Protocol Title	The Role of Social Partners in Buffering Physiological and Brain Indicators of Stress in Children and Adolescents: An MRI Study of Stress and Social Support
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Scientific Assessment	Nationally-based, federal funding organizations
IND/IDE # (if applicable)	N/A
IND/IDE Holder	N/A
Investigational Drug Services # (if applicable)	N/A
Version Number/Date:	Version 5.5 / 2022.09.20

PROTOCOL COVER PAGE

REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?
1 (v2)	May 29, 2019	Response to reviewer concerns	Yes
2 (v2.01)	Aug 1, 2019	Addition of Safety Screen worded for Minors. No other change to protocol document.	No
3 (v2.02)	Aug 29, 2019	Clarified that no HIPAA data will be given to CMRR; corrected misloaded file documenting CMRR pre-approval for IRB review	No
4 (v3.0)	Nov 21, 2019	Added option for online consent for parent of "friend" via Redcap – changed in protocol and in Consent_Friend form; updated MRI screening form for minors per CMRR requirement; added additional self-report questionnaire measures for participant.	Yes (Consent_Friend form is updated to include option of online completion)
5 (v4.0)	Jan 28, 2020	Added option for research participants to complete questionnaires online using an iPad and Redcap.	No.
6 (v4.01)	Nov 11, 2020	Response to required modifications: Addition of clearer rationale for deception used in test procedures and indication that participants may withdraw their data and records when they are debriefed on this deception.	No
7 (v5.0)	Nov 30, 2020	Removed urine + oxytocin testing, added Certificate of Confidentiality language to consent form. Changes required for COVID safety and budget limits: added online consent and assent for all subjects, session 1 completely online, removed nurse exam, removed friend condition	Yes
8 (v5.01)	Jan 4, 2021	Made required changes: removed references to nurse exam from	No

		section 17.2 and removed references to involvement of a friend in section 13.1	
9 (v5.1)	Jan 15, 2021	Added video recording of sessions. Consent form updated to clarify recording.	Yes
10 (v5.2)	May 25, 2021	Added new NRI questionnaire version and contacting past participants to complete this addition, with payment of $10 via online gift card or ClinCard.	
11 (v5.3)	Oct 27, 2021	Added recruitment method of contacting organizations through email.	No
12 (v5.4)	Aug 18, 2022	Added recruitment methods of mailing paper flyers and contacting people through organization listserv.	No
13 (v5.5)	Sep 20, 2022	Increased child payment to $100 total and parent payment to $50 total.	Yes

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ABBREVIATIONS/DEFINITIONS

- fMRI: Functional Magnetic Resonance Imaging
- MISTiC: Minnesota Imaging Stress Test in Children
- TSST: Trier Social Stress Test
- CORT, AUCi: salivary cortisol, area under the curve from intercept
- DHEA: dehydroepiandrosterone
- EKG: Electrocardiogram
- vmPFC: ventromedial prefrontal cortex
- ICD: Institute of Child Development

1.0 Objectives

1.1 Purpose:
The goal of this project is to examine the neural systems underlying the physiological responses to stress in children and adolescents, and to examine the effectiveness of various social partners (parent, stranger) in buffering youth from the physiological and brain effects of stress.

2.0 Background

2.1 Significance of Research Question/Purpose & Existing Literature:
Social buffering, a key concept in the psychobiology of stress, describes a phenomenon in which the presence and availability of social partners reduces activity of threat- and stress-mediating neurobiological and neuroendocrine systems (Gunnar, 2017). It is a key pathway through which social support reduces stress (Uchino et al., 2012) and enhances health (Kaplan et al., 1977). Impaired social buffering is a primary pathway through which adverse childhood experiences get under the skin to affect development (Hostinar et al, 2014). Despite its centrality to stress regulation, we know relatively little about the normative development of social buffering beyond the infant years.

2.2 Preliminary Data:
Prior behavioral data from our laboratories demonstrates that parents remain effective social buffers beyond infancy and throughout childhood. Among 9- and 10-year-olds, preparing for the Trier Social Stress Test (TSST) with a parent completely blocked elevations in cortisol to this social evaluative stressor (Hostinar et al., 2015). However, our results suggest that puberty results in a waning of parental HPA stress buffering effectiveness. Preparing for the TSST with the parent vs experimenter had no effect on 15- and 16-year-olds or on 11- to 14-year-olds who were at more advanced pubertal stages (Doom et al., 2016).

2.3 Study Aims:
The current study examines the neural response, as well as the cortisol hormonal response to social evaluative stress across the transition to puberty. We use a modified version of the Trier Social Stress Test (TSST) in the MRI scanner (called the Minnesota Imaging Stress Test in Children; MISTiC) to elicit social evaluative stress. In this project, we will randomly assign youth to complete this testing under one of three social buffering conditions: presence of a parent, presence of an experimenter (stranger), or no social support present (standard imaging condition).

The **long-term goal** of our research is to enrich understanding of stress regulation and its role in human development. The **overall objective** of this project is to understand normative processes in stress regulation and their neurobiological correlates as children leave childhood and become more autonomous in adolescence. The **central hypothesis** of this project is that early adolescence is a period of time when parental buffering wanes in efficacy, leaving many youth experiencing a relative dearth of relationships that can provide powerful stress buffers.

The aim of the current study is to delineate changes in brain activation in response to social buffering by parents over the pubertal transition.

Hypothesis #1: Parental social buffering will result in increased activation in brain regions associated with safety (e.g. vmPFC) and lower activation in brain regions involved in fear and pain (e.g., insula, amygdala, hypothalamus), as well as tighter coupling between the vmPFC and amygdala activity, but these relationships will wane with pubertal development.

Hypothesis #2: Neural activity associated with social buffering will mediate the effectiveness of physiological buffering by social partners.

3.0 Study Endpoints/Events/Outcomes

3.1 Primary Endpoint/Event/Outcome:
Neural activity in vmPFC, insula, amygdala, and hypothalamus; salivary cortisol, heart rate, and respiration

3.2 Secondary Endpoint(s)/Event(s)/Outcome(s):
Exploration of possible sex differences in different conditions later in puberty but not earlier in puberty.

4.0 Study Intervention(s)/Investigational Agent(s)

4.1 Description: Note that there are no investigational agent(s) (e.g., drug, device) being evaluated. The study qualifies as an intervention due to random assignment into social buffering condition, to determine the effect of social partner on stress response.

Child participants (11- to 14-year-olds) will be randomly assigned to one of three conditions during an fMRI version of a social evaluative stress paradigm: (1) no social buffer, (2) presence of experimenter/stranger, or (3) presence of a parent.

4.2 Drug/Device Handling: N/A

4.3 Biosafety: N/A

4.4 Stem Cells: N/A

5.0 Procedures Involved

5.1 Study Design: The study design is cross-sectional. 200 target children (11- to 14-year-olds) will be randomly assigned to one of three conditions: (1) no social support (standard MRI scanning condition); (2) presence of a stranger (experimenter); or (3) presence of a parent. Outcome measures will be compared across the three social buffering conditions. Pubertal stage will be included as a continuous variable in analyses of physiological and/or brain responses.

Study Overview:
Visit 1: Parent and child will connect with a research staff member via video conferencing for a session lasting approximately 2 hours. Following a detailed overview of study and consent/assent procedures, participants will be asked to complete the following measures:

Parent:

- Questionnaire regarding family income, education level, and family composition
- Parent-report questionnaire about child's general health, behavior, and development (MacArthur Health Behavior Questionnaire; HBQ)
- Parent-report MRI safety screening questionnaire about the child to rule out any contraindications for MRI scanning

Children/adolescents:

- Questionnaire about general health, behavior, and development (MacArthur Health Behavior Questionnaire; HBQ)
- Questionnaire about their relationships with their parents and their best friend (Network of Relationships Questionnaire)
- Pubertal development questionnaire (Morris and Udry, 1980)
- Safety screening questionnaire to confirm safety in the MRI scanner

Visit 2: Parent and child will come to the Center for Magnetic Resonance Research for a session lasting approximately 2 hours. Following a session overview and consent/assent procedures, participants will be asked to complete the following measures:

Parent:

- Required safety screening questionnaire to confirm child's safety in the MRI scanner
- *[If the child is randomized to the parent-present buffering condition*], sit in a separate room while connected via video conference call to research staff in the MRI console room for duration of MRI scan (approx. 1 hour)

Child:

- Required safety screening questionnaire to confirm child's safety in the MRI scanner
- Saliva sampling at 10 time points throughout the testing session to assess levels of stress-sensitive compounds, including cortisol, alpha amylase, and DHEA.
- Children chew on an absorbent sponge for 1-2 minutes to acquire each saliva sample.
- Measure height and weight using a stadiometer.
- Practice in a pretend MRI machine to gain familiarity with the scanning environment and assess comfort in the scanner bore (i.e., rule out claustrophobia)
- Standard non-invasive structural and functional MRI scanning for 1 hour at 3-Tesla (clinical standard) field strength.
- Collection of physiological data during the MRI scan (respiration belt, and pulse oximeter and/or 3 EKG leads).
- Performance of a social evaluative stress test during MRI scanning. (1) Five minutes of silent preparation of a speech about oneself, (2) Oral delivery of the speech to two judges in white lab coats, visible by video feed via video conference call, and (3) Performance of challenging, but age-appropriate math problems under timed limited conditions, while observed by the same two judges. To increase the social evaluative component of the stress, youth are told that we will be video recording their speech and will show it to a panel of kids their age who will rate their performance. Although, we do video record, we will not show anyone the child's performance. The social evaluative stress created by this test depends upon both the visible judges and the belief of a recording that is shown to peers. Without one of these aspects, the stress test does not reliably produce a stress response in the studied population. The research goals of this study cannot be met without a reliable and standard social evaluative stress test. This is standard practice of the Trier Social Stress Test. This recording will be used for training and data purposes only.
- Completion of a post-scan survey to assess perceived stress at various points throughout the session.
- Debriefing of participant to reveal deception (no kids evaluating the recording, two judges were also not actually evaluating performance).

5.2 Study Procedures:

Social Buffering Manipulations: Will include 3 between-subjects conditions and both sexes. Participants will be randomly assigned to No Social Partner, Stranger/Experimenter, or Parent.

Stressor Paradigm: MISTiC paradigm while the child is lying in the MRI scanner; includes two judges (one male, one female). After consent, youth are separated from their parents and taken for completion of questionnaires (approximately 20 minutes). Youth are taken to a private changing room to change into hospital scrubs prior to moving to the MRI scanner. An MRI-safe respiration belt and pulse oximeter and/or EKG electrodes are positioned for autonomic assessment and the child is given ear plugs, a button response box for math task, and an emergency call button. Youth rest and/or watch a video of their choosing during structural MRI sequences (6-10 minutes), then lie quietly with eyes open for a 5-minute scan with no video or audio material. They are then given an overview of the speech task and are told that they have 5 minutes to prepare. MRI data are collected during the 5 minutes of speech prep, following which the child is introduced to two judges who are visible on the child's video screen and who ask the child to perform their 5 min speech. No MRI data are collected during speech performance. At the end of the speech, the judges give the child instructions for the math performance section, and the child performs multiple-choice math problems that appear on the video screen using the button response box. MRI data are collected during math performance and judges remain visible on the screen during math performance. Following the judged math, judges leave the screen and youth rest or watch a video for 10 minutes during diffusion scanning. Finally, children perform another set of multiple-choice math problems with no judges present (photo of the same judges turned away) while MRI data are collected. Children are removed from the scanner and immediately complete a stress questionnaire and a debriefing session. Saliva samples are collected every 10-20 minutes throughout the session, including between scan segments, to assess dynamic changes in stress-related hormones. In the scanner, this involves handing a long collection sponge to the child which hangs out of the mouth to avoid any possibility of choking since the child is lying supine. We have used this method successfully in a previous study collecting saliva in the scanner environment. Following debriefing, the child will change back into their own clothing and return to the lobby to complete additional questionnaires for 30 min while being sampled for cortisol every 10 min.

Note: As part of the stress challenge, we tell youth that their speech and judged math performance will be video recorded and rated by a group of kids their age. However, we will not actually share their performance with anyone.

Pubertal Development: We will create a summary pubertal development index from several measures. All youth will complete the Morris and Udry (1980) pubertal development questionnaire, which allows placement in the gold-standard Tanner stages for hair, testicles, and breast development (Tanner, 1962). In all studies target participants will complete the Morris and Udry questionnaire within 2 weeks before the experimental sessions described below. We will also analyze salivary DHEA to augment our measures of puberty (Shirtcliff, Dahl, & Pollak, 2009).

Questionnaires.

Forms will be completed either on paper or online via Redcap.

Demographic Questionnaire: Parents will complete information on pre-tax family income, education level of parent(s), composition of the household, and medications that the child regularly takes.

Daily Diary: The parent and the youth both will report the following for the child that day: the time of wake up, medication usage, illness/fever, physical activity, and caffeine consumption.

Relationship Quality: Participants will complete the Network of Relationships Inventory - Behavioral Systems Version (NRI-BSV, Furman & Buhrmester, 2009) for the parent who accompanies them and their close friend. This questionnaire is based on an integration of attachment and Sullivanian theory and assesses three systems that are expected to be key in close relationships: attachment, caregiving and affiliation. The scales are: companionship, seek safe haven, seek secure base, provide safe haven, provide secure base, conflict, antagonism and criticism. We will also add 3 scales from the NRI-Relationship Qualities Version: Emotional Support, Approval and Satisfaction. Each scale consists of 3 items scored on a 5-point Likert scale. Psychometric qualities are high. Condition differences in quality of relationships will be examined and included as covariates if groups differ significantly. Otherwise, in follow-up analyses we will examine whether social buffering effects are stronger in relationships the child views as more supportive.

Four additional NRI scales (Seek Safe Haven, Seek Secure Base, Provide Safe Haven, Provide Secure Base) will be collected to better measure relationship quality. Completed participants will be contacted to complete these additional 12 questions and will be compensated for their time.

MacArthur Health and Behavior Questionnaire (HBQ) for 9-18 Year-Olds (Parent and Child, 2.1): This questionnaire was developed by Marilyn Essex based on the original HBQ for 4-8 year olds. We have used it many

times. The scales we proposed to use here have high reliabilities (Cronbach alpha's> .8). We will use the peer scales (acceptance/rejection, bullied, relational victimization, asocial, behavioral inhibition) and broad-band symptoms scales (Internalizing, Externalizing). These measures will be examined for balance across groups and if differences by condition, pubertal stage, or sex are found, will be entered as covariates.

Self-Report of Stress: As a manipulation check, participants will rate their arousal and emotional state at various points during Visit 2.

Version 3.0 addition: Added Child-self-report RSQ for Peer, Family, & Academic Stress, and PALS Student report of adaptation to challenge in environment.

Physiological Measures.

Stress Hormones: We will assay saliva samples for stress sensitive hormones: cortisol and alpha-amylase. Saliva will be collected using SalivaBio Oral Swabs (SOS) or passive drool and stored frozen at −20°C until shipped for assay. All testing will be conducted in the late afternoon to control for circadian rhythm. Medications will be recorded from parent report and classes of drug codes will be used to group meds by presumed mechanisms of action and will be entered as covariates (Granger et al., 2009). Systemic glucocorticoid medications are an exclusion criterion. For each analyte, we will calculate area under the curve from intercept (AUCi) to provide a single measure for the main analysis.

Pubertal Hormones - DHEA, Testosterone, Estradiol: Sufficient saliva will be obtained at sampling to assay for these additional pubertal hormones. These assays will be performed if we obtain a sex by condition by pubertal development (3-way) interaction on key outcome measures. If so, these measures may provide insight into the mechanism of these interactions (Butler et al., 1989; Mehta, Mor, Yap, & Prasad, 2015).

Physiological Recording: Participants will wear standard Siemens physiological recording equipment for the MRI environment (respiration belt, and pulse oximeter or EKG leads). Samples will be collected throughout the entire MRI scan. Response measures will be computed by regressing the stress condition values on comparison condition values and saving the residual as the response measure.

All questionnaires and surveys are included in the ETHOS portal. Contact information and child age will be obtained from an existing registry of families who have expressed interest in child development research (specifically, parent name(s), address, phone number, email address, child

name, child sex, and child birthdate). All other data will be acquired directly from the participants, and information obtained from the participant registry will be verified verbally with the parent.

5.3 Study Duration: Participants will attend two sessions. Each session will last approximately 2 hours.

- All participants will be enrolled and assessed within a 30-month period.
- Primary data analysis endpoints will be available within 1 year of the end of data collection (i.e., 42 months from initial enrollment).

5.4 Individually Identifiable Health Information: This study will collect health information directly from participants. A HIPCO survey has been uploaded in the ETHOS portal.

5.5 Use of radiation*:* N/A

5.6 Use of Center for Magnetic Resonance Research*:* All MRI scanning will occur on a 3-Tesla MRI scanner located at the CMRR. Scans will be conducted by the PI or her study team. No PHI data will be entered into any CMRR computer or equipment; scans will be collected in de-identified form. The CMRR will not have access to any PHI from any participant in this study. Therefore, HIPAA authorization is not applicable in this case.

6.0 Data and Specimen Banking

6.1 Storage and Access: Saliva samples will be stored in a -20 degree freezer until they can be batched with other samples for hormonal assay. Any material remaining after the planned assays will be discarded. No tissue or biological samples will be stored for future use or shared.

Biospecimen samples will be identified only with a unique study ID, child age, child sex, and sample number. No PHI will be associated with any biospecimen samples. Samples will not identify the study condition of interest (i.e., social buffering condition).

6.2 Sharing: De-identified data will be shared with qualified researchers when requested for scientific purposes including replication of results, use in meta-analyses, or appropriate extension of analyses. No biospecimen samples will be saved or shared.

7.0 Sharing of Results with Participants

7.1 Results will only be shared with participants in aggregate, with the following exceptions:

We will take anthropomorphic measures (such as height and weight) as well as pubertal staging and these figures will be shared with parents at the time of the session. We deliver the figures on a post-card, with links to national pediatric norms provided by the CDC.

Regarding other hormonal or physiological measures in this study, abnormal values are commonly due to an interfering substance present in the sample (e.g., milk for salivary cortisol) or motion artifact in the case of EKG. Without a body of evidence, it seems unethical to alarm families given an out-of-range assay value and therefore we do not plan to share individual results with participant.

In the event that the scanner operator or study personnel see something unusual in the MRI scan, the images will be submitted for review by a radiologist. Images contain only the study ID number, participant's age in years, participant sex, and test date. In the event that the radiologist determines that the incidental finding should receive clinical follow-up, we will share this information with the parent(s) of the minor child participant with the recommendation to consult with the child's primary care physician. Incidental findings from research scans are not clinically diagnostic and the images may not be added to the child's medical record.

8.0 Study Population

8.1 Inclusion Criteria: Healthy 11- to 14-year-olds. Additional inclusion criteria ensure that youth will be able to follow the study procedures (have sufficient vision, hearing, and language skills to provide verbal and written assent, see and read stimuli presented on the computer screen, and hear verbal instructions provided by the experimenter and/or judges).

8.2 Exclusion Criteria for child participation: Premature birth (less than 37 weeks), congenital and/or chromosomal disorders (e.g. cerebral palsy, FAS, mental retardation, Turner Syndrome, Down Syndrome, Fragile X), Autism Spectrum Disorders, history of serious medical illness (e.g., cancer, organ transplant), youth using systemic glucocorticoids or beta-adrenergic medications, diagnosed psychiatric illness or psychotropic medication, seizure disorder or other neurological disorder, academic delay or individualized education plan (IEP), contraindications for MRI (implanted medical device; presence of non-removal metal in or on the body, including piercings, orthodontic braces or certain permanent retainers), known pregnancy, tattoo, or history of significant claustrophobia.

8.3 Screening: Screening will occur by asking parents questions at the time of telephone recruitment. To reduce confounding of age and pubertal stage, we will use a stratified recruitment method. During recruitment, parents will be asked 3 questions about their child's physical development, and children will be initially categorized into Pre/Early and Mid/Late Puberty. As

cells fill, participants will no longer be enrolled into those cells. Also, to increase minority representation we will sample across zip codes with higher racial and ethnic minority representation.

9.0 Vulnerable Populations

9.1 Vulnerable Populations:

Population / Group	Identify whether any of the following populations will be targeted, included (not necessarily targeted) or excluded from participation in the study.
Children	Targeted Population
Pregnant women/fetuses/neonates	Excluded from Participation
Prisoners	Excluded from Participation
Adults lacking capacity to consent and/or adults with diminished capacity to consent, including, but not limited to, those with acute medical conditions, psychiatric disorders, neurologic disorders, developmental disorders, and behavioral disorders	Excluded from Participation
Non-English speakers	Excluded from Participation
Those unable to read (illiterate)	Excluded from Participation
Employees of the researcher	Excluded from Participation
Students of the researcher	Excluded from Participation
Undervalued or disenfranchised social group	Included/Allowed to Participate
Active members of the military (service members), DoD personnel (including civilian employees)	Included/Allowed to Participate
Individual or group that is approached for participation in	Excluded from Participation

research during a stressful situation such as emergency room setting, childbirth (labor), etc.	
Individual or group that is disadvantaged in the distribution of social goods and services such as income, housing, or healthcare.	Included/Allowed to Participate
Individual or group with a serious health condition for which there are no satisfactory standard treatments.	Excluded from Participation
Individual or group with a fear of negative consequences for not participating in the research (e.g. institutionalization, deportation, disclosure of stigmatizing behavior).	Included/Allowed to Participate
Any other circumstance/dynamic that could increase vulnerability to coercion or exploitation that might influence consent to research or decision to continue in research.	Excluded from Participation

9.2 Additional Safeguards:

Children are the targeted population for this study. Others that have been checked as "allowed" above are not being intentionally included or excluded. For example, we will not inquire about someone's membership in a disenfranchised group, nor whether a target child's parent is on active duty in the military. Our careful consent/assent procedures, designed to handle research with children, should be sufficient to cover other vulnerable participants who may inadvertently enter our study.

All of the subjects will be children, ranging 11-14 years old. This study focuses on adolescents because our goal is an analysis of the impact of social buffering and puberty on stress-mediating systems. Children have been the focus of our research for the past 30+ years. Our staff members are highly trained in working with children and families. The graduate students working on the project will be Child Psychology and/or Child Clinical Psychology Ph.D. students. The undergraduate research assistants will, for the most part, be majoring in child development.

Much of the work will be performed online over video conferencing. Staff will guide parents and children through the study procedures and participating families will likely be comfortable in their own homes.

Our laboratory has been scanning both typically developing children and adolescents (ages 4-17) and those at risk due to early health or environmental risks for over two decades. We have conducted NIH funded research with child participants at the CMRR for the past 16 years. We are very experienced in putting children at ease in the MRI scanner and ensuring that children are aware that they can stop at any time. We have completed a prior MRI study with 11- to 14-year-olds using the MISTiC social stress paradigm; therefore, we are confident that we are well positioned to properly inform children about the procedures and are not stressing children beyond normal everyday levels that they experience in school or in extracurricular activities (sports competitions; music, dance, or drama performances; etc.).

Parents provide consent, youth provide assent, and see below where that process is fully described to ensure that youth have full understanding and are free to decline participation.

10.0 Local Number of Participants

10.1 Local Number of Participants to be Consented: We plan to analyze data from at least 200 target participants. To account for attrition between sessions/replacement for incomplete data, up to 300 children may be consented/enrolled.

11.0 Local Recruitment Methods

11.1 Recruitment Process: Families will be initially contacted by phone, email, or paper mail to invite them to participate. Paper mail will include IRB-approved recruitment flyers. Interested families will be provided a more detailed overview of the study procedures in a telephone call. If the family is interested in participating, the parent will verbally complete a screening interview to assess inclusion and exclusion criteria, and for those who are eligible, the first visit will be scheduled. Copies of the consent and assent forms will be sent to families prior to the visit, along with directions to the University, and a reminder call will be a chance for families to confirm their interest in participation after having seen the consent forms. The formal consent process will occur at both of the two sessions.

11.2 Identification of Potential Participants: Potential participants will be identified using the ICD registries of families interested in being contacted about research. These families have agreed to be contacted, and once ICD Registry procedures have been followed, including proof of IRB approval,

an encrypted list is provided to study staff. No protected records are involved. Study staff will make initial contact.

11.3 Recruitment Materials: We will recruit via telephone, email, and paper mail (as parents have given us these three means of contacting them). We will also post flyers and put the flyer information on our website so that families can contact us. We will also email organizations what may be able to assist with sharing study contact information and spreading interest, using the Letter to Organizations template. We will also email organizations' listservs when approved to do so by the listserv administrators. Materials are uploaded in ETHOS.

11.4 Payment:

- Child Participant: $40 debit or gift card for Visit 1; $60 in debit or gift card for Visit 2
- Parent of Child Participant: $25 in debit or gift card for Visit 1; $25 in debit or gift card for Visit 2

Cards will be given at the session to parent & child as specified, though we will have parents sign receipts for payment. We intend to use Greenphire ClinCard prepaid debit cards for compensation and have included appropriate language in the consent document. We will provide a Target gift card instead of the ClinCard if parents object to the terms and conditions of the ClinCard.

12.0 Withdrawal of Participants

12.1 Withdrawal Circumstances: If participants are enrolled (consent signed) and subsequently determined to meet exclusion criteria, their data will be excluded from analyses. Children may be withdrawn from the study without their consent if we discover a safety concern for MRI scanning after consent/assent. Children may also be withdrawn if the experimenter believes that the child is excessively distressed by the stress paradigm or MRI scanning procedures. Participants will receive full compensation for the session at which they are withdrawn.

12.2 Withdrawal Procedures: If participants voluntarily withdraw, we will verify permission to retain and use any data collected to that point. If participants wish to withdraw all permission, we will destroy any data collected from the participant but will keep their name in our study records with a note that they withdrew. This procedure prevents us from inadvertently enrolling participants more than once. If the participant is withdrawn by the researcher, we will retain and use any data collected from the participant.

12.3 Termination Procedures: Data will not be used after termination.

13.0 Risks to Participants

Foreseeable Risks: Puberty Staging: There is the risk of embarrassment. Both for the youth's well-being and to prevent study drop out, we are motivated to reduce any sense of embarrassment as much as possible in a study that requires the measurement of pubertal changes. The Morris and Udry questionnaire will be completed in privacy and in the comfort of the subject's home while connected via video conference.

Questionnaires: Youth or parents may experience concerns when completing questionnaires dealing with symptoms of behavior problems. Any time one works with youth there is the possibility that they will reveal information about abuse and/or indicate that they are thinking of hurting themselves or others. We are mandated reporters, a fact that is revealed in both consent and assent forms. However, in over a decade of working with children and youth drawn from our participant files, we have never encountered a reportable incident, so we consider this risk to be very low.

Minnesota Imaging Stress Test in Children: The MISTiC is a social evaluative stressor. Public speaking evokes anxiety in many individuals.

The MISTiC activates stress-mediating systems because it serves as a threat to the social self. Public speaking produces anxiety in most people. However, answering questions in class, speaking in front of the class or school, and solving problems in front of teachers and classmates is a common occurrence in children's lives. Thus, the MISTiC standardizes a common everyday stressor. To reduce risk, youth are told that they can stop without prejudice if they choose. Because they are told they are being evaluated (and this is a necessary part of the procedure), after the MISTiC session, the youth will be debriefed to make it clear that there were no judgements made about the speech or math performed. It will be explained that performance in a social evaluation task was used in order to elicit a physical stress response. The youth will never receive any negative evaluations from adults as part of being in this study. As a part of this debriefing, participants are told that they may withdraw their data and record of participation.

MRI Scanning: The primary risks associated with magnetic resonance scanning involve risk of injury or burn in the event that unsafe metallic objects are introduced into the scanner environment. Loose items have the potential to become projectiles while stationary metallic surfaces could build heat over time and cause a burn. In addition, implanted medical devices could become non-functional or experience torque that could move metal implants through body tissue. All participants are screened multiple times for the presence of metal in or on the body. Final screening occurs immediately prior to MRI scanning.

There is no evidence for any long-term negative effect of MRI procedures on the body. The guidelines from the Bureau of Radiological Health (Food and Drug Administration) will be followed with regard to specific absorption rate (SAR) of energy (rf) and time-variant magnetic fields (dB/dt). All safety features of the clinically approved Siemens 3T scanner will be used to ensure that levels are well below the peripheral nerve stimulation threshold for both children and adults.

The MRI procedure involves exposure to a high level of noise while in the scanner environment. Participants are required to wear sound attenuating earplugs to protect their hearing. Beyond the risk to hearing, many participants find the scanner sounds annoying and monotonous. We provide additional headphones or foam padding over the top of the earplugs to further attenuate the background noise. This additional padding can become uncomfortable after long periods of time.

To reduce anticipatory stress for the scanner experience and to ensure that children feel comfortable with all MRI procedures, youth will complete a mock MRI scan in an MRI simulator. This pretend scanner has all of the primary features of the actual scanner, including an identifcally sized tunnel, the peripheral recording equipment, earplugs, headphones, video screen, audio system, button response box, and a speaker to play the scanner sounds around the tunnel, as if we were scanning, but with no magnetic field and no images produced.

Saliva Sampling. The saliva is collected on an absorbent swab. Any time anything is put in the mouth there is the risk of choking. This is a greater concern when participants are lying supine in the MRI environment. However, we deliberately use long sponge rolls that that hang out of the mouth during saliva collection. The child is instructed to hold on to the end sticking out of the mouth while chewing on the other end until it is soggy. The age of participants, 11-14 years, also reduces the risk of choking as these youth are better able to follow experimenter instructions and to realize when they are placing the sponge too far into their mouths to be comfortable.

13.1 Reproduction Risks:

The risks of strong magnetic fields for the developing human fetus are unknown. To protect against these unknown but potential risks, we will screen all female youth who have started menstruating for possible pregnancy.

13.2 Risks to Others: N/A

14.0 Potential Benefits to Participants

14.1 Potential Benefits: This study confers no direct benefits to participants.

15.0 Statistical Considerations

15.1 Data Analysis Plan:

Preliminary Analyses to Determine Covariates Needed for Primary Analyses. The purpose of collecting data on potential covariates is to identify variables that are correlated with both the independent variable (social buffering condition, sex, pubertal stage) and the primary outcome measures. Those covariates that are associated with the independent and outcome variables will be controlled for in the primary analyses. Those that are not will be used to describe the sample, but will not need to be included in the analyses to obtain unbiased estimates. For each potential covariate, we will conduct correlations or t-tests with pubertal stage, and t-tests, ANOVAs, or chi-square tests (as appropriate) with sex and condition. If data do not confirm to modeling assumptions, we will use nonparametric analogs instead.

Preliminary Analyses of Imaging Data to Yield Primary Outcome Measures: fMRI data will be analyzed in FSL software (version 5.0.10) to generate voxel-wise contrast maps. Mean signal values will be extracted from these maps in a priori regions of interest for every participant using the methods described below. These preliminary analyses are used to generate the fMRI outcome variables of interest. The values of these outcome variables for each participant will be used in the general statistical analyses described in the next section. In total, these preliminary analyses will yield 6 primary task-related outcome variables, and 6 primary connectivity-related outcome variables.

Task-based analysis: Math under social evaluative stress In first level analyses (single subject, single-run), math performance will be modeled for each participant using a voxel-wise multiple regression analysis with a task predictor (math trials vs. blank trials), 24 regressors for head motion (Satterthwaite et al., 2013) and additional regressors to model censored data points (motion spikes). In subsequent analyses, we will explore the utility of including other time varying individual differences variables (e.g., heart rate, respiration) that are known to contribute to the BOLD signal. However, since these measures are expected to vary by stress condition, removing the effects of these physiological variables may unintentionally remove effects of interest. Particular care will be taken to reduce the confound of head motion by censoring (identifying as artifact) any data point with absolute motion greater than 2 mm (1 voxel) or a framewise displacement greater than 0.5 mm, as well as the immediately preceding and following data points. Data points with DVARS values exceeding the

individual's 75th percentile plus two times the interquartile range will also be censored. Participants with more than 25% of data points censored for artifact will be excluded from further analyses.

Second level analyses (single-subject, multiple runs) will test the within-subject contrast of math performed with judges (social evaluative stress) relative to math performed alone (without social evaluation) to identify brain regions differentially activated by social evaluative stress. Based on prior literature, we will examine activation in 5 a priori regions of interest, including "pain-related" brain regions (dorsal anterior cingulate [dACC], left and right anterior insula) and "safety-related" or regulatory brain regions (ventromedial prefrontal cortex [vmPFC], and left and right dorsolateral prefrontal cortex [dlPFC]). For each subject, the mean percent change in MR signal (averaged across voxels) between judged math and math alone will be extracted from each of these 6 regions. This analysis will generate the 6 task-related brain activation outcome variables (listed in section 4.3) for each participant.

Given that the MISTiC paradigm has not been used previously, it is possible that the most prominent regions of activity for this task may not match the *a priori* regions identified from the prior stress and social buffering literature. Therefore, we will also conduct an exploratory group-level analysis to identify primary regions of activation for the same socially evaluative math vs. non-evaluative math conditions (judged math vs. math alone, cluster corrected $p<.005$). Because we expect that social buffering will alter the activity difference between social evaluative and non-evaluative math periods, we will use only the control condition (no social partner) to identify regions activated by this task. In the resultant group-level map, we will identify up to 4 primary regions of activation by selecting clusters that meet the cluster-corrected $p<.005$ threshold, and then rank-ordering those regions by maximal z-statistic. Mean percent change in signal will be extracted for each participant (across all four buffering conditions) using anatomical masks derived from this analysis conducted in the control (no social partner) group. Therefore, exploratory analyses may generate up to 4 additional task-related MRI outcome variables.

Connectivity analysis: Speech preparation We are also interested in the neural correlates of anticipatory stress during the speech preparation period. However, the speech preparation period does not contain distinctive event markers needed for task-based fMRI analyses. Thus, to address the neural correlates of stress during speech preparation, we will use a seed-based connectivity approach to examine strength of functional connectivity between a priori seed regions of interest in the limbic system (amygdala, insula, hypothalamus) and target regions in the prefrontal

cortex (left and right vmPFC and dlPFC). To determine whether connectivity varies as a function of stress, we will compare connectivity during the speech preparation scan to connectivity during the baseline resting state scan. Preprocessing steps will include motion correction, unwarping, and eddy-current correction. An initial voxel-wise multiple regression analysis will be performed to remove effects of head motion 24 predictors), and estimates of CSF and white matter signals (2 predictors) from each subject's data. Initial analyses will not include a predictor for global signal given evidence that the inclusion of global signal may falsely introduce anticorrelations in the data (Murphy et al., 2009; Saad et al., 2012). However, in exploratory analyses, we will examine the degree to which the results change when global signal is included in the model.

First-level (single-subject, single run) connectivity analyses on the residualized data will include the time course for a seed region and predictors for censored data points. Two separate regression models will be run, one using the bilateral amygdala as the seed region, and the other seeding connectivity in the hypothalamus.

Second-level (single-subject, multiple-runs) analyses will test the within-subject contrast of connectivity during anticipatory stress (speech preparation) relative to baseline resting state for each seed-region. Masks of a priori target brain regions of interest will be applied to resultant contrast maps to extract stress-related connectivity in the bilateral vmPFC and left and right dlPFC for each individual. This analysis will generate the 6 connectivity-related brain outcome variables (2 seed regions x 3 target regions) for each participant.Because difference scores (speech preparation minus baseline) can mask the direction of connectivity effects (positive vs. negative correlations), follow-up simple-effects analyses will assess connectivity separately in the speech preparation and resting state periods, using the same a priori seed and target regions. These follow-up simple-effects analyses will allow us to interpret the directionality of connectivity effects.

15.2 Power Analysis: For the power analysis for the primary analyses, we calculated the minimum detectable effect size (MDES) assuming n = 200, a = .05, and power = .80 with 12 covariates in our model. Power was estimated in R (R Core Team) using the pwr library (Champely, 2018). A power analysis was performed to quantify the minimum detectable effect size (f2) for a three-way interaction between social buffering condition, sex, and pubertal stage within a multiple regression model controlling for the 12 covariates, all two-way interactions, and the main effects (the most complex model considered in our primary analysis, see Equation 1 below). Assuming these conditions, we should be able to detect an f2 of 0.056. In

other words, adding the three-way interaction to a model containing the covariates and all lower order interactions and main effects, the three-way interaction would need to explain an additional 5.6% of the variability to be detected. This falls between Cohen's criteria for a small (f2 = .02) and medium effect (f2 = .15) (Cohen, 1988). With a sample size of 200, even if we lose 40 of the participants to movement artifact for the imaging analyses, assuming all the previous conditions but a sample size of 160, we will still have an f2 of .070, or still within a small-medium effect size. As the number of covariates decreases, the size of the MDES will decrease. Therefore, this represents a conservative estimate of the power for our primary analyses as we are likely to have fewer covariates in our models and will use multiple imputation to correct for missing data. For the two-way interaction and main effects models, described below, the power will be larger than these reported values as the models will be simpler.

15.3 Statistical Analysis of MRI and non-MRI Outcome Variables:

The following is a list of the analyses that we will perform on the outcome measures. They are classified as to whether they are the primary or mediational analyses. All statistical analyses will be performed in R.

The primary analyses will consist of a series of multiple regression models. For each dependent variable, variation in neural activity, cortisol response, and heart rate response, we will begin by testing the three-way interaction between social buffering condition, sex, and pubertal stage for all outcomes. If the three-way interaction is not significant, we will then examine all two-way interactions. If the two-way interactions are not significant, we will then examine main effects only models. If the three-way interaction is significant, we will not examine any other model. If condition is significant as an interaction or as a main effect, we will examine all pairwise comparisons using Tukey's Honestly Significant Difference test.

Multiplicity of testing. We will correct for multiple comparisons using the Benjamini-Hochberg (BH) correction. The BH method works by controlling the false discovery rate, and relative to the Bonferroni's correction is more powerful, while still adequately protecting against Type I error (Williams, Jones, & Tukey, 1999).

Mediational Analysis. When changes in activity of brain regions differ by condition or condition by pubertal status ***and*** we also see condition or condition by pubertal status differences for cortisol, or heart rate, then we will examine whether brain activity differences mediate the neuroendocrine or autonomic differences. This will be examined using path analysis, while controlling for covariates identified in the preliminary

covariate analysis, and will be estimated using the lavaan package in R (Rosseel, 2012).

The significance of all indirect effects will be assessed using bias-corrected 95% confidence intervals (Efron & Tibshirani, 1986), where confidence intervals that are non-overlapping with zero will be considered significant.

Power Analysis for the Mediational Analysis: To assess power to detect mediation, we performed a Monte Carlo-based power analysis using simulated data. We varied the parameter values to roughly correspond to Cohen's criteria for small, medium, and large effects. This resulted in a total of 27 conditions, which we examined at our target sample size of 200. Each condition was replicated 2000 times and we calculated empirical power, which we defined as the proportion of times out of 2000 where we rejected the null hypothesis at $\alpha = .05$. Given the exploratory nature of this analysis and to simplify the simulation for the power analysis, we omitted covariates and did not vary the effects for the other condition contrasts (i.e., they were always fixed to 0). Next, we fit a path model using the lavaan package in R.

Our findings suggest that in order to detect mediation, the effect of condition on the mediator must be large and the effect of the mediator on the outcome must be medium or large. In addition, if the effect of condition on the outcome is not large, but small or medium sized, then we will be underpowered and would likely conclude complete mediation, when mediation may only be partial or non-existent. Based on this power analysis, it is likely that the MDES for the moderated mediation models will need to be at least medium to large as well.

Missing data. Because we will replace participants who do not come in for session 2 after doing session 1 (which is rare in our experience), we should have 200 completed participants. Missing data, however, could arise from problems in obtaining good imaging data. Participants also often fail to fully complete questionnaires and/or refuse to provide information on household income. If there is missingness in any of the dependent or independent variables, multiple imputation will be used (Little & Rubin, 2014; Peng, Harwell, Liou, & Ehman, 2006; Schafer & Graham, 2002). Variables related to the missingness and the variables missing data will be included in the missing data model. In the event that missingness is not at random (MNAR), we will investigate the use of models for non-ignorable missing data.

15.4 Data Integrity: Questionnaire data will be double-entered to reduce the likelihood of errors in data entry. Biospecimen data will be assayed in labs

that have standard quality control procedures. MRI data reviewed for raw data quality and artifact detection.

16.0 Confidentiality

Data Security: Only authorized research staff will have access to the data, and all data will be stored in REDcap databases, secure servers (CMRR and Minnesota Supercomputing Institute) or in locked file cabinets. Names and contact information, and chid birth date will be maintained in REDcap database to avoid enrolling participants more than once, to calculate age at test, and to allow us to contact families who have participated with overall study findings. IRB and CMRR policies require that we maintain the MRI safety screening information for the same duration that we maintain consent documents. These will be stored in locked file cabinets and/or uploaded to a secure REDcap database.

A password-protected document linking name and ID number will be kept in University of Minnesota Secure Box storage until the conclusion of the study in case we must contact a family for any reason, including identification of an incidental MRI finding. This document will be destroyed at the conclusion of the study.

All data files are de-identified, password protected, or maintained in secure storage locations (REDcap and/or University Box). All paper copies are stored in locked cabinets.

16.1 No documents will be placed in participants' medical, employment, or educational records.

17.0 Provisions to Monitor the Data to Ensure the Safety of Participants

17.1 Data Integrity Monitoring:

All of the planned procedures meet the NIH definition of minimal risk. The PI and Study Coordinator will meet regularly to ensure that all study protocols are followed and best practices are applied. The Study Coordinator will bring any identified protocol violations to the PI in a timely fashion and team members will be retrained, if needed.

17.2 Data Safety Monitoring:

This project is considered a clinical trial because it involves a) random assignment to condition, and b) physiology is being measured.

There is no risk associated with the assignment to conditions (No Social Buffer, Stranger/Experimenter, Parent). All conditions resemble naturally occurring social situations that children might experience, and there is no inherent danger to one condition over another as there would be for, say, a drug vs placebo trial.

Further, in two of the conditions the presence of another person is expected to provide a social buffer whose stress reduction potency by condition is what is being tested. All target youth experience the same stressor, the MISTiC. The MISTiC mimics a naturally occurring stressor, as when the youth is asked to speak in front of the class and his/her performance is being evaluated. This can provoke anxiety.

The other potential risks of this study to which all of the participants are exposed regardless of condition are low, including embarrassment in the assessment of pubertal status, claustrophobia or fear of the MRI tunnel, loud noises in the scanner, and risk of choking during saliva collection

The steps taken to reduce or prevent these risks include: allowing participants to refuse the puberty questionnaire and use of proper saliva collection materials, and by completing thorough safety screening prior to MRI scanning, and providing youth with a simulated MRI experience first. These risks are all managed by the routine processes monitored by our IRB and the CMRR Safety Committee. Adverse Events (AEs), including Serious Adverse Events (SAEs) such as deaths, hospitalizations, and life-threatening events and Unanticipated Problems (UPs), will be managed and reported, as required, to the IRB and relevant monitoring authorities. All members of the project have received human subjects training and certification in FDA Good Clinical Practice through the Collaborative Institutional Training Initiative (CITI) curriculum.

<u>The following information will be collected to monitor risk:</u>

- The number of youth refusing the puberty questionnaire.
- The number of youth refusing the MRI scan.
- Any instance of gagging during the saliva collection.

<u>How often will risks be assessed?</u> Every three months we will examine the number of youth refusing the MRI scan and adjust procedures, if needed, to improve participant tolerance. In our experience, few youth of this age who have agreed to come in for testing end up refusing the MRI scan once they are here.

<u>The individuals responsible for trial monitoring and advising the appointing entity</u>. Because of the low level of risk in this study, the PI, Dr. Kathleen Thomas will be responsible for monitoring and reporting events for this project.

18.0 Provisions to Protect the Privacy Interests of Participants

18.1 Protecting Privacy: Once the participant has been tested, identifying information will be removed, and all materials will be identified with a participant number only. A master list with participant names, addresses and contact information will be maintained in secure REDcap database, separately from the data and study materials. A master list connecting study ID to participant name will be maintained in a password-protected database on REDCap. Only researchers involved in the study and having a legitimate reason for connecting data to an individual participant (e.g., in the event of a clinically-relevant incidental MRI finding) will have access to the file that merges participant number and personal information. Non-data study materials (e.g., consent forms, payment forms, MRI safety screening forms) will be maintained in a secure REDcap database separately from the de-identified data.

Considerable efforts are made to make participants comfortable, despite the nature of the stressor in the second visit. Consent and all study procedures will be conducted in private rooms. During consent/assent multiple efforts are made to make it clear that participation is optional and that the child is in control of refusal; for example, payment is promised at the start of the session rather than upon completion. Collection of biological measures, placement of electrodes, and administration of sensitive questionnaires are all done with a "matter of fact" attitude to minimize any feeling of embarrasment. Should a participant need to skip a portion or end a session early, experimenters are trained to accept this as a standard procedure when working with children rather than a problematic ending to the session. We have successfully guided hundreds of youth through these very procedures without undue problem, including longitudinal studies where the participants continue to return.

Female participants who have begun menstruation will be verbally screened for possible pregnancy. If a child thinks she could be pregnant, we will not scan her. By Minnesota Statue, we are not allowed to break confidentiality to inform parents of a teen pregnancy. If a child tells us that she might be pregnant, we will offer her a urine pregnancy test to give her more information but will not require anyone to take a pregnancy test. If the test is positive, we will encourage her to tell her parents, and will offer to sit with the child while she informs a parent. If a child thinks she may be pregnant or has a positive pregnancy test, we will provide her with information on services that she can access to receive pre-natal care and counseling.

18.2 Access to Participants: This study does not involve access to participant medical records or other private records. Any personal information collected in this study will be provided directly by the participants

19.0 Compensation for Research-Related Injury

19.1 Compensation for Research-Related Injury: All of the proposed procedures meet the NIH definition of minimal risk. In the event of an injury, costs will be charged to the participant or the participant's medical insurance plan.

19.2 Contract Language: N/A

20.0 Consent Process

20.1 Consent Process (when consent will be obtained):

- All participants will be children, recruited by contacting parents. Age will be verified with parent.
- Parental permission will be obtained from:
  - One parent, even if the other parent is alive, known, competent, reasonably available, and shares legal responsibility for the care and custody of the child.
- All consent and assent will be collected online via the e-consent framework on REDCap. Staff will be present on a video conference call with the parent and child for the full consent and assent process and will both explain the study and answer all questions.
- On infrequent occasions, a parent requests that a grandparent or adult nanny be allowed to accompany the youth to the session. In those cases, we will request the parent sign a consent form to send in with the child. We will conduct the full consent & assent process as usual with the grandparent or adult nanny on site.
- All youth will be part of the consent process, will be asked questions to verify understanding, including the question of "When are you allowed to skip a task or end the session?". All youth will provide assent online.
- Only those individuals listed as personnel on the study will be allowed to obtain consent. All listed researchers have completed HIPAA and CITI training, and have watched an in-house video of an ICD professor explaining and modeling appropriate consenting procedures. Further, all consenters have experience consenting/assenting families and children in this age range for studies of similar procedures. Consenters are trained both to follow a bullet-pointed list of key items as well as to sensitively discuss a range of concerns that parents or youth commonly raise. The consent process is a regular topic of weekly staff meetings, where researchers share experiences (without providing identifiable information) in order to help others learn novel approaches or gain tips for success.

20.2 Waiver or Alteration of Consent Process (when consent will not be obtained): N/A

20.3 Waiver of Written/Signed Documentation of Consent (when written/signed consent will not be obtained): N/A

20.4 Non-English Speaking Participants: N/A

20.5 Participants Who Are Not Yet Adults (infants, children, teenagers under 18 years of age):

- All participants will be children, recruited by contacting parents. Age will be verified by the parent.
- Parental permission will be obtained from:
  - One parent, even if the other parent is alive, known, competent, reasonably available, and shares legal responsibility for the care and custody of the child.
- On infrequent occasion, a parent requests that a grandparent be allowed to accompany the youth to the session. In those cases, we will request the parent sign a consent form to send in with the child. We will conduct the full consent & assent process as usual with the grandparent on site.
- All participating youth will complete an onlinevassent form to accompany the online parental consent described above, in accordance with Minnesota statutes. The investigator will review the consent form with both the youth and the parent, and will ask the youth to tell them in their own words what will occur during the session ("In your own words, what is going to happen in today's session?"). The investigator will then answer any questions the youth has and describe in more detail anything that she/he believes the adolescent does not fully understand. Each youth is reminded that they can stop at any time. We give them an "out" by saying, "Some people change their minds and decide this isn't something they want to do right now, and that's okay with us. Do you still want to go on?" If the youth does agree to participate in the study, s/he will sign an assent form. Similar procedures will be followed with the parents. Parents will be asked to describe in their own words what the child will be asked to do. If the parent seems unsure, the investigator will describe the procedures in another way and will provide corrective feedback.

  Recruitment procedures will make it clear that participation is voluntary and that families will still be able to participate in future research if they decline to participate in the current project. They may also remove their name from the participant database without prejudice if they wish. Parents will be mailed or emailed the consent forms and directions to the lab so that they have further time to make their decision before the session. Investigators will stress that parents and children are free to discontinue participation at any time

without penalty. Further, compensation will be promised at the time of consent/assent, eliminating the chance that someone may feel compelled to continue for the compensation.

20.6 Cognitively Impaired Adults, or adults with fluctuating or diminished capacity to consent: N/A

20.7 Adults Unable to Consent: N/A

21.0 Setting

21.1 Research Sites:

- Participants will be recruited from the ICD registry of potential participants, or through direct response to posted advertisements.
- Research will be conducted online via video conference, and at the Center for Magnetic Resonance Research (CMRR).

21.2 International Research: N/A

22.0 Multi-Site Research: N/A

23.0 Resources Available

23.1 Resources Available:

- There are sufficient potential participants on the registry to meet the needs of the study design/cell count. In the age range we would need over the years of this proposal there are 14,529 girls and 15,284 boys from which to draw.
- We anticipate that it will take approximately 2.5 years to complete recruitment and testing. We anticipate an additional 12 months needed to complete primary analyses of the MRI and physiological data.
- The Center for Magnetic Resonance Research are ideal facilities to collect these data, with appropriate rooms, parking, private consent areas, and sibling/family-friendly waiting areas. The CMRR has a realistic MRI simulator that will be critical for this study. The CMRR houses 7 research-dedicated scanners, three of which are 3-Tesla scanners appropriate for the current study. Full-time staff maintain the scanners to ensure high-quality data acquisition, as well as linen services to provide necessary sheets, blankets, and hospital scrubs for participant safety and comfort. The CMRR has a -80 degree freezer for secure storage of biospecimen samples until samples can be batched for shipping to the assay labs.
- We do not anticipate the need for either medical or psychological resources as risk is minimal in the current study. However, the CMRR

has First Aid and AED kits on-site, and islocated at on the medical campus of the University of Minnesota, providing close proximity to emergency medical services.

- All staff & lab personnel, including undergraduate students working with de-identified data, undergo required and available ethics training. The study coordinator has over 25 years of experience training staff and conducting behavioral and psychophysiological research with children and adolescents. Dr. Thomas has over 20 years of experience in designing and running MRI studies with child participants, and in mentoring junior scientists in MRI methods. The staff scientist has 16 years of experience scanning children and supervising MRI data processing and analysis in Dr. Thomas' lab. Weekly project meetings keep us adequately informed about any changes in protocol, updates to research procedures, or distribution of staff duties and functions.

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