i. Cover Sheet:

Title of Project: Modulating repetitive negative thinking related brain networks in young adults with depression

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
Inclusion and exclusion	Expanded age range from 18 to 35 (previously from 18 to 25). Eliminated the inclusion criteria of moderate depression (QIDS ≥ 11) Eliminated the exclusion criteria of use of antipsychotic medication	To broaden the inclusion criteria and enhance the recruitment process
Trial design	Added the option to conduct the baseline visit prior to Visit 1, and included a one-month follow-up.	To reduce the time burden of Visit 1, we split it into two separate visits. A one-month follow-up has been added to better understand and monitor participants' symptom changes over a longer term.

Trial design	Changed from using the PANAS-X (extended version) to the I-PANAS-SF (short form version). Added a one-month follow-up survey (original to the study).	To reduce the time burden to answer extended version. To better understand and monitor participants' symptom changes over a longer term.
Trial design	Adjusted of participants compensation	In response to the above changes and to fairly compensate participants for their commitment.
Screening	Added study-specific recruitment materials.	To enhance the recruitment process.

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A. Purpose of the study

Young adult mental health is crucial, as 1 in 10 young adults (ages 18-25) suffer from major depressive disorder (MDD) (Czeisler et al., 2020; Giedd et al., 1999; Paus et al., 2008), impacting long-term outcomes such as comorbid mental disorders, unemployment and suicide (Arnett, 2014). With increasing rates of MDD among young adults (Twenge et al., 2019), new explanatory disease models focusing on targetable disease-modifying processes (DMPs) are needed. Repetitive negative thinking (RNT) is a key DMP in MDD, involving difficulty controlling distressing thoughts and past events (Ehring & Watkins, 2008), and predicting depression severity and suicidal ideation/attempts in early adulthood (Krajniak et al., 2013; Law & Tucker, 2018; Rogers & Joiner, 2018; Surrence et al., 2009; Watkins & Roberts, 2020). Current treatments, such as medication and psychotherapy including cognitive-behavioral therapy (CBT), often have limited effectiveness for MDD (Cain, 2007), with higher levels of RNT associated with slower and poorer response rates (Jones et al., 2008; Schmaling et al., 2002). In theory, novel treatments designed to modify the neurobiological mechanisms underlying RNT could facilitate recovery in MDD. Real-time functional Magnetic Resonance Imaging neurofeedback (rtfMRI-nf) is a non-invasive method, providing individuals with feedback concerning their brain activity in order to facilitate one's self-control.

Our preliminary studies identified a circuit with stronger connectivity between the right anterior insular (rAI, emotional salience processing hub) and the right superior temporal sulcus (rSTS, episodic memory/language processing area), correlating with the severity of RNT apart from depression (Tsuchiyagaito, Sanchez, et al., 2022). This finding aligns with the framework that views RNT as heightened evaluative and dialogic inner speech, resulting from an inability to disengage from self-critical and threatening interpretations of episodic memories. We propose that excessive functional connectivity between the rAI and STS may contribute to RNT in young adults with MDD.

This project is a research project of CoBRE phase II at the Laureate Institute for Brain Research (LIBR), where participants will complete the Neuroscience-Based Mental Health Assessment and Prediction (NeuroMAP) core protocol (LIBR protocol #2018-010-00, PI: Martin Paulus) as a part of CoBRE. In this project, we use rtfMRI-nf to causally relate dysfunction of rAI-rSTS connectivity with the intensity of RNT. We hypothesize that rtfMRI-nf reducing rAI-rSTS connectivity would reduce RNT. We propose a randomized double-blind, sham-controlled trial of rtfMRI-nf with 110 young adults (n=55/arm) with MDD and high trait-RNT levels. Primary outcome will be active vs. sham rtfMRI-nf's effect on rAI-rSTS connectivity. Secondary outcomes will be active vs. sham rtfMRI-nf's effect on state-RNT (Brief State Rumination Inventory, BSRI) and depression severity (Montgomery-Asberg Depression Rating Scale, MADRS). Exploratory outcomes will be the relationship between reducing rAI-rSTS connectivity and BSRI scores. Participants will perform a self-regulation task involving neurofeedback, receiving either real-time feedback on rAI-rSTS connectivity (active group) or artificial feedback unrelated to rAI-rSTS connectivity (sham group). We will collect data across two visits, one week apart. The first visit will include five runs of the self-regulation task, the middle three containing the neurofeedback condition (Visit 1). The second visit will include one run of the self-regulation task without the neurofeedback condition 1-week later (Visit 2). The primary analysis is to investigate acute changes in rAI-rSTS connectivity (Visit 1), while the secondary analyses evaluate acute effects on RNT and sub-acute effects on depression 1-week later (Visit 2).

Aim 1 (Primary Outcome): Assess active vs. sham rtfMRI-nf's acute effect on rAI-rSTS connectivity (Visit 1).

Hypothesis: Active rtfMRI-nf will yield a greater reduction in rAI-rSTS connectivity compared to sham.

Aim 2 (Secondary Outcomes): Evaluate active vs. sham rtfMRI-nf's acute effect on RNT (Visit 1) and sub-acute effect on depression (Visit 2).

Hypotheses: Active rtfMRI-nf will lead to a) an immediate RNT reduction and b) reduced depression 1-week post-rtfMRI-nf.

Aim 3 (Exploratory Aim): Investigate if acute modulation of rAI-rSTS connectivity (Visit 1) results in acute changes in RNT (Visit 1) and sub-acute changes in depression and rAI-rSTS connectivity 1-week post-rtfMRI-nf (Visit 2).

Exploratory hypotheses: Greater reduction in rAI-rSTS connectivity at Visit 1 will correlate with a) immediate RNT reduction, b) decreased depression, and c) reduced rAI-rSTS connectivity 1-week later (Visit 2).

B. Background and Significance

B1. Importance of young adult mental health

About 1 in 10 young adults (ages 18-25) (Czeisler et al., 2020; Giedd et al., 1999; Paus et al., 2008) suffer from major depressive disorder (MDD), which if not effectively treated, has serious effects on long-term outcomes such as comorbid mental disorders, unemployment and suicide (Arnett, 2014). Rates of major depressive episodes increased 52% (2005–2017) among adolescents aged 12 to 17 and 63% (2009–2017) among young adults aged 18 to 25, with less consistent and weaker increases among adults ages 26 and over (Twenge et al., 2019). To develop new explanatory disease models for young adults with MDD, it will be important to delineate targetable disease-modifying processes (DMP), i.e., processes based on circuitry, behavior, or other levels of analysis, which, when modulated, change the risk for, the severity of, or the recurrence of a disease.

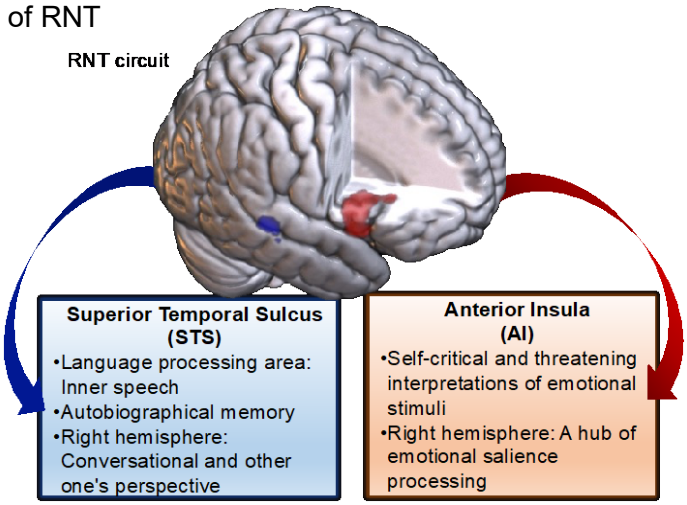
B2. Targetable disease-modifying process, Repetitive Negative Thinking (RNT) brain circuit.

Repetitive negative thinking (RNT), i.e., the difficulty controlling recurrent distressing personal thoughts and past events (Ehring & Watkins, 2008), is a significant predictor of the severity of depression and suicidal ideation/attempts in early adulthood (Krajniak et al., 2013; Law & Tucker, 2018; Rogers & Joiner, 2018; Surrence et al., 2009; Watkins & Roberts, 2020). Although effective medication and psychotherapy treatments for MDD have been established, nearly two-thirds of patients will not respond (Cain, 2007), and increased RNT is linked to a slower and poorer response to both antidepressant medication and cognitive-behavioral therapy (CBT) (Jones et al., 2008; Schmaling et al., 2002). In theory, novel treatments designed to modify the neurobiological mechanisms underlying RNT could facilitate recovery in MDD. Real-time functional Magnetic Resonance Imaging neurofeedback (rtfMRI-nf) is an ideal non-invasive method for providing individuals with feedback concerning their brain activity in order to facilitate one’s self-control.

B3. Gaps in our knowledge and technical hurdles we will overcome in this proposal.

There are critical gaps in our knowledge and technical hurdles that may explain poor treatment response. First, the neural basis of RNT is still not well understood. Functional connectivity patterns associated with RNT in MDD are inconsistent. For example, some fMRI studies showed that increased recruitment of the default mode network (DMN) is linked to the severity of RNT; however, recent meta-analyses reported slightly reduced DMN activity in depression and RNT(Tozzi et al., 2021; Yan et al., 2019). Second, it is not clear whether neurobiological features of RNT reported in prior studies are specific to RNT or related to the general severity of depression or anxiety since these phenotypes are highly correlated. Third, it is not clear whether the brain processes that support RNT are also ideal targets for interventions. Even if we identify brain activity/circuits associated with levels of RNT, it does not necessarily mean that reversing the RNT-associated brain processes would improve RNT. To overcome these gaps, we conducted a voxel-wise search to identify an RNT-specific circuit after controlling for depression/anxiety severity and related variables (**C. Preliminary Data**). We found that stronger connectivity between the right anterior insular (rAI) and the right superior temporal sulcus (rSTS) was highly correlated with the severity of RNT measured by the Ruminative Response Scale, brooding score (RRS-B) (Tsuchiyagaito, Sanchez, et al., 2022)(**Fig 1**). In this proposal, we use rtfMRI-nf to causally relate dysfunction of rAI-rSTS connectivity with the intensity of RNT.

Fig 1. Conceptual framework of neural mechanism of RNT



B4. RtfMRI-nf is a targeted and robust experimental manipulation.

Most studies have utilized region-of-interest (ROI) based techniques where feedback from the activity in a single ROI as the training target. These investigations have demonstrated that self-regulation of target brain activity is possible (Zotev et al., 2011), and concomitant symptom relief can be observed (Scheinost et al., 2013; Young et al., 2016). For example, in a recent, randomized, sham-controlled clinical trial (RCT) in participants with MDD showed greater left amygdala activity and significant symptom reduction in the active rtfMRI-nf group compared to the sham group (Young et al., 2016). Recently, there has been a shift towards functional connectivity-based techniques, with early findings indicating the potential self-regulation in functional connectivity accompanied by symptom changes (Bauer et al., 2020; Morgenroth et al., 2020; Zhao et al., 2019). Connectivity-based rtfMRI-nf holds significant potential, particularly for developing transdiagnostic treatments addressing certain neural circuits associated with specific DMP (e.g., dysfunction of a particular network, coupled with deficits in self-referential processing represented by RNT) (Berman et al., 2011b; Hamilton et al., 2015; Jacob et al., 2020; Jacobs et al., 2014; Rosenbaum et al., 2017; Zhou et al., 2020; Zhu et al., 2017). rtfMRI-nf is not only a promising therapeutic approach but can be employed as a powerful, experimental tool to target particular neural systems with a high-degree of specificity.

B5. Strength of the scientific rigor utilizing the sham condition.

Most rtfMRI-nf studies have utilized a sham-control from an alternative (ROI) (Mehler et al., 2018; Young et al., 2017; Young et al., 2014; Zotev et al., 2011; Zotev et al., 2018; Zotev et al., 2013); however, they did not control the sense of achievement experienced by participants when they believed they were controlling their brain activity. In fact, studies showed that a general sense of achievement during rtfMRI-nf was correlated with symptom changes(Mehler et al., 2018). To delineate the neurobiological mechanisms underlying symptom changes caused by rtfMRI-nf, it is necessary to equalize the sense of achievement experienced across active and sham-control groups. Thus, this project uses simulated feedback from the active group data to match the feeling of achievement across groups. Simulated feedback will be generated from the probabilities of positive

feedback from the active group with Bayesian probabilities, and will be used for the sham group. This sham condition has been successfully employed in our prior rtfMRI-nf study (Tsuchiyagaito et al., 2021).

C. Innovation

This project is innovative in several aspects:

1. Our approach targets functional connectivity using real-time fMRI with advanced processing methods.
2. It focuses on RNT as a specific, transdiagnostic psychological phenomenon that is closely linked to neural circuitry compared to broader constructs like depression symptomatology.
3. We focus on young adults, a population at risk for depression, where modulation of neural circuitry may have long-lasting benefits due to brain development.
4. It uses sham conditions that control not only context but also the impact on reward experiences or self-efficacy.
5. By evaluating symptom and brain changes in a randomized controlled design, the project will establish the causal role of rAI-rSTS connectivity and lay the groundwork for future grant applications.

D. Preliminary Data

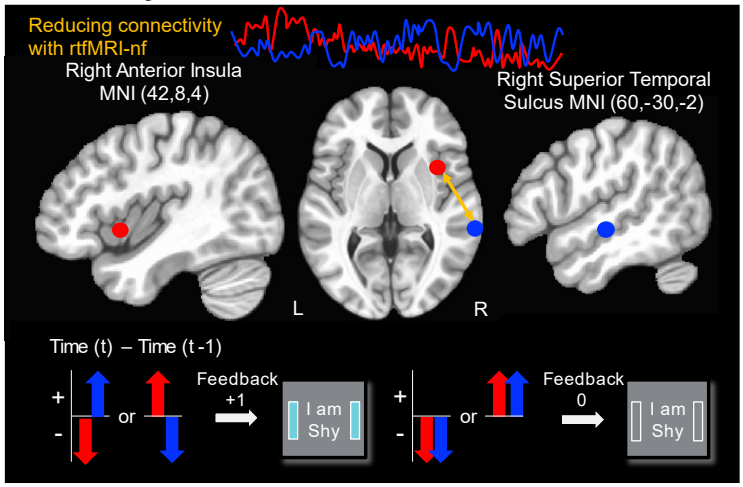
D1. RNT-Specific Target Connectivity for rtfMRI-nf.

We identified a pattern of connectivity linked to RNT in individuals with MDD in resting-state fMRI dataset (Kuplicki et al., 2021; Tsuchiyagaito, Sanchez, et al., 2022). Using a propensity score matching approach, we matched high and low RNT groups (n=50/group) on the severity of depression, anxiety, and demographic characteristics and conducted a whole-brain voxel-wise connectivity pattern analysis (Anteraper et al., 2020; Anteraper et al., 2019; Byun et al., 2021; Guell et al., 2020; Kazumata et al., 2017; Morris et al., 2021; Muehlhan et al., 2020; Takamiya et al., 2021; Wang et al., 2019; Westfall et al., 2020; Whitfield-Gabrieli et al., 2016). We found that individuals with high relative to low RNT showed stronger connectivity between areas involved in emotional salience (right anterior insula, rAI) and memory/semantic processing (right superior temporal sulcus, rSTS) regardless of depression or anxiety severity (d = 1.19). This finding aligns with the framework that conceptualizes RNT as increased evaluative and dialogic inner speech resulting from the inability to disengage from self-critical and threatening interpretations of episodic memories (**Fig 1**). Therefore, rAI-rSTS connectivity is a plausible candidate for a circuit underlying RNT and a promising target for rtfMRI-nf. This study aims to test whether modulating rAI-rSTS connectivity can reduce RNT in the context of MDD.

D2. Developing Functional Connectivity-based rtfMRI-nf.

One challenge in connectivity-based rtfMRI-nf is to calculate online connectivity with adequate noise suppression while providing timely feedback. We optimized the number of time points to calculate connectivity using a two-point method (Ramot et al., 2017), based on a simulation analysis (n=223, mood and anxiety disorder) (Misaki et al., 2020). The two-point method calculates a signal change direction in a consecutive two-time point window and compares the change in direction of the two target ROIs as a proxy measure of online connectivity. The feedback signal is calculated as a binary value. When the BOLD signals from ROI1 and ROI2 move in different directions (i.e., ROI1 BOLD signal change increases but ROI2 BOLD signal change decreases, or vice versa, see example in **Fig 2**), participants see blue bars displayed on both sides of a negative self-referential word stimulus (**Fig 2**) as positive neurofeedback (coded as +1). When ROI1 and ROI2 BOLD signals change in the same direction, participants see blank sidebars, indicating the absence of feedback (coded as 0). The two-point method can provide time-sensitive feedback for the training (Misaki et al., 2020).

Fig 2. Two-point method to calculate functional connectivity in real-time



E. Research Design and Methods

E1. Screening

Participants will be recruited, screened and enrolled from the community at the Laureate Institute for Brain Research (LIBR) using the Screening Evaluation for Patients with Neuropsychiatric and Substance Use Disorders and Healthy Controls protocol (LIBR protocol #2020-001-03, PI: Martin Paulus). This protocol allows for careful screening of potential research participants to determine eligibility for inclusion in research protocols at LIBR. Screening evaluation may include psychiatric interview, diagnostic interview, questionnaires, rating scales, medical history, physical exam, substance use testing, and request for medical records. After screening, participants will either be offered participation in a research protocol or deemed unsuitable for LIBR research and referred back into the community. The screening protocol serves as an entry point for individuals with psychiatric disorders or healthy volunteers to enter IRB-approved LIBR protocols, including the current protocol. Participants' data will be saved in the LIBR database, and they will be contacted for future studies as long as they provide an agreement during the screen. Additionally, participants will be recruited through study-specific radio and Facebook advertisements. The study-specific advertisement materials are provided with this protocol.

E2. Baseline assessment

Before participating in the proposed rtfMRI-nf study (CoBRE, research project), participants will complete the Neuroscience-Based Mental Health Assessment and Prediction (NeuroMAP) core protocol (LIBR protocol #2018-010-00, PI: Martin Paulus) as a part of CoBRE Core. This evaluates the baseline assessment including behavioral testing, neuroimaging and biomarker testing. Participants who complete CoBRE Core and meet criteria for the current study will be invited to participate. Potential subjects will be screened by trained study staff or the PI to determine eligibility, such as the absence of any major medical or psychiatric illness that would preclude the use of rtfMRI-nf and or MRI.

E3. Participants

Detailed inclusion and exclusion criteria are described in **I. Human Participants section**. The presence of a current major depressive episode will be assessed using the Mini International Neuropsychiatric Interview (MINI). Participants between 18 to 35 years old, with high trait-RNT (Ruminative Response Scale, brooding score: RRS-B ≥ 13 (Nolen-Hoeksema & Morrow, 1991; Shihata et al., 2021)) will be included in this study. If more than one month elapses between the baseline assessment and the rtfMRI-nf session, participants will complete the MINI, QIDS-SR, and RRS again at an additional reassessment session.

Rationale for inclusion and exclusion. Depression and RNT severity: All MDD participants will have at least RRS-B ≥ 13 (Nolen-Hoeksema & Morrow, 1991; Shihata et al., 2021)) so that they are not already at the low end of RNT when enrolled in the study. The RRS-B is a self-rated measure collected in the CoBRE Core. Treatment history: To study brain functioning, it is ideal to recruit participants who are naïve to psychotherapy or medication-free; however, our target population is individuals with moderate depression and RNT, who are generally treatment-resistant or display recurrent illness with histories of pharmacotherapy and/or psychotherapy use (Conway et al., 2017). Thus, limiting participants to those who are unmedicated and treatment-naïve will make recruitment infeasible and will reduce the generalization of our results. Comorbidity: Anxiety disorders are very common in MDD with RNT (McLaughlin & Nolen-Hoeksema, 2011). Thus, to enhance recruitment feasibility and the generalizability of findings, current/lifetime diagnoses of anxiety disorders will be allowed. We will characterize the other symptoms that co-occur with MDD, allowing us to explore the specificity of RNT to MDD vs. MDD comorbid with anxiety. Overall, these inclusion criteria will ensure that our preliminary data will inform future transdiagnostic studies in real world populations with MDD, who are not unmedicated or treatment-naïve and commonly present with other anxiety disorders.

E4. Consent

Each preliminarily eligible participant will be scheduled for a remote or in-person meeting. The purpose, content, duration, and expected risks and benefits of the study will be reviewed with each potential subject by the trained study staff. If they pass the screening, written consent will also be obtained before subjects can participate in any portion of the study. The actual consent form will either be given to the potential participant by mail, email or in-person, depending on their preference. Subjects will be encouraged to be open with the investigator and/or staff about any questions or concerns they have either before, during, or after the study.

E5. Trial Design

To test the central premise that functional connectivity between rAI and rSTS plays a causal role in RNT among young adults with MDD, we will measure rAI-rSTS connectivity, RNT and depressive symptoms in these individuals. In addition to the screening visit, this study consists of two visits (Table 1 and Fig. 3). Visit 1 includes five runs of the self-regulation task, with the middle three containing the neurofeedback condition. Visit 2 (one week later) includes one run of the self-regulation task without the neurofeedback condition. The primary outcome is the change in rAI-rSTS connectivity at Visit 1. The secondary outcomes are the change in state-RNT measured by the Brief State Rumination Inventory (BSRI) (Marchetti et al., 2018) from pre- to post-rtfMRI-nf at Visit 1; and the change in depression severity measured by Montgomery–Åsberg Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979) from Visit 1 to Visit 2 (one-week follow-up).
This randomized, parallel design study uses active or sham rtfMRI-nf scans with 110 young adults (ages 18-35) with MDD (100 completers, assuming a 10% drop-out rate) with high trait-RNT (RRS-B ≥ 13). Participants will be randomized to receive an active (n=55, 50 completers) or a sham (n=55, 50 completers) rt-fMRI-nf in a parallel group design. Randomization will be performed by Data Management and Statistics Core staff as a part of CoBRE Core service, independent from data collection and PI.

Table1. Procedures.

Procedures/Assessments	Baseline Day 0	Visit 1 Day 0 ~7	Visit 2 Day7~14	Follow-up Day 28~35

	(remote)	Before MRI	During MRI	After MRI	Before MRI	During MRI	After MRI	(remote)
Informed Consent	X							
Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998)	X							
MRI environmental screening form		X			X			
Montgomery–Åsberg Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979)		X			X			
Hamilton – Anxiety Rating Scale (SIGH-A) (Hamilton, 1959)		X			X			
16-item Quick Inventory of Depressive Symptomatology (QIDS-SR) (Rush et al., 2003)		X			X			X
Overall Anxiety Severity and Impairment Scale (OASIS) (Norman et al., 2006)		X			X			
Ruminative Response Scale (RRS) (Nolen-Hoeksema & Morrow, 1991)		X			X			X
Repetitive Thinking Questionnaire (RTQ-10) (McEvoy et al., 2010; McEvoy et al., 2014)	X				X			
Perseverative Thinking Questionnaire (PTQ) (Ehring et al., 2011)	X				X			
Brief State Rumination Inventory (BSRI) (Marchetti et al., 2018)		X		X	X		X	X
Penn State Worry Questionnaire (PSWQ) (Meyer et al., 1990)	X				X			
Emotion Regulation Questionnaire (ERQ) (Gross & John, 2003)	X				X			
Metacognition Questionnaire-30 (MCQ-30) (Wells & Cartwright-Hatton, 2004)	X				X			
PROMIS Sleep Impairment and Disturbance (Cella et al., 2010)	X				X			
International positive and negative affect schedule short-form (I-PANAS-SF) (Karim et al., 2011)		X		X	X		X	
Thought collection work sheet (study original)	X				X			
Visual Analogue Scale (VAS, distress about repetitive negative thinking) (study original)		X			X			
Negative Trait Word List (study original)	X				X			
Neurofeedback Practice Work Sheet (study original)		X			X			
Visual Analogue Scale (VAS-fMRI session) (study original)			X			X		
Post-Neurofeedback Questionnaire-Part1 (study original)				X				
Post-Neurofeedback Questionnaire-Part2 (study original)							X	
One-month follow-up survey (study original)								X
Physiological measurement (ECG, Respiration)			X			X		
Anatomical scan			X			X		

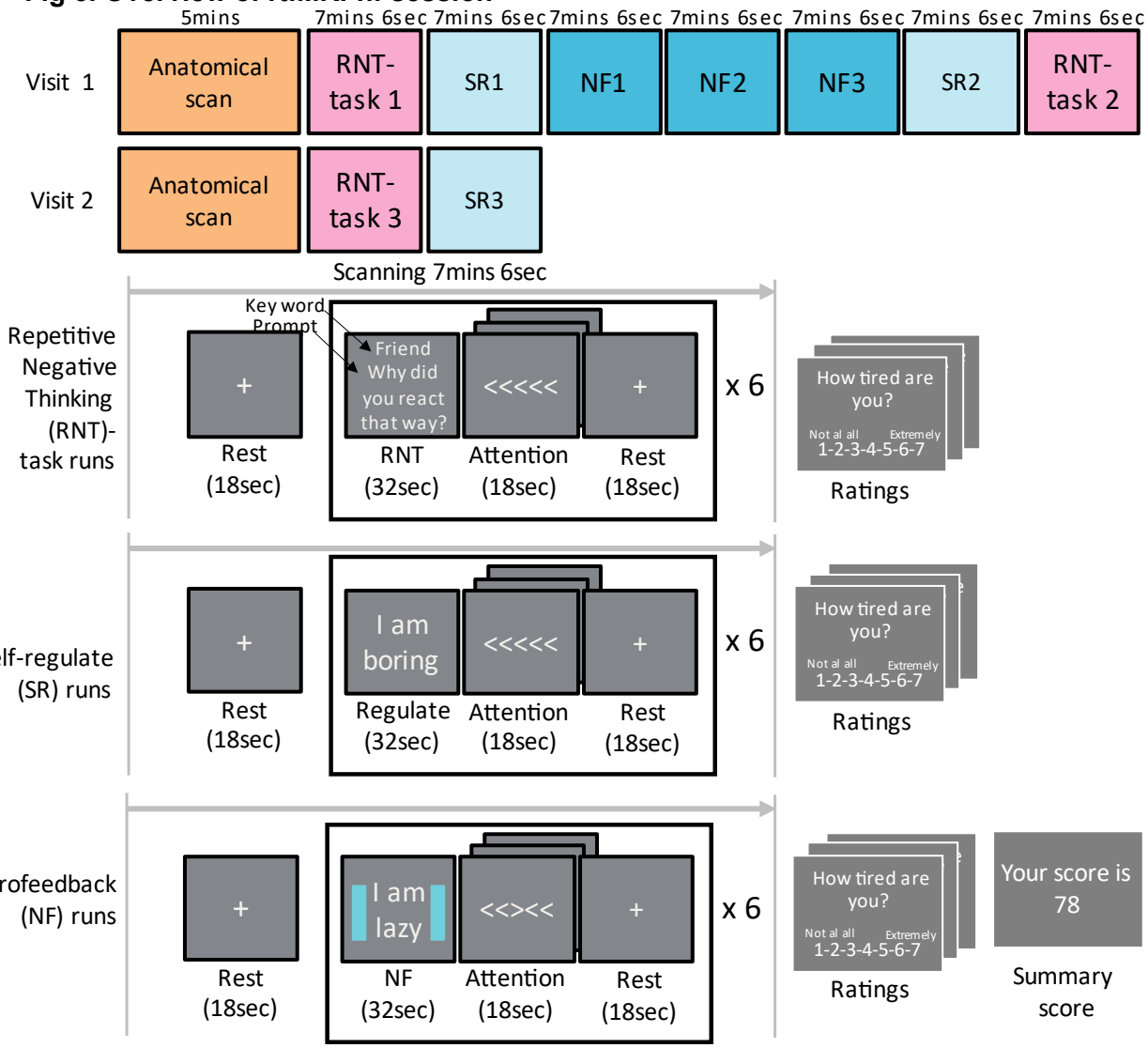
RNT-task fMRI scan		X	X	
Self-regulation without rtfMRI-nf scan (SR)		X	X	
Self-regulation with rtfMRI-nf scan (NF)		X		

Table1. Procedures.

Procedures/Assessments	Baseline Day 0 (remote)	Visit 1 Day 0 ~7			Visit 2 Day7~14			Follow-up Day 28~35 (remote)
		Before MRI	During MRI	After MRI	Before MRI	During MRI	After MRI	
Informed Consent	X							
Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998)	X							
MRI environmental screening form		X			X			
Montgomery–Åsberg Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979)		X			X			
Hamilton – Anxiety Rating Scale (SIGH-A) (Hamilton, 1959)		X			X			
16-item Quick Inventory of Depressive Symptomatology (QIDS-SR) (Rush et al., 2003)		X			X			X
Overall Anxiety Severity and Impairment Scale (OASIS) (Norman et al., 2006)		X			X			
Ruminative Response Scale (RRS) (Nolen-Hoeksema & Morrow, 1991)		X			X			X
Repetitive Thinking Questionnaire (RTQ-10) (McEvoy et al., 2010; McEvoy et al., 2014)	X				X			
Perseverative Thinking Questionnaire (PTQ) (Ehring et al., 2011)	X				X			
Brief State Rumination Inventory (BSRI) (Marchetti et al., 2018)		X		X	X		X	X
Penn State Worry Questionnaire (PSWQ) (Meyer et al., 1990)	X				X			
Emotion Regulation Questionnaire (ERQ) (Gross & John, 2003)	X				X			
Metacognition Questionnaire-30 (MCQ-30) (Wells & Cartwright-Hatton, 2004)	X				X			
PROMIS Sleep Impairment and Disturbance (Cella et al., 2010)	X				X			
International positive and negative affect schedule short-form (I-PANAS-SF) (Karim et al., 2011)		X		X	X		X	
Thought collection work sheet (study original)	X				X			
Visual Analogue Scale (VAS, distress about repetitive negative thinking) (study original)		X			X			
Negative Trait Word List (study original)	X				X			
Neurofeedback Practice Work Sheet (study original)		X			X			
Visual Analogue Scale (VAS-fMRI session) (study original)			X			X		
Post-Neurofeedback Questionnaire-Part1 (study original)				X				
Post-Neurofeedback Questionnaire-Part2 (study original)							X	

One-month follow-up survey (study original)				X
Physiological measurement (ECG, Respiration)		X	X	
Anatomical scan		X	X	
RNT-task fMRI scan		X	X	
Self-regulation without rtfMRI-nf scan (SR)		X	X	
Self-regulation with rtfMRI-nf scan (NF)		X		

Fig 3. Overview of rtfMRI-nf session



E6. Procedures

Procedure for Assignment to a Study Group. Participants will be randomly assigned to either the active rtfMRI-nf or sham group.

Randomization: Participants will be randomly assigned in a double-blind fashion via a central database hosted at the LIBR using a block size of 2. The research staff of the Data Management and Statistics Core works as a randomizing center, independent from PI and the research team.

Blinding: Blindness of participants, investigators, experimenters, and care-providers (clinical interviewers) will be maintained by ensuring that (i) randomization codes will be held by LIBR staff not involved in the trial until study completion, and (ii) the graphical image display during the neurofeedback of the active rtfMRI-nf group and the sham group are identical except for the source of neurofeedback signal.

MRI Acquisition. Participants will be scanned using either a GE Discovery MR750 3.0 Tesla scanner (GE Medical Systems) or a Siemens Magnetom Prisma–NX system (Siemens Healthineers) at LIBR. The GE scanner is equipped with 8- or 32-channel phase array receive-only head coils, while the Siemens scanner comes with 32- or 64-channel radiofrequency (RF) head coils. These multi-element head arrays serve as highly sensitive MRI signal detectors, offering vastly improved sensitivity for detecting functional activation-induced brain signal changes at high spatial and temporal resolutions, as well as anatomical MRI at very high spatial resolutions. The multi-channel brain signal reception allows for shorter readout times, reduced signal dropout, and minimized image distortions.

Each MRI session will include the following scans:

- A T1-w magnetization-prepared rapid gradient-echo sequence will be collected for anatomical reference.
- fMRI: Cognitive/behavioral task will be collected for interventions and brain functional evaluations.

MRI Preparation. Before scanning, task instructions will be presented, and the participants' understanding will be ascertained. Participants may be presented with a pre-scanning task training session to confirm the participant's understanding of the task requirements when it is appropriate. This training session will last from 15 minutes to 1 hour. Training will take place in a location within the LIBR scanning facilities.

MRI eligibility will be confirmed prior to study entry. In addition, an MRI Environment Screening Form for Individuals will be completed prior to scanner entry. The MRI screening questionnaire was developed and distributed by the Institute for Magnetic Resonance Safety, Education, and Research (IMRSER) in Los Angeles, CA*. The safety-screening questionnaire probes for possible occupational exposure to metal slivers or shavings (remnants of which may remain lodged in a participant’s head or neck), surgical clips or shrapnel, cochlear implants, or any other form of ferrous metal implanted in or on the participant’s body. Participants answering in the affirmative to any of these conditions will be excluded. All subjects with any form of implanted wires, metal, or electronic devices will be excluded. All persons involved in this protocol will receive MR safety training conducted at the LIBR.
*INSTITUTE FOR MAGNETIC RESONANCE SAFETY, EDUCATION, AND RESEARCH 7511 McConnell Avenue, Suite 100, Los Angeles, CA 90045, http://www.imrser.org

The participant will be trained on all fMRI tasks and given time to practice. If the participant requires vision correction, he or she will be fitted with plastic glasses closely matching his/her optic prescription. A urine sample will be collected for a pregnancy test for female participants. The participant will be asked to remove all ferromagnetic items prior to entering the scanner room. The audio system will be explained so that the participant will know how to communicate with the scanner operator. Ear plugs will be inserted into and headphones will be placed over the ears. The participant will be reminded of the importance of staying still in the scanner. To minimize motion, the participant’s head will be stabilized with a specially designed pillow under the neck. Wedge-shaped cushions will be placed between the sides of the head and head-holder. Padding will be arranged to maximize comfort; a pillow will be placed under the knees to provide lower back relief. The MRI operator will put the head coil in position over the participant’s head, localize the head position on the scanner, and ensure that the participant can fully view the display screen at the end of the scanner gurney, where task images will be displayed by a mirror directly above the participant’s eyes. The response box will be positioned in the participant’s dominant hand; the participant will test the buttons. The pulse oximeter will be placed on one finger of the participant’s non-dominant hand. The participant will be provided the emergency squeeze ball.

Once the participant is comfortable, the gurney will move into the magnet. The MRI technician will secure the scan room door, dim the scan room lights, and communicate with the participant through the console.

Physiological Monitoring. Physiological signals will be acquired through physiological monitoring of pulse rate using a finger-tip pulse oximeter throughout the MRI session (Mulert & Lemieux, 2009). The scanner is equipped with a finger pulse oximeter measuring blood oxygenation for cardiography and a respiration belt that fits comfortably around the participant's chest to measure respiratory changes. An elevated pulse rate will generate a query from the scanner operator. Pulse rates above 90 beats per minute will prompt the scanner operator to ask participant if they are okay. The pulse oximeter sensors will be connected to an In Vivo physiological monitoring system. The concurrent information on pulse rate will be used to remove physiological fluctuations from the fMRI data.

Structural MRI Sequence. A series of structural MRI scans will be performed (voxel-wise maps: T1, T2 relaxations times).

Functional MRI Imaging. Prior to task-relevant functional scans subjects will be reminded of the task instructions. Typical functional scans will involve a series of 5- to 7-minute-long scans with short breaks (~1 minute) between scans. During imaging breaks the participant's comfort level will be assessed and instructions will be provided again. The number of functional scans will range from 5 to 10 with the total functional scanning time not to exceed 1.5 hours. Participants will also be asked to rate their comfort level in the scanner and to describe any discomfort that may have occurred. These ratings will be recorded on the session scanning log. The participant's total amount of time spent in the scanner will not exceed 2 hours.

Real-time fMRI Neurofeedback Procedure. Processing includes slice-timing correction, motion correction, spatial smoothing with 6mm-FWHM Gaussian kernel within the brain mask, scaling to a percent change relative to the average for the first 28 TRs, and regressing out noise components (Misaki et al., 2015) (six motion parameters, eight RETROICOR (Glover et al., 2000) regressors [four cardiac and four respiration], white matter mean signal, ventricle mean signal, and Legendre polynomial models of slow signal fluctuation). This noise reduction is performed in real-time (less than 400 ms) (Misaki et al., 2015; Misaki & Bodurka, 2021; Misaki et al., 2022). Masks for white matter and ventricles and the ROIs are defined in the MNI template brain: for example, spheres of a 6~7 mm radius at the rAI (MNI: 42, 8, 4) and the rSTS (MNI: 60, -30, -2) (Tsuchiyagaito, Sánchez, et al., 2022); and will be warped to the individual subject's brain for real-time signal calculation. Specific real-time scanning parameters may differ based on the scanner model used, either GE or Siemens; Figure 3 illustrates an example of the parameters for the GE scanner.

Experimental Procedures.

Repetitive Negative Thinking Task (RNT-task)

Preparation for RNT-task

Prior to MRI scanning, subjects will be instructed to recall four autobiographical events which the participant can clearly associate with RNT (**Repetitive Negative Thought Collection Work sheet, see Appendix**). The participant will be asked to generate up to six events and to write down a short description (key words) to remember those events inside MRI (i.e., *We would like you to try and remember four different thoughts you have had about the past that make you feel bad, embarrassed, sad, or afraid when you think about them, and that may be hard to stop thinking about*). They will answer the visual analog scale (**VAS-Distress about Repetitive Negative Thinking, see Appendix**) to rate the emotional load (i.e., how distressing is the thought these keywords are referencing?), and Brief State Rumination Inventory (BSRI). Through the process, research staff will be available for a participant to ask any questions or concerns. We will use the most distressing RNT event for the RNT-task.

RNT-task with fMRI

The RNT-task with fMRI scanning is performed to measure brain activations while participants are engaging with RNT. This task will be used to evaluate participant's brain function during RNT before (RNT-task1, Visit1), after (RNT-task2, Visit1) and at 1-week after the rtfMRI-nf session (RNT-task3, Visit2). The task will last about 7mins and 6second (**Fig. 3**).

1) **Repetitive Negative Thinking block (RNT)**, in which a participant will see a short description of their autobiographical events and will engage with self-relevant RNT.

2) **Flanker block (Attention)**, in which an array of five arrows is shown, and a participant is required to respond to the direction of the center arrow. A participant will have a 3-sec to make a response for each trial, and six trials will be presented in one block. We use a flanker task (Eriksen & Eriksen, 1974) to minimize the carry over effect of the Regulation block on the Rest block.

3) **Rest block (Rest)**, in which a participant will see a cross sign and will be instructed to clear their mind and not to think anything in particular.

After the task, they will answer the visual analog scale (**VAS-fMRI session, see Appendix**) to rate the emotional load.

Self-regulation Task with and without rtfMRI-nf (SR1, NF1, NF2, NF3, SR2, and SR3)

SR1, SR2, and SR3 are self-regulation (SR) task without rtfMRI-nf signals, and SR1 serves as a pre-evaluation, SR2 as a post-evaluation, and SR3 as a follow-up evaluation. NF1 to NF3 are all neurofeedback runs with rtfMRI-nf signals. This task was previously used in published papers (Tsuchiyagaito et al., 2023; Tsuchiyagaito et al., 2021).

Preparation for the Self-regulation Task

Prior to MRI scanning, subjects will be instructed to select 36 words that describe their negative traits or personality from a 160-word list (**Negative Trait Word List, see Appendix**), which was taken from 555 sets of personality trait words (Anderson, 1968). In order to balance the valence, they were asked to select nine words in each of four columns, which included 36 words in total (the average valences of each column were statistically the same). Six words will be randomly presented within each run, and six runs will be performed (five runs at Visit 1 and one run at Visit 2).

During the regulation block (see below), participants are encouraged to apply emotion regulation strategies to reduce negative thinking as they view negative trait or personality words. Three recommended emotion regulation strategies are:

- Reinterpreting Negative Thoughts
- Focusing on Your Breath
- Distracting from Negative Thoughts

Participants will be instructed to try all or just one of these strategies, and they are encouraged to utilize the neurofeedback signals to determine which works best for them. They will undergo a practice session prior to the fMRI scan using **Neurofeedback Practice Work Sheet (see Appendix)**.

Self-regulation Task without rtfMRI-nf (SR1, SR2, and SR3)

1) **Self-Regulation block (SR)**, in which a participant attempts to regulate RNT using emotion regulation (e.g., reinterpretation: apply an emotion regulation strategy to reinterpret their negative self-perspective) while viewing negative words describing their personality traits.

2) **Flanker block (Attention)**, in which an array of five arrows is shown, and a participant is required to respond to the direction of the center arrow. A participant will have a 3-sec to make a response for each trial, and six trials will be presented in one block. We use a flanker task (Eriksen & Eriksen, 1974) to minimize the carry over effect of the Regulation block on the Rest block.

3) **Rest block (Rest)**, in which a participant will see a cross sign and will be instructed to clear their mind and not to think anything in particular.

These blocks will be repeated six times in one fMRI scan run (SR1, SR2, and SR3). SR1 will be performed before NF runs, and SR2 will be performed after NF runs at Visit1. A week later, participants will undergo another MRI session including SR3 as a follow-up evaluation. Negative personality words collected prior to the scan (see above) will be presented in the Self-Regulation block in a randomized order.

After the task, they will answer the visual analog scale (**VAS-fMRI session, see Appendix**) to rate the emotional load.

Self-regulation Task with rtfMRI-nf (NF1, NF2, and NF3)

1) **Neurofeedback block (NF)**, in which a participant attempts to regulate RNT using emotion regulation (e.g., reinterpretation: apply an emotion regulation strategy to reinterpret their negative self-perspective) while viewing negative words describing their personality traits. During this NF run, rAI-rSTS connectivity will be fedback to a subject in the active group so that they are able to learn how to modulate the rAI-rSTS circuit, while sham feedback will be fedback to a subject in the sham group.

2) **Flanker block (Attention)**, in which an array of five arrows is shown, and a participant is required to respond to the direction of the center arrow. A participant will have a 3-sec to make a response for each trial, and six trials will be presented in one block.

3) **Rest block (Rest)**, in which a participant will see a cross sign and will be instructed to clear their mind and not to think anything in particular.

These blocks will be repeated six times in one fMRI scan run, and three runs will be performed (rtfMRI-nf training, NF1, NF2, and NF3). Similar to SR runs, negative personality words collected prior to the scan (see above) will be presented in the Regulation block in a randomized order.

After the task, they will answer the visual analog scale (**VAS-fMRI session, see Appendix**) to rate the emotional load, and participants will be given a summary score of the neurofeedback success (e.g., percentage of successful regulation).

F. Outcomes

Neural outcomes

Primary outcome (Visit 1). The primary outcome is the change in rAI-rSTS connectivity at Visit 1 (five time points: SR1, NF1, NF2, NF3, and SR2). This will be assessed with a generalized psychophysiological interaction (gPPI)(McLaren et al., 2012) (i.e., changes in functional connectivity strength covarying with the task (Self-Regulation block described **D5**) of self-regulation and rtfMRI-nf tasks). Standardized beta coefficients

associated with the interaction term (task block by rAI seed) within rSTS ROI will be used as task-related rAI-rSTS connectivity values.

Exploratory outcome (Visit 2): An exploratory outcome is the change in rAI-rSTS connectivity during a self-regulation task without neurofeedback 1-week after the rtfMRI-nf relative to baseline (three time points: SR1, SR2 at Visit 1, and SR3 at Visit 2); and during a RNT-task 1-week after the rtfMRI-nf relative to baseline (three time points: RNT-task1, RNT-task2 at Visit 1, and RNT-task3 at Visit 2).

Clinical outcomes

Secondary outcome. State-RNT (Visit 1). Acute effects of rtfMRI-nf on state-RNT will be measured by the Brief State Rumination Inventory (BSRI) (Marchetti et al., 2018). The BSRI is an 8-item measure of state-RNT assessed on a VAS from strongly disagree (0 mm) to strongly agree (100 mm). The BSRI has been demonstrated to be sensitive to the experimental manipulation of RNT, and showed one factor with a good convergent validity with RRS-B (Marchetti et al., 2018) even after controlling for depression and anxiety. **Depression severity (Visit 2).** Montgomery–Åsberg Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979) will be collected before and 1-week after the rtfMRI-nf.

Exploratory outcomes. Trait-RNT (Visit 2). Effects at 1-week after rtfMRI-nf on trait-RNT will be measured by the brooding subscale of the RRS (RRS-B). The RRS (Nolen-Hoeksema & Morrow, 1991), was found to have three distinct subtypes: brooding (RRS-B), depression-related (RRS-D), and reflective RNT (RRS-R). Studies have been demonstrated that RRS-D overlaps with general levels of depression (Berman et al., 2011a), and that RRS-B refers to a passive comparison of one's situation with unachieved standards and is considered to be a pathological form of RNT (Treynor et al., 2003), whereas RRS-R involves reflective self-focus with the aim of dealing with depressive feelings and is considered to be an adaptive coping strategy (Treynor et al., 2003). Thus, we will use RRS-B to measure pathological aspects of trait-RNT. Since the original RRS did not specify the time frame (e.g., "Please indicate what you *generally* do"), we will modify the instruction as "Please indicate what you did *over the last week*. **Self-rated depression, anxiety, and other cognitive processes.** Participants will complete other measures listed in Table 1.

G. Analytic Plan

All the collected data and preprocessed structural and fMRI data (BIDS format) will be stored in the data management center provided by the Data Management and Statistics Core. The primary (rAI-rSTS connectivity) and secondary outcomes (state-RNT and depression) will be collected as longitudinal data. To initially test for effects of active rtfMRI-nf compared to sham, each repeated outcome measure will be analyzed using linear mixed effects models (LMEs) with per-participant random adjustment to the fixed intercept (random intercept) and, when appropriate, per-participant adjustment to time (random slope) (Feehley et al., 2019). Pre-defined hypotheses will be tested rigorously as follows:

Aim 1 (Primary Outcome). To determine an acute effect of active vs. sham rtfMRI-nf on rAI-rSTS connectivity (Visit 1). Hypothesis: 1**)** Individuals undergoing active rtfMRI-nf compared to sham will show a greater reduction in rAI-rSTS connectivity at Visit 1. The change in rAI-rSTS connectivity during an rtfMRI-nf session at Visit 1 will be assessed using a Linear Mixed-Effects model (LME), including fixed effects of rtfMRI run (SR1, NF1, NF2, NF3, and SR2 at Visit 1), group (active vs. sham), run-by-group interaction, age, sex, and a random effect of subject. A *post hoc* analysis will be conducted by comparing changes in rAI-rSTS connectivity (from SR1 to SR2 at Visit 1) between active and sham groups.

Aim 2 (Secondary Outcome). To determine an acute effect of active vs. sham rtfMRI-nf on RNT (Visit 1) and a sub-acute effect on depression (Visit 2). Hypotheses: 2a) Individuals undergoing active rtfMRI-nf compared to sham will show a reduction of RNT immediately after rtfMRI-nf. 2b) Individuals undergoing active

rtfMRI-nf compared to sham will show a reduction of depression 1-week after rtfMRI-nf. The change in RNT measured by BSRI at Visit 1 and in depression measured by MADRS at Visit 2 will be assessed by an LME including fixed effects of time (baseline and post-rtfMRI-nf within Visit 1 for BSRI, or Visit 1 and Visit 2 for MADRS), group (active vs. sham), time-by-group interaction, age, sex, and a random effect of the subject on intercept.

Aim 3 (Exploratory Aim). To explore the degree to which acute modulation of rAI-rSTS connectivity (Visit 1) leads to acute changes in RNT (Visit 1) as well as sub-acute changes in depression and rAI-rSTS connectivity 1-week after rtfMRI-nf (Visit 2). Exploratory hypotheses: Individuals with a greater reduction in rAI-rSTS connectivity at Visit 1 will show 3a) a greater immediate reduction in RNT (Visit 1), 3b) followed by a reduction in depression and 3c) rAI-rSTS connectivity 1-week later (Visit 2). The changes in BSRI (within Visit 1) and MADRS (from Visit 1 to Visit 2) will be regressed with change in rAI-rSTS connectivity (within Visit 1) by linear regression analysis, respectively. We will also explore if decoupled rAI-rSTS connectivity at Visit 1 would be maintained at Visit 2 in active vs. sham by an LME including fixed effects of self-regulation runs (SR1, SR2 at Visit 1, and SR3 at Visit 2), group (active vs. sham), run-by-group interaction, age, sex, and a random effect of the subject on intercept. The similar exploratory analysis will be performed to investigate if active vs. sham rtfMRI-nf would reduce rAI-rSTS connectivity at Visit 2 by an LME including fixed effects of RNT-task runs (RNT-task1, RNT-task2 at Visit 1, and RNT-task3 at Visit 2), group (active vs. sham), run-by-group interaction, age, sex, and a random effect of the subject on intercept.

Potential Problems and Alternative Analysis Plans.

(1) Some of the participants may not be able to reduce rAI-rSTS connectivity at all at Visit 1. Possible factors of non-regulators may be the use of inappropriate strategies (irritation, anger, anxiety during the rtfMRI-nf) or the time spent to find successful strategies for them and not focusing on the rtfMRI-nf. We also acknowledge that higher trait-RNT could be a predictor of poor training outcomes. We will analyze predictive factors including brain activation or connectivity during the RNT-task, sleep-related impairment and disturbance, and other behavioral and cognitive measures by comparing successful versus poor rtfMRI-nf responders after the completion of data collection.

(2) It is possible that rtfMRI-nf could be effective, not due to the mechanisms we hypothesized, but instead due to increased ability to regulate any brain areas, a general increase in self-efficacy, or a general impact of engaging in reappraisal. In this case, we may see a reduction in depressive scores or general negative mood scores in both groups. By examining changes in both the hypothesized as well as adaptive aspects of RNT and more general psychological constructs (e.g., reflective thinking, emotion regulation), we will be able to elucidate central mechanisms and inform future clinical trials.

H. Compensation for time spent and inconvenience of participating

Table 2. Study compensation

	Approximate Time in Hours	Estimated Compensation	Incentive Earned
Screening			
Consent	0.5	$10	
Assessments	1.0	$20	
Total for Screening	2.5	$30	
Visit 1			
Assessments (pre MRI)	0.75	$15	

Practice (outside of MRI)	0.5	$10	
MRI scan	1.0	$50	
Assessments (post MRI)	0.25	$5	
Total for Visit 1	2.5	$80	
Visit 2			
Assessments (pre MRI)	0.75	$15	
MRI scan	0.5	$25	
Assessments (post MRI)	0.25	$5	
Total for Visit 2	1.5	$45	
Follow-up			
Assessments	0.25	$5	$15
Total for Follow-up	0.25	$20	
Total for completing the study		$175	

For participation time, participants will be compensated at a rate of $20 per hour. While participants are in the scanner, they will be compensated at a rate of $50 per hour of participation. Participants will never spend more than 2 hours in the scanner in a single session and may have an additional 1 hour of participation spent immediately prior to, or after, the scan. As such, a typical visit for an MRI session may last as long as 3 hours. If a participant withdraws prior to the study completion, they will be compensated for whatever portion of the study they have completed. Participants who completed both Visits 1 and 2 will earn an extra $50 as an incentive.

I. Human Participants

Over a three-year period, a total of 110 young MDD individuals (ages 18-35) with high RNT (Brooding subscale of Ruminative Response Scale, RRS-B ≥ 13) will be recruited in this randomized experimental medicine design study to either receive active rtfMRI-nf (n=55) or sham (neurofeedback with artificially generated feedback signals; n=55) feedback. Participants will be recruited through a variety of sources focused primarily on outreach efforts by the recruitment team at LIBR; as well as through newspaper, flyers, radio, and Facebook or other media advertisements in the Tulsa metropolitan area. All efforts will be made to ensure that our subject population closely resembles the gender, ethnicity, race and age composition of adults in the Tulsa area. All participants will be between the ages of 18 and 35 years to focus on young adults with MDD. Potential participants will complete a phone screen to determine initial eligibility by the Recruitment and Assessment Core. After informed consent and before completing any other study procedures, the presence of inclusion/exclusion criteria will be obtained using both an unstructured and diagnostic interview performed by interviewers trained in the assessment of substance use, eating disorders depression and anxiety, and confirmed by a psychiatrist or clinical psychologist.

I1. Inclusion Criteria

General conditions:

- Young adults ages 18-35
- Participants who are able to give written informed consent prior to participation

Psychiatric Disorders:

- Meeting DSM-5 diagnostic criteria for MDD who are currently depressed defined by the MINI

Rating scores:

- Participants who have RNT symptoms (Brooding subscale of Ruminative Response Scale: RRS-B ≥ 13)

I2. Exclusion Criteria

Medical Conditions:

- Moderate to severe traumatic brain injury (>30 min. loss of consciousness or >24 hours posttraumatic amnesia) or other neurocognitive disorder with evidence of neurological deficits
- Presence of co-morbid medical conditions not limited to but including cardiovascular (e.g., history of acute coronary event, stroke), pulmonary, endocrine, neurological diseases (e.g., Parkinson's disease), or gastrointestinal illness, as well as pain disorders

Psychiatric Disorders:

- Current significant suicidal ideation or suicide attempt within the previous 12 months
- Current psychosis
- Schizophrenia or schizoaffective disorder
- Substance use disorder within the previous 12 months, except for mild alcohol, cannabis, or tobacco use disorder defined as less than 4 symptoms of the criteria for substance use disorder according to the MINI
- Current diagnosis of post-traumatic disorder (PTSD) defined by the MINI

Contraindications for MRI:

- Severe claustrophobia
- Bodily implants of unsafe paramagnetic materials such as pacemakers and aneurysm clips
- Pregnancy

Medications and psychotherapies:

- Current regular use of cardiovascular medications with a direct vasomotor effect, namely beta- or alpha-beta-blockers, clonidine, and antianginal agents.
- Current use of more than three psychotropic medications
- Evidence of recreational drug use from a urine test
- Commencement of psychotropic medication for depression and/or anxiety less than a month before the study enrollment
- Commencement of psychological therapy less than a month before the study enrollment

Health Factors:

- Participants who have a clinically significant or unstable cardiovascular, pulmonary, endocrine, neurological, gastrointestinal illness or unstable medical disorder will be excluded
- Participants who, on arrival to the study, have a temperature greater than 100.4°F will not be allowed to initiate the study

Non-English speaking participants:

- The majority of the assessments proposed for this study have not been translated from English, thus, non-English speaking volunteers will be excluded

J. Justification of the sampling, recruitment, and retention plans:

The project focus on understanding neural and behavioral mechanisms related to repetitive negative thinking (RNT) in young adults with RNT and depression. Thus, our recruitment efforts focus on enrolling individuals with RNT and depression to collect these data. No information will be collected from potential subjects until they initiate contact with the LIBR assessment team for the purposes of research participation. Participation in the study is strictly voluntary and the subjects will be able to withdraw from the study at any time.

Sample size calculations for the primary outcome were based on the meta-analysis of rtfMIR-nf where they provided an estimate of d=0.59 (medium effect) of brain activation or connectivity difference between active and sham groups. With n=100 evaluable participants (50 per arm), we have 80% power to detect a medium effect size of rtfMRI-nf between active and sham groups. With a 10% attrition rate, we will need to enroll 110 subjects overall. We aim to recruit n=35-40/year. The Recruitment and Assessment Core has successful records in recruiting approximately 70 young adults with MDD (ages 18-25) per year. In order to minimize attrition we will maintain regular contact which includes updating contact information, appointment reminders with MRI preparation instructions, and driving and parking directions; offer incentive payments for completing the study; and offer a LIBR original keepsake item (e.g., water bottle) at study completion.

Gender/Minority/Pediatric Inclusion for Research. We will include participants age 18-35 for our study purpose. Women and minorities will be included in the study without prejudice according to their representation in the study population. Adult subjects will be recruited from the greater Metro Tulsa area and should thus share the racial and ethnic composition of this area. All efforts will be made to ensure that our participant population closely resembles the ethnic and racial composition of the greater Tulsa area.

K. Data Storage and Confidentiality

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study. Study data records and urine samples will be assigned code numbers and will not be individually identifiable. Code numbers are a combination of numbers and letters. A centralized data repository will be used to store all acquired data. The data repository employs a rapidly emerging and widely accepted Brain Imaging Data Structure (BIDS) database standard. The BIDS format facilitates data sharing between projects, exporting data and validating data integrity. The data will be collected in REDCap (Research Electronic Data Capture), a software toolset and workflow methodology for electronic collection and management of research and clinical trial data, developed by Vanderbilt University, through collaboration from a consortium of institutional partners. The electronic data will be kept in a firewalled and password protected database on a secure server managed by LIBR. REDCap data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team with planning assistance from the information technology staff. The iterative development and testing process results in a well-planned data collection strategy for individual studies. REDCap servers are housed in a local data center at LIBR and all web-based information transmission is encrypted. REDCap was developed specifically around HIPAA-Security guidelines and is recommended to LIBR researchers by both our Privacy Office and the Western Institutional Review Board (WIRB). REDCap has been disseminated for use locally at other institutions and currently supports 240+ academic/non-profit consortium partners on six continents and over 26,000 research end-users (**www.project-redcap.org**).

Participants will not be identified in any reports or publications.

Records of the participant's participation in this study will be held confidential except as disclosure is required by law or as described in the informed consent document (under "Confidentiality"). The study doctor, the sponsor or persons working on behalf of the sponsor, and under certain circumstances, the United States Food and Drug Administration (FDA) and WIRB will be able to inspect and copy confidential study-related records that identify the participant by name. Therefore, absolute confidentiality cannot be guaranteed. If the results of this study are published or presented at meetings, the participant will not be identified. Paper copies of consents, screening forms, the Research Privacy Form, and any other forms, testing results or papers containing Personally Identifiable Information (PII) will be stored in a secured medical records room with access granted only to authorized personnel.

Linkages to subjects and access to identities: The minimum amount of personal identifiable information necessary to conduct this study will be collected from participants including full name, contact information (address, phone numbers, email), sex, age, and social security number (for payment purposes only). All data will be obtained from participants during individual sessions and each participant will be given a unique subject ID code. All collected data will only be labeled with the unique subject code and will not include any identifying information. For example, identifying information that links subjects to their data will be removed (e.g., name will be replaced with a code). Only the PI and a limited number of trained research staff who have completed CITI online training in Human Subjects and Good Clinical Practice have access to the database that links names with subject codes. All hardcopy data are stored in locked file cabinets in locked offices and on password protected computers located behind secure and maintained firewalls.

How data are collected and whether data will be collected specifically for proposed research: Data are collected by trained research assistants (RAs) who have completed CITI online training in Human Subjects and Good Clinical Practice. Data will be collected specifically for this proposed research project.

Data sharing and publications: This study will be conducted in accordance with the following publication and data sharing policies and regulations:

This study will be registered at ClinicalTrials.gov and resulting information from this study will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers after the completion of the primary endpoint by contacting the Laureate Institute for Brain Research.

Consent procedures to be followed: All participant interactions including consenting will be conducted in private interview/exam rooms. These exam rooms are secured from public areas via combination locked doors that are only accessible to authorized personnel. Consent will be obtained by the PI or a member of the LIBR research staff trained and approved by the IRB. Written informed consent will be obtained from each participant after they have been provided a full verbal and written explanation of the study purpose, procedures, risks and benefits, and after they have been allowed sufficient opportunity to review this information and ask questions concerning any aspect of the study.

L. Risk/Benefit Assessment

L1. Potential Benefits to the Participants

There is no immediate personal or medical benefit derived from participation. The results of these studies, however, will further the scientific community's understanding of the neuropathophysiology of mental health disorders and may ultimately lead to better treatments. As such, participants may derive personal satisfaction from their contribution to the discovery process.

L2. Risks Associated with Screening, Interviewing, and Assessments

The risks of the screening evaluation and assessments are minimal and most likely risks include fatigue and discomfort or irritability with the nature of questions and/or testing procedures. Some of the questions in the interviews may be distressing or uncomfortable to answer and the most likely risk is psychological distress, and subjects have the right to refuse to answer.

L3. Risks Associated with Delaying Treatment

Participants can continue any current medications and/or psychotherapies. Subjects will NOT undergo any "washout" procedures and will NOT be asked to discontinue any medications for the purposes of this study. Further, they will not participate in the study if they do not wish to delay treatment or if, in the opinion of a LIBR physician, it is inadvisable to delay treatment.

L4. Risks Associated with MRI Scanning

MRI scanning uses powerful magnetic fields and weak radio frequency pulses (electromagnetic radiation), neither of which has been associated with adverse effects in patients or laboratory animals when studied under clinical imaging protocols. However, as in the clinical setting, subjects must be free of any external or implanted ferrous material. People are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Because MRI is performed in confined quarters, the feeling of being isolated or confined may cause some healthy subjects and patients to request termination of their participation. It is expected that a very low percentage of subjects will be unable or unwilling to complete participation. Because the noise from the scanner is loud enough to damage hearing, participants will be fitted with hearing protection prior to MRI scanning and will wear hearing protection devices at all times during scanning.

L5. Risks Associated with Neurofeedback

Neurofeedback involves providing subjects information concerning the changes in functional connectivity in brain regions while engaged in the cognitive process. In this project, subjects are provided feedback about changes in functional connectivity during self-referential processing. The most likely risks associated with these procedures would be frustration or psychological distress due to difficulties in either engaging with self-referential processing while viewing negative words related to individuals or regulating the target brain region. There is a theoretical risk that some subjects may experience a worsening of depressive symptoms and/or an increase in negative thinking, although no adverse events have occurred in over 140 individuals with major depressive disorder (MDD) and post-traumatic stress disorder (PTSD) that have completed previous neurofeedback studies at LIBR using the similar paradigm to that proposed in this grant application.

All volunteers who are deemed a serious suicide risk are excluded from the study during the state-of-the-art screening and diagnostic assessments. These assessments are conducted by a clinician interviewer who has received extensive training and experience in the evaluation and management of patients with major psychiatric disorders. In the evaluation of suicidal risk, we specifically will exclude from participation any volunteer who endorses having developed a plan or intent to attempt suicide or has made a suicide attempt within the preceding six months. Any volunteer who is excluded from participation for these reasons is referred for emergency care according to written LIBR policies for managing potentially suicidal patients, as described below.

At any time during the study, subjects may report increased psychological distress, suicidal ideation, or intent to harm self or others. If this occurs, PI, LIBR psychiatrists, Dr. Guinjoan (NeuroMAP Core, board-certified psychiatrist), LIBR psychiatrists, LIBR physicians, or their licensed designee, will be contacted immediately to ensure appropriate care and compliance with mandated reporting to authorities. Subjects may be referred for professional intervention, as deemed appropriate, including calling emergency personnel (911) if needed.

If concerns arise when the subject is physically present at LIBR, a LIBR psychiatrist will be available on site to address any concerns that arise.

L6. Risks of Loss of Confidentiality

As with other data from the study, data with sensitive information such as psychiatric status will be stored on restricted-access servers and/or in filing cabinets in a locked room, to which only the PI and a restricted number of trained research staff who have completed CITI online training in Human Subjects and Good Clinical Practice have access. These procedures will minimize the risk of personal information being made available to those who would use it for other than its intended purposes. There are no known less risky alternatives to any of the procedures proposed in these experiments that would provide comparable scientific information.

L7. Risks Associated with Research Participation During the COVID-19 Pandemic

Due to the ongoing COVID-19 pandemic, there are additional risks posed by the requirement of in-person research participation for numerous aspects of the proposed study at LIBR. Participants will interact directly with LIBR personnel, and therefore it is possible that they may acquire infection with COVID-19 during the study participation. The level of this risk is judged to be very low based on the historical case rates of COVID-19 in the Tulsa area.

L8. Minimizing Risks of Loss of Confidentiality Around Sensitive Information (e.g., psychiatric status, history of substance abuse, etc.)

Data will be collected by trained staff who have completed training in human subjects' research and training to criterion on the project protocol. To protect the confidentiality, no personally identifying information will be coded on questionnaires, interviews, bioassays, brain imaging data, or other scoring forms. Unique subject identification numbers are assigned to each participant. Only the PI and trained project personnel who have completed CITI online training in Human Subjects and Good Clinical Practice have access to the file that links names with subject numbers, which will be stored separately from the de-identified data. All paper data will be stored in locked file cabinets in a locked office and all electronic data will be stored in password-protected computers located behind a secure and maintained firewall.

L9. Contingency Plans for Monitoring Suicidality

All volunteers who are deemed a serious suicide risk will be excluded from the study. All research volunteers will undergo routine, state-of-the-art screening and diagnostic assessments at LIBR. The screening will be conducted by a clinician interviewer (who holds either a nursing or MD degree, or a MA or PhD degree in clinical or counseling psychology and has received extensive training and experience in the evaluation and management of patients with major psychiatric disorders). In the evaluation of suicidal risk, we specifically will exclude from participation any volunteer who endorses having developed a plan or intent to attempt suicide. Any volunteer who is excluded from participation for these reasons will be referred for emergency care according to written LIBR policies for managing potentially suicidal patients, as described below.

While the participants are in this study, they will be monitored for the development of suicide risk or for worsening in their clinical illness as follows:

Monitoring for suicide risk over the course of the study will be assessed through: the Montgomery-Åsberg Depression Rating Scale (MADRS) item #10. The MADRS item #10 score ≥ 4 would increase concern for suicidal behavior and trigger a conversation among the PI and the research staff, as to whether to withdraw the participant from the study.

If concerns arise when the subject is physically present at LIBR, a board-certified psychiatrist, Sahib Khalsa, M.D., Ph.D., Director of Clinical Operations at LIBR is available on site to address any concerns that arise. In the event that Dr. Khalsa is unavailable, Salvador Guinjoan, M.D., Ph.D., a board-certified psychiatrist with 25 years of experience in assessing and managing patients with mood disorders including suicide assessment and management will serve as back-up for Dr. Khalsa.

For patients who are at the LIBR facility and are deemed to constitute a serious risk for suicide, the LIBR policy requires the subject be referred to the Clinical Assessment Department (CAD) at Laureate Psychiatric Clinic and Hospital (LPCH) when there are safety concerns. If the participant agrees, a LIBR staff member may accompany them to CAD (which is located approximately 100 yards down a sidewalk from the LIBR facility). If participants refuse to be escorted to this facility and leave the LIBR premises, the study clinician will contact the Community Outreach Psychiatric Emergency Services (COPES – 918-744-4800), which is available 24 hours per day to send a mobile unit to the person's home.

For participants who develop serious suicidal ideation while not on the LIBR premises, these participants will be instructed to call 911 or to go to the nearest emergency room if they feel they are a threat to themselves or others. They will also be given the contact details of the Tulsa Community Outreach Psychiatric Emergency Services (COPES – 918 744 4800).

For participants who have clinical issues that do not reflect a psychiatric emergency, they will be given a telephone number where they can reach a LIBR clinician during those hours when LIBR is officially staffed by clinicians (weekdays between 0800 and 1700). For weekends and off-hours (evenings and nights) they are provided a second telephone number where they can reach the 24-hour per day on call service for LIBR, which is provided through the Call Center of Laureate Psychiatric Clinic and Hospital. Upon study completion or early withdrawal, patients who are currently under treatment will be referred to their own clinician. Patients who complete the study and are not under the care of a clinician, will be provided a referral to a psychiatrist or other mental health professional at one of the following clinics:

Outpatient	
Insured: Laureate Psychiatric Clinic and Hospital (LPCH) 6655 South Yale Ave Tulsa OK, 74136 (918) 481-4000	Uninsured (sliding scale): Tulsa Center for Behavioral Health 2323 South Harvard Ave Tulsa OK, 74114 (918) 293 2140
Sliding scale:	
Department of Psychiatry University of Oklahoma College of Medicine Tulsa OK, 74136 (918) 619 4400	Associated Centers for Therapy 7010 South Yale Ave, Suite 215 Tulsa OK, 74136 (918) 492 2554
Inpatient:	
Laureate Psychiatric Clinic and Hospital (LPCH) 6655 South Yale Ave Tulsa OK, 74136 (918) 481-4000	Tulsa Center for Behavioral Health 2323 South Harvard Ave Tulsa OK, 74114 (918) 293 2140

Individually tailored referrals will be made for subjects who reside outside of the Tulsa region, so that they will be referred to psychiatric services that are located near their home or workplace.

L10. Plan of Action for Incidental Scan Findings

In addition to standard MPRAGE and EPI sequences used for the research component of the study, we will also obtain T2-weighted FLAIR images which are sensitive to CNS and white matter abnormalities. For each subject, a set of T1- and T2 weighted images will be sent via PACS and evaluated by a neuroradiologist (Integris Radiology Group, Oklahoma City, OK) for the presence of unanticipated abnormalities (e.g., tumors). The neuroradiologist will recommend referral to a physician, if incidental findings are discovered which warrant follow-up evaluation. Upon detection of such incidental findings during MRI scanning, a LIBR physician will verbally communicate the discovery to the participant. The LIBR physician will guide the participant to make the discovery known to their physician, and provide a copy of the clinical MRI report to their physician upon receiving a signed release of information letter. Participants will be informed that they will only be made aware of incidental findings and that the clinical scans do not constitute or replace routine medical care.

L11. Protection for Vulnerable Subjects

All subjects enrolled in this study will be of legal age and have the capacity to provide fully informed consent. All female subjects must have a negative pregnancy test result in order to participate in the MRI. This project will not enroll prisoners and will not include persons at risk for suicide.

The exclusion/inclusion criteria, research design, informed consent, and procedures for clinical assessment and monitoring were developed with the aim towards enhancing the potential benefits gained from the study in balance with minimizing the potential risks. Each participant will have at least a 50/50 chance of getting randomized to an active intervention (rtfMRI-nf). Those in the sham-control condition will not receive feedback of functional connectivity thought to be involved in the RNT cognitive process, and they will be informed about this randomization process during the consent. Informed consent will involve discussion about the relative risks and benefits of the study, as well as other treatment opportunities outside of the study that they can seek concurrently (in addition to the current care they are required to be receiving external to the study).

L12. Reporting Mechanisms for Adverse Events to the IRB and NIGMS

Any serious adverse events (SAE) and/or reportable deviations will be reported within one business day to the LIBR Human Protection Administrators at (918) 502-5155 or via email at hpa@laureateinstitute.org. Reporting to the IRB will be completed within five business days; reporting to the NIGMS will be made according to their respective regulations governing SAE reporting. Any unanticipated adverse events will be reported immediately to the IRB. All adverse events will be included in the annual IRB report. In addition, an Independent Medical Monitor will review the study adverse events and make recommendations to the PI regarding safe continuation of the study.

L13. Potential Benefits of the Proposed Research to Research Participants and Others

There is no immediate personal or medical benefit derived from participation. The results of this study, however, will further the scientific community's understanding of mood and anxiety disorder pathophysiology and ultimately may lead to better treatment. As such, participants may derive personal satisfaction from their contribution to the discovery process.

Why risks are reasonable in relation to benefits: MRI procedures generally hold few risks to persons, other than to individuals listed in the exclusion criteria. Functional MRI provides information about the function of the human brain that cannot be obtained using any other non-invasive technique. By increasing understanding of

pathophysiology and/or aiding in the development of novel treatments, this research experiment has the potential to improve diagnostic capabilities and treatment modalities. The importance of the knowledge to be gained from this study exceeds the associated potential risks.

L14. Importance of the Knowledge to be Gained

Given the major public health burden imposed by depression, the limited effectiveness of current antidepressant therapies, and the lack of clarity of how precisely to conceptualize depression severity and stage of illness, there is a critical need for non-invasive biomarkers of disease severity to assess prognosis, monitor therapeutic progress, and predict response to existing and future treatments. This experimental project uses an acute manipulation of the RNT-related brain circuit (functional connectivity) in order to begin to understand how modulations of the RNT brain circuit influence the recovery from depression.

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