Project report: Multi-focal ultrasound neuromodulation to the dorsal anterior cingulate cortex disrupts behavioural and neural pain processing

Contributors: Sophie Clarke[1], Samuel Mugglestone[1], Sam Hughes[2] and Elsa Fouragnan[1]

[1]University of Plymouth, [2]University of Exeter

Summary: Transcranial ultrasound stimulation (TUS) is a promising non-invasive technique for modulating deep brain regions involved in pain. TUS applied to the dorsal anterior cingulate cortex (dACC), a region implicated in chronic pain and established target for deep brain stimulation, has shown potential for reducing pain. This study aimed to investigate the neural mechanisms underlying TUS effects on pain in healthy participants using neuroimaging. Thirty-two participants underwent two double-blind, randomised TUS-fMRI sessions (active or sham). A tonic cold stimulus was applied during multifocal dACC-TUS and during fMRI and magnetic resonance spectroscopy (MRS) blocks. While no significant main effect of TUS on pain intensity was observed, active TUS showed a significantly greater reduction in pain ratings between 28- and 55-minutes post-stimulation, suggesting a delayed analgesic effect. Active TUS also disrupted the typical relationship between stimulus temperature and reported pain intensity, indicating altered sensory encoding. There was increased functional connectivity between the dACC and the supplementary motor area, pre-motor cortex, mid-ACC and the supramarginal gyrus, along with decreased coupling with the periaqueductal grey (PAG), and altered salience network connectivity. Overall, these findings suggest TUS to the dACC has multidimensional effects across behavioural and neural aspects of pain processing, supporting its potential therapeutic value.

Methodology: This was a double-blind, randomised, cross-over study conducted in healthy humans. Participants attended three visits: an initial MRI session for neuronavigation and acoustic modelling, followed by two counterbalanced sessions involving active or sham TUS to the dACC. During each main session, participants received multi-focal, neuronavigated TUS to three anatomically defined dACC targets. This was followed by resting-state fMRI during baseline, tonic pain and recovery, and MRS of the dACC. Tonic pain was induced by submerging the hand into a cold gel, with pain intensity rated on a numerical scale. TUS was delivered using a 500 kHz transducer with a 10 Hz, 10% duty-cycle protocol designed to induce inhibitory neuromodulation, with individualised acoustic and thermal simulations ensuring accurate targeting and adherence to safety guidelines. Sham stimulation consisted of matched auditory control. MRI data were acquired on a 3T scanner and included BOLD fMRI and MEGA-PRESS MRS to quantify GABA and glutamate concentrations. Behavioural data were analysed using linear mixed-effects models, temperature–pain relationships with linear and robust regression, fMRI connectivity using seed-based and independent component analyses, and MRS data using standardised spectral modelling. Control and specificity analyses were conducted to confirm localisation and state-dependence of TUS effects.

Results: Multi-focal transcranial ultrasound stimulation (TUS) targeting the dACC achieved good target engagement while remaining within established acoustic and thermal safety limits. Behaviourally, there were no significant differences in pain ratings between active and sham TUS at individual time points; however, exploratory analyses revealed a significantly greater reduction in pain ratings between the two latest time points following active TUS, suggesting a delayed analgesic effect. Moreover, while pain ratings in the sham condition showed the expected sensitivity to stimulus temperature, this relationship was no longer present following active TUS, indicating altered encoding of nociceptive input. Imaging analyses demonstrated that during tonic cold pain, active TUS selectively modulated dACC functional connectivity, increasing coupling with pain-related motor and salience regions including the supplementary motor and premotor cortices, alongside altered connectivity with the anterior insula and reduced coupling with the periaqueductal grey. These effects were specific to the dACC, absent in control regions, and state-dependent, occurring only during pain and not during rest or post-pain periods. Independent component analysis further showed

reduced connectivity between the salience network and prefrontal, cerebellar and hippocampal regions following active TUS. Although MRS revealed no overall session effects on GABA or Glx concentrations, greater reductions in GABA following active TUS were associated with stronger analgesic effects, linking neurochemical changes to behavioural outcomes. Finally, TUS was well tolerated, with only mild, transient symptoms reported at comparable rates across active and sham conditions, supporting a favourable safety profile.

Project impact and relevance to Neuromod+ network aims: This study advances understanding of both pain neurobiology and neuromodulation mechanisms by providing causal evidence that TUS can selectively and state-dependently modulate dACC functional connectivity during pain, linking circuit-level changes to behavioural and neurochemical outcomes. The study design embodies a true multidisciplinary approach, integrating several techniques, including TUS, fMRI and MRS. The use of a sham-controlled, cross-over design, and multiple outcome measures aims to address variability and replicability issues in neuromodulation research, providing a transparent framework for defining mechanistic and behavioural endpoints. Importantly, the demonstration of target-specific, context-dependent effects with a favourable safety profile strengthens the translational relevance of TUS, informing dose selection, target engagement metrics and trial design for future clinical studies in chronic pain.

Dissemination and reception: 1) This work is currently under review at *Nature Communications* and has received generally positive and supportive reviewer feedback. **2)** Sophie Clarke has received a Poster Prize at the Focused Ultrasound Neuromodulation conference in 2025 for a poster on this work. **3)** Sophie Clarke and Elsa Fouragnan have shared the results at multiple conferences in the format of talks or posters.

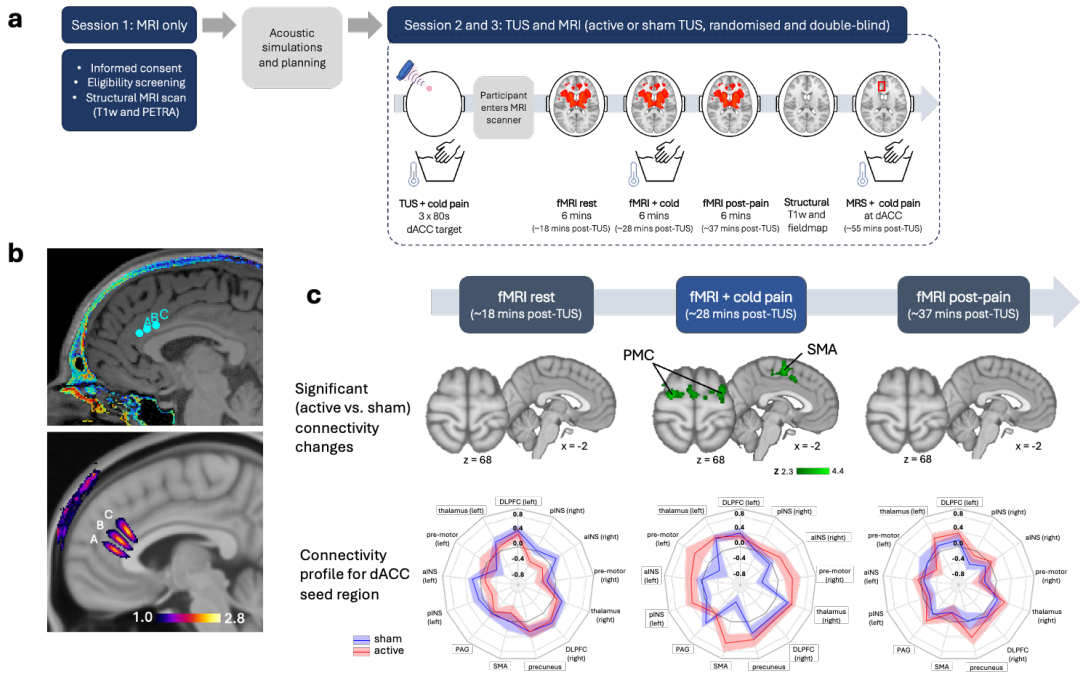

Figure 1: a *Participants completed 3 sessions; in the first session, T1-weighted and PETRA scans were acquired for neuronavigation and acoustic planning, then at sessions 2 and 3 received either active TUS to the dACC or sham followed by completing fMRI and MRS scans.* ***b*** *This multi-focal TUS intervention involved application of TUS to 3 target sites within the dACC, which are shown in the top panel labelled A, B and C. Average stimulation intensity (I_{SPPA}) simulated using k-Plan software (BrainBox, Inc.) across all participants is shown in the bottom panel.* ***c*** *There was altered functional connectivity between the dACC seed region and the supplementary motor area and the pre-motor cortex during tonic pain, but not during rest or post-pain recovery, following active TUS compared to sham.*