22nd of February 2025

Neuromod+ grant report

Project title: State-dependency of deep-brain transcranial ultrasonic neurostimulation

The interest in transcranial ultrasound stimulation (TUS) as a new neuromodulation technique that can target deep brain regions has grown fast. For the first time in humans, neural activity in deep brain regions can be modulated non-invasively and with millimetre precision. This opens the possibility for interventions for brain disorders with abnormal activity in subcortical regions, and where current treatments include invasive deep brain stimulation or ablation (e.g., mood disorders, epilepsy, Parkinson's disease etc).

However, despite several hundred ultrasound users world-wide, the development of reliable and effective protocols is lagging behind. This is partly because little is known about how sonication interacts with the functional brain state – an ongoing question for any neuromodulation technique. The default for TUS protocols applied "offline" (i.e., to induce neuroplasticity) is to sonicate at rest. A few studies have instead used a theta-burst-patterned *offline* protocol with effects lasting ~1h (Zeng et al., 2022, Yaakub/Fouragnan, Nat Comms 2022). Such plasticity-inducing offline protocols may have great clinical potential. Yet, TUS application *at rest* creates an ill-defined state with potentially large inter-subject variability, and which may not be optimal for modulating brain activity. In vitro, TUS effects show clear state-dependency (Prieto et al., 2020), which is also supported by related work in humans using other neurostimulation techniques (e.g., TMS). However, whether TUS effects in humans are state-dependent remains unclear and stimulating at rest may not be the most effective way to interact with specific circuits and induce long-term changes.

Using the pilot funds provided by Neuromod+, we ran a pilot study to examine the state-dependency of offline-TUS in a well-established emotion processing task. **Our objectives were to** establish whether TUS effects are modulated by the functional state of the targeted brain circuit. Our working hypothesis was that knowing this will help develop more reliable and effective protocols to induce brain plasticity in deep targets and improve the replicability and reduce the variability of TUS (which will also extend to other neurostimulation techniques)

Methods: We ran a small pilot study using within-subject single-blind sham-controlled design. We applied TUS to the amygdala (or sham) either while emotional stimuli were shown to the participant, or while the participant was at rest (2 x 2). We then probed the behavioural effects of amygdala TUS offline as changes in an emotional approach/avoid during the ~1h following TUS.

Expenditure Neuromod+ kindly provided costs for a 2-month research assistant costed at 50% (extending a current RA's contract; this RA was already trained in TUS). The RA assisted in setting up the study, submitted the ethics application, and helped with initial advertising, recruiting and testing. Costs were also received to cover MRI scan fees and participant reimbursement.

Results and Impact: Even though this pilot study yielded only preliminary results, they showed promising effects in the expected direction. The RA on the project was invited to present these results at the key focused ultrasound conference (in Toronto in 2024), and he received full funding for his PhD during which he is now continuing this work in a full study. We learnt several lessons from the pilot study thanks to the Neuromod+ funding which allowed us to improve the design. For example, we are now using M1 task engagement rather than amygdala task engagement as our read-out, simply because behavioural read-outs are easier from this region. The final jury on the state-dependency of TUS effects is still out, but the pilot study funded here provided the first important stepping stone towards developing the full study which we hope to complete this year (2025). A secondary outcome was that this funding fostered collaborations with people in other UK institutions (e.g. Elsa Fouragnan in Plymouth) which also led to us writing and publishing an Editorial together (Brain Stim in 2024).

The full study that is currently running and was informed by and built on the pilot study funded here by Neuromod+ is hoped to increase the efficacy of future clinical applications of TUS, shape the best standards of a growing field, help understand TUS biomechanisms, and impact potential future clinical applications of TUS. The potential applications of TUS in both clinical and basic science settings are vast, yet reliable TUS protocols are currently scarce. Therefore, establishing whether TUS effects are state-dependent, and whether applying TUS to task-active circuits might increase its efficacy is expected to have large impact on future studies. The knowledge gained from our pilot has now already been translated to a new studies in healthy participants and will be translated to clinical populations following this first full study (and to studies targeting different subcortical or deep cortical brain circuits or focusing on different behaviours of interest). Thus, we hope to provide proof-of-principle that considering the brain-state *during* TUS sonication is important. Our findings will build on and extend prior work on brain state-dependent neurostimulation with TMS (e.g. Bergmann, 2018) and shape the field going forwards.