

Probing the Dorsolateral Prefrontal Cortex and Central Executive Network
for Improving Neuromodulation in Depression

Study Protocol and Statistical Analysis Plan

NCT05224063

July 2, 2021

Overview. A computer engineer's effectiveness is only as good as their ability to *observe*, *probe*, and *modulate* a circuit. Neuroimaging techniques can *observe* a human brain circuit, but without the ability to *probe* or *modulate*, our understanding of *causality*, *directionality*, and *malleability*, and their role in complex behavior and disease is severely limited. In recent years, the PIs have developed two different methodologies to causally *probe* human brain networks. One tool measures a circuit from inside the brain with high spatial and temporal resolution but is only attainable in a specific neurological population. The other tool is non-invasive but suffers from off-target effects, rendering interpretation difficult. This research combines modalities to produce a novel technique to *observe*, *probe*, and *modulate* human brain circuits with a clear biological basis and high spatial and temporal resolution. Specifically, this research will define and model the functional neural properties of the central executive network (CEN), a circuit critical for decision making and implicated in many psychiatric disorders. A greater understanding will enhance our ability to modulate the CEN, the target of FDA-approved brain stimulation treatments like transcranial magnetic stimulation (TMS). The proposed research involves a series of experiments to be performed at Stanford University and Aix-Marseille University, building on a data sharing platform and stimulation methodologies developed at these two sites. The project consists of three clear steps: (1) *Investigate the excitability, connectivity, and neuronal properties of each subregion within the CEN using intracerebral measures*, (2) *Identify the components of non-invasive TMS responses in the CEN that relate to intracerebral measures, and utilize these biologically-grounded TMS responses to study the CEN and its abnormalities in depression*, and (3) *To identify inter-individual variability of the CEN and test if localization at the individual level can more effectively modulate this circuit*.

Intellectual Merit. This novel approach first utilizes a large database of brain stimulation recordings to map the circuit of interest at an unparalleled level of detail, develop the transfer function to validate non-invasive measures of connectivity, and apply these insights to improve targeted stimulation treatments. One scientific outcome is to produce the **most precise, electrophysiologically-grounded, causal, functional parcellation maps of the human CEN to date**. These maps will quantify the *directionality, causality, and neuronal properties* (axon delay, excitability and inhibitory time constants) to and from each subunit of the CEN. This level of precision derived from direct brain recordings from >1000 individuals, is unattainable from any single laboratory and requires cross-collaboration data sharing initiatives. These maps will be freely available using an already established robust data sharing infrastructure and will be highly impactful to researchers interested in understanding, modeling, and predicting complex frontal lobe behavior. A second scientific outcome are **novel non-invasive measures of connectivity grounded in neurophysiology**. These measures will be freely available to enable other researchers to *causally probe* brain circuits and study brain-behavior relationships and disease pathophysiology. Future work will apply similar analytics to derive biologically grounded non-invasive measures from each brain circuit.

Broader Impact. This research has a broader impact by testing the implementation of a USA-France collaboration using data sharing infrastructure developed from the European branch of the Human Brain Project. Although data sharing is standard in neuroimaging, it is unfortunately rare among electrophysiologists; thus, there is great potential to move towards large knowledge and data sharing initiatives. This research also has public health relevance as it focuses on developing **more targeted and effective treatments for mental health disorders**. These disorders have a lifetime incidence of nearly 50%. Furthermore, the pandemic has increased those suffering from depressive or anxiety symptoms to over 40%, and preliminary data show a high incidence of post-infection new onset mental health disorders. This research identifies CEN abnormalities in depression and tests a novel method to localize and *modulate* the CEN on an individual level. If successful, this individualized approach would represent a paradigm shift in brain stimulation. Finally, as TMS is now FDA-approved or being investigated for treatment of almost all psychiatric and neurological disorders, this novel approach of circuit dissection and individualized brain stimulation has broad treatment and societal implications.

BACKGROUND AND SIGNIFICANCE

A1. The need for novel treatments for mental health disorders. Nearly 50% of all Americans will suffer from a mental health disorder during our lifetimes¹. Recently, coronavirus has caused a 'mental health pandemic' across the world, with 40% of Americans in 2020 struggling with some symptoms of depression or anxiety². Furthermore, emerging data suggests nearly 20% of coronavirus cases have an associated post-infection new onset of a mental health disorder³. Outside of the pandemic, depression is the leading cause of disability worldwide⁴. Medications are currently ineffective or not tolerated for nearly half of depressed patients (5.3 million patients in the US)⁴, fueling the need for alternative treatments. Non-pharmacologic FDA-approved brain stimulation treatments for depression include electro-convulsive therapy (ECT) and rTMS. These interventions attempt to normalize dysfunctional brain networks associated with depression and other psychiatric disorders⁵. ECT is clinically effective (50-80% response rate)⁶, but requires general anesthesia, has significant stigma and side effects, and cannot be implemented in outpatient clinics. In contrast, rTMS is an outpatient procedure with minimal stigma or side effects. rTMS uses strong focused magnetic fields to induce electrical currents in neural tissue and elicit neuronal firing⁷. When applied repetitively, rTMS can strengthen or weaken brain excitability and if utilized effectively will represent a critical front-line network-based treatment for mental health disorders⁸.

A2. The need for a detailed understanding of the human dIPFC and its connectivity profile within the associated CEN. rTMS is

FDA-approved and moderately effective for depression, obsessive compulsive disorder (OCD), migraines, and smoking cessation, with clinical trials underway for Alzheimer's Disease, epilepsy, and post-traumatic stress disorder (PTSD). The main treatment target of rTMS is the dIPFC⁹, a functionally-defined brain region confined by the middle frontal gyrus (Brodmann's area 9 and 46). The dIPFC critically lies at the nexus between two large-scale brain networks, the *fronto-parietal central executive network* (CEN) and the *salience network* (SN, Fig 1). The CEN plays a critical role in working memory, decision making, and cognitive control through connections between the dIPFC and the posterior parietal cortex (PPC)^{10,11}. Importantly, the CEN is weakly connected in major depressive disorder¹²⁻¹⁴, and cognitive theories propose that this results in top-down regulation of aberrant emotional processing, perpetuating a bias toward negative affect and depressed mood¹⁵. Furthermore, dIPFC rTMS is thought to improve depressive symptoms through normalization of the CEN by increasing local dIPFC excitability and enhancing downstream connectivity^{16,17}. However, evidence of this (and any other) mechanism by which rTMS acts is critically lacking and remission rates for rTMS remain suboptimal (20-40%)¹⁸⁻²⁰. In order to enhance understanding of and develop tools to probe the human CEN, we need to 1) investigate dIPFC excitability and CEN connectivity, 2) delineate the CEN and its abnormalities in depression, and 3) identify inter-individual CEN variability to improve network modulation.

A3. A multi-modal approach to causally probe human brain networks. A typical evaluation of human brain network activity

measures statistical associations between brain regions (e.g., functional MRI, EEG). Critically, it does not directly assess the causal influence of one region on another²¹. In contrast, we have developed methods to probe human brain networks in a 'stimulate-and-measure' manner, providing critical insight about the *causality* and *directional flow of neural information*²²⁻³². Intracranially, single electrical pulses create a cortico-cortical evoked potential (CCEP) that directly reflects causal brain connectivity at high spatiotemporal resolution (Fig 2A). However, CCEPs can only be obtained in epilepsy patients who are candidates for resective surgery. Non-invasively, single TMS pulses yield a slightly noisier but still causal and whole-brain TMS-evoked potential (TEP) that can be measured in any population (Fig 2B). Both methods, when used in combination to draw insights, can thus be critically valuable to dissect large brain networks and optimize treatments across a variety of mental health disorders. Here, for the first time we

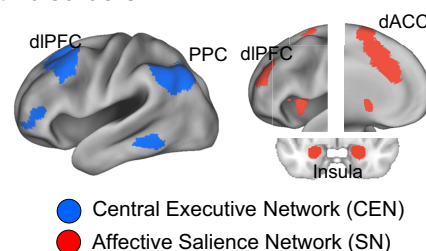


Figure 1 - Large scale brain networks. The dIPFC intersects with the CEN and the SN. The dIPFC and posterior parietal cortex (PPC) make up the CEN; the dIPFC, anterior cingulate, and insula make up the SN.

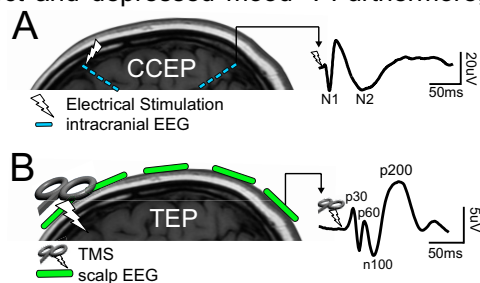


Figure 2 - Causal methods to probe human brain networks. A) Single electrical pulses induce cortico-cortical evoked potentials (CCEPs) measured from intracranial EEG. B) Single TMS pulses induce TMS evoked potentials (TEPs) measured from scalp EEG.

propose to combine CCEPs and TEPs. We will first derive precise, causal mechanistic insights in the CEN using computational modeling of CCEPs. We will then translate these findings non-invasively using multivariate analysis of TEPs, in order to characterize the CEN, identify its abnormalities in depression, and improve methods to modulate the CEN.

A3.1 Intracranial approach: Cortico-cortical evoked potentials. To find cortical areas where seizures start and propagate³³, epilepsy surgery requires intracranial electrode implantation in ~25% of cases, either at the surface or deep inside the brain (stereo-electroencephalography, SEEG)³⁴. The application of extra-operative direct pulses of current between two adjacent electrodes induces an electrophysiological response at other electrodes termed the CCEP^{35,36}. CCEPs are composed of a sharp early deflection (<80ms, termed the 'N1') followed by a slow wave (80-250ms, termed the 'N2') (Fig 2). The amplitude and latency of the N1 at every electrode can be used to estimate neuroanatomical functional pathways to and from the site of stimulation^{26,37} (Fig 3). Since 2013, O. David's team has used CCEPs to develop a functional whole-brain atlas of cortico-cortical connectivity (F-TRACT atlas: f-tract.eu)^{26,37} and study the plasticity of epileptogenic circuits²⁷. C. Keller's team has utilized CCEPs to study the functional brain infrastructure^{22,23,38}, causally examine visual brain networks³⁹, and monitor brain changes during and after repetitive stimulation^{24,40}. The F-TRACT database currently contains raw CCEP datasets from >1200 patients across 25 centers. The atlas, which is iteratively updated in response to incoming patient data or improved processing pipelines, uses several international parcellation schemes with spatial resolution ranging from coarse (e.g. Desikan atlas of FreeSurfer⁴¹), medium (e.g. HCP parcellation⁴²), or fine (e.g. Lausanne atlas⁴³). The atlas currently contains processed information from 60% of patients. This proposal will 1) improve the spatial resolution of the atlas by processing the remaining patients; 2) design new methods to extract CCEP basis functions for each ROI; 3) test the face validity of inferred neuronal parameters from computational modeling and if necessary improve their biological plausibility; and 4) develop specific group analyses to visualize and quantify CEN connectivity.

A3.2 Transcranial approach: TMS evoked potentials. TMS paired with high-density scalp electroencephalography (TMS-EEG) is a non-invasive technique that provides a whole brain causal readout of neural activity with millisecond resolution. Moreover, TMS-EEG can be generalized, standardized, and translated to the clinic. TEPs⁴⁴⁻⁴⁶ are the TMS-EEG response phase-locked to the TMS pulse. Similar to the CCEP, TEPs consist of an early potential (<80ms) that likely reflects increased spiking activity^{7,47} in pyramidal neurons, followed by later potentials (80-250ms) that likely reflect decreased pyramidal neuron activity and increased interneuron activity⁴⁷⁻⁵¹. TEPs have been used to monitor consciousness⁵², study sleep^{46,53}, identify brain signatures of mental disorders^{54,55}, and track brain changes after ECT⁵⁶. To increase the processing speed and reliability of TEPs, C. Keller co-developed and validated the first artifact rejection algorithm³¹ that automatically removes non-neural components (e.g., pulse artifact, muscle

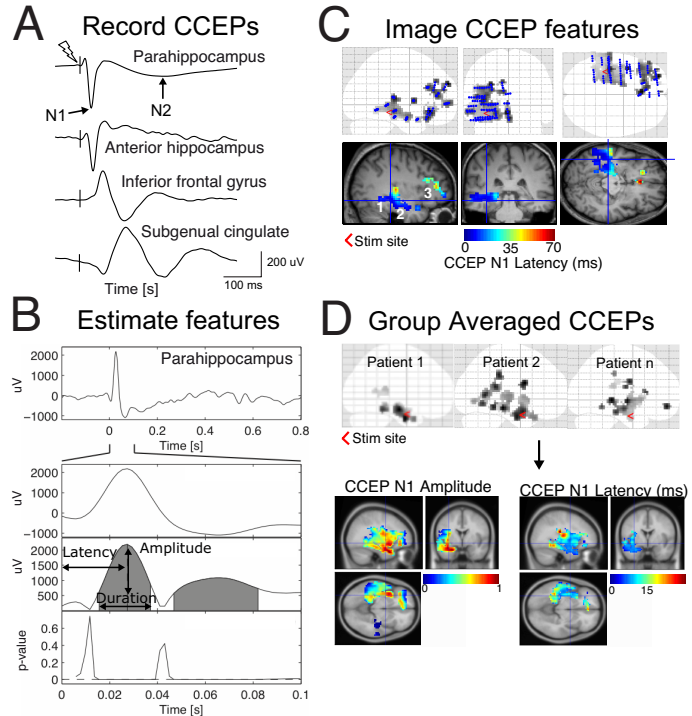


Figure 3 - CCEP group analysis A) CCEPs have an early N1 response reflecting direct connectivity between the stimulation and recording sites, and a late N2 response. B) N1 features include CCEP amplitude, latency, and duration. C) CCEPs propagate and the N1 latency can be mapped. D) When stimulating a specific ROI across multiple subjects, one can use these features to quantify the spatio-temporal connectivity properties of that ROI.

twitches, blinking)^{44–46} from the TEP. C. Keller also demonstrated high test-retest reliability of TEPs across sessions³². O. David's team has robotized TMS-EEG to develop cortical excitability imaging from multisite TEP in single subjects²⁹ (Fig 4). Despite these significant advances in TMS-EEG methodology, results are difficult to interpret because in addition to targeting specific brain networks, TMS induces off-target auditory and somatosensory effects that cannot be fully controlled experimentally^{57–59}. In summary, TMS-EEG is ideally suited for the causal, directional monitoring of brain networks in the clinic, but critically lacks neurophysiological validation.

INNOVATION (EXPECTED IMPACT)

Brain stimulation targets specific brain networks and represents an exciting alternative treatment to medication. However, there has been minimal change in clinical rTMS since its FDA-approval for depression more than 10 years ago. Now with recent approvals for OCD (August 2018), smoking cessation (August 2019), and multiple phase III clinical trials underway for bipolar disorder, substance use disorders, PTSD, Alzheimer's and Parkinson's disease -- many of which stimulate the dlPFC in a non-personalized manner -- without intervening we risk a suboptimal, one-size-fits-all treatment. We have developed intracranial (CCEPs) and transcranial (TEPs) causal brain mapping techniques and expanded these to incorporate automated methods for acquisition²⁹, data-driven algorithms for analysis²³, and open-source data sharing platforms^{26,30}. Now is the time to harness the power of these causal brain mapping and data sharing tools to better understand the properties of large-scale brain networks, refine biologically-validated tools to causally probe these regions, and use these tools to improve focal brain stimulation techniques. Besides its overarching novelty through this multimodal and multiscale translational approach, this proposal has several unique characteristics:

1) The most detailed and direct investigation into the excitability of the dlPFC and connectivity of the CEN. Typical functional parcellations of the human cortex are derived from measures of indirect brain activity (i.e. fMRI), and thus both the causality and the biological basis of these maps are unclear. Here, using data driven analysis and computational modeling from direct electrical recordings with >1 million stimulation recordings across >1200 patients, we will create the most detailed causal parcellation maps of the excitability of the dlPFC and connectivity of the associated CEN to date. Importantly, these maps will be unique in that they are 1) derived from >1M direct brain recordings and 2) causal and directional in nature. Furthermore, based on neural mass computational modeling, they will include information on network 3) excitatory and inhibitory time constants as well as 4) axonal conduction latency and speed. This unparalleled level of neurophysiology within the dlPFC and CEN in humans will lead to important insights from a brain network critical for working memory, decision making, cognitive control, and implicated in myriad psychiatric disorders. This part of the proposal will utilize the international multicenter CCEP database developed by O. David in the F-TRACT project (f-tract.eu), and will be implemented on the newly (2019) created Human Intracerebral Platform (HIP, humanbrainproject.eu/en/medicine/human-intracerebral-eeeg-platform) of the European flagship of the Human Brain Project. This platform is co-led by P. Ryvlin (CHUV, Lausanne, Switzerland, see letter of collaboration) and O. David (co-PI of the proposal) and aims at integrating large multicenter databases and open-source research software solutions for human intracerebral data. HIP strictly follows BIDS-iEEG requirements⁶⁰ for streamlined data sharing in a GDPR compliant manner, the European standard for data privacy protection. HIP is part of EBRAINS, the common platform of HBP, which integrate the development of the multimodal human brain atlas of HBP (led by Dr. Timo Dickscheid, Juelich, Germany, see letter of collaboration), that includes cytoarchitecture, micro and macrostructure measurements, tractography, fMRI, genetics and neurophysiology

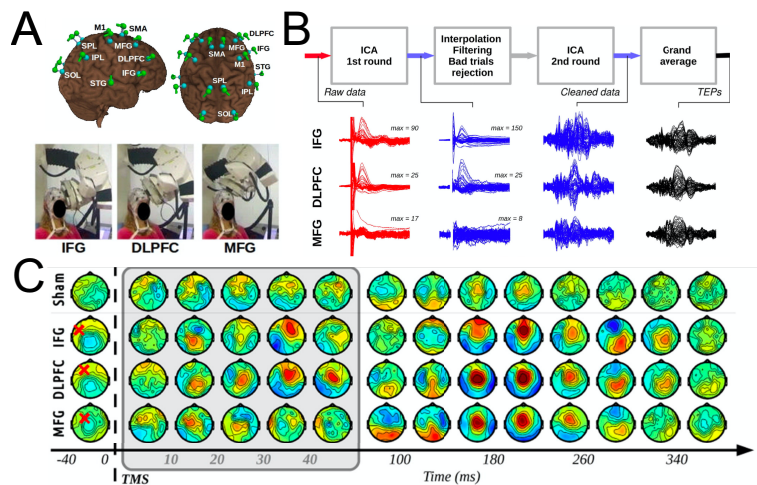


Figure 4 - TMS evoked potentials (TEPs). A) Robotized TMS increases reliability of stimulation targets. B) Pre-processing independent component analysis (ICA) to remove non-neuronal artifacts. C) Scalp topographies of TEPs. The gray area emphasizes the early TEP components.

(ebrains.eu/services/atlasses/brain-atlasses). Thus, while this proposal focuses on CCEPs and TEPs, multimodal data integrated in the HBP framework can be easily merged with our measures to aid in the characterization of the CEN (and eventually other brain networks). This integration across multiple datasets will lead to subsequent future lines of research. The current small-size project will thus not only focus on the functional details of CEN anatomy, which is novel, but will also provide an opportunity to test in real conditions the implementation of a USA-France collaboration using infrastructure from the large European brain initiative, which is eager to further develop interactions between international large brain initiatives. There is thus great potential to move towards large knowledge and data sharing initiatives.

2) The development of novel connectivity measures grounded in neurophysiology. Non-invasive connectivity measures are limited by the inability to separate signal from noise, leading to biological ambiguity and skepticism of findings that severely hinders the field of human neuroscience⁵⁷. By using a multivariate constrained ordination technique (CCA), we will map direct measurements from intracranial methodology onto non-invasive measurements and define for the first time non-invasive causal connectivity measurements grounded in neurophysiology. Using these biologically-grounded TEP measures, we then can further delineate the dlPFC excitability and CEN connectivity in healthy subjects and identify abnormalities in depression. Future work includes expanding our project to integrate a user-friendly, automated toolbox that outputs the CCEP-derived biologically-grounded TEP features for a given brain network (CCEP manager, see Methods). These TEP features will be useful for a variety of applications, including studying cortical information flow within and between brain networks in physiological and pathological brain states.

3) A more effective method to stimulate human brain networks. Non-invasive brain stimulation is currently applied without a detailed functional understanding of the underlying cortical region (i.e. subregions of the dlPFC that connect to other regions of the CEN) and without consideration of individual differences in the CEN. Doing so should greatly enhance target engagement and lead to increased efficacy of these treatments. Here, we objectively test the target engagement component of this hypothesis, using validated non-invasive methodologies (Aim 2) to more accurately target the portion of the dlPFC connected to the CEN and evaluate the degree of rTMS-induced change in the CEN. This data driven, automated, network-based individualized approach to brain stimulation using tools that can be implemented in the clinic will provide a clear path towards a clinical trial for a novel, more effective treatment for depression. More broadly, this type of work will hopefully lead to a paradigm shift in neuromodulation techniques, from one lacking precise network targeting tools in the clinic and accepting a black box approach to treatment (no monitoring of brain changes for treatment) to one of precision network-based targeting coupled with frequent monitoring of network changes and quantification of brain (i.e. CEN network change) – behavior (i.e. change in clinical symptoms and cognition) relationships, driving algorithmic fine-tuning of treatment parameters to improve clinical outcome.

Critically, the approach taken in this proposal is one that can be generalized to any brain region and disorder. The approach utilizes a large database of direct brain recordings to map a brain network at an unparalleled level of detail, develop the transfer function to yield validated non-invasive connectivity measures, and apply these insights to individually localize the network and improve targeted brain stimulation. In follow-up studies, we plan to translate these ideas to other critical brain regions and associated networks in psychiatry including the *ventrolateral prefrontal cortex and ventral attentional network (VAN)*, involved in attentional control and response inhibition, the *orbitofrontal cortex and frontostriatal network*, involved in fear, pleasure, and motivation, and the *medial prefrontal cortex and default mode network (DMN)*, involved in emotion regulation and negative perceptions of the self.

APPROACH

C1. Aim 1: To investigate the excitability, connectivity, and neuronal properties within the CEN using intracerebral CCEPs.

Rationale and significance: Critically lacking across all large-scale brain networks in human neuroscience is a detailed understanding of their directional and causal intra- and inter-regional connectivity properties. Here, our main goal is to create a ‘functional roadmap’ of the dlPFC and the associated CEN, regions essential in decision making, cognitive control and implicated in myriad psychiatric disorders. Specifically, we will advance knowledge by using computational approaches and biophysical modeling to define causality, directionality, and neuronal properties (axon delay, excitability and inhibitory time constants,

plasticity) of the CEN. We extend our current atlas by 1) developing custom scripts to focus analysis and visualization on the CEN); 2) processing data from ~400 patients, targeting to double the atlas's spatial resolution; and 3) testing the face validity of current neuronal models and expanding the type of models inferred from computational modeling. This work will result in the most precise electrophysiologically-grounded causal functional maps of the human dIPFC and CEN to date.

Preliminary Data: CCEP feature maps processed from 321 patients were published in 2019: f-tract.eu/f-tract_v1910_Lausanne2008. There, each region of interest (ROI) is labeled with CCEP N1 features (e.g. *peak latency*, *amplitude*, *duration*; see Fig 3) and inferred neural parameters (e.g. axonal conduction delay and velocity). Figure 5 demonstrates the spatial and temporal distribution of CCEP responses following dIPFC stimulation. Though preliminary, dIPFC stimulation induces a notable N1 response in parietal areas (black arrow, Fig 5A), which is moderately fast (60 ms N1 peak latency; Fig 5B). The spatial precision and significance of the atlas will continue to grow with increasing sample size. A version of the atlas from 650 patients is in preparation and an updated version with >1200 patients is expected to be used for this proposal. To define critical neuronal properties of the CEN, we plan to implement neural mass models to explain CCEPs. Using Bayesian model parameter inversion based on the Dynamic Causal Modeling formalism⁶¹, we will derive cortical excitatory and inhibitory properties and axonal propagation delay (Fig 6). Very preliminary data, using unrealistic but computationally manageable connectivity profiles for the whole brain, suggest some variability in the estimated neural parameters within the dIPFC (Fig 6C). We plan to greatly refine this computational

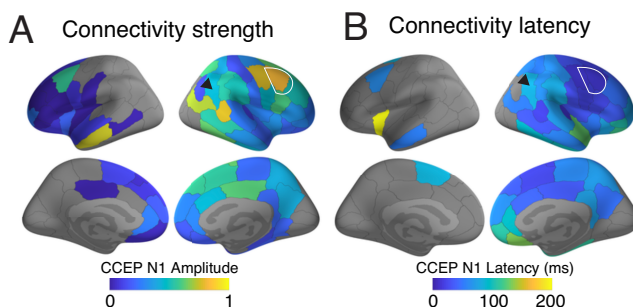


Figure 5 – CCEP maps after single pulse stimulation of the dIPFC. A) *Connectivity probability* is the probability of measuring a significant CCEP N1 when stimulating the dIPFC ROI). B) *Connectivity latency* is the time to N1 peak. Note that after stimulation of the dIPFC (outlined in white), parietal regions (black arrow) demonstrate significant (A) CCEP N1 connectivity and (B) relatively low N1 latency.

modeling work by focusing it within the CEN and with more biological realism.

Overview of methods:

The methods outlined in this proposal harness the F-TRACT infrastructure (database and processing pipeline), which is compliant with the BIDS format for facilitating data and code exchange⁶⁰, as part of

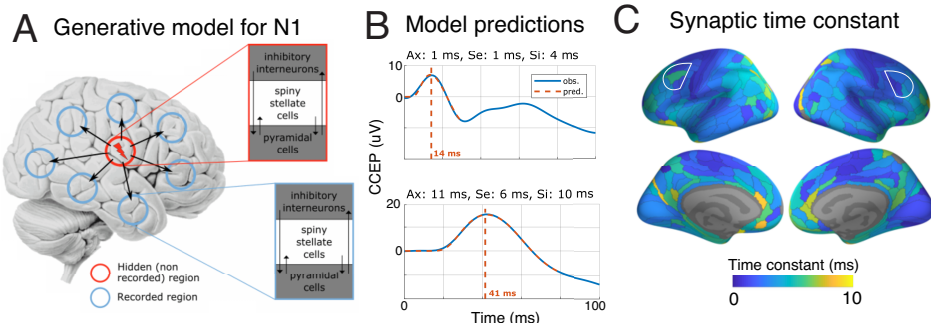


Figure 6 - Estimation of CCEPs neuronal parameters. A) Architecture of a generative model underlying the early N1 component of CCEPs. Each of the stimulation (red circle) and recording (blue circles) sites are modeled with local neural mass models. B) Model predictions (red) of CCEP observations (blue) for increasing N1 peak latencies (vertical line). Estimated axonal conduction delays (Ax), excitatory (Se) and inhibitory (Si) synaptic time constants are indicated on top of each panel. C) Excitatory synaptic time constant mapped on the HCP parcellation for 321 patients.

our platform for human intracerebral data within the Human Brain Project. To this end, we are developing a BIDS app called 'CCEP manager' (see Methods) that handles processing pipelines (based on open source software from the international community, e.g. SPM, Brainstorm, Fieldtrip, MNE). We will first aggregate all patients that received stimulation within the CEN and will quantify the dIPFC-dIPFC local excitability and the dIPFC-parietal inter-connections between nodes of the CEN. This will be performed using custom SEEG-specific software and scripts⁶² and expanded to focus on the CEN. To explore intra-regional differences of connectivity and neuronal properties within the dIPFC, we will next overlap CCEP maps onto the Lausanne atlas, which segments the dIPFC into ~20 parcels⁴³. Here, for each anatomical ROI, we will quantify the functional properties on a group level.

Aim 1.1: The goal of this subaim is to create detailed parcellation maps for dIPFC *excitability* and directional CEN *connectivity* matrices between each subunit of frontal (dIPFC) and parietal regions within the CEN. To

this end, O. David's team will pre-process data from the remaining 400+ patients and incorporate these CCEP features into the database. The methods to quantify *excitability* and *connectivity* are based on processing of the N1 component of the CCEP. *Excitability* is defined within a region as the strength of the local CCEP N1 (after stimulation of that same or nearby region), whereas *connectivity* is defined between two regions within the CEN and quantified as the strength, latency, and direction of CCEP response at the second region following stimulation of the first region^{26,37}. The steps for generating *excitability* and *connectivity* measures are similar. We will first detect significant N1 responses for each stimulation-recording pair in the CEN (Fig 3). Group excitability and connectivity matrices will then be generated based on group averaging of features measured for each stimulation-recording pair using methods described previously³⁰. These matrices will be mapped onto each ROI within the CEN. Finally, using principal component analysis (PCA), CCEP basis functions will be generated at each CEN ROI for the TEP/CCEP comparison in Aim 2. Potential confounders such as demographics and epilepsy type will be accounted for with selection of subject parameters (see Potential pitfalls).

Aim 1.2: The goal of this subaim is to identify the *neuronal properties* of each subunit within the CEN. Specifically, neural parameters including *propagation latency* and *excitatory and inhibitory time constants* will be inferred from neural mass modelling of CCEPs at each ROI. This modeling will be first based on coupled Jansen and Rit units⁶³, whose parameters will be estimated using a Bayesian inversion scheme as implemented in Dynamical Causal Modeling (DCM)⁶⁴. Second, to improve biological plausibility, additional models will be tested using either DCM or other methods implemented on The Virtual Brain platform (thevirtualbrain.org). These alternative models will propose regionally specific macrocolumnar organization that will mimic differences of cytoarchitecture between frontal and parietal regions⁶⁵. Because of the size of our database, we will balance the trade-off between model complexity, realism, identifiability, and computational time.

Potential pitfalls: The technological risk level of this task is low as a robust computational infrastructure linked to a large CCEP database exists. The main scientific pitfall is a large inter-individual variability within the dIPFC that would smooth group spatial maps and thus limit spatial resolution. We will thus decompose the database into homogenous demographic subgroups (based on age, gender, hand laterality, epilepsy etiology) and test for the existence of confounding factors. We estimate that inter-subject variability will not significantly confound results as we have observed that splitting our database into groups over and under age 18 produced similar CCEP maps (Lemarechal et al. in preparation). Interestingly, adults had shorter latency N1 connectivity across brain networks, consistent with brain maturation processes⁶⁶, suggesting that within-group variability is lower than between-group variability. Another potential confound is the effect of epilepsy on inferred neural properties that would diminish the translational value of this dataset to other populations. To limit this potential confound, we will quantify (and remove from analysis) brain regions with significant abnormal interictal events (spikes and ripples) using a published algorithm⁶⁷.

C2. To delineate the CEN in healthy subjects and its abnormalities in depression using biologically-grounded TEPs.

Rationale and significance: Non-invasive TEPs are powerful clinic-ready tools that measure whole brain connectivity in a causal and directional manner. However, significant off-target auditory and somatosensory artifacts^{58,59} limit the ability to interpret TEPs as true measures of brain connectivity. In contrast, CCEPs lack auditory and somatosensory artifacts and directly measure brain connectivity in a mechanistically unambiguous or 'biologically-grounded' manner²⁵. The first subaim focuses on deriving causal, non-invasive, TEPs grounded in direct intracranial electrophysiology (CCEPs) that can be used to study brain network physiology and pathophysiology. Here, we will compare mechanistically ambiguous TEPs to biologically-grounded CCEPs using a multivariate machine learning approach. We predict that components of the TEP will relate to invasive CCEPs because 1) CCEPs are positively correlated with other non-invasive brain measures including functional MRI²² and diffusion tensor imaging⁶⁸, and 2) preliminary evidence (Fig 8, 9) and previous studies^{17,24,40} demonstrate that single pulse stimulation of the dIPFC elicits parietal CCEPs invasively as well as TEPs non-invasively, suggesting similarities between stimulation modalities. Developing a set of non-invasive, causal, clinic-ready, and biologically-grounded brain connectivity measurements would provide fundamental knowledge of the *causality, latency, strength, directionality, stability, and variability* of human brain networks.

Aim 1 focuses on the detailed dissection of the CEN using direct brain measurements from >1200 individuals, obtainable only from epilepsy patients. In Aim 2, we will relate these findings to non-invasive TEPs in healthy subjects. We see this as a novel approach and somewhat similar to a previous undertaking wherein functional MRI observations from thousands of individuals were morphed onto an individual brain for subsequent analyses⁶⁹⁻⁷¹, thus increasing signal-to-noise. Here, we will perform a similar morphing procedure to make use of both the direct brain recordings and the large sample size to enhance our understanding of non-invasive connectivity measures.

The second subaim focuses on utilizing these biologically-grounded TEPs to investigate the CEN in healthy subjects and depressed patients. To date, differences in TEPs have been observed in depression⁷², but it is unclear if these differences originate from the CEN or from off-target effects of TMS^{58,59}. Here, we directly test this by performing group analysis using biologically-grounded (as opposed to standard mechanistically ambiguous) TEPs. Identifying TEP abnormalities grounded in neurophysiology will help elucidate the network pathophysiology of depression.

Overview of methods: In Aim 2.1, to derive the biological signal underlying the TEP, using multivariate machine learning methods, C. Keller will increase the specificity of the TEP by relating each component of the TEP to the biologically-grounded CCEP (Fig 7). Specifically, he will compare dIPFC stimulation-induced parietal TEPs in the CEN from healthy subjects to biologically grounded dIPFC stimulation-induced parietal CCEPs in the CEN from O. David's CCEP database. Using biologically-grounded TEPs from Aim 2.1, in Aim 2.2 C. Keller will identify dIPFC excitability and CEN connectivity differences between depressed patients and healthy subjects. In parallel, to identify the structural correlates of electrophysiology measurements, using a small cohort of healthy subjects and robotized TMS, O. David will map the microstructure of the dIPFC at high-field MRI (7T) and identify MRI features that correlate with dIPFC stimulation-induced CCEPs and TEPs in the CEN.

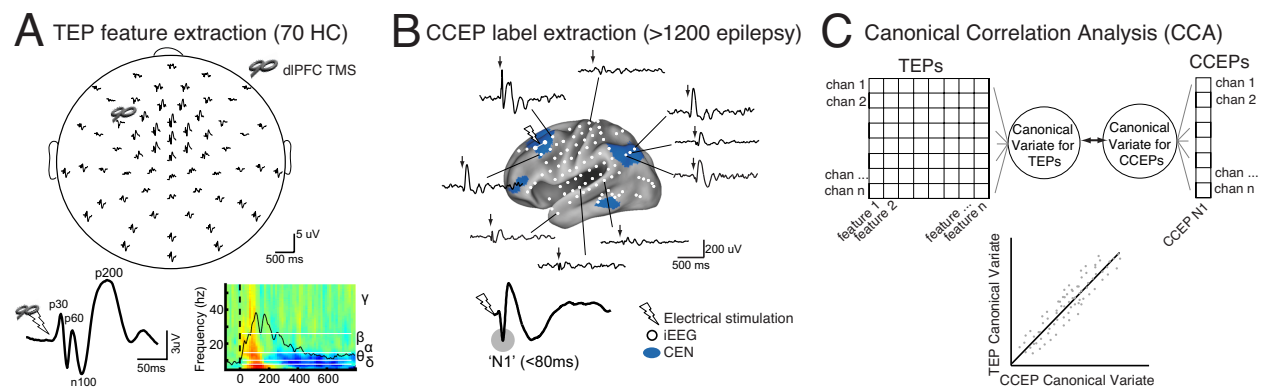


Figure 7 - Overview of Aim 2. A) *TEP feature extraction.* In 70 healthy controls, single pulse TMS applied to the dIPFC induces TEPs measured with scalp EEG recordings. Top: TEPs at each channel. Bottom: For each channel, the standard evoked potential responses (p30/n45/p60/n100/p200) and spectral power changes (δ 1-4Hz, θ 4-8Hz, α 8-12Hz, β 12-25Hz, γ 25-50Hz) will be quantified and used as features to compare to CCEPs. B) *CCEP label extraction.* In >1200 epilepsy patients, single pulse intracranial electrical stimulation applied to the dIPFC yields CCEPs at each intracranial electrode. Bottom: As the early N1 (<80ms) peak represents the most direct measure of cortico-cortical connectivity, the N1 responses in the CEN (blue regions) will be the biologically-grounded 'label' for CEN connectivity. Arrow denotes time of single pulse stimulation. Note the strong CCEP in parietal CEN elicited after dIPFC stimulation. C) *Canonical correlation analysis (CCA).* CCA connects two sets of variables (here, TEP and CCEP maps of brain responses elicited following dIPFC stimulation) by finding canonical latent variates that represent linear combinations of variables that maximally correlate. Adapted from⁶⁹.

Aim 2.1: The goal of this subaim is to identify non-invasive functional correlates of invasive electrophysiology. Specifically, we will increase the specificity of the dIPFC-stimulated TEP by relating each temporal and frequency component of the TEP to biologically-grounded dIPFC-stimulation CCEPs in the CEN. First, O. David will provide CCEP N1 features and basis functions for excitability and connectivity within the CEN (Aim 1). Next, C. Keller will analyze a dataset of 70 healthy subjects already collected in his laboratory that received single TMS pulses to the dIPFC. He will extract the source-level TEPs across the brain and compute standard typical temporal TEP features, including the positive deflection at 30ms 'p30', negative deflection at 45ms 'n45', p60, n100, p200. He will also extract spectral power TEP features (δ 1-4Hz, θ 4-8Hz, α 8-12Hz, β 12-25Hz, γ 25-50Hz). Next, each dIPFC-stimulated TEP feature across the brain in 70 healthy subjects will be compared to the biologically-grounded dIPFC-stimulated CCEP N1 in the

CEN, with the goal of identifying the transfer function between the two methodologies. Our hypothesis is that because temporal features close to the stimulation (<60ms) and higher in frequency (>20Hz) typically reflect more local processes^{73,74}, these features of the TEP will better reflect underlying biology (CCEP N1) in the CEN. To compare these variables, we will apply canonical correlation analysis (CCA), a type of machine learning multivariate constrained ordination technique commonly used to infer information from cross-covariance matrices⁷⁵⁻⁷⁸. CCA describes a 'many-to-many' relationship, which is advantageous over a 'one-to-one' relationship (i.e. Pearson's correlation) or a 'many-to-one' relationship (i.e. ordinary multiple regression) when comparing electrophysiological measurements over multiple channels. CCA connects two sets of variables (here, TEPs and CCEPs elicited following dlPFC stimulation) by finding features (canonical latent variates) that maximally correlate, while using a small number of linear combinations. 70% of subjects will be used to identify the canonical latent variables and the remaining 30% of subjects will be used to test the strength of these latent variables. For each dataset, 10-fold cross validation will be used to determine accuracy, sensitivity, and specificity. A negative CCA control will also be run, comparing non-TEP features to CCEP N1, expecting the non-TEP/CCEP relationship to be at chance level. Non-TEP features in the control analysis will include pre-TMS brain activity (-500ms to -250ms, where no evoked potentials are observed), non-neural spectral changes (>100Hz, where no meaningful neural activity is measured non-invasively), and sham single pulse TMS (where any measured evoked potential should not reflect dlPFC excitability).

In parallel, on a separate small cohort of healthy subjects (n=20), to identify the non-invasive structural correlates of intracranial electrophysiology, O. David will acquire dlPFC microstructure markers acquired at high field MRI (7T) and high spatial resolution (0.5 mm cubic) using multiparametric imaging, with the expertise of the Centre for Magnetic Resonance in Biology and Medicine at Aix-Marseille University (see support letter)^{79,80}. Data processing will include grey/white matter segmentation at high-resolution from T1 images, surface reconstruction, and an equivolume layering process to track local differences in cortical thickness and layer distribution of T1 parameter. Other MR contrasts will be used to add optional discriminative power between ROIs within the dlPFC and between frontal and parietal nodes of the CEN. First, this MRI-based microstructural information will be correlated to CCEP maps in O. David's database to cross-validate neural parameter mapping (excitatory and inhibitory time constants of neural mass models) (Fig 6C, Aim 1). It will be performed by 1) superimposing microstructure maps and CCEP neural parameter maps in a stereotactic reference space (MNI space) to visualize relationships on a large scale, and 2) comparing all multimodal measures for each ROI to precisely quantify within dlPFC changes. Second, in the same subjects, local TEPs responses will be acquired on a neuronavigated and robotized TMS system (Cobot, Axilum Robotics, France) using a grid positioned over the dlPFC centered on CCEP maps for subsequent coregistration of TEP and CCEP features. For each of 20 ROIs within the dlPFC, we will apply single pulse TMS and measure local TEPs. This procedure will allow us to align TEPs and CCEPs. Spectral and temporal components of TEPs will then be processed and correlated to microstructure to assess whether TEPs reflect the functional cytoarchitecture through region specific time-frequency signature of the TEP, as we and others recently proposed⁸¹.

Aim 2.2: After identifying components of the TEP that reflect biologically-grounded CCEPs, C. Keller will use these features to evaluate CEN connectivity in healthy subjects and depressed patients. For each of these neurophysiologically-validated TEP components, C. Keller will compare the single pulse dlPFC-induced parietal TEP in 70 healthy subjects (the same subjects as in Aim 2.1) and 70 depressed patients also collected previously in C. Keller's laboratory. Analysis will be performed in a split-half manner to ensure the robustness of the finding, and FDR correction will be applied for multiple comparisons⁸². In addition to performing contrasts between healthy subjects and depressed patients, we will also relate the severity of baseline depressive symptoms, as measured by the Quick Inventory of Depressive Symptomatology self-report (QIDS-SR)⁸³, to the degree of CEN connectivity as measured by the TEP. Our hypothesis is that because of the positive relationship between depressive symptoms and ischemic events in the dlPFC (leading to decreased neuronal activity)^{84,85}, depressed patients will have *lower* dlPFC excitability and *lower* CEN connectivity. Thus, the severity of depressive symptoms will be inversely related to CEN connectivity (more depressed = less connected CEN). Negative controls will include sham TMS and features of the TEP that did not relate to connectivity (and thus likely relate to off-target effects and would likely not show a difference between healthy subjects and depressed patients).

Potential pitfalls:

While it would be simpler to obtain direct brain measurements in the population of interest (healthy subjects or depressed patients), this is not feasible. We will instead utilize O. David's CCEP database of 1200 epilepsy patients and remove components that may relate to seizure pathology. The assumption is that the group averages will yield novel insights and be translatable to other populations given the etiologic and seizure onset heterogeneity of these patients. Thus, we will remove from analysis 1) all patients whose seizures arise from either frontal or parietal lobes (areas housing nodes of the CEN); and 2) all electrodes from each patient where epileptic spikes or ripples were observed⁶⁷. As the majority of these patients have temporal lobe epilepsy, removing those with frontal or parietal lobe seizures will leave >60% of patients for analysis.

dIPFC-induced TEPs do not predict dIPFC-induced CCEPs in the CEN. This is unlikely given the fact that 1) both measures are derived using similar methodology (single pulse dIPFC stimulation and evoked potential measurement); 2) previous work demonstrated similar intracranial and scalp CCEPs after single pulse electrical stimulation^{86,87}; 3) C. Keller demonstrated that CCEP maps relate to typical large-scale resting fMRI networks²²; and 4) intact CEN in epilepsy patients has been reported by C. Keller using CCEPs²⁴ and resting fMRI⁸⁸. If we do not observe TEP features predictive of CCEP excitability, it is possible that pre-processing algorithms can be improved to separate neural versus non-neural and off-target effects. Thus, we will re-process TEP data using different preprocessing techniques^{89,90}. If still there are no predictive TEP features using the CCA approach, it is possible that alternative methods to relate TEPs to CCEPs are warranted. As such, C. Keller will apply other standard machine learning methods (i.e., logistic regression) to evaluate the accuracy of TEP feature maps to predict CCEP maps. If still no TEP features map onto CCEPs, we will conclude that standard TEP features used in research do not reflect dIPFC excitability or CEN connectivity as measured by CCEPs. In this case, using convolutional neural networks^{91,92}, we will perform a data driven feature learning approach to better understand how each datapoint within the TEP (not just standard features) maps onto CCEPs. If still no components of typical or learned TEP features map to dIPFC CCEPs, we will conclude that either differences in acquisition (TMS vs electrical, epilepsy vs healthy subjects) may be contributing, or that the TEP may only represent off-target effects unrelated to brain excitability and connectivity. To rule out the fact that our lack of findings may be related to analyzing two different populations, we will process a small subset of data (N=6) from C. Keller's collaborator that has collected dIPFC stimulation-induced TEPs and CCEPs within the same epilepsy patients. This multimodal acquisition is still performed in a non-simultaneous manner. However, a within-subject comparison will reduce the potential confounds of analysis across populations (albeit now in a small sample compared to the previous large sample from the CCEP database).

dIPFC-induced TEPs and CCEPs do not correlate with cortical microstructure. The absence of clear correlation between TEP/CCEP features and microstructure, though not expected *a priori*, would be an interesting finding for the interpretation of TEP/CCEP. It would suggest that those measures have moderate local value but best reflect large network integration processes. This negative result will not modify our plan per se but may influence the translatability of our findings for understanding the pathophysiology of the CEN in depression.

C3. Aim 3: To evaluate the effect of individualized rTMS targeting of the CEN.

Rationale and significance: Current dIPFC targeting methods for rTMS treatment involve either 1) scalp-based estimation of the dIPFC, which lacks anatomical specificity⁹³; 2) anatomical targeting using MRI, which lacks functional specificity^{94,95}; or 3) network-based targeting using functional MRI⁹⁶, which suffers from low signal-to-noise for an individual subject, is costly, and cannot be implemented in the clinic. While targeting of the stimulation site (i.e. dIPFC) and downstream regions (i.e., parietal node of the CEN) on an individualized basis to account for inter-individual variability would make intuitive sense, evidence for or against this approach is currently lacking. This aim focuses on testing the hypothesis that rTMS targeted to

the CEN on an individualized basis will more effectively engage the targeted brain network. If this target engagement aim is successful, it will lead to a randomized controlled trial to test the clinical efficacy of individualized rTMS targeting of the CEN in depression. More broadly, this localization method for each individual will enhance our ability to modulate brain networks, a critical component in studying any normal or pathological brain-behavior relationship.

Preliminary Data. For this aim to yield insights, the CEN needs to be (1) capable of being localized on an individual basis and (2) modifiable with a single session of rTMS. Intracranially, C. Keller recently demonstrated that single pulses of dIPFC electrical stimulation induces CCEPs in the parietal CEN on an individual basis (Fig 8A,B). We furthermore showed that a single session of 10Hz dIPFC intracranial electrical stimulation – patterned to mimic rTMS (10Hz, 5s on, 10s off, 3000 pulses) – suppressed the p30 parietal CCEP in the CEN for ~20 minutes following stimulation^{24,40} (Fig 8C,D).

Non-invasively, in 2019 C. Keller completed a double-blind randomized clinical trial (NCT01829165) treating depressed patients with four weeks of daily 10Hz rTMS (typical treatment frequency and duration) or sham (no TMS) to the left dIPFC. TEPs were measured only before and after all 20 sessions of rTMS. rTMS and not sham suppressed the p30 of the TEP in the CEN (Fig 9A,B). The degree of post-rTMS p30 suppression in the CEN importantly predicted over 50% of clinical response¹⁷. Since these changes reflected an entire treatment course and we are interested in single session changes in this aim, C. Keller then measured TEPs after a single dIPFC rTMS session (10Hz, 5s on, 10s off, 3000 total pulses) in eight healthy participants. dIPFC rTMS induced a parietal TEP reduction in the p30 (Fig 9C,D). As additional evidence that dIPFC rTMS modulates the CEN, in a small cohort of depressed patients, O. David's group demonstrated that following 10Hz dIPFC rTMS, resting EEG was more strongly modulated in clinical responders. Specifically, clinical responders demonstrated higher delta and theta power in parietal regions and weaker frontal alpha power, when compared to non-responders^{97,98}. In summary, our preliminary data suggest that a single session of dIPFC stimulation modulates the CEN and the degree of rTMS-induced CEN modulation relates to clinical outcome.

Overview of methods. C. Keller will enroll 50 patients with major depression and in a single rTMS 'dose,' prospective, randomized, double-blind, cross-over design will test if rTMS using individualized targeting of the CEN will more effectively engage the targeted brain network. In Aim 3.1, C. Keller will develop an automated software algorithm to individually target the CEN using TEPs. In Aim 3.2, he will directly compare this method to standard methods by evaluating the strength, duration, and specificity of CEN modulation after a single session of dIPFC rTMS (Fig 10). Our primary outcome is on pre/post p30 TEP changes in the CEN for reasons described above and previously^{17,24}, but exploratory analysis will determine

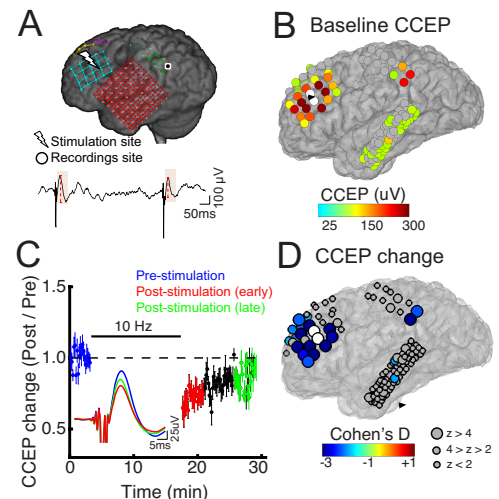


Figure 8 – 10Hz intracranial repetitive stimulation suppresses the CCEP p30 in the CEN A) Example of single trial CCEP showing a single trial p30 (shaded region). B) Baseline p30 CCEP. Note the strong p30 elicited in the parietal region after dIPFC stimulation (white electrodes). C) Change in CCEP p30 after 10Hz dIPFC repetitive stimulation. Insert: p30 waveform before and after repetitive stimulation. D) Post-stimulation CCEP change. Note the strong CCEP modulation in the CEN. Adapted from Keller et al., 2018.

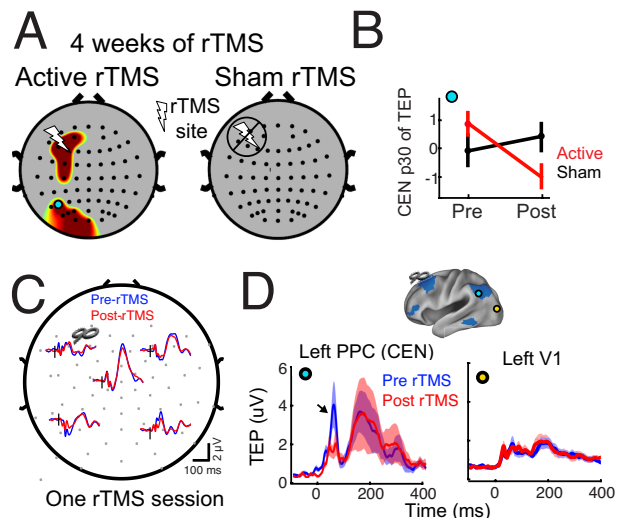


Figure 9 – Daily active 10Hz rTMS treatment for depression suppresses the TEP p30 in the CEN. A) Scalp topographic plot of the main effect of time (pre/post four weeks of rTMS) for p30 TEP change (n=25 depressed patients, $p < .01$ effect in red). Note the effect of left dIPFC rTMS treatment on regions overlying the CEN. B) CEN p30 was suppressed in active but not sham rTMS. C) Sensor level TEP changes following a single session of 10Hz dIPFC rTMS (n=8 healthy subjects). D) Source-level TEP changes after single session rTMS in the parietal region of the CEN and in V1. The arrow denotes the p30.

if other time and frequency features of the TEP (and how these relate to biologically-grounded components from Aim 2) are differentially modulated as a function of targeting method.

Aim 3.1: C. Keller will develop an automated, data driven calibration procedure to individually target the CEN. This goal of the procedure is to refine the dIPFC target based on parietal CEN connectivity. On an individual level, C. Keller will identify regions of the dIPFC strongly connected to the parietal CEN by applying single TMS pulses in grid-like fashion to ROIs within the dIPFC. For each anatomical dIPFC subunit probed with TMS, the p30 of the TEP will be quantified in the parietal region of the CEN. The dIPFC subunit that demonstrates the strongest p30 of the TEP in parietal cortex will be chosen for assessment in Aim 3.2 (Fig 10).

Aim 3.2: After developing the method to optimize dIPFC targeting for individual CEN localization (Aim 3.1), C. Keller will directly assess the effect of dIPFC rTMS targeting on CEN modulation in depression. 50 depressed patients will be prospectively enrolled into a cross-over design. For each condition, a single session of rTMS at standard parameters (10Hz, 5s on, 10s off, 3,000 total pulses, 15 min duration) will be performed and changes in CEN connectivity will be quantified

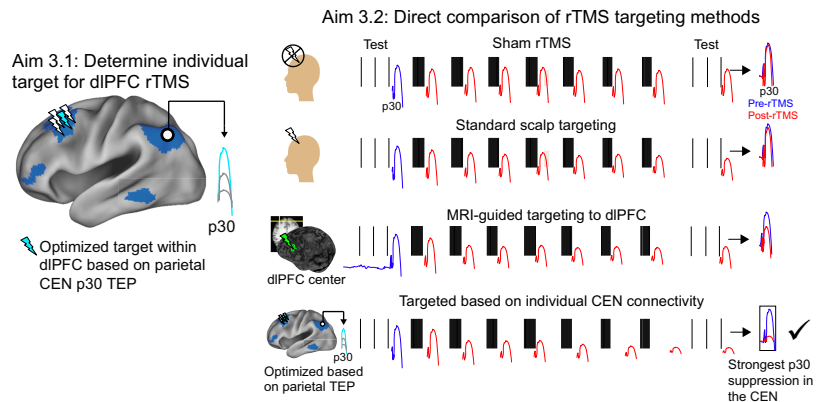


Figure 10 - Overview of Aim 3. Aim 3.1: Determination of the optimal dIPFC site for CEN target engagement. Aim 3.2: Comparison of targeted dIPFC rTMS and its effects on the strength and specificity of CEN modulation.

using pre/post dIPFC-stimulated parietal TEPs (Fig 10). The dIPFC will be targeted for rTMS using three methods: (a) MRI-guided with individual CEN optimization using TEPs as described above, (b) MRI-guided alone, and (c) standard scalp targeting (Beam F3 method⁹⁹). Additionally, sham rTMS will be applied to control for off-target effects. We hypothesize that while each active rTMS method (condition a-c) will suppress the p30 of the TEP in the CEN, optimized CEN localization using individual TEPs (condition a) will induce the *strongest* and most *specific* change in the CEN for the longest *duration*. Our primary outcome will be parietal p30 CEN modulation directly following rTMS. Secondary outcomes will assess parietal p30 changes in the parietal node of the CEN *during* rTMS (quantifying the p30 after the last pulse in each stimulation train) as well as 15 and 30 min following rTMS. We will also assess pre/post rTMS behavioral changes in attention with a standard continuous performance task and working memory using an N-back task, both of which have been implicated in the CEN and depression^{100,101}. Although it is unlikely that changes in mood will be observed after a single session of rTMS¹⁰², we will evaluate pre/post rTMS changes in depressive and anxiety symptoms using self-report questionnaires (QIDS-SR¹⁰³ and GAD7¹⁰⁴, respectively). rTMS sessions will be triple-blinded to operator, participant, and statistician. To blind the study to the operator, all targets will be determined by a separate researcher and inputted into the neuronavigation software, labeled only as #1-4, thus blinding to the manner in which these targets were determined. rTMS sessions will be separated by at least two days to remove potential lasting effects >24 hours, and rTMS session order will be randomized and counterbalanced to reduce any potential bias. Negative control conditions will include sham rTMS, standard non-MRI guided rTMS, and anatomical MRI-guided rTMS. Negative control analysis within each condition will include evaluating p30 change in the regions outside the CEN and the n100 and p200 change in the CEN.

Potential pitfalls:

Lack of CEN modulation for any targeting method. This is unlikely given our preliminary results demonstrating single session modulation of the p30 CEN both intracranially^{24,40} and transcranially. If, however, this is the case, because other TMS-elicited EEG measures such as oscillatory activity in the frequency domain (*event-related spectral perturbation* or *ERSP*) are also sensitive to external perturbation¹⁰⁵, we will analyze the time-frequency components of the TMS response before and after rTMS. We will also collect resting EEG before and after rTMS as these measures have been shown to predict treatment outcome in depression^{98,106,107}. If no CEN change is observed in any of these EEG features, we will characterize the dose-response of CEN modulation by performing 2-3 daily sequential

sessions of rTMS for each targeting method in order to determine the smallest number of sessions sufficient to induce CEN changes.

Lack of active/sham rTMS separation. A lack of active/sham rTMS separation would suggest that a single session either induces neural changes from non-specific sources (e.g., sensations or auditory click due to TMS), or induces non-neural changes (e.g., from coil pressure or electrode vibration). Experimental controls attempt to minimize these non-neural contributions¹⁰⁸, but if this lack of separation is observed we will revisit these design choices to minimize these effects.

Individualized CEN targeting does not outperform standard methods. If there is an observable p30 CEN modulation with active but not sham rTMS, but individualized CEN targeting does not outperform standard methods, we will conclude that this targeting method is not helpful with respect to engaging the CEN. It could still be helpful clinically but given that our go/no-go decision will be based on CEN target engagement, we will not pursue a clinical efficacy trial. Due to small sample sizes in the previous trial (NCT01829165), which suggested that the p30 is a predictive biomarker for depression outcome, it is possible that another TEP feature may differentially be modulated with individualized but not standard rTMS, and that this feature may correlate with clinical outcome. Thus, in parallel to this work to optimize rTMS targeting, C. Keller will perform an independent replication of the p30 CEN biomarker finding¹⁷ based on an ongoing large naturalistic rTMS clinical trial (NCT02843373).

METHODS

C4. CCEPs. CCEPs will be processed on the Human Intracerebral Platform of the Human Brain Project developed by O. David's team, using a dedicated tool, the CCEP manager. CCEP manager is a BIDS application written in Python that queries the F-TRACT database stored in BIDS-iEEG format defined by an USA/Europe consortium to which we participated⁶⁰. The CCEP manager includes a status tool to monitor subject data at each quality control and processing step within the F-TRACT pipeline. This includes raw data conversion to CCEP feature quantification used for brain atlasing. The CCEP manager directly integrates multiple BIDS-formatted open source software solutions from labs with expertise in functional mapping of SEEG signals^{109,110}. For the proposal, the CCEP manager will be extended to output CCEP basis functions for further TEP comparisons.

C5. TMS-EEG studies. For all TMS studies in this proposal, the following methods will be used:

Robotized TMS: Experiments in France will use a robotised system (Cobot, Axilum Robotics) coupled to a neuronavigation system that precisely controls the TMS coil's relative position to the subject's head location. Stanford experiments will use neuronavigation to anatomically target TMS to specific brain regions.

Single Pulse TMS. TMS pulses will be applied every 2 seconds (jittered to prevent entrainment or expectation effects) at 120% of motor threshold (MT) for a total of 200 trials prior to and after rTMS. Participants will be instructed to keep their eyes open and gaze relaxed. Stimulator recharge will be delayed to prevent EEG artifact¹¹¹. A thin (0.5mm) foam pad will be attached to the TMS coil to minimize electrode movement and bone-conducted auditory artifact. White noise matching the frequency of the TMS click⁸¹ will be provided with noise cancellation headphones to reduce auditory artifact^{111,112}.

EEG Recordings. To record TEPs, we will use a 64-channel high-density EEG (Brain Products, Germany) with pre-amplification and 25KHz sampling rate that reduces noise and stimulation artifact. This system has a large dynamic range to avoid amplifier saturation by the TMS pulse. The amplifier is attached to an active EEG cap (actiCAP, Brain Products) with flat electrodes designed to record without overheating from TMS. Electrode impedances will be less than 5 kΩ to reduce stimulation-related artifact.

Repetitive TMS (rTMS). rTMS will be applied to the left dlPFC at standard stimulation parameters (10Hz for 5s, 10s rest, 15s duty cycle, 15 minutes and 3000 pulses total, 120% resting motor threshold), with a MagVenture X100 TMS and a liquid-cooled B65 A/P butterfly coil (MagVenture, Denmark). For Aim 2, the TMS coil was placed above the left dlPFC identified by structural MRI. For Aim 3, the TMS coil will be placed above the left dlPFC depending on the targeting method (See Aim 3.2). Sham rTMS will include 1) a direction-sensor TMS coil that alerts the operators to flip the coil if the wrong side is being used, and 2) low-intensity 10Hz electrical stimulation applied to scalp electrodes under the coil for sham and placed but not activated in the active arm, as is standard in the field¹¹³.

TMS-EEG and Statistical Analysis. All data analysis will be carried out in MATLAB (Mathworks, MA) with custom scripts. As the TMS pulse introduces large-amplitude artifacts, multiple stages of artifact rejection are needed before resolving the TEP. Data will be preprocessed using an automated TMS-EEG artifact

rejection algorithm C. Keller co-developed³¹. The algorithm involves: 1) interpolation of large stimulation artifact; 2) downsampling (1kHz); 3) independent component analysis (ICA)-based removal of muscle and vibration artifacts; 4) 60Hz line noise removal; 5) high-pass (0.1Hz cutoff) filter to remove slow drift; 6) re-referencing to the common average and epoching (-500 to 1000ms); 7) bad channel and trial rejection; and 8) ICA-based removal of remaining artifacts (e.g., eye blink, EKG). The algorithm showed 96% accuracy compared to manual rejection by EEG experts and outperformed current semi-automated methods. Cleaned TEPs will then be baseline corrected, averaged, source localized¹¹⁴, and grouped by regions of interest to reduce the number of statistical tests performed. For Aim 2, standard TEP features will be quantified at each channel, including the p30, n45, p60, n100, p200, and spectral power features, and related to CCEP measures. For Aim 3, p30 change in the CEN will be the primary outcome. Data will be analyzed using a longitudinal linear mixed-effects model¹¹⁵ to account for the pre/post TEP measures for each participant, and identify TEP changes that are specific to the active rTMS conditions. We will use a 4 condition x 3 site multivariate analysis of variance (MANOVA) to determine the main effects and interactions between rTMS condition (CEN-targeted, MRI-based, scalp-based, sham) and TEP site (dIPFC, parietal, V1 control). We will correct for multiple comparisons⁸² as necessary.

C6. Participants and Study Structure

For Aim 2. O. David will recruit 20 right-handed men and women (50/50), ages 18-40, without history of psychiatry and neurological disorders or risk of seizures, and with no contraindication for MRI. Participants will first complete a screening procedure to determine eligibility. Eligible participants will then complete a structural multimodal MRI session at 7T, followed by a robotized TMS-EEG acquisition as outlined above.

For Aim 3. C. Keller will recruit 50 English-speaking, right-handed men and women (50/50), ages 18-65, without history of neurological disorders or risk of seizures. Participants require a principal diagnosis of MDD, made by a Structured Clinical Interview for DSM-5 (SCID-I¹¹⁶) and a PHQ9¹¹⁷ >10 to ensure disorder severity. Exclusion criteria are as follows: contraindications for MRIs (e.g. implanted metal), history of head trauma with loss of consciousness, history of seizures or on medications that reduce seizure threshold, neurological or uncontrolled medical disease, history of suicide attempt in past year, active substance abuse, psychotic or bipolar disorders, or a prior history of electroconvulsive therapy or rTMS failure.

Participants will first complete a screening procedure to determine eligibility based on inclusion/exclusion criteria. Eligible participants will then complete a structural and functional MRI followed by four rTMS-EEG sessions based on the targeting methods outlined in Aim 3. For each rTMS session, self-report symptom scales (QIDS-SR for depression and GAD7 for anxiety), behavioral tasks (continuous performance attention and N-back working memory), single pulse TMS-EEG, and resting EEG will be performed before and after the rTMS session.

C7. Power Calculations.

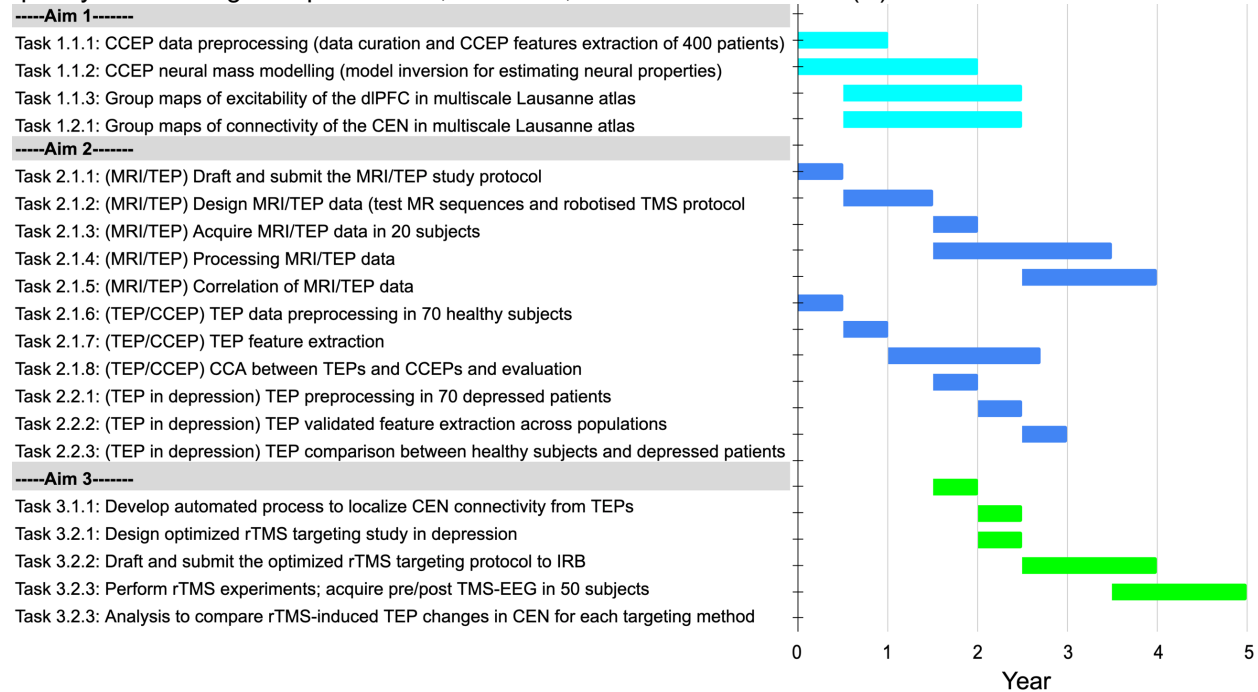
Aim 1 will use all available data from the F-TRACT database showing responses in the CEN. Bootstrapping methods on the group data will be used to assess statistical significance of estimated group CCEP features. According to estimated variability, the spatial resolution of the CCEP maps will be adjusted to ensure that a sufficient amount of data is used for each ROI.

Aim 2: Power calculation was based on the comparison of TEP features in the CEN between depressed patients and healthy subjects. Power analyses were conducted using G*Power¹¹⁸ (at 80% power and $\alpha = 0.05$). Empirically-obtained effect size estimates from a study comparing TEPs in depressed patients demonstrated an effect size greater than one⁷². As the sample size was low in this study and with multiple limitations, a more conservative estimate for this aim is an effect size of 0.5. A study with an effect size of 0.5 and a power of 80% will require a total sample of 127 patients to test the association at 5% levels using two-tailed test. Assuming we will remove ~10% of subjects due to poor data quality, 70 subjects in each study group will provide sufficient power for primary aims.

Aim 3: Power calculation was based on the comparison of *optimal individualized CEN targeting vs MRI-based dIPFC targeting*. Sample sizes were based on effect size estimates for p30 TEP group differences in the CEN. Empirically-obtained effect size p30 TEP suppression estimates following a single session of rTMS (Keller et al., *in preparation*) ranged from 20-40% for MRI-based targeting. We will assume a 20% larger p30 suppression for optimally-targeted rTMS compared to standard rTMS. A study with an effect size of 0.3 and a power of 80% will require a sample of 45 patients to test the association at 5% levels using two-tailed test. Assuming ~10% attrition rate, 50 patients will provide sufficient power for primary aims.

C8. Consideration of sex as a biological variable in experiments. We have observed no effect from biological sex on CCEPs, TEPs, change in TEPs, or clinical change following rTMS. For all experiments, we will enroll equal distribution of sexes and will continue to monitor sex as a variable.

C9. Timelines and Milestones. We decomposed each aim into tasks and defined Milestones (M) early to quickly initiate mitigation procedures, if needed, and main Deliverables (D) at the end of Tasks.



Aim 1.1:	
M1.1.1: Preliminary dIPFC excitability and CEN connectivity inferred from 600 CCEP datasets	M8
D1.1.1: Final CCEP excitability and connectivity maps	M30
Aim 1.2:	
M1.2.1: Preliminary inversion of dIPFC neural mass modeling inferred from 300 CCEP datasets	M8
D1.2.1: Final CCEP neural parameter maps	M30
Aim 2.1:	
M2.1.1: (MRI/TEP) MRI/TEP study protocol submitted	M6
M2.1.2: Multiparametric MRI sequences fully validated on phantoms	M12
M2.1.3: Complete MRI/TEP dataset curated for one subject	M20
D2.1.1: Final CCEP/TEP/MRI feature comparison	M48
M2.1.4: (TEP/CCEP) TEP healthy control data preprocessed	M6
M2.1.5: TEP feature extraction complete (M12); M2.1.6: Preliminary comparison between CCEPs and TEPs	M18
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