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Modulating Reward Function Using Focused Ultrasound Aimed at the Nucleus Accumbens:

Swedish: Undersökning av belöningsfunktioner med fokuserat ultraljudsstimulering riktad mot belöningscentrum

Purpose

There is an urgent need to identify new depression therapies, as a substantial proportion of patients with this common and disabling disorder do not respond to conventional treatments, often referred to as having a treatment resistant depression (TRD). Depression, and especially TRD, is closely related to anhedonia, which is a loss of pleasure and motivation for pleasurable sensations. Pleasure and motivation are thought to be regulated through the brain's reward system. To date, knowledge of how this system operates in detail is lacking. In order for the mental health field to move forward, a deeper understanding of basic brain functions and systems is much needed. Through an enhanced understanding of how the healthy brain functions, we might increase our understanding of how and why dysfunctions emerge and how they can be treated.

Background

Depression is a main contributor to the global burden of disease with devastating consequences for the afflicted patients, peers and society (1). Approximately one third of patients do not respond to the first two trials with antidepressants, and are referred to as having treatment resistant depression (TRD) (2). The next treatment step for these patients is usually brain stimulation with repetitive transcranial magnetic stimulation (rTMS) or electroconvulsive therapy (ECT). However, at present only a minority of patients benefit from rTMS, while ECT have cognitive side-effects and requires anaesthesia (3,4) and both methods suffer from limited spatial targeting. Thus, new effective and feasible treatments are called for, that allow for precision targeting of brain regions directly involved in the TRD pathology.

Anhedonia and nucleus accumbens

One of the two core symptoms of depression, and an especially prominent feature in TRD, is anhedonia, i.e. decreased ability to experience pleasure and joy. Anhedonia has neurobiologically been associated with disturbed reward-circuit functioning, including the core nexus in the brain's reward-circuitry, the Nucleus Accumbens (NAcc) (5). Paralleling the dorsal striatum, the NAcc is part of a fronto-striatal circuit including the ventral anterior cingulate cortex (ACC) (6), is implicated in motivational behaviour (7), and modulated by mesolimbic dopamine (8). Importantly, the NAcc is involved in anticipatory aspects of hedonic processing (9).

Consequently, there are several pieces of evidence pointing towards a key role of the NAcc in depression in general, and anhedonia specifically (10). The NAcc is smaller in severely depressed patients compared to healthy individuals (11), and is hypo-reactive to negatively valenced stimuli (12). From a network point of view, fronto-striatal connectivity is correlated to anhedonia (13), and the resting functional connectivity in NAcc based networks is predictive of response to antidepressants (14).

There is evidence that many of the current clinical brain stimulation techniques exert their effect through modulation of the NAcc. Deep brain stimulation (DBS) directly targeting the NAcc has shown profound anti-anhedonic effects in patients with very treatment-resistant depression (15,16). Similarly, non-invasive brain stimulation techniques such as rTMS and ECT are also dependent on NAcc engagement, but through indirect network modulation (11,17–19).

However, the understanding of NAcc´s role in the healthy human brain is severely limited due to technical limitations and ethical consideration. Traditional non-invasive techniques such as rTMS and ECT lack the spatial specificity and dept to target the NAcc. DBS on the other hand requires neurosurgery with risks of intracranial infections or irreparable brain damage when introducing the electrodes. Thus it has so far not been possible to conduct any experimental studies of the direct effects of NAcc stimulation in a healthy population, which would be necessary to draw any definite conclusions of its functions. However, with new neuromodulation techniques emerging, new possibilities are opening up.

Transcranial Ultrasound Stimulation

A novel technique for deep and focal non-invasive brain modulation is low-intensity Transcranial Ultrasound Stimulation (TUS) which has opened a whole new field of research to increase our understanding of subcortical brain functioning. By the use of concave transducers, the mechanical energy of the ultrasound waves can be focused deep within the brain to selectively and reversibly modulate subcortical structures (20). One proposed mechanism of action for TUS is that the mechanical force changes the electrical properties of the cell membrane either by thermal effects or by changing its conformation when rhythmically deforming it (20). Another, complementary, theory is that part of the effect is exerted through manipulation of mechano-receptors in the neural cell membrane, whereby transmembrane ion-flows are triggered (21).

Considerable work has demonstrated neurophysiological, clinical and histological safety and tolerability of TUS in animals and humans (22–26). The recently formed International Transcranial Ultrasonic Stimulation Safety and Standards (ITRUSST) has developed a consensus guideline for biophysics safety (26). As there is currently no official regulation for focused ultrasound, these guidelines are based on regulations previously set for diagnostic ultrasound. The most important safety limits are the upper limit for mechanical index at 1.9 and the upper limit for the delta temperature change in the brain. Three distinct metrics can be used to assess a safe temperature change (26), hence the precise limits vary according to which of the three metrics that is being used. The limits are set conservatively to prevent any potentially harmful mechanical and/or thermal effects on tissue in humans.

Within these safety limits several recent human studies on healthy participants have shown that TUS over the motor cortex could increase corticospinal excitability (27–30), with a duration of more than 30 minutes (28,30), and at the same time increase motor function (28,29). Further, excitability of deeper brain regions such as the cingulate cortex could be modulated with TUS resulting in changes in gamma-aminobutyric acid (GABA) and functional connectivity (31). There are preliminary data supporting that fear-conditioning can be modulated by TUS targeting the amygdala (32), a finding that parallels a previous pivotal study in non-human primates showing that brain network connectivity could be selectively manipulated when applying TUS over the amygdala (33). Together, these findings show that in-human selective neuromodulation is feasible, targeting a range of regions, including subcortical structures.

Creating a feasible sham sonication with TUS has proven challenging, as TUS can sometimes induce sensations in the skull and can have an audible component due to the sonication pulses. To improve blinding, the state-of the art is to use a control area of sonication within a similar depth of the sonication, eliminating any confounders induced by the sonication. Recently, the Lateral Ventricles (LV) have been proposed as a safe gold standard control site in TUS studies, due to its absence of any neural tissue (34). However, as this control condition is yet to be extensively tested outside of the initial study, the authors suggest further validation in future studies using LV.

Feasibility of the approach

To date, only a small amount of studies have investigated NAcc function through TUS modulation. A recent study using fMRI showed that delivering TUS to NAcc can modulate brain activations and functional connectivity within the reward network (35). A pre-print using offline behavioural outcomes reported significant alterations in reward-related behaviours when using TUS to target NAcc, indicating an enhanced reward sensitivity (31). The TUS protocol used in this pre-print has also been reported to increase corticospinal excitability outlasting the sonication period (28,30), indicating potential plastic excitatory properties of the protocol.

Although not yet used in combination with TUS-NAcc, EEG offers excellent temporal resolution with the possibility to measure online change in subcortical activation (36). Furthermore, EEG is practical to employ in conjunction with psychological paradigms. One previous study observed a modulation of the reward related ERP positivity as an index of emotional reward function following stimulation (19), using functional connectivity to indirectly target NAcc via a cortical entry point with rTMS. In one of the few studies directly manipulating the NAcc with DBS, a flanker test was performed while measuring error-related negativity which decreased during DBS (16). As such, we have reasons to believe that EEG might be a feasible neurophysiological measure for TUS-NAcc.

Pupil dilation has been associated with uncertainty signaling, which is relevant for learning during decision making (37). Although mainly driven by noradrenaline rather than dopamine, uncertainty signalling and expectancy violation are often components of the psychological tasks assessing reward processes. For instance, the ERP component of P300, related to reward valence and expectancy outcome (38), shows a strong positive correlation with phasic

pupil dilation (39). Moreover, reduced pupillary responses have been associated with higher depressive symptom scores during both reward anticipation and feedback (40,41). Furthermore, pupil dilation can easily be measured in conjunction with such tasks. However, preliminary findings suggest no correlation between NAcc activation and pupil dilation (42). If replicated, this dissociation could provide valuable insights into the differential functional contributions of the NAcc.

Non-invasive brain stimulation offers a promising avenue for developing personalized interventions within a precision medicine framework (43). Given its novelty, individual predictors of TUS effects are still unknown. Drawing on insights from the broader NIBS literature, it is therefore essential to investigate individual variations that may predict NAcc function. Due to the complexity of brain networks, such work must consider factors ranging from transient brain states (44, 45) to structural and functional connectivity traits (46) and illness-related phenotypes (47,43). Evidence that functional connectivity and NAcc volume correlate with psychometric assessments of anhedonia (48, 13), and that anhedonia ratings might predict NIBS responsiveness in depression (49), suggests the plausibility that self-reported reward responsiveness may also be indicative of NAcc functions. Furthermore, specific EEG microstates have been identified as potential biomarkers for guiding personalized rTMS treatment in depression (50), making resting EEG a potential personalized predictor for TUS effects as well.

Introduction summary

In summary, NAcc is a central subcortical structure in the brain´s reward circuit, however our understanding of the exact role of NAcc in the reward network is still limited. Hence, NAcc is a promising target for reversible neuromodulation to study reward network effects. TUS is a novel technique enabling safe, direct, and selective targeting of subcortical structures for reversible neuromodulation. By modulating the NAcc with TUS in healthy participants, whilst at the same time monitoring individual variations at baseline, one can investigate the precise functions of NAcc's role in the reward circuit, providing knowledge which could potentially be used to develop novel treatments for several serious psychiatric disorders.

Project description

Aims

In this project we will use low-intensity TUS to investigate subcortical functions in the reward circuit. The aim is to investigate the role of NAcc in reward functions by brief modulation of the NAcc with TUS in healthy participants, using active LV sonication and inactive sham sonication as controls. We aim to do so by considering both behavioural and neurophysiological outcome measures, as well as investigating several potential predictors of NAcc functioning. In the long term we believe that using this technique to further our understanding of the healthy brain's reward system might pave the way for novel treatment methods for TRD and other psychiatric disorders.

Hypotheses

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One session of TUS-NAcc compared to TUS-LV and TUS-sham will modulate:

1. reward responsiveness as measured in a probabilistic reward learning task
2. the amplitude of the reward-related ERP component RewP measured in conjunction with a doors gambling task
3. microstates and aperiodic activity measured in resting EEG

We further hypothesize that the above effects will be predicted by:

4. baseline NAcc connectivity (rsfMRI)
5. resting state EEG biomarkers relating to baseline theta and alpha oscillations
6. self assessments of reward responsiveness using SHAPS

For explorative purposes we will also investigate how NAcc function is related to pupil dilation.

Inclusion criteria

18-60 years old and written informed consent to participation. All participants must provide written informed consent prior to participation.

Exclusion criteria

Contraindication to brain MRI, neurological disorders, any current or previous psychiatric or neurological disorders, current medication for a psychiatric disorder, any current or previous alcohol and/or substance abuse disorder, insufficient proficiency in Swedish, inability to sit straight in a chair for 2 hours (TUS administration) and pregnancy (current or planned in the near future).

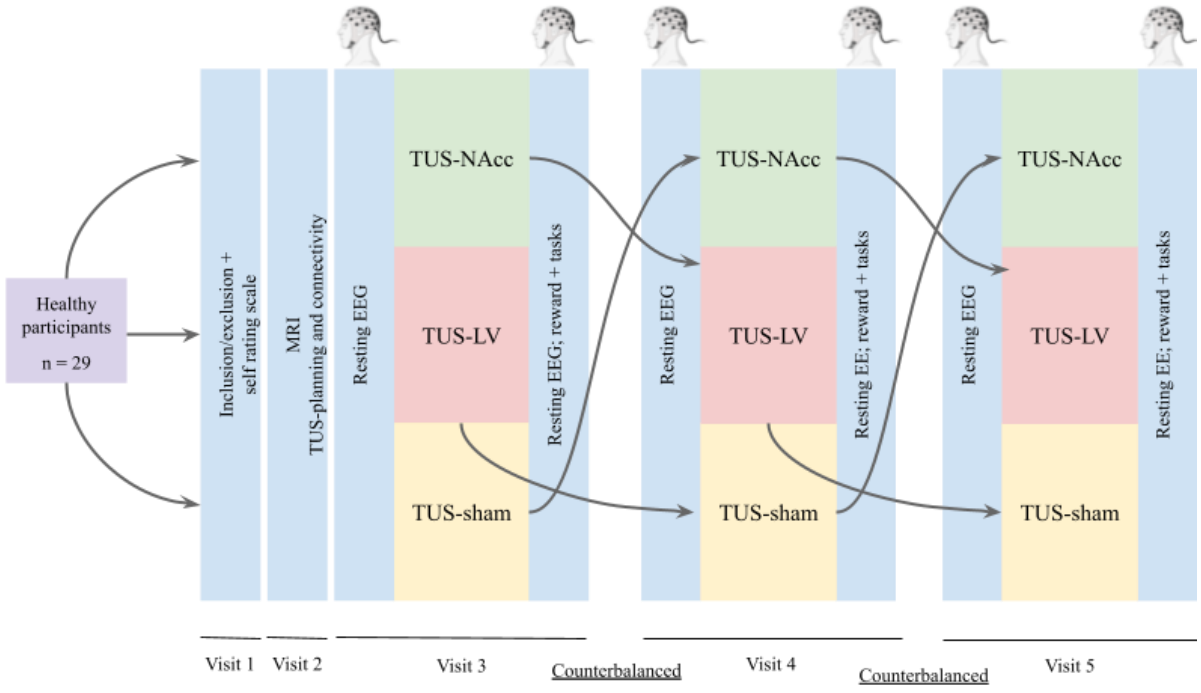
Sample size and recruitment

Available studies to inform a sample size calculation are limited, especially given the novel use of outcome measures in relation to TUS employed in the present study. Hence, our sample size calculation must be based on assumptions of analogies. Yaakub et al (31) achieved significant results ($p=0.011$, Cohen $D = 0.53$) on one of their behavioural outcome measures with 26 participants, using a similar TUS protocol. The paradigm used by Yaakub et al is different from ours, however similar in that it is also related to reward learning. Their neurophysiological (fMRI BOLD signal in the region of sonication) findings were also significant with ($p = 0.024$; Cohens $D = 0.47$) when comparing NAcc sonication with sham. In the current study, assuming similar effect sizes as Yaakub and compensating for potential attrition rate of 10%, would need to recruit 29 participants with acceptable data quality. The participants will primarily be recruited through an online research recruitment platform.

Design

This study utilizes a randomized experimental repeated measures design (see picture below for a visualization). Participants will receive bilateral, sequential TUS-NAcc, TUS-LV (active control) or TUS-sham (inactive control) in a randomized counterbalanced order one

week apart. TUS-sham is operationalized as placing the transducer on the participant's scalp without turning it on, mimicking the active conditions. It is primarily used for validation of the other control condition, TUS-LV, as the LV is a novel and relatively untested control stimulation site. All three conditions are treated equally in the subsequent data analysis. The procedures following the administration will remain identical between the three sessions and the intervention will be double-blinded. To ensure double-blinding, preparations of the neuro navigation software and calibration of the TUS sonication parameters is done by a separate person than the one administering TUS. TUS administrator will state his/her guess for active/control before the participant is asked the same question. It will include a total of five visits.



Procedure

Visit 1 - Inclusion/ exclusion

Participants have received information of the research project prior to the first visit. During the first visit, the information is repeated manually to the participants and they are asked to provide written consent if they remain interested in being part of the study. Participants will be given the opportunity to postpone consent up to two weeks after the first visit, which could change the total number of visits to 6. If the participants consent, a structured diagnostic interview (MINI) will be conducted along with contraindications for MRI. A self-assessment of anhedonic symptoms is administered. If the participants pass the exclusion criteria, dates for the upcoming visits are scheduled. The visit will take up to 2 hours.

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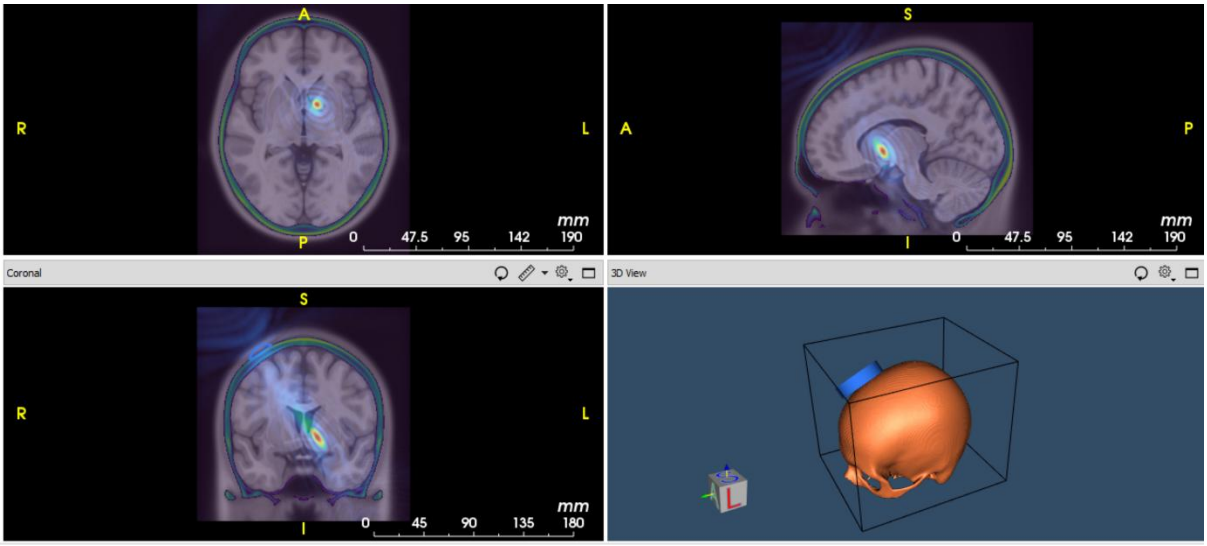
Visit 2 – MR:

Neuroimaging using MRI will be conducted at baseline. The protocol consists of anatomical T1- and T2-weighted images, which are necessary for planning the transducer placement and perform acoustic simulations prior to the administration of TUS. Additional MRI measurements include resting-state functional MRI and diffusion weighted imaging to assess structural and functional connectivity of the nucleus accumbens and its associated reward network, as well as MR spectroscopy with a sequences that allows for the simultaneous measurement of gamma-aminobutyric acid (GABA) and glutamate within the reward network. Total scanning time is approximately 1 hour.

Targeting and simulation procedures

To plan the current intervention with TUS-NAcc we run simulations of how the cigar-shaped TUS-beam can be positioned within current safety limits (see picture below of left NAcc targeting). The MR imaging of the skull bone is used to take into account the attenuation and distortion of the ultrasound as well as scalp heating. MR techniques to visualize the mechanical focus of the ultrasound have previously been used to validate the simulations. The simulations produce several potential target specific entry points for each sonication. The final entry points are selected based on several factors; shortest distance from skull to target, least tangential angle between transducer and scalp and highest possible simulated ISPPA (intensity spatial peak average) whilst maintaining a safe temperature change in the brain and a MI below 1.9, as stipulated by the ITRUSST safety limits (26,30). Simulations are conducted individually for each participant. A total of two entry points are produced for each active condition (TUS-LV and TUS-NAcc) and hemisphere, giving room for eventual practical limitations of transducer placement during the sessions. The TUS-sham will borrow the entry points from one of the other conditions using a randomization script.



Visit 3-5 – TUS administration

The participant is seated in a chair in front of a computer screen and given instructions of the procedure. The procedure will start by measuring resting-state EEG for 8 minutes. TUS preparations and procedure is then carried out in concordance with practical recommendations (51) and include eliminating the risk of air bubbles forming by applying a coupling agent (gel) between scalp and transducer. TUS sonication is active during 2 minutes, giving 4 minutes of total bilateral sonication time (see TUS protocol further down).

Post TUS administration, another resting-EEG is conducted identical to the pre-sonication measurement. The EEG cap stays on as the participant is given instructions on the screen of the forthcoming reward-related computer tasks. The participant will perform two tasks, measuring both reward-related behaviour and neural underpinnings of reward response, for a duration of 30 minutes. The tasks include a monetary reward which is handed out to participants at the end of the study, together with the monetary compensation. All participants receive the same amount of total compensation. The session ends with a registration in the neuro-navigation software of electrode placement on the head. One session will take approximately two hours in total.

Measurements

Symptom ratings and other scales:

- Mini-International Neuropsychiatric Interview (M.I.N.I.) – clinical screening of psychiatric disorders.
- Snaith-Hamilton Pleasure Scale (SHAPS), a 14-item scale that measures anhedonia. Items cover the domains of: social interaction, food and drink, sensory experience, and interest/pastimes (52). The scale will be used to explore how individual variation of anhedonia might predict primary outcome measures.
- Positive Valence Systems Scale, 21 items (Swedish version) (PVSS-21) (53). Used for measuring the RDoC domain Positive Valence System through a wide range of responses to reward.
- For tracking and follow up of eventual side effects of the procedure, a translated version of a TUS safety report is filled by the participant at the end of every TUS session (31).
- AUDIT
- DUDIT
- Sociodemographic information

Neurophysiological measurements:

- Resting-EEG, measuring EEG microstates, connectivity and spectral analysis, specifically analysing theta power, individual alpha band frequency and aperiodic activity. Used as a primary outcome measure (pre vs post sonication) as well as for finding predictors of individual ERP and PRT results. Can also be used for a secondary analysis on source localisation.
- The event related potential (ERP) component Reward Positivity (RewP), related to immediate reward response. RewP is measured with EEG in conjunction with The Doors Task, a gambling task where the participant receives random monetary

feedback of loss or win following a choice between one of two "doors" to open on the screen. (54)

- Pupil dilation will be measured using a Tobii Pro Spectrum eye tracker, for exploring the relationship between NAcc function and pupil dilation.

MR protocol (total scanning time: 1h):

- T1W for generating a Pseudo-CT for acoustic simulations
- T2W + T1W for skull segmentation for planning transducer placement
- DWI for structural connectivity
- fMRI (resting state) for functional connectivity
- Reward task-fMRI for probing reward network function
- MEGA-PRESS over dACC for assessing GABA and glutamate levels
- Field map estimations for DWI and fMRI for distortion correction

Behavioural measures:

- The probabilistic reward task (PRT), originally developed as a way to objectively characterise the anhedonia phenotype of depression (55), will measure the RDoC subconstruct of probabilistic and reinforcement learning. It measures response bias towards a more frequently rewarded stimuli compared to a less frequently rewarded stimulus.

TUS Protocol

The active TUS sonication protocol in use is a slightly improved version of the 5Hz-protocol from Zeng et al, described in the introduction. It is well within the suggested safety limits from iTRUSST (26,30): 120-second train of 30-millisecond pulses of ultrasound (0.5MHz) with a ramp up/-down period of 10ms, repeated every 200 milliseconds (5Hz, 600 pulses) with a duty cycle of 10%, and a target free-field intensity of 30W/cm^2.

Analysis plan

Preprocessing of EEG data will be performed using established EEGLab pipelines optimized for resting EEG and ERP analysis respectively. ERP analysis is conducted using the EEGLab and ERPLab toolboxes in MATLAB, with a time window of 250-350 ms for RewP analysis. FOOOF (for separating periodic and aperiodic spectra) and LORETA (algorithm for deducing source localization) is used for the resting EEG. Pre-processing of rsfMRI data will be performed with the fMRIPrep pipeline and further denoising and calculation of connectivity indices using XCP-D. DTI data will undergo pre-processing and reconstruction, including calculation of connectomes, using QSIPrep. Group level modeling of functional connectivity will be performed in the conn toolbox or equivalent software and modelling of both functional and structural connectomes using network-based statistics. MRS data will be pre-processed and modelled in Osprey, a Matlab toolbox. GABA and glutamate concentrations will be derived from water referenced spectra. Response bias and accuracy from the Probabilistic Reward Task is calculated with a MATLAB script. A repeated measures ANOVA followed by post-hoc t-tests will be conducted for the PRT data. A paired

samples t-test will be used for between-condition comparisons for the ERP and resting-state data.

Time plan and implementation

Inclusion of participants will commence immediately upon receiving ethical approval, with a target sample size of 29 participants. Data collection will proceed continuously and, together with recruitment, is expected to be concluded by the end of 2026. Data analysis and manuscript preparation will begin shortly thereafter, with the aim of submitting the paper for publication by the beginning of 2027.

Project organization

The principal investigator is professor Robert Bodén. Associate professor Jonas Persson will oversee the neurophysiology investigations, and design the neuroimaging protocols. Jonas Persson will also perform most of the pre-processing and analyses of the data, and act as a project leader for the neuroimaging part of the project. A PhD student, Olle Lundin, will be in charge of project management and conducting the study. A psychiatric research nurse will aid in administering the treatments and the neurophysiological investigations.

We also have collaborators from adjacent disciplines serving as advisory board: professor Dag Nyholm, neurology, associate professor Elham Rostami, neurosurgery, and associate professor David Fällmar, neuroradiology. The latter will also give advice on technical aspects of the MRI parts and is also responsible for screening of the morphological brain scans for potential pathology prior to TUS.

Research group prior experience and competence

Our psychiatric brain stimulation research group has been performing studies on anhedonia and TRD using non-invasive brain modulation techniques such as rTMS and ECT since almost a decade. We have conducted a sham-controlled RCT funded by the Swedish Research Council where we found that dorsomedial prefrontal rTMS was effective in alleviating anhedonia and related symptoms in patients with depression, but not in schizophrenia (56). We have also contributed to the current understanding of the neurobiology of anhedonia and the mechanisms of rTMS by combining different neuroimaging modalities and neurophysiological techniques including rsfMRI, task-based MRI, diffusion-tensor imaging (DTI), magnetic resonance spectroscopy (MRS), positron

emission tomography (PET), functional near-infrared spectroscopy (fNIRS), and EEG (57–59).

In several of our rTMS studies we have been using an advanced infrared neuronavigation system which allows precise targeting with rTMS and we now use the very same equipment for targeting with TUS. As several of our research staff are highly skilled in performing neuro-navigated rTMS, the transition from rTMS to TUS during the recent months of pilot testing of the technique has been smooth. During the pilot testing we successfully sonicated ourselves within the research group with NAcc and LV TUS, while performing the neurophysiological examinations described above within stipulated time-frame to capture the effects (starting 15-20 minutes post sonication), with good EEG-data quality, and excellent tolerability, proving the feasibility of the approach.

Being the first team in Sweden to adopt low-intensity focused TUS, we have established a collaboration with several of the world-leading TUS-labs that performs stimulation of subcortical structures in humans. For example, the team at the Donders Institute in the Netherlands lead by Lennart Verhagen, has been key in planning the TUS-intervention in the current project. His lab has preliminary data showing that it is possible and feasible to modulate fear conditioning by applying a single TUS targeting the amygdala (32). Other established collaborations with front-line researchers in this field include: Maximillian Lueckel, in the Bergmann Neurostimulation Group, Leibniz Institute for Resilience Research (LIR), Mainz, Germany, and Keith Murphy, at the Department of Psychiatry and Behavioral Sciences, Stanford, USA. Furthermore, several members of our team have participated in international TUS workshops focused on the practical administration of TUS.

Our team recently acquired all necessary equipment to conduct TUS research: a cutting-edge TUS device, a NeuroFUS Pro 2.0, including a 500 DPX transducer capable of reaching a focal depth of 36-148mm, a neuronavigation equipment (Localite) for accurate positioning of the transducers and the software K-plan for simulations and treatment planning for each participant. During recent years we have acquired the necessary equipment and skill set to measure 32-channel EEG. We also have access to a 3T scanner for MRI through the Academic Hospital in Uppsala.

Significance

Thus far only two studies have investigated reward function using TUS-NAcc. As such, this will be one of the first studies applying TUS-NAcc in the world and the first to investigate the technique in general in Sweden. As the core pathology underlying most severe psychiatric disorders include subcortical brain structures this means that TUS has potential as a novel treatment in an array of other psychiatric disorders with poor current treatment options such as addiction, negative symptoms in schizophrenia, OCD, and eating disorders including obesity, to name a few. In an even wider context this study may also pave the way for future

treatment studies of other brain disorders with subcortical pathology such as tremor, Parkinson, disorders of consciousness, traumatic brain injury rehabilitation and chronic pain.

In conclusion, study healthy reward function by directly modulating the NAcc with TUS is vital in the pursuit of mapping the neural reward network and developing novel treatments for TRD as well as other disorders. In a wider perspective this study will be pivotal to show the potential of direct modulation with low-intensity focused TUS for the study of subcortical human brain functions.

Ethical considerations

Although in its early phases, TUS is considered safe with only mild side-effects being observed in about 3.4% of healthy subjects and clinical patients (60). These effects include perceived headache, deterioration of well-being, pain and painless sensations of pressure on the skull. However, similar side effects have also occurred as a consequence of placebo treatment. All these effects have been temporary. The safety limits stipulated by ITRUSST will be strictly adhered to. These limits remain well within margins of safety, far from levels that could be considered hazardous. Given the low risks associated with the intervention for the participants, combined with the potential scientific value of this project, we consider the interventions planned in this project ethically justifiable.

References

1. GBD 2019 Diseases and Injuries Collaborators (2020) Lancet, 396, 1204–1222.

2. Rush, A. J. et al. (2006) Am J Psychiatry, 163, 1905–17.

3. Ekman, C. J. et al. (2023) Journal of Affective Disorders, 329, 50–54.

4. Brus, O. et al. (2017) The Journal of ECT, 33, 96.

5. Cole, S. L. et al. (2018) PLoS One, 13, e0207694.

6. Cummings, J. L. (1993) Arch Neurol, 50, 873–880.

7. Gallo, E. F. et al. (2018) Nat Commun, 9, 1086.

8. Nicola, S. M. et al. (2005) Neuroscience, 135, 1025–1033.

9. Knutson, B. et al. (2001) J. Neurosci., 21, RC159–RC159.

10. Russo, S. J. and Nestler, E. J. (2013) Nat Rev Neurosci, 14, 609–25.

11. Wade, B. S. C. et al. (2016) Neuropsychopharmacol, 41, 2481–2491.

12. Connolly, M. E. et al. (2015) Journal of Psychiatric Research, 68, 384–391.

13. Liu, R. et al. (2021) Neuroimage Clin, 30, 102599.

14. Hou, Z. et al. (2018) Brain Imaging Behav, 12, 1042–1052.

15. Bewernick, B. H. et al. (2010) Biological Psychiatry, 67, 110–116.

16. Sildatke, E. et al. (2022) Neuromodulation, 25, 245–252.

17. Van Cauwenberge, M. G. A. et al. (2021) Transl Psychiatry, 11, 1–10.

18. Wang, X. et al. (2021) Depression and Anxiety, n/a.

19. Ryan, J. et al. (2022) Biological Psychiatry, 7, 833–840.

20. Darmani, G. et al. (2022) Clinical Neurophysiology, 135, 51–73.

21. Ridone, P. et al. (2019) Biophys Rev, 11, 795–805.

22. Qin, P. P. et al. (2024) Neuroscience & Biobehavioral Reviews, 156, 105501.

23. Stern, J. M. et al. (2021) Brain Stimulation, 14, 1022–1031.

24. Bubrick, E. J. et al. (2024) Brain Stimulation, 17, 7–9.

25. Mahoney, J. J. et al. (2023) Front Psychiatry, 14, 1211566.

26. Aubry, J.-F. et al. ITRUSST Consensus on Biophysical Safety for Transcranial Ultrasonic Stimulation 2023.

27. Samuel, N. et al. (2022) Brain Stimulation, 15, 1337–1347.

28. Zeng, K. et al. (2022) Annals of Neurology, 91, 238–252.

29. Zhang, M.-F. et al. (2022) Frontiers in Neurology, 13.

30. Zeng, K. et al. (2024) Brain Stimulation, 17, 258–268.

31. Yaakub, S. N. et al. (2023) Nat Commun, 14, 5318.

32. Carpino, E. et al. London, 2023.

33. Folloni, D. et al. (2019) Neuron, 101, 1109–1116.e5.

34. Atkinson-Clement, C. et al. (2024) Brain Stimulation, 17, 1328–1330.

35. Peng, X. et al. (2024) Front Hum Neurosci, 18, 1359396.

36. Seeber, M. et al. (2019) Nat Commun, 10, 753.

37. Lavín, C. et al. (2014) Front Behav Neurosci, 7, 218.

38. Wu, Y. et al. (2009) Brain Res, 1286, 114–122.

39. Menicucci, D. et al. (2024) Psychophysiology, 61, e14550.

40. Schneider, M. et al. (2020) Brain Sci, 10, 906.

41. Guath, M. et al. (2023) Behav Brain Res, 436, 114060.

42. Brendler, A. et al. (2024) Sci Rep, 14, 344.
43. Padberg, F. et al. (2021) Exp Neurol, 341, 113713.
44. Sack, A. T. et al. (2024) Biol Psychiatry, 95, 536–544.
45. Bradley, C. et al. (2022) Nat Rev Neurosci, 23, 459–475.
46. Silvanto, J. et al. (2008) Trends Cogn Sci, 12, 447–454.
47. Drysdale, A. T. et al. (2017) Nat Med, 23, 28–38.
48. Auerbach, R. P. et al. (2017) Neuropsychopharmacol, 42, 2087–2095.
49. Rezaei, M. et al. (2023) Journal of Affective Disorders, 339, 756–762.
50. Che, Q. et al. (2025) Behav Brain Res, 483, 115463.
51. Murphy, K. R. et al. (2025) Clin Neurophysiol, 171, 192–226.
52. Snaith, R. P. et al. (1995) Br J Psychiatry, 167, 99–103.
53. Khazanov, G. K. et al. (2020) Assessment, 27, 1045–1069.
54. Proudfit, G. H. (2015) Psychophysiol, 52, 449–459.
55. Pizzagalli, D. A. et al. (2005) Biol Psychiatry, 57, 319–327.
56. Bodén, R. et al. (2021) Journal of Affective Disorders, 290, 308–315.
57. Struckmann, W. et al. (2022) Neuroimage Clin, 34, 103028.
58. Persson, J. et al. (2020) Behav Brain Res, 394, 112834.
59. Persson, J. et al. (2021) Psychiatry Research: Neuroimaging, 315, 111327.
60. Sarica, C. et al. (2022) Brain Stimulation, 15, 737–746.