected from the monkeys. I made a mesh for a third monkey, though data for this monkey was not collected in time for this project. If there is a bigger pool of data to be modelled, the results will be more accurate and conclusions could be drawn about the relationship between the field angle and strength, the baseline entrainment, and the change in entrainment in neurons. The range of the independent variables is not expected to change, namely because the angle difference between the field and neuron's preferred direction is limited between 0 and 90, and the field strength is limited by how much current we apply, which is already at the highest it should go for macaque research before the current begins to bother the animals, making research impossible. Also, the range of base PPC of neurons is not expected to go higher than about 0.4. The range of the effect of tACS on entrainment should also not change, and would likely remain as a maximum increase of about 0.1 on average at its most effective, with some higher outliers, and a maximum decrease of about 0.4, a complete extinction of rhythmicity in the strongest baseline entrainment neurons.

Another important improvement would be to automatically determine the preferred direction of each neuron on the scan using a software technique rather than manual placement of the line. Such a technique could more accurately determine the line from the point in the grey matter to the nearest white matter surface. This would remove a source of possible human error.

A possible future experiment would be to analyze within-neuron effects. One analysis would be changing the electrode positions while recording and examining the effect of the changing angle and strength of the field on individual neurons. Another analysis would be on the within-neuron effect of frequency of stimulation. That is, changing just the frequency of stimulation without changing electrode position while recording and seeing if it has any effect on how the entrainment changes with respect to angle. Since the frequencies we stimulate at with tACS, 5-40 Hz, are relatively low compared to high frequencies at which the conductivity of tissues significantly changes (Gabriel et al., 1996), I would hypothesize that there is no difference in the electric field within our range of stimulating frequencies, and also no change in how neurons respond to changes in the field, i.e. all the change in how neurons are entrained can be explained by the difference in baseline PPC with respect to the changing frequency. There were 84 neurons in the data set which were recorded from under different stimulation frequencies, and these could be part of the data set for this experiment. These neurons were not part of the data set included in my analysis.

It is important for simulations of electrical stimulation to be as accurate as possible, so our mesh models of the subjects' heads should be created to produce the most similar results to real stimulation. This is why I want to perform comparisons of different model types in order to see if there are significant differences in the simulations. The half-head model is a model which involves segmenting only the tissue from the top of the head to the bottom of the brain and slightly below. It is a tempting compromise for non-human primate research, considering it requires significantly less manual work on the segmentation. However, due to the significant lack of elements of skin/flesh below the skull in this half-head model, there is a lack of current absorption by lower regions, which may cause a significant change in the resulting simulated electric field strength in the areas of interest. Aligning with previous results (Mantell et al., 2022), I would hypothesize that a full head model would produce significantly different electric fields when simulated than a half head model. I intended to perform this experiment as part of my thesis but did not due to time constraints. The methodology would involve making two different meshes for the same subject, one half-head and one full-head, and then comparing the field strength at either specific identical points of interest on the brain, which would be easy to match up between both models since the brain is fully constructed in both cases, or comparing just the average field strength in grey matter. Another useful comparison would be for the difference in angle between the field and preferred direction of the neurons between the two models at locations of neurons which were recorded from.

Another experiment which could highlight the importance of accurate modelling for simulations would be comparison of the field strength and angle at locations of recorded neurons between the normal model and one where the surgical components in the monkey's head have been added. I would hypothesize that the addition of new material to the model would significantly change the result of the simulations. The headpost in particular is made of conductive metal which could have a strong effect on the current flow through the head. If it is significant, it should be added to head models when researchers want accurate results for their simulations when doing monkey experiments with these surgical components.

One last experiment we theorized but did not carry out would involve creating 3D models of a variety of human heads, sourced from the Human Connectome Project. This would give us access to a large data set of healthy adult head MRI scans. With these models, we would put electrode montages in identical relative positions. That is, using software to assign standardized 10-10 electrode placement and stimulating all the heads with electrodes in the same position, then measuring the electric field strength and angle relative to the curvature of the grey matter in multiple locations we would assign as dummy neuron locations, corresponding to areas we might desire to stimulate in patients. From this, we could see how much variation there is in field strength and angle just due to the different head shapes and sizes. It has been shown that inter-individual variability is linked to variability in the outcome of tACS (Kasten et al., 2019). It is possible that there is such an effect of angle as well, or perhaps that the two might be related, such that the variability in the outcome of tACS is linked to the strength of the field in the preferred direction of the neurons in the relevant area. This project would show simply how much variability there is, and similar work with scans of human subjects could link the importance of variability of angle to the outcome of tACS just as the previously mentioned paper does for variability of field strength.

13. Conclusion

The conclusion to be drawn from this data is that electric field angle does play a significant effect on the magnitude of the effect of tACS, though there are other factors of the electric field and neurons that affect the change in entrainment by tACS, particularly the strength of the electric field and the baseline entrainment to the frequency of stimulation. The effect that electric field angle plays is most likely a result of the fact that neurons are more strongly affected by tACS when it produces stronger electric field components in the neurons' preferred directions. More data will need to be collected to see the real effect of the angle between the electric field and neuron's preferred direction and the efficacy of tACS. What can be concluded at the moment is that the angle of the electric field relative to the neurons does have a significant effect on the ability of tACS to entrain or detrain neurons *in vivo*, and as such researchers and clinicians should look to account for this by minimizing this angle difference when optimizing tACS delivery in order to achieve the best possible results.

14. Figures

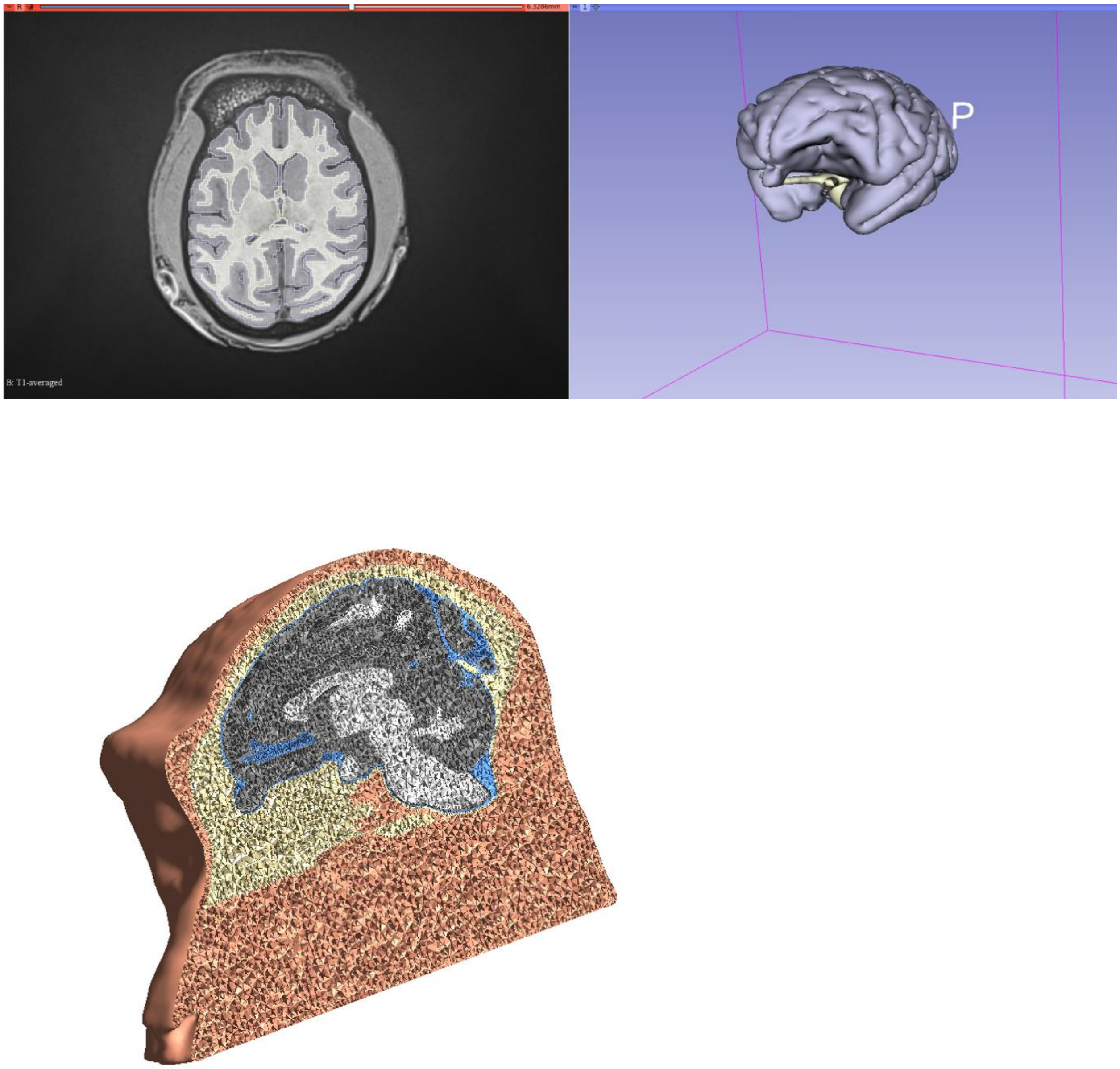

Figure 1. Processing MRI data to create 3D finite element models. Top: An MRI scan of one monkey is displayed in the 3D Slicer software with segments of the grey and white matter on the left, with an approximate rendering of the resulting 3D model on the right. Bottom: The 3D finite element model produced for one of the monkeys, clipped in half to show the different segments: skin, bone, CSF, grey and white matter, eyes (not shown).

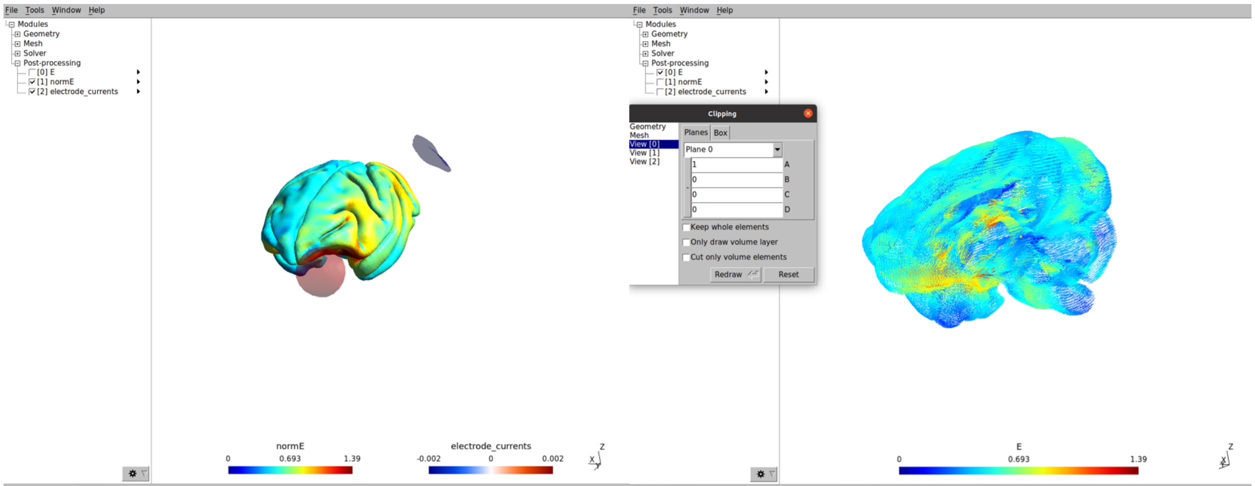

Figure 2. 3D model representation of a simulation of tACS at peak amplitude. SimNIBS software is used to simulate the electric fields at every element in the 3D finite element model. Left: the magnitude of the electric field on the grey matter surface is shown with electrodes placed on the scalp (not visible). Right: Visualization of electric field with strength and direction displayed.

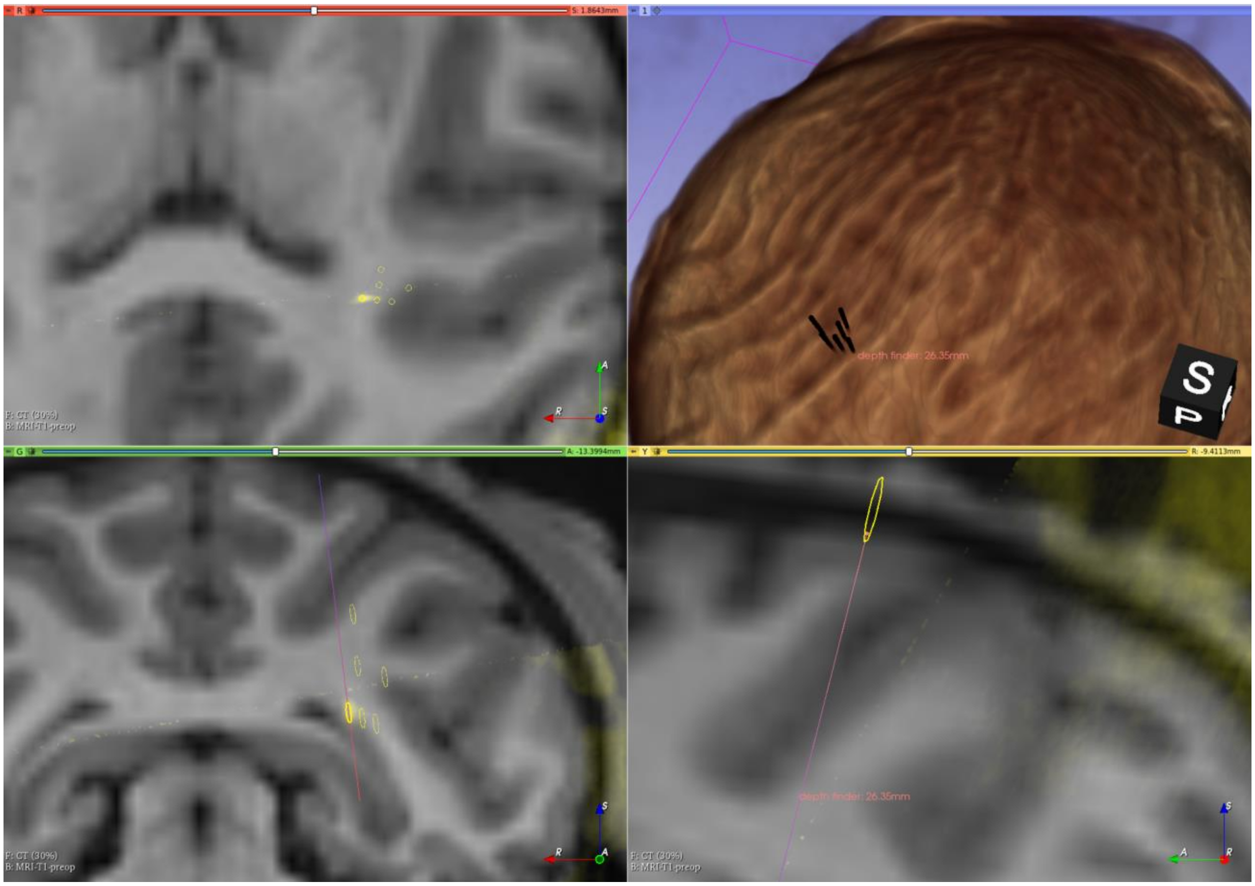

Figure 3. Locating individual neurons on an MRI. The top-right window is a 3D visualization of the monkey's head with modelled guide tubes displayed. The other windows show three views of the MRI scan with the modelled guide tubes in their grid positions. A line from the grey matter surface extends downward 26.35mm in the direction of a guide tube to locate a neuron at that depth in that grid position on the scan.

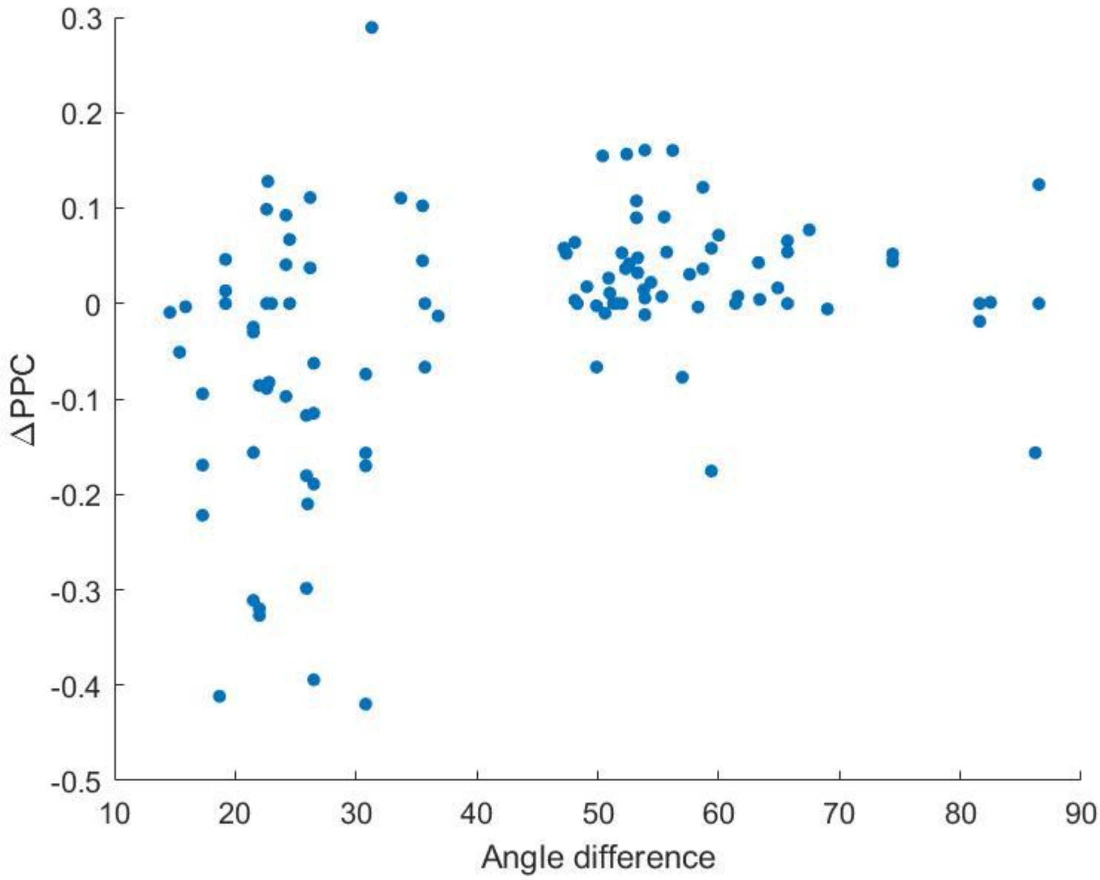

Figure 4. Scatter plot of isolated neurons' change in entrainment with respect to the difference in angle between their preferred direction and the electric field at their position. A wider range of entrainment and detrainment values can be observed at lower angle differences, indicating that neurons are preferentially affected by fields along their longest axis.

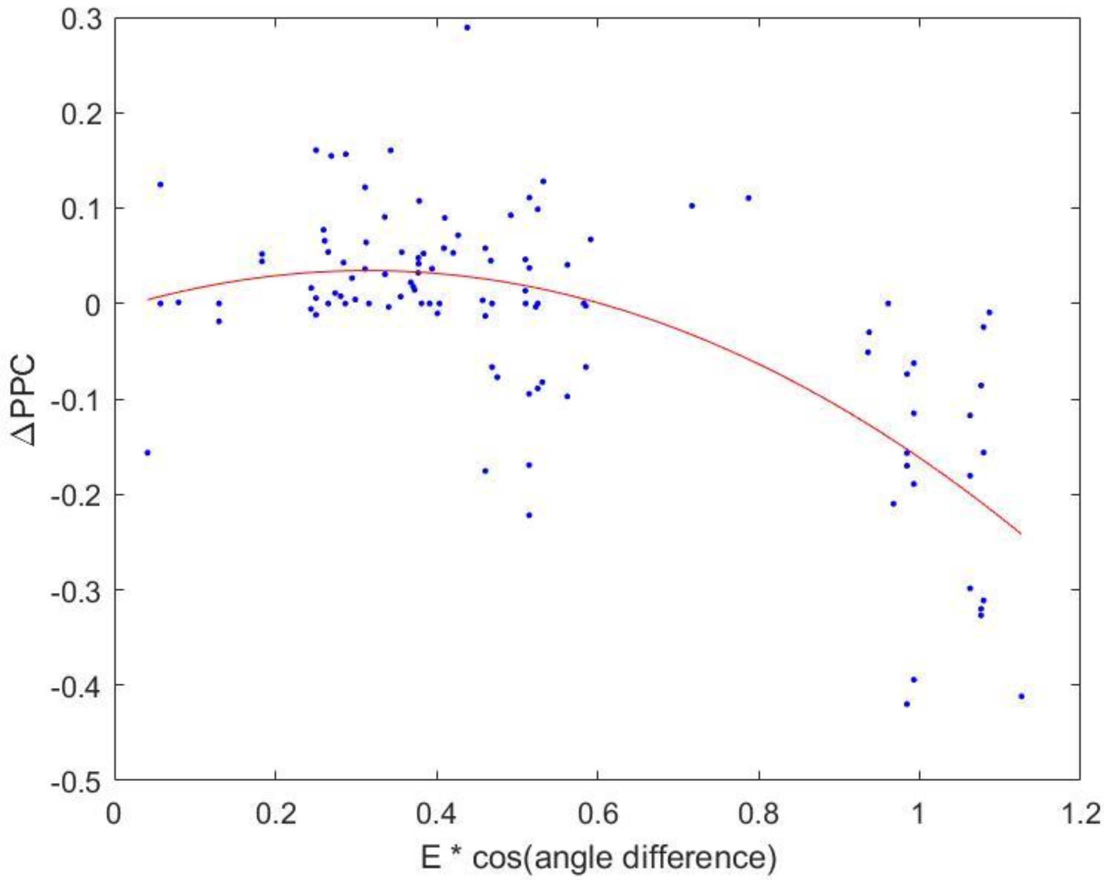

Figure 5. Polynomial regression of the effect of field strength in the neuron's preferred direction on the change in PPC. (R^2 = 0.47, p = 1.9984×10^{-15}, F-test)

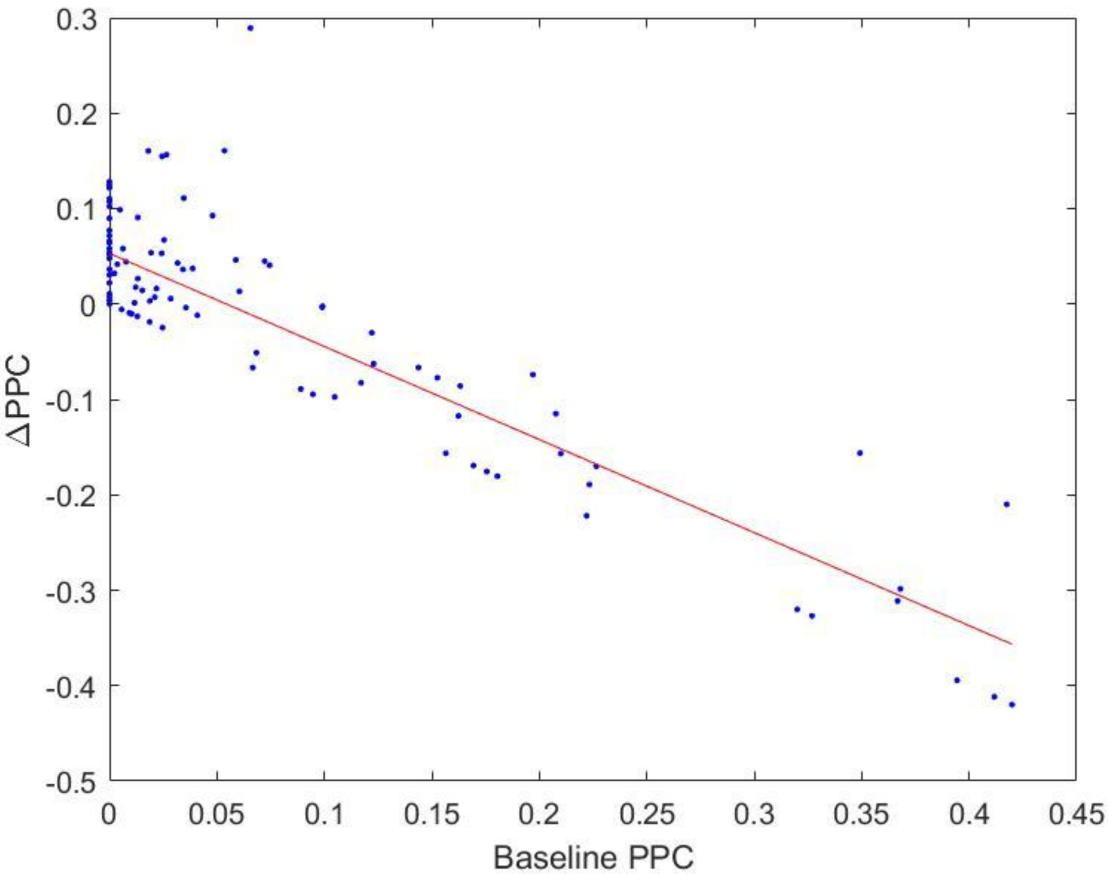

Figure 6. Polynomial regression of the effect of baseline entrainment on the change in PPC.

($R^2 = 0.7593$, $p = 0$, F-test).

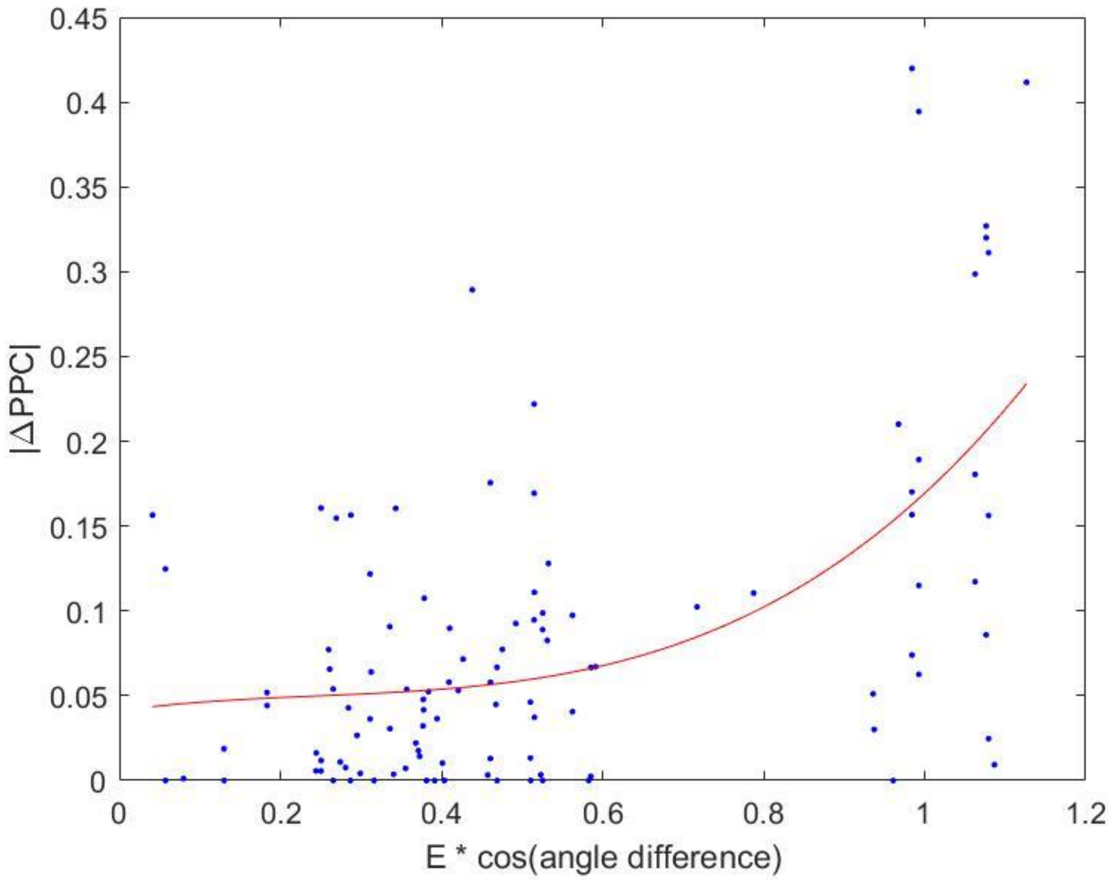

Figure 7. Polynomial regression of the effect of field strength in the neuron's preferred direction on the magnitude of the change in PPC. ($R^2 = 0.3265$, $p = 4.65e\text{-}9$, F-test)

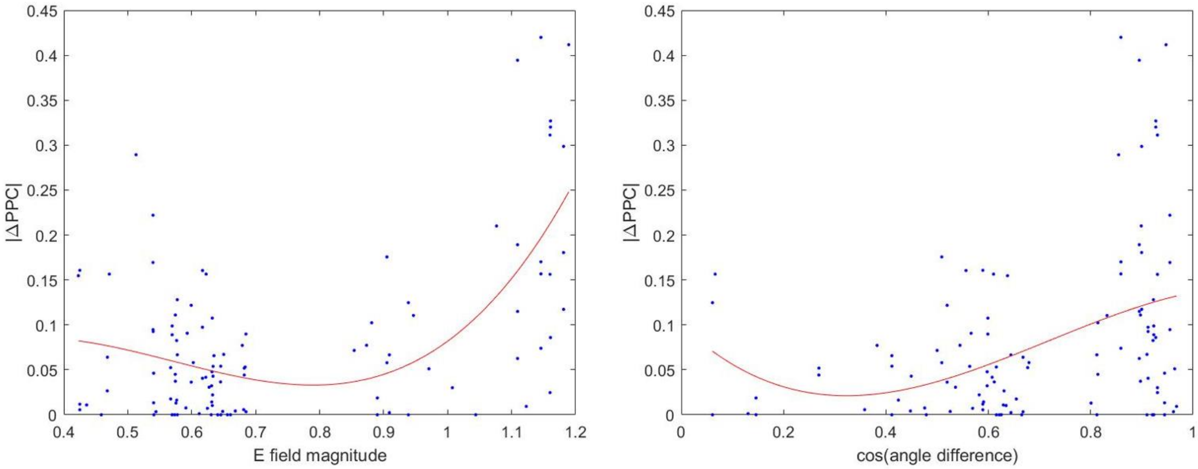

Figure 8. Polynomial regressions of the effect of electric field properties on the magnitude of the change in PPC. Left: the effect of electric field magnitude ($R^2 = 0.38$, $p = 5.82x10^{-11}$, F-test). Right: the effect of the angle ($R^2 = 0.16$, $p = 3.41x10^{-4}$, F-test).

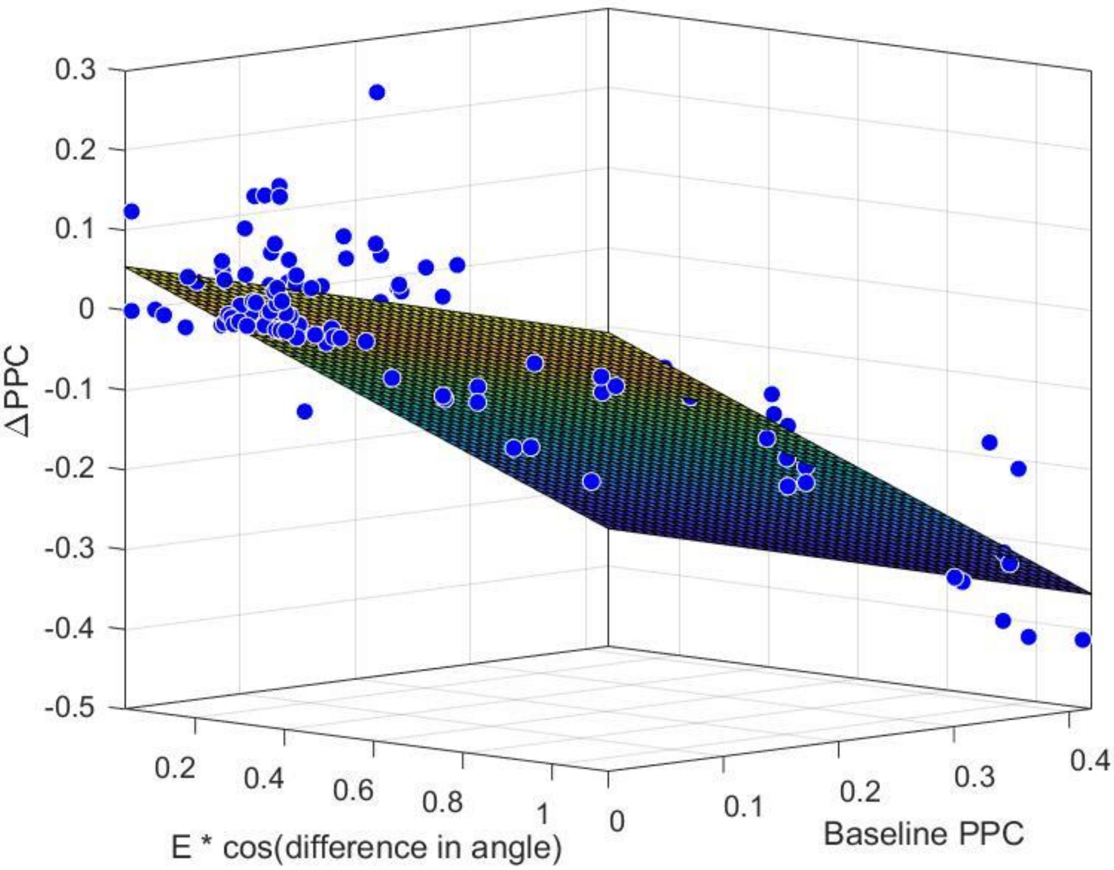

Figure 9. Polynomial regression of the effect of baseline PPC and electric field strength in the neuron's preferred direction on the change in PPC. (R^2 = 0.7593, p = 0, F-test)

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