Modulation of Motor Cortex Excitability by Glucose Administration

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MODULATION OF MOTOR CORTEX EXCITABILITY BY GLUCOSE ADMINISTRATION

A pilot and single-site study for investigating neuronal excitability of blood glucose dynamics.

Study Code: Glucose

Funding Mechanism:	*NORC Pilot Grant*
Principal Investigator:	*Flavio Frohlich, PhD*
Co-Investigator:	*John Buse, MD PhD; Kai Xia, PhD*
Protocol Number:	*19-1451*
Version Number:	*1.6*
Date:	*3 March 2020*

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BIS/BAS	Behavioral Inhibition System / Behavioral Approach System
CFR	Code of Federal Regulations
CI	Confidence interval
Co-I	Co-Investigator
CRF	Case Report Form
DHHS	Department of Health and Human Services
DMV	Department of Motor Vehicles
DSM-5	Diagnostic and Statistical Manual of Mental Disorders (Version 5)
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
EEG	Electroencephalogram
EMG	Electromyography
EOG	Electrooculography
FDA	Food and Drug Administration
FDI	First Dorsal Interosseous
GCP	Good Clinical Practice
GTT	Glucose Tolerance Test
Hz	Hertz
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IDE	Investigational Device Exemption
IRI	Interpersonal Reactivity Index
NAMI	National Alliance on Mental Illness
NIH	National Institutes of Health
NIMH	National Institute of Mental Health
NORC	Nutrition Obesity Research Center
OHRE	Office of Human Research Ethics
OHRP	Office for Human Research Protections
PHI	Protected Health Information
PI	Principal Investigator
PMA	Premarket Approval
SMC	Safety Monitoring Committee
SPI	Serial Peripheral Interface
SOP	Standard Operating Procedure
TEP	TMS-evoked potential
TMS	Transcranial Magnetic Stimulation
UE	Unexpected Event
UNC	University of North Carolina
UNC-CH	University of North Carolina at Chapel Hill
US	United States

STUDY SUMMARY

Title	*Modulation of motor cortex excitability by glucose administration*
Short Title	*Glucose*
Protocol Number	*19-1451*
Phase	*Pilot*
Methodology	*Single-site*
Study Duration	*This study is expected to last 1 year.*
Study Center(s)	*This is a single-site study performed at The University of North Carolina at Chapel Hill.*
Objectives (Purpose)	*Purpose of this pilot study is to investigate how changes in blood glucose level modulates neuronal excitability and brain network dynamics.*
Number of Subjects	*20*
Diagnosis and Main Inclusion Criteria	*Eligible participants will be healthy individual without any severe illness and mental disorder.*
Description of Intervention (Procedures/methods)	*Each participant will be seated in a reclining chair and receive transcranial magnetic stimulation (TMS) to determine resting motor threshold (RMT). At baseline, participants will receive 10 TMS pulses (120% intensity relative to RMT) while wearing an electroencephalography (EEG) net to record brain activity on the scalp. TMS-induced motor-evoked potentials (MEPs) and EEG data will be recorded. In addition, blood glucose level testing will be performed by finger prick to measure baseline glucose level. In an intervention step, glucose drink (75g of sugar) or drinking water (control condition) will be administered. Both blood glucose level testing and TMS-induced MEPs and EEG data collection will be performed at 0, 30, 60, 120, and 180 minutes after drink administration.*
Related IRB Applications	*17-0149*

1 KEY ROLES

1.1 INDIVIDUALS

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Medical Monitor	

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1.2 INSTITUTIONS

The University of North Carolina at Chapel Hill

1.3 OPTIONAL

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The University of North Carolina – Chapel Hill
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113

1.4 FUNDING SOURCES

Please list below the funding sources for this project:

Sponsor Name	UNC Ramses Number	Sponsor Type	Prime Sponsor Name	Prime Sponsor Type	Sponsor/Grant Number
UNC NORC			NIH/NIDDK	Federal	P30 DK056350

External Funding: This project is externally funded and UNC-CH is the direct recipient of federal funds.

UNC-CH Funding: This project is funded through UNC-CH Nutrition Obesity Research Center.

Classified: This project is not classified.

2 INTRODUCTION

This document is a protocol for a human research study. This study is to be conducted according to U.S. and international standards of Good Clinical Practice (FDA Title 21 Part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

2.1 BACKGROUND

More than a third of the adult US population has impaired glucose tolerance, which is tightly linked to obesity and a risk factor for the development of diabetes and disorders of the central nervous system. In particular, diabetes exhibits a bidirectional causal association with depression. A growing literature delineates the pathophysiology underlying mood disorders at the level of large-scale activity patterns in brain circuits (Davidson, 1985; Nusslock et al., 2015; Smart et al., 2015; Sutton and Davidson, 1997), which has enabled the development of novel brain stimulation treatments such as transcranial magnetic stimulation (TMS) (George et al., 1995). TMS is one of the non-invasive brain stimulation tools used to deliver electrical stimuli through the scalp in humans. In general, single-pulse TMS (including paired-pulse TMS) is used to explore brain functioning. TMS on the motor cortex leads to a twitch in the target muscle evoking motor-evoked potentials (MEPs) on electromyography (EMG) electrodes. The MEP is usually used to assess corticospinal tract excitability (Tofts, 1990). In addition, combining TMS with electroencephalography (EEG) can capture cortical excitability beyond corticospinal excitability since EEG mainly captures neural activity of cortical neurons (Farzan et al., 2016). The concurrent combination of TMS with EEG can be a powerful technology for characterizing and modulating brain networks.

2.2 DOSE RATIONALE

In this study, our primary purpose is to investigate how changes in blood glucose level affect neuronal excitability. We will administer a 75g load of glucose (oral glucose tolerance test, OGTT) that complies with the standard guidelines for diagnosing diabetes

2.3 STUDY AIMS/HYPOTHESES

2.3.1 SPECIFIC AIMS

Aim 1. Examine how glucose levels gate neuronal excitability measured by the response to TMS.

Aim 2. Delineate how brain network dynamics are modulated by experimentally induced elevated blood glucose levels.

2.3.2 RESEARCH HYPOTHESES

We hypothesize that glucose administration modulates neuronal excitability measured by TMS-induced MEPs and TEPs. We also hypothesize that frontal theta (4-8Hz) and left frontal alpha (8-12Hz) oscillations, which represent an engaged (theta) and disengaged (alpha) cortical state, are modulated in response to glucose administration compared to water drink. The modulation of neuronal excitability and brain network dynamics is depending on blood glucose level

3 SUBJECT SELECTION AND WITHDRAWAL

A total of 20 healthy participants will be recruited for this study and all data will be collected at UNC-CH. No specific plans have been made to enroll participants from vulnerable populations.

3.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, a participant must meet all of the following criteria:

- Between the ages of 35 and 65
- Right-handed
- Body mass index (BMI) < 30
- Fasting blood glucose test level < 95 mg/dL
- Capacity to understand all relevant risks and potential benefits of the study (informed consent)
- Willing to comply with all study procedures and be available for the duration of the study
- Speak and understand English

3.2 EXCLUSION CRITERIA

A potential participant who meets any of the following criteria will be excluded from participation in the study:

- Diagnosis of diabetes or pre-diabetes
- Neurological disorders and conditions, including, but not limited to:
    - History of epilepsy
    - Seizures (except childhood febrile seizures and ECT-induced seizures)
    - Dementia
    - History of stroke
    - Parkinson's disease
    - Multiple sclerosis
    - Cerebral aneurysm
    - Brain tumors
- Medical or neurological illness or treatment for a medical disorder that could interfere with study participation (e.g., unstable cardiac disease, HIV/AIDS, malignancy, liver or renal impairment)
- Prior concussion
- Prior brain surgery
- Any brain devices/implants, including cochlear implants and aneurysm clips
- Traumatic brain injury
- Anything that, in the opinion of the investigator, would place the participant at increased risk or preclude the participant's full compliance with or completion of the study

Justifications for any exclusions based on race, gender, or ethnicity: Non-English speaking individuals are excluded because the ability to accurately and completely communicate study information, answer questions about the study, and obtain consent are necessary.

Justification for excluding women or women who become pregnant

Pregnant participants will be excluded despite the fact that theoretical risk to mother or fetus is exceedingly small, since no safety data for pregnancy is known to exist for TMS studies. Furthermore, the TMS device description indicates that the device should not be applied to the head, neck, or abdomen of pregnant women. We will verify pregnancy status via a urine pregnancy test for all female participants prior to receiving treatment on the first session of the study. Women will be asked prior to each test sessions about the likelihood they could be pregnant. Any woman who indicates there may be a possibility or is unsure will be reminded the testing is contraindicated in pregnant women and will be offered the opportunity to have pregnancy testing repeated prior to TMS.

3.3 STRATEGIES FOR RECRUITMENT AND RETENTION

3.3.1 RECRUITMENT

We intend to recruit 20 healthy participants at the University of North Carolina at Chapel Hill. Study coordinator will be informed of the inclusion and exclusion criteria and will be asked to discuss the study with their subjects. Study coordinator will identify subjects they believe to be appropriate for this study based on the information we will provide them about the study. Study coordinator will ask subjects whether they are willing to be contacted by the research team regarding participation.

3.3.2 RETENTION

Our retention strategy includes monetary compensation for the time and effort required to participate in the study. The participant will receive payment at the stimulation session. The research staff will also give each participant a reminder call or email for the stimulation session. Each research staff member will be easily available for the participants to contact via email or phone.

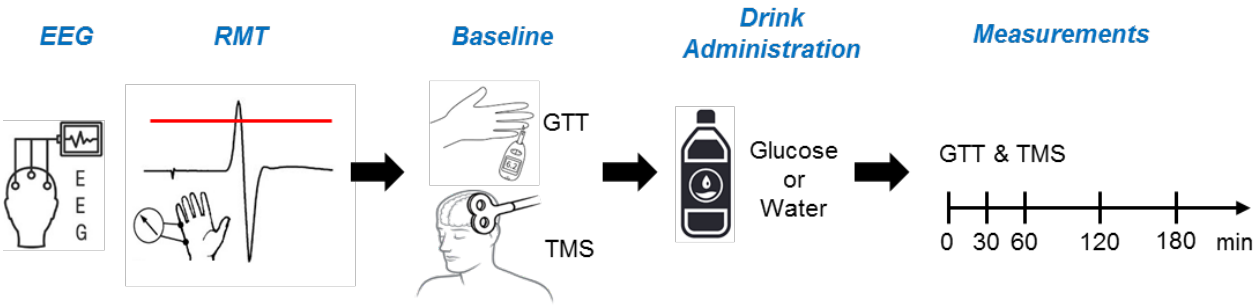
The inclusion criteria state that each participant must be able to understand all risks and benefits associated with this study. We will be asking each participant to answer questions about the consent form to determine that the study process and the duration of participation are completely understood by all participants. We will aim to have a specific research team member assigned to complete all sessions with the same participant to establish rapport and encourage the participant to continue attending sessions.

4 BASIC STUDY DESIGN

Participants who pass an initial screening by phone are asked to attend a screening visit where consent is provided, eligibility for study participation is confirmed and demographic information is collected. Participants who are eligible to participate will then be asked to return for two study sessions after an MRI scan. Sessions will start at 8.00 am and study participants will be asked to fast for the preceding 12 hours and to consume approximately 150g of carbohydrates each day for the 3 days prior to the session. The study procedures are identical for both study visits. The order of the two sessions is equally randomized across participants.

In one visit, an oral 3-hour GTT with a 75g load of glucose will be administered. In the other visit, pure drinking water will be used. Both study visits have the following time-line:

1. Determined the target (hand area of the left precentral gyrus, left primary motor cortex) based on MR images using a TMS neuro-navigation system.
2. Fitting of an EEG electrode net.
3. Baseline EEG and MEP measurements elicited by TMS
4. Measurement of fasting blood glucose level by finger prick
5. Administration of drink (glucose drink or water, 296mL of H_2O for both drinks)
6. Capillary blood draw (finger prick) for glucose level testing and TMS-induced EEG and MEP measurements at 0, 30, 60, 120, 180 minutes after drink administration
7. Digitization of EEG electrodes on the scalp using a camera-type scanner



4.1 TREATMENT ASSIGNMENT PROCEDURES

Each participant will experience two experimental conditions (glucose drink and water). There are 2 ways to order the two conditions (glucose drink and then water, water and then glucose drink). By randomization, each participant will be assigned to one of the sequences. Ten participants will be assigned to each of these 2 sequence groups. Each session has at least a 3-day gap to minimize the presence of outlasting effects of either the GTT or the TMS. The randomization schedule will be computed prior to recruitment of subjects and the random assignments will be concealed from the personnel who screen potential participants until the moment that the participant is enrolled and ready for baseline evaluations. To accomplish this, the randomization schedule will be used to create a set of sequentially-numbered opaque sealed envelopes.

Glucose drink → Drinking water (10 participants)
Drinking water → Glucose drink (10 participants)

5 STUDY SCHEDULE

In order to increase data quality, the assessments for an individual participant will be administered by the same researcher. It is important to note that consent, scales, and experiments will all take place in a private room. Any phone calls will take place in a private lab environment as well.

5.1 SCREENING

- Phone screening

The phone screening allows researchers to screen out participants based on self-report responses and for potential participants to become familiar with the study schedule, including procedures. During the telephone screening, researchers will provide a brief background about GTT, TMS, and EEG. The timeline will be explained to the participants and the participant will be informed of compensation, both amount and payment schedule. The participant will be asked if they have any questions. Once all questions have been answered, the participant will be asked if he/she is still interested in participating in the study. If yes, the researcher will ask if the participant will provide verbal consent to begin the initial phone screening which will determine eligibility for the stimulation session. A telephone script, which includes the screening questions, is provided in *Appendix E.*

- Screening visit (Visit 1)

Participants who pass an initial screening by a telephone call are asked to attend a screening visit where consent is provided, eligibility for study participation is confirmed and demographic information is collected. To ensure that all aspects of the research are understood, participants may be asked a series of questions about the research they are about to take part in (*Appendix C*). After then, participants are asked to provide weight and height for calculating body mass index (cut-off level < 30). Fasting blood sugar test level will be collected (cut-off level < 95 mg/dL). If participants meet the inclusion/exclusion criteria, they will be asked to visit 2 study sessions after an MRI scan. Also, participants will perform a cognitive control task to measure baseline cognitive function. At the screening session, the participants will complete the STAI form Y-2 and the IDAS to determine their trait levels of anxiety and depression. This data will be used to explore how a participant's trait levels of anxiety and depression affect their corticospinal activity. The BIS/BAS and STAI form Y-1 questionnaires will be administered twice at each experimental session, once before the drink administration and once three hours after. The PANAS questionnaire will be administered before the drink administration, as well as 0, 30, 60, 120, and 180 minutes after the drink administration. The scores on these questionnaires will be compared before and after the drink administration to determine how the drink affected the participants' psychological state over time. For each participant, scores on these questionnaires will be compared between the session during which they consumed glucose and the session during which they consumed water.

- Questionnaires (Appendix F)

Participants will be asked to complete the following questionnaires.
1. State-Trait Anxiety Inventory (STAI): a 40-item self-report questionnaire for assessing state and trait anxiety). This questionnaire will be used to determine each participant's level of both state and trait anxiety
2. The Positive and Negative Affect Schedule (PANAS): a self-report questionnaire that consists of two 10-item

scales to measure both positive and negative affect. This questionnaire will be used to measure participants' positive and negative effect.
3. The Edinburgh Handedness Inventory: a well-known questionnaire for determining objectively whether one is left or right handed. This will be used to confirm that all participants are right-handed.
4. Behavioral avoidance/inhibition (BIS/BAS) scales: a questionnaire that consists of 24-item for assessing behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment. This questionnaire measures each participant's motivation to avoid adverse events and approach goal-directed activity. Since a loss of motivation is a key element of depression and other psychological illnesses, this questionnaire can indicate psychological well-being.

5. Inventory of Depression and Anxiety Symptoms (IDAS): a questionnaire that consists of 64 items to assess specific symptom dimensions of major depression and related anxiety disorders. The IDAS consists of ten symptom scales including suicidality, lassitude, insomnia, appetite loss, appetite gain, ill temper, well-being, panic, social anxiety, and traumatic intrusions.

5.2 STUDY SESSIONS

- MRI scan (Visit 2)

We will ask participants to come in and complete an MRI scan. MRI scan imaging will take place at the Biomedical Research Imaging Center (BRIC), located on the first floor in Marsico Hall. Screening questions will be completed prior to the MRI scan (attached) and all preparations for the scan will take place in a facility area behind a locked door, where only research study personnel have access. When subjects are required to change their clothing, they will be allowed to do so alone, in a room with a door that locks. MRI scan will be performed by trained study personnel or designees of the PI that are approved to complete an MRI scan.

- Study sessions (Visit 3-4)

All participants will undergo two study sessions (glucose or water administration) with at least 3-day gap to minimize the effect of either GTT or TMS. In each visit, all participants will be asked to fill out the questionnaires at the beginning and end of the session. Participants undergo a two-minute eyes-open resting-state EEG recorded with high-density, 128 channels EEG system (Netamps 410, Electrical Geodesics Inc, EGI) using Geodesic Sensor Nets (sampled at 1000 Hz). EEG nets will be fitted at the beginning of the two study visits and remain in place until completion of the study visit. Also, participants will perform a cognitive control task to investigate if changes in glucose blood level could modulate cognition.
Single-pulse TMS on the motor cortex leads to a twitch in the target muscle evoking a motor-evoked potential (MEP) measured by electromyography (EMG) electrodes. Participants will be seated in a comfortable armchair with their hands positioned on the armrest. Two disposable electrodes (15x21mm, Ambu Neuroline) will be placed in a tendon-belly arrangement over the first dorsal interosseous muscle and a ground electrode will be placed over the styloid process of the ulna. A figure-eight coil (C-B60, MagVenture) will be used for stimulation and EMG signals will be recorded by an amplifier (MagPro X100, MagVenture). The coil will be placed tangentially over the scalp with the handle pointing backwards and laterally at 45° from the mid-sagittal line. Biphasic TMS pulses will be applied over the motor cortex and the resting motor threshold (RMT) will be defined by the minimum TMS intensity required to evoke MEP of at least 50 uV in 50% of 10 consecutive trials. At baseline, 10 TMS pulses will be applied on the scalp and EEG and EMG data will be collected. At the same time, blood glucose level will be measured by 5uL of finger prick blood sampling as a baseline glucose level. In an intervention step, we will administer a 75g load of glucose drink or drinking water at each study visit. After then, glucose level testing and EMG/EEG data collection will be performed at 0, 30, 60, 120, and 180 minutes after the drink administration. Once participants complete the first study visit, a study coordinator will schedule their next visit with at least 3-day gap.

6 STUDY PROCEDURES/EVALUATIONS

6.1 SELF-REPORT MEASURES

Study coordinators will consider using a standard safety questionnaire to screen TMS candidates. The following questions represent the basic information required. Additional information may change according to particular demands. Consensus has been reached for this questionnaire (Rossi et al., 2009).

1. Did you ever have a concussion?
2. Do you have or have you ever had diabetes?
3. Do you have or have you ever had anorexia?
4. Do you have or have you ever had OCD?
5. Do you have or have you ever had ADHD?
6. Have you ever had a fainting spell or syncope? If yes, please describe in which occasion(s)
7. Have you ever had severe (i.e., followed by loss of consciousness) head trauma?
8. Do you have any hearing problems or ringing in your ears?
9. Are you pregnant or is there any chance that you might be?
10. Do you have metal in the brain/skull (except titanium)? (e.g.,splinters, fragments, clips, etc.)
11. Do you have cochlear implants?
12. Do you have an implanted neurostimulator? (e.g., DBS, epidural/subdural, VNS)
13. Do you have a cardiac pacemaker or intracardiac lines or metal in your body?
14. Do you have a medication infusion device?
15. Are you taking any medications? (Please list)
16. Did you ever have a surgical procedures to your spinal cord?
17. Do you have any disorder related to spinal cord or ventricle?
18. Did you ever undergo TMS in the past?

We will ask these questions in order to follow the standard safety guidelines for TMS studies. Answers from the questions will help us to exclude study participants regarding the safety of TMS

6.2 SPECIAL ASSAYS OR PROCEDURES

6.3.1 GLUCOSE TOLERANCE TEST

All participants will be asked to fast 12 hours prior to the test. In addition, we will ask participants to consume approximately 150 g of carbohydrates per day on the 3 days prior to the test to stabilize the participant's glucose response (See Appendix G for instruction sheet). We will perform a capillary blood draw by finger prick to obtain approximately 5uL of blood using a microcuvette. The blood sample will be immediately analyzed by an FDA-cleared glucose analyzer or stored in a refrigerator (below 46°F). We will be using HemoCue Glucose 201 system (HemoCue, Angelholm, Sweden).

6.3.2 MOTOR-EVOKED POTENTIALS AND TMS-EVOKED POTENTIALS

We will collect TMS-induced motor-evoked potentials (MEPs) and EEG potentials using EMG electrodes and a high-density EEG. Resting motor threshold (RMT) is determined while the target test muscle is at rest. EEG will be collected using Geodesic 400 system (EGI Inc., Eugene, OR, USA).

7 STUDY INVESTIGATIONAL PRODUCT

- Glucose solution

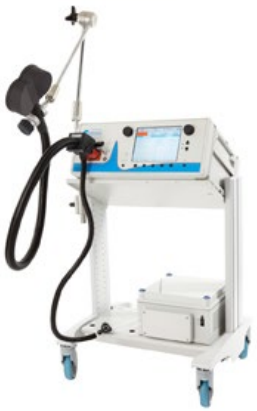
We will use a FDA-cleared glucose drink (Azer Scientific Inc., Morgantown, PA) contains the following ingredients: dextrose (source: corn), citric acid, artificial flavoring, sodium benzoate (0.1%), and purified water. The glucose solution is manufactured according to WHO and ADA standards, and it is produced in a FDA certified, pharmaceutical facility. The glucose drink is 10 fluid ounces (296mL), so the participants will receive 10 fluid ounces of water during the other session.

- Glucose tolerance test (GTT)

We will use the Glucose 201 system (HemoCue, Angelholm, Sweden) for measuring blood glucose level. The HemoCue Glucose 201 System is based on a glucose dehydrogenase method and consists of a small dedicated analyzer and a unique disposable microcuvette. The system combines the precision and accuracy of a central laboratory with the speed and convenience of obtaining results at the point-of-care. With just a fraction of a drop of whole blood and three simple steps, the HemoCue Glucose 201 System will produce immediate, accurate results for diagnosing or monitoring diabetes, as well as monitoring neonatal blood glucose levels.
The system has several features:

- Factory calibrated and traceable to ID GC-MS method, needs no further calibration and no coding
- Approximately 5 µL whole blood sampling
- Sample material: Capillary, venous or arterial whole blood including neonatal blood
- Display range: Plasma equivalent values 0-444 mg/dL
- Result calculation time: 40-240 seconds

- Transcranial magnetic stimulation (TMS)



We will use the MagPro X100 system (MagVenture Inc., Alpharetta, Georgia, USA) for magnetic stimulation. The MagPro X100 is an advanced, high performance magnetic stimulator designed primarily for research purposes. It is a high-quality tool for researchers with a large choice of stimulating parameters and has stimulation rates up to 100 pps at high intensities and the possibility to combine waveforms and pulse modes.

The simulator has several features:

- 3 waveforms: Biphasic, Biphasic Burst and Monophasic.
- Selectable current direction.
- Stimulation rates up to 100 pulses per second.
- Easily connects to external equipment via programmable input/output triggers.
- System operation control via a built-in computer, eliminating the need for an external computer to set up and control the timing of stimulus sequences.

- Controllable from an external device.

7.1 SAFETY FEATURES

- GTT

According to the Hazard Communication Standard 29 CFR 1910.1200 and the Guidance for Hazard determination, chemicals that falls under the category "articles" does not apply to this standard.
"Article" means a manufactured item other than a fluid or particle:
(i) which is formed to a specific shape or design during manufacture;
(ii) which has end use function(s) dependent in whole or in part upon its shape or design during end use; and
(iii) which under normal conditions of use does not release more than very small quantities, e.g., minute or trace amounts of a hazardous chemical and does not pose a physical hazard or health risk to employees. All HemoCue Microcuvettes are articles, therefore this standard is not applicable.

- TMS

In the USA federal law regulates the sale of Medical Devices through the US Food and Drug Administration (FDA). This is done to ensure safety and effectiveness. Devices which are permitted to be marketed for their intended use must either have a 510(k) or PMA clearance. The use of devices for other than their FDA cleared intended use is considered as investigational. Such use is only permitted if the Investigational Device Exemption (IDE) guidelines have been followed. All investigational devices must be labeled in accordance with the labeling provisions of the IDE regulation (§ 812.5) and must bear a label with this statement:

7.2 PREPARATION AND ADMINISTRATION OF STUDY INVESTIGATIONAL PRODUCT

After participants have completed the questionnaire, they will be comfortably seated. The study coordinator and/or the research assistant will be thoroughly trained and have trainings documented on the glucose blood sampling and TMS device and will be present during all sessions. To monitor side effects of blood sampling and stimulation, a questionnaire will be administered after each session.

7.3 ASSESSMENT OF PARTICIPANT COMPLIANCE WITH STUDY INVESTIGATIONAL PRODUCT

Compliance for this study includes making 2 study sessions. Participants have the ability to schedule their second visit 3 days after the first visit. Follow-up emails will be sent out 1 day before the scheduled second visit.

8 POTENTIAL RISKS AND BENEFITS

8.1 BENEFITS TO SUBJECTS AND SOCIETY

There is no direct benefit to participants but the research outcome will help to design new study for understanding the underlying mechanism of brain network excitability and diabetes.

8.2 POTENTIAL RISKS

8.2.1 PSYCHOLOGICAL

Risk of Embarrassment: Self-report questionnaire contains questions regarding personal information. This risk is necessary in order to assess symptomology and associated psychopathology. Participants will be assured upon intake that only study personnel will see any answers.

Risk of Confidentiality Breach. To avoid breaches in confidentiality, study documents that contain personal information, including the informed consent, and the document that links study ID numbers to personal identifying information are kept in locked filing cabinets in locked rooms, separate from any source documents containing participating dummy identifiers. All data is stored in locked cabinets inside locked offices; electronic data will be stored only on password-protected computers, and data encryption methods will be used during communication between investigators. Only study personnel will have access to these data. All study staff participate in annual human participating training that includes education about responsibilities to minimize the risk of confidentiality breach. In the unlikely event of a breach of confidentiality, people might discover that an individual was involved in this research study and some people might not agree with the principle of participating in research or of changing natural brain activity.

8.2.1 PHYSICAL

Risk of Injury and Discomfort: Non-invasive brain stimulation has been used without any reports of serious side-effects for more than a decade and TMS has been cleared for use in the USA by the FDA. The stimulation made nothing to do with electroconvulsive therapy that applies many orders of magnitude higher stimulation current. Some participants report a scalp pain, tingling, burning, or itching on the stimulation site but no other side effects have been noted. In order to monitor these side-effects, we will be administering side effects stimulation questionnaire after each stimulation session to determine whether these effects were experienced. Research personnel present during these sessions will also check in with the participant periodically during the stimulation to see whether they are comfortable. If any side-effect occurs (rated by the participant as stronger than “moderate”) or the participant is experiencing severe discomfort, the stimulation will be immediately stopped.

Risk of Bleeding: The glucose tolerance test is a medical test in which glucose is given and blood samples taken afterward to determine how quickly it is cleared from the blood. The test is usually used to test for diabetes, insulin resistance, impaired beta cell function, and sometimes reactive hypoglycemia and acromegaly, or rarer disorders of carbohydrate metabolism. We strictly follow the medical guidelines using an FDA-cleared device and sampler and blood sampling will be immediately stopped if any unexpected bleeding occurs.

Risk of Glucose Ingestion: The risks associated with glucose ingestion in healthy participants are minimal.

The potential side effects include nausea, sweating, light-headedness, vomiting, bloating, headache, diarrhea, and constipation. Serious side effects from the test are very uncommon.

8.3 REFERRALS FOR MEDICAL FOLLOW-UP OR PSYCHOLOGICAL COUNSELING

There is a purely theoretical likelihood that stimulation of neuronal circuits can lead to epileptic discharge. To minimize this occurrence, we screen and exclude subjects with personal history of neurological conditions from the study. If abnormalities or a seizure is witnessed during the course of the study, a referral will be made to the UNC Department of Neurology for follow-up. In the theoretical event that a seizure is witnessed that involves the loss of consciousness, the subjects will be told not to operate a motor vehicle until cleared by the DMV.

There is an additional albeit small likelihood that a participants blood glucose levels at 2 hours may be indicative of impaired glucose tolerance and a possible undiagnosed status of diabetes or prediabetes. Since the GTT is not administered in a medical context or under the supervision of an MD, this is not taken as a conclusive result. Therefore in the event that this occurs, the participant will be contacted by our study diabetes doctor (Dr. John Buse, MD, PhD) or the participant may be referred to follow up with his or her own primary care doctor.

To ensure participant comfort, a study coordinator or research assistant will periodically check in with the participant about any side-effects he/she may be experiencing during each stimulation.

9 DATA AND SAFETY MONITORING

9.1 FROHLICH LAB MONITORING PLAN

The purpose of this monitoring plan is to present the Frohlich Lab's approach to monitoring clinical trials. The plan facilitates compliance with good clinical practice (GCP):

a. The rights and well-being of human subjects are protected.

b. The reported trial data are accurate, complete, and verifiable from source documents

c. The conduct of the trial is in compliance with the currently approved protocol/amendment(s) with GCP, and with applicable regulatory requirement(s)

This section identifies key monitoring activities and specifies the data to be reviewed over the course of a clinical trial. This is a single site, investigator-initiated, clinical trial, so there will be no site monitoring plan in place.

The latest version of the approved IRB application for this clinical trial will be followed at all times. This responsibility falls into the hands of the study coordinator and research assistants. If at any time there is a deviation from protocol, the deviation from protocol log will be filled out. All team members will be trained on how and when to use this log. The most up-to-date IRB application will be on file in the Clinical Trials office in Room 233 of the Medical School Wing C. Deviations will be sent to the IRB every 4-6 weeks (if necessary).

Periodically, study staff should review 3 randomly selected informed consent forms to ensure that (1) these forms have been filled out appropriately, and (2) the consent form process was followed and properly documented. Should any consent form be in violation, the research team will perform and document a complete review of all consent forms.

AE and SAE are clearly defined in this document. Documents of AE and SAE can be found in the study binder on file in the Clinical Trials office in Room 233 of the Medical School Wing C. It is the responsibility of the study coordinator to report all events to the PI in a timely manner (see 9.3 Reporting Procedures). All AEs and SAEs will be discussed with the PI. For our practices, we have adapted the decision tree provided by the UNC-CH IRB to assist with reporting of such events.

Periodically, the study coordinator should also choose one CRF/eCRF and Source Document to assess for completion and maintenance. In addition, the PI will assess completeness of data on REDCap. The PI has read-only access. This allows the PI to view reports that provide information on any missing data on an individual participant basis, but does not allow the PI to add, change, or input any data. A data safety monitor will review blinded AEs every month.

9.2 SAFETY OVERSIGHT BY THE DSMB

A data safety monitoring board will not be used for this study.

9.3 EARLY WITHDRAWAL OF PARTICIPANTS

9.3.1 REASONS FOR WITHDRAWAL

A study participant will be discontinued from further participation if:

- The participant meets any exclusion criteria (either newly developed or not previously recognized).
- Anything that, in the opinion of the investigator, would place the participant at increased risk or preclude the participant's full compliance with or completion of the study.

Participants are free to withdraw from participation in the study at any time upon request.

9.3.2 DATA COLLECTION FOR WITHDRAWN PARTICIPANTS

We will collect safety data on any participant discontinued because of side effect. In any case, every effort will be made to undertake protocol-specific follow-up procedures. If voluntary withdrawal occurs, the participant will be asked to continue scheduled evaluations and complete an end-of-study evaluation. In the case of an early withdrawal, the researcher will make a note to file indicating this.

9.4 TERMINATION OF STUDY

Participants will be informed of their right to discontinue the study at any time with no penalty. If a participant decides to drop out of the study, either because they cannot tolerate the 75 grams of glucose or they are uncomfortable with the stimulation, the experiment will be immediately stopped. The participant will still be compensated for the entire session. The data from that participant will not be used in analysis. If the participant has already completed either a blood draw or TMS stimulation when they decide to drop out of the study, they will be asked to complete the blood sampling and stimulation questionnaire.

If a seizure occurs at the time of a study visit, a temporary hold will be placed over the study and further investigation will ensue. This could lead to stopping the study prematurely or continuing on with further safety measures in place. If two seizures are witnessed during the study visit, the entire study will be stopped prematurely. These individuals would be referred for further medical attention. It is very unlikely that a seizure will occur.

The study will also be stopped (at least temporarily) if studies provide evidence that transcranial current/magnetic stimulation caused brain damage or other harmful effects on subjects, either short-term or long-term

The IRB will also be informed promptly and provided the reason(s) for the termination of suspension of by the investigator, as specified by the applicable regulatory requirement(s).

10 SAFETY & REPORTING

It is important to assess safety over the course of this study. This section describes in detail how safety is assessed, reporting of side effects and unanticipated problems. This section is a reference for internal use.

10.1 SAFETY PARAMETERS

STIMULATION SIDE EFFECTS. The side effects listed are headache, neck pain, scalp pain, tingling, itching, ringing/buzzing noise, burning sensation, local redness, sleepiness, trouble concentrating, improved mood, worsening of mood, dizziness, flickering lights, and other (specify). Participants are also asked to rate on a 5 point likert scale how related they believe the side effects to be to stimulation (1 = no relation, 2 = remote, 3 = possible, 4 = probable, 5 = definite).

10.2 METHODS AND TIMING FOR ASSESSING, RECORDING, AND ANALYZING SAFETY PARAMETERS

10.2.1 UNANTICIPATED PROBLEMS

Unexpected Events (UE) will be recorded) and will include information such as the date of the event, when the study team was informed of this event, details of the event, when the IRB was notified. The IRB will be notified of each UE that may occur during the study.

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in the guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

If an UE occurs the IRB will be notified and the study will be adjusted as needed to protect the health and safety of the participants. Depending on the nature of the UE, the research protocol, inclusion/exclusion criteria, and informed consent will be changed to reflect the possibility of this event reoccurring. During this time, no new participants will be recruited and the research procedures for currently enrolled participants will be stopped. Each UE will be recorded and reported throughout the study.

11 STATISTICAL PLAN

The statistician for this study is Dr. Kai Xia.

11.1 STATISTICAL ANALYSIS STRATEGIES

Outcome variables include:
Aim 1
1) motor-evoked potentials to measure corticospinal excitability (unit: uV)
2) TMS-evoked potentials to measure cortical excitability (unit: uV)
Aim 2
1) EEG spectral power in the theta-band (4-8Hz) averaged over frontal EEG channels to measure engaged brain network dynamics (unit: dB).
2) EEG spectral power in the alpha-band (8-12Hz) averaged over left frontal EEG channels to measure disengaged brain network dynamics (unit: dB).

For all analyses, outcome measures are analyzed as the difference from baseline metrics before the GTT. The repeated measures of primary outcome will be fitted into general linear mixed effect model where baseline measures (continuous), time of measures (categorical), treatment method (binary) and time by treatment as fixed effects, while the within-subject effect among different time point (0, 30, 60, 120, 180 minutes) is modeled as random intercept assuming autoregression model (AR(1)) as covariance structure. The two sided Wald-test of treatment method (fixed effect) between the verum GTT versus control GTT condition is our primary result of interest. We hypothesize a significant main effect for factor GTT but no effect for factor time nor the interaction of GTT by time. We hypothesize that verum-GTT will modulate corticospinal and cortical excitability. In case we do find a significant interaction of GTT by time, we will use post-hoc contrast test to compare the overall differences between verum-GTT versus control-GTT at each time point and correct for multiple comparisons using false discovery rate (FDR) method. All hypothesis tests that are observed to be not statistically significant will be reported as being inconclusive. Based on the nature of our study design, we assume missing data to be minimal, where linear mixed effect model is robust to handle such missing value situations without creating any obvious bias. For all the result tables of statistical analysis, statistical estimates of population parameters will be tabulated along with corresponding confidence intervals (CIs). Given the lack of any comparable study in the literature, we performed a power analysis guided by the number of participants we are able to enroll based on the size of the grant. Briefly, with a sample of N=20 participants, we will be able to detect an effect size of dz = 0.66 with alpha = 0.05 and beta = 0.2. Power analysis was performed with G*Power.

Questionnaire data
The level of trait anxiety, as indicated by the STAI form Y-2, will be compared to the participant's corticospinal excitability to investigate whether a correlation between the two factors exists. Lower motivation, as measured by the BIS/BAS, is hypothesized after glucose consumption as compared to after water consumption. Greater state anxiety, as measured by the STAI form Y-1, is hypothesized after glucose consumption as compared to after water consumption. Greater negative affect and lesser positive affect, as measured by the PANAS, is hypothesized after glucose consumption as compared to after water consumption.

11.2 CHOICE OF SAMPLE SIZE

The choice of sample size was based on expert opinion and funding limit; we conjecture that 20 participants may be sufficient to provide adequate precision for the estimators of variance components and other parameters for which information is needed to plan a future study. As a rough and indirect assessment of the precision afforded by studying a sample of 20 subjects, we considered that an estimate of the proportion of subjects in the target population that would experience a greater decrease in MEP and TEP amplitude with verum-GTT (Glucose), relative to control-GTT(water) for an observed estimate of 50%, the 95% confidence interval would span [50% ± 20%]. Or, if 80% was the observed estimate, then the 95% CI would span [80% ± 16%].

11.3 DATA MANAGEMENT

The glucose solution will be acquired from Azer Scientific and stored in a secure air-conditioned facility. The solution will be packaged and shipped in shatter-proof 10 fluid ounce plastic bottles with clear labels

Data will be stored in a password-protected cloud-based data system that does not contain any participant information. Individual records are referred to by dummy identifiers that cannot be traced back to the study participants except with the master code list that is stored separately in a secured location.

12 DATA HANDLING AND RECORD KEEPING

The study coordinator and research assistants are responsible for the accuracy, completeness, legibility, and timeliness of the data reported.

12.1 PHI AND HIPAA

Information about study participants will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from the participants in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a participant revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization. For participants that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e., that the subject is alive) at the end of their scheduled study period.

12.2 CONFIDENTIALITY

12.2.1 ACCESS TO SOURCE DOCUMENTS

The research coordinator, research assistants, and PI will have access to all of the source documents collected over the course of the study. The Co-I and medical monitor will have access to files upon request, as they will need access to the locked rooms and filing cabinets in which these documents are located.

Data will stay on a password-protected computer. Subsequently, a copy will be processed on a separate, password-protected desktop computer in the Frohlich Lab (Neuroscience Research Building 4129).

12.2.3 OTHER

Please note that there is no significant risk of deductive disclosure in this study. In addition, none of the groupings or subgroupings used in analysis will be small enough to allow individuals to be identified.

12.3 SOURCE DOCUMENTS

Source data is all information, original records of clinical findings, observations, or other activities in a study necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Source data include:

PARTNERS HUMAN RESEARCH COMMITTEE (IRB).

- All IRB correspondences are documented
- The study staff is IRB approved prior to performing any study procedures
- All versions of the IRB protocols and informed consent forms are on file

INFORMED CONSENT.

- Ensure that participant identification is not recorded on the ICF (i.e., no participant ID)
- There is documentation that the participant is given a copy of the consent form
- The participant and study representative signed and dated the consent form for him/herself
- The participant initialed and dated all appropriate pages on the informed consent form
- Note to file (Appendix D) made for any informed consent deviations
- Ensure a valid (current version date) copy of the consent form was used

PROTOCOL DEVIATIONS.

- Any and all protocol deviations (exceptions and violations) are documented in the participant folder and reported to the IRB as required

OTHER SOURCE DOCUMENTS.

- Each participant folder will contain a checklist to ensure that all source documents are administered and collected properly. The checklist will be dated by the researcher for each time an assessment is administered
- Review participant folders to ensure the accuracy, completeness, and legibility of the data.
- Any correction made to the source documents is dated, initialed, and explained. The original entry should not be obscured.
- The protocol-specific source documents are on file.
- Source documents are completed in ink.
- Note to files (Appendix D) are made for missing or incomplete data and to explain any discrepancies or additional comments.

12.4 DATA MANAGEMENT RESPONSIBILITIES

The responsibilities designated to each member of the research team are documented on the Delegation of Authority Form. The study coordinator and research assistants will be responsible for the informed consent process, review for eligibility, questionnaire administration, data entry, device administration, EEG administration, and CRF entries. The study coordinator will be responsible for documentation and reporting, while the PI will be responsible for review of the documentation forms, and overview of the research staff.

12.5 DATA CAPTURE METHODS (REDCAP)

All data will be entered into a data capture system provided by REDCap. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

All assessments completed by the participant at home will be completed via REDCap as well, ensuring participant security and confidentiality.

12.6 PROTOCOL DEVIATIONS

All deviations from the protocol will be addressed in study participant source documents. The researcher will complete a Protocol Deviation Log using the participant code as the identifier. This form will collect information such as the date the deviation occurred, details of what the deviation consisted of, any corrective and preventative actions that were taken as a result of the deviation, and the date that the PI and IRB were notified. The PI will

review the information and initial once approved. A completed copy of the Protocol Deviation Form will be maintained in the regulatory file, as well as in the participant's source document. Protocol deviations will be sent to the IRB per their guidelines. The site PI/study staff will be responsible for knowing and adhering to their IRB requirements.

12.7 RECORD RETENTION

According to the University of North Carolina at Chapel Hill's Archives and Record Management Services schedule for General Records Retention and Disposition Schedule 6.10, records will be kept for 5 years after the completion of the study or grant end date, whichever is later.

13 ETHICAL CONSIDERATIONS

13.1 ETHICAL STANDARD

The investigator will ensure that this study is conducted in full conformity with the principles set forth in the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

13.2 INSTITUTIONAL REVIEW BOARD (IRB)

The Office of Human Research Ethics is responsible for ethical and regulatory oversight of research at UNC-Chapel Hill that involves human participants. The OHRE administers, supports, and guides the work of the Institutional Review Boards and all related activities. Any research involving human participants proposed by faculty, staff, or students must be reviewed and approved by an IRB before research may begin, and before related grants may be funded. OHRE and the IRBs are critical components of the coordinated Human Research Protection Program, which serves to protect the rights and welfare of human participants. All components of this program must work together to ensure institutional compliance with ethical principles and regulatory requirements. The following is a mission statement for the coordinated Human Research Protection Program:

The University of North Carolina at Chapel Hill is committed to expanding and disseminating knowledge for the benefit of the people of North Carolina and the world. An important part of that commitment to knowledge is research of the highest quality on all aspects of the health and behavior of people, and such research is only possible through the participation of humans as research participants. Human participants are partners in research and a precious resource to the university. At UNC-Chapel Hill, human participant research is a privilege, but not a right. Consistent with that philosophy, it is the mission of the UNC-Chapel Hill Human Research Protection Program to ensure that:

a. The rights and welfare of human participants are paramount in the research process;
b. The highest standards of ethical conduct are employed in all research involving human participants;
c. Research investigators are properly trained in the ethical and regulatory aspects of research with human participants;
d. Research investigators deal honestly and fairly with human participants, informing them fully of procedures to be followed, and the risks and benefits of participating in research; and
e. Research using human participants at UNC-CH conforms to applicable local, state, and federal laws and regulations and the policies of the university.

13.3 INFORMED CONSENT PROCESS

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks of TMS and GTT will be provided to the participants. Consent forms describing, in detail, the study intervention, device, procedures, and risks are given to the participant and written documentation of informed consent is required prior to the administration of any assessments used in this study. All consent forms will be IRB-approved and updated with any new information as modifications are made throughout the study.

Together, the researcher and potential participants will review the study in its entirety by reviewing the consent form together in a private location. At several intervals during the consent review, the researcher will ask the

participant questions that will assess the comprehension of the information in the consent. If the participant is unsure or does not know, the researcher will return to that section and more carefully explain the information. Participants must sign the informed consent document prior to any procedures taking place. If needed, the participants will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. Participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records.

13.4 EXCLUSION OF WOMEN, MINORITIES, AND CHILDREN (SPECIAL POPULATIONS)

Non-English speaking individuals are excluded because the ability to accurately and complete communicate study information, answer questions about the study, and obtain consent is necessary.

13.5 PARTICIPANT CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the research team. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants.

All data will only be referenced by dummy identifier code. Data will be stored on a password protected computer. A key connecting names and code numbers will be kept in a locked cabinet, accessible only by research personnel. All data will be stored and analyzed on password protected computers, also only accessible by research personnel. Participants will not be identified in any report or publication about this study. See *10 Data Handling and Record Keeping* for more information on source documentation storage and security.

13.6 STUDY DISCONTINUATION

In the event that the study is discontinued, participants who have completed or who are still enrolled in the study will be notified. Any new information gained during the course of the study that might affect participant's willingness to continue will be communicated within 2 days of the PI learning this information.

14 PUBLICATION POLICY

There are no restrictions on publications since this is an investigator-initiated study funded by a grant agency that has no influence on the publications resulting from this study.

15 LITERATURE REFERENCES

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APPENDIX A: SCHEDULE OF EVENTS

A detailed schematic describing phone screening and study visits.

Procedures	Phone Screening	On-Site Screening	Study Sessions
Provide Verbal Consent	x		
Signed Consent Form		x	
Assessment of Eligibility Criteria	x	x	
Review of Medical History	x	x	
Review of Concomitant Medications	x	x	
Behavioral and Mood Questionnaires		x	x
Glucose Drink Administration			x
Capillary blood sampling (finger prick)			x
Non-Invasive Brain Stimulation			x
Side-Effects Questionnaires			x

APPENDIX B: IRB AMENDMENT TRACKING LOG

Change Initiated By:	Description of IRB: Type and Brief Summary	Date Submitted to IRB	Date of IRB Response	Requires Stipulations? (Y/N)	Requires Updated Consent Form? (Y/N)	Stipulation Submission Date	IRB Approval Date
Initials	Reference ID						

APPENDIX C: INFORMED CONSENT QUIZ

Name of Research Study:

You have been asked to be in a research study. This sheet will help you think of questions to ask but you may have other questions. This is not a test. We want to be sure you understand what it means to be in this research study. You should understand the research before you decide whether or not to participate.

1. What is the purpose of the research?

2. What are the possible risks of the research?

3. Does in the research cost me anything extra?

4. Can you stop being in the research once you've started?

5. Who will view your medical records?

6. Who do you call if I have questions about being a research subject?

7. Any questions?

APPENDIX D: NOTE TO FILE

IRB#: 19-XXXX PI: Flavio Frohlich

Study Title: [Insert Short Name]

Researcher: _____ Date of Occurrence: _____

Participant ID: _____

Reason for Note:

_____
_____

Note:

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Corrective action (if applicable):

_____
_____
_____

Signature: _____ Date: _____

APPENDIX E: TELEPHONE SCRIPT

Hello, my name is _____ and I am a _____ from the University of North Carolina at Chapel Hill conducting a research study about investigation of blood glucose level and neuronal excitability. Based on your history, you may be eligible to participate in our study.

Do you have time now to hear about the study, answer a few screening questions?

(If 'No', ask for a good time to call back)

(If 'Yes', proceed)

Great! This study is investigating how changes in blood glucose level modulate neuronal excitability. Findings from this study will help the development of new study. In this study, a very weak electric current will be applied to your scalp. Some people report a tingling, itching because of this stimulation, but no other side effects have been found. It is not a shock and should not cause pain. Also, we will collect your 5uL blood on your finger by pricking to measure your blood glucose level. This procedure is widely used for diagnosing diabetes in hospitals thus there is no other side effects for blood sampling.

Participation in this study includes 1 on-site screening visit and 2 study sessions. You will be compensated for your time spent participating in the study. The maximum compensation for this study is $200 for completing all sessions. Are you still interested in participating?

(If 'No', thank them for their time; if 'Yes', proceed)

Great! In order to make sure you're eligible for the study, I need to ask you a few questions. Please answer yes or no. You do not need to provide any further details.

(If the answer given is not the same as the answer shown, thank the individual for his or her interest and say, unfortunately, they do not qualify for the current study)

- Are you between 35 to 65 years old and right-handed? (Yes)
- Have you ever, or are you currently being diagnosed with diabetes and pre-diabetes?
- Have you ever, or are you currently being treated for a neurological condition (e.g., epilepsy, migraines)? (No)
- Are you currently taking medication for any other psychiatric illness? (No)
- Have you ever had brain surgery? (No)
- Do you have any brain devices or implants, including a cochlear implant or aneurysm clip? (No)
- Have you ever been diagnosed with a traumatic brain injury? (No)

Phone Screening:

☐ Pass
☐ Fail

Reason for failing: _____

You are eligible for participation in the stimulation session of the study.

Scheduled Session

Date: _____

Time: _____

I will send you an email confirming this time, and providing directions on how to find the specific location of your session. We will also send you an email to confirm your appointment 24 hours beforehand. Please respond to this email so we know you are still coming. If you have any questions before then, please don't hesitate to contact us at this phone number or at christopher_walker@med.unc.edu. Thank you for your time.

APPENDIX F: QUESIONNAIRES

1. STAI

SELF-EVALUATION QUESTIONNAIRE STAI Form Y-1

Please provide the following information:

Name_____Date_____S_____

Age_____ Gender (*Circle*) **M** **F** T_____

DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right* now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

STAIP-AD Test Form Y
www.mindgarden.com

SELF-EVALUATION QUESTIONNAIRE

STAI Form Y-2

Name_____Date_____

DIRECTIONS

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you *generally* feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
21. I feel pleasant	1	2	3	4
22. I feel nervous and restless	1	2	3	4
23. I feel satisfied with myself	1	2	3	4
24. I wish I could be as happy as others seem to be	1	2	3	4
25. I feel like a failure	1	2	3	4
26. I feel rested	1	2	3	4
27. I am "calm, cool, and collected"	1	2	3	4
28. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
29. I worry too much over something that really doesn't matter	1	2	3	4
30. I am happy	1	2	3	4
31. I have disturbing thoughts	1	2	3	4
32. I lack self-confidence	1	2	3	4
33. I feel secure	1	2	3	4
34. I make decisions easily	1	2	3	4
35. I feel inadequate	1	2	3	4
36. I am content	1	2	3	4
37. Some unimportant thought runs through my mind and bothers me	1	2	3	4
38. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
39. I am a steady person	1	2	3	4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

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2. PANAS

Date: _____ Time: _____ Subject ID: _____ Session Number: _____

This scale consists of a number of words that describe different feelings and emotions. Please indicate to what extent you feel this way right now – that is, at this present moment – according to the numbers in the scale below.

1	2	3	4	5
Very Slightly or Not At All	A Little	Moderately	Quite a Bit	Extremely

_____	1. Interested	_____	11. Irritable
_____	2. Distressed	_____	12. Alert
_____	3. Excited	_____	13. Ashamed
_____	4. Upset	_____	14. Inspired
_____	5. Strong	_____	15. Nervous
_____	6. Guilty	_____	16. Determined
_____	7. Scared	_____	17. Attentive
_____	8. Hostile	_____	18. Jittery
_____	9. Enthusiastic	_____	19. Active
_____	10. Proud	_____	20. Afraid

3. Edinburgh Handedness Inventory

Edinburgh Handedness Inventory[1]

Your Initials:

Please indicate with a check (✓) your preference in using your left or right hand in the following tasks.

Where the preference is so strong you would never use the other hand, unless absolutely forced to, put two checks (✓✓).

If you are indifferent, put one check in each column (✓ | ✓).

Some of the activities require both hands. In these cases, the part of the task or object for which hand preference is wanted is indicated in parentheses.

Task / Object	Left Hand	Right Hand
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking a Match (match)		
10. Opening a Box (lid)		
Total checks:	LH =	RH =
Cumulative Total	CT = LH + RH =	
Difference	D = RH – LH =	
Result	R = (D / CT) × 100 =	
Interpretation: (Left Handed: R < -40) (Ambidextrous: -40 ≤ R ≤ +40) (Right Handed: R > +40)		

[1] Oldfield, R. C. (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsycholoigia*, *9*, 97-113.

4. BIS/BAS

BIS/BAS

Each item of this questionnaire is a statement that a person may either agree with or disagree with. For each item, indicate how much you agree or disagree with what the item says. Please respond to all the items; do not leave any blank. Choose only one response to each statement. Please be as accurate and honest as you can be. Respond to each item as if it were the only item. That is, don't worry about being "consistent" in your responses. Choose from the following four response options:

1 = very true for me
2 = somewhat true for me
3 = somewhat false for me
4 = very false for me

1. A person's family is the most important thing in life.	
2. Even if something bad is about to happen to me, I rarely experience fear or nervousness.	
3. I go out of my way to get things I want.	
4. When I'm doing well at something I love to keep at it.	
5. I'm always willing to try something new if I think it will be fun.	
6. How I dress is important to me.	
7. When I get something I want, I feel excited and energized.	
8. Criticism or scolding hurts me quite a bit.	
9. When I want something I usually go all-out to get it.	
10. I will often do things for no other reason than that they might be fun.	
11. It's hard for me to find the time to do things such as get a haircut.	
12. If I see a chance to get something I want I move on it right away.	
13. I feel pretty worried or upset when I think or know somebody is angry at me.	
14. When I see an opportunity for something I like I get excited right away.	
15. I often act on the spur of the moment.	
16. If I think something unpleasant is going to happen I usually get pretty "worked up."	

BIS/BAS

1 = very true for me
2 = somewhat true for me
3 = somewhat false for me
4 = very false for me

17. I often wonder why people act the way they do.	
18. When good things happen to me, it affects me strongly.	
19. I feel worried when I think I have done poorly at something important.	
20. I crave excitement and new sensations.	
21. When I go after something I use a "no holds barred" approach.	
22. I have very few fears compared to my friends.	
23. It would excite me to win a contest.	
24. I worry about making mistakes.	

5. IDAS

Inventory of Depression and Anxiety Symptoms (IDAS)

Below is a list of feelings, sensations, problems, and experiences that people sometimes have. Read each item to determine how well it describes your recent feelings and experiences. Then select the option that best describes **how much** you have felt or experienced things this way **during the past week, including today**. Use this scale when answering:

1	2	3	4	5
Not at all	A little bit	Moderately	Quite a bit	Extremely

_____ 1. I was proud of myself

_____ 2. I felt exhausted

_____ 3. I felt depressed

_____ 4. I felt inadequate

_____ 5. I slept less than usual

_____ 6. I felt fidgety, restless

_____ 7. I had thoughts of suicide

_____ 8. I slept more than usual

_____ 9. I hurt myself purposely

_____ 10. I slept very poorly

_____ 11. I blamed myself for things

_____ 12. I had trouble falling asleep

_____ 13. I felt discouraged about things

_____ 14. I thought about my own death

_____ 15. I thought about hurting myself

_____ 16. I did not have much of an appetite

_____ 17. I felt like eating less than usual

_____ 18. I thought a lot about food

_____ 19. I did not feel much like eating

_____ 20. I ate when I wasn't hungry

_____ 21. I felt optimistic

_____ 22. I ate more than usual

_____ 23. I felt that I had accomplished a lot

_____ 24. I looked forward to things with enjoyment

_____ 25. I was furious

_____ 26. I felt hopeful about the future

_____ 27. I felt that I had a lot to look forward to

_____ 28. I felt like breaking things

_____ 29. I had disturbing thoughts of something bad that happened to me

_____ 30. Little things made me mad

_____ 31. I felt enraged

_____ 32. I had nightmares that reminded me of something bad that happened

_____ 33. I lost my temper and yelled at people

_____ 34. I felt like I had a lot of interesting things to do

_____ 35. I felt like I had a lot of energy

_____ 36. I had memories of something scary that happened

_____ 37. I felt self-conscious knowing that others were watching me

IDAS – 2

1	2	3	4	5
Not at all	A little bit	Moderately	Quite a bit	Extremely

_____ 38. I felt a pain in my chest

_____ 39. I was worried about embarrassing myself socially

_____ 40. I felt dizzy or light headed

_____ 41. I cut or burned myself on purpose

_____ 42. I had little interest in my usual hobbies or activities

_____ 43. I thought that the world would be better off without me

_____ 44. I felt much worse in the morning than later in the day

_____ 45. I felt drowsy, sleepy

_____ 46. I woke up early and could not get back to sleep

_____ 47. I had trouble concentrating

_____ 48. I had trouble making up my mind

_____ 49. I talked more slowly than usual

_____ 50. I had trouble waking up in the morning

_____ 51. I found myself worrying all the time

_____ 52. I woke up frequently during the night

_____ 53. It took a lot of effort for me to get going

_____ 54. I woke up much earlier than usual

_____ 55. I was trembling or shaking

_____ 56. I became anxious in a crowded public setting

_____ 57. I felt faint

_____ 58. I found it difficult to make eye contact with people

_____ 59. My heart was racing or pounding

_____ 60. I got upset thinking about something bad that happened

_____ 61. I found it difficult to talk with people I did not know well

_____ 62. I had a very dry mouth

_____ 63. I was short of breath

_____ 64. I felt like I was choking

APPENDIX G: FASTING INSTRUCTIONS FOR GLUCOSE TOLERANCE TEST

Preparing for the oral glucose challenge test:

For three days before the test, you should eat a diet that contains at least 150 grams of carbohydrates a day and particularly eat at least 50 grams of carbohydrates in the meal the evening before the test.

Foods that contain 15 grams of carbs include:

A small piece of fruit

1 slice of bread

1/2 cup cooked oatmeal

1/3 cup cooked pasta or rice

4 to 6 crackers

1/2 cup black beans or other starchy vegetable

1/4 large baked potato

2/3 cup nonfat yogurt

2 small cookies

1/2 cup ice cream or sherbet

6 chicken nuggets

1/2 cup of casserole

1/4 serving of medium french fries

You should have at least 3-4 servings of these at each meal for 3 days before the test. You can have more if you like. The meal before the test is the most important meal to eat plenty of carbohydrates

These are food groups and other examples of carbohydrate containing foods:

Dairy: Milk, yogurt, and ice cream

Fruit: Whole fruit and fruit juice

Grains: Bread, rice, crackers, and cereal

Legumes: Beans and other plant-based proteins

Starchy Vegetables: Potatoes and corn

Sugary Sweets: Soda, candy, cookies, cakes

You should have nothing by mouth after 10PM the night before the test except for water, which you should drink freely to ensure that you stay well hydrated. You should not smoke after 9PM. No coffee, juice or other beverages in the morning except for water.