Protocol Title	The Role of Social Partners in Buffering Physiological and Brain Indicators of Threat-Related Stress in Children and Adolescents: An MRI Study of Learning and Social Support
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PROTOCOL COVER PAGE

REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?
1 (v2)	May 29, 2019	Response to reviewer concerns	Yes
2 (v3)	July 12, 2022	The original protocol was written pre-grant award, with a planned mid-grant launch. As a result of changes due to covid etc, the protocol has been updated. Re-tooled for e-consent, remote buffers, and rather than counterbalancing two tasks, we will pilot and select one of the two tasks previously proposed; some measures were dropped for budgetary reasons. In response to the CR stipulations, we also updated consent/assent forms to conform to current language regarding: contact info, pregnancy info. We already noted that we will neither target nor exclude youth who have family members who are active members of military.	Yes
3 (v4)	May 16, 2023	Reduced manipulated conditions from 4 to 3 (alone, parent, friend). Removed unnecessary questionnaire. Updated fMRI task details. Updated biological samples. Increased participant payment.	Yes
4 (v4)	Sept 21, 2023	Added optional actigraphy measure. Added new recruitment methods.	Yes
6 (v6)	Oct 27, 2023	Added parent and child questionnaires.	No
7 (v7)	Dec 7, 2023	Added child questionnaire.	No
8 (v8)	Jan 25, 2024	Updated session length and hair assay language in consent form and protocol. Created consent addendum for participants who	Yes

		have already completed participation.	
9 (v9)	March 7, 2024	Added recruitment method of mailing paper flyers.	No
10 (v10)	December 18th, 2024	Increasing number of participants to enroll	No

Table of Contents

ABBREVIATIONS/DEFINITIONS

- fMRI: Functional Magnetic Resonance Imaging
- CORT, AUCi: salivary cortisol, area under the curve from intercept
- DHEA: dehydroepiandrosterone
- EKG: Electrocardiogram
- SCR: Skin conductance response
- 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 threat in children and adolescents, and to examine the effectiveness of various social partners (parent, friend) in buffering youth from the physiological and brain effects of threat.

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 or threat 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).

Supportive friendships are associated with many positive outcomes for children, while their lack is associated with negative emotionality (Laursen & Hartl, 2013). However, counter to our initial expectations, our results showed that preparing for the TSST with a friend *increased* rather than reduced the cortisol response for adolescents (Doom et al., 2015). Having the friend “help” may actually have amplified effects of social evaluation.

2.3 Study Aims:
The current study examines the neural response, as well as the physiological responses to threat-related stress across the transition to puberty. We use two standard threat learning paradigms that have been

used previously with adolescents. 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 a close friend, or no social support present (standard imaging condition).

The **long-term goal** of our research is to enrich understanding of threat-related stress regulation and its role in human development. The **overall objective** of this project is to understand normative processes in threat-related 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 and friends 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: Friend social buffering will result in less attenuation in the same brain regions, and less mPFC-Amygdala coupling than parental buffering, but will also become less effective with pubertal development.

Hypothesis #3: 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, respiration, skin conductance

3.2 Secondary Endpoint(s)/Event(s)/Outcome(s):
Exploration of possible sex differences in across buffering 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 threat-related stress responses.

Child participants (10- to 15-year-olds) will be randomly assigned to one of three conditions during an fMRI version of a threat learning paradigm: (1) no social buffer, (2) presence of a parent, or (3) presence of a same-sex/similar-age close friend.

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. 150 target children (10- to 15-year-olds) will be randomly assigned to one of three conditions: (1) no social support (standard MRI scanning condition); (2) presence of a parent; or (3) presence of a close friend. 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.

Two tasks will be used during the MRI scanning. Both have been shown to activate stress-related regions of the brain in past studies and have been used by our labs. The order of these two tasks will be counterbalanced.

Based on the results of piloting social buffering will EITHER be done with the presence of a buffer in person, or, displayed on screen via a live Zoom feed. All conditions will use the same modality.

Study Overview:
Visit 1: Parent and child will participate in an online video session (via Zoom) lasting approximately 2 hours. Following a detailed overview of study and consent/assent procedures, participants will be asked to complete the following measures:

<u>Parent:</u>

- 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
- *[If the child is randomized to the parent-present buffering condition*], self-report MRI safety screening questionnaire to rule out any contraindications for sitting in the MRI area

- Child life challenges question

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)
- Questionnaire about anxiety in different contexts (State-Trait Anxiety Inventory for Children)
- Pubertal development questionnaire (Morris and Udry, 1980)
- Safety screening questionnaire to confirm safety in the MRI scanner
- Perceived stress questionnaire
- Child life challenges questionnaire

Visit 2: Parent and child [*and sometimes, child's close friend*] will come to the Center for Magnetic Resonance Research for a session lasting approximately 2 hours. Note that if buffering occurs in person, we will invite the close friend to attend in person, and if buffering occurs on-line, we will invite them to attend via zoom. 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*], self-report MRI safety screening questionnaire to rule out any contraindications for sitting in the MRI area
- *[If the child is randomized to the parent-present buffering condition*], sit next to scanner bed and listen to music, radio, audio book, podcast, etc. or read a book for duration of MRI scan (approx. 1 hour) OR sit in a separate room while connected via video conference call to research staff in the MRI console room for duration of MRI scan, chatting with the child periodically.

Child:

- Required safety screening questionnaire to confirm child's safety in the MRI scanner
- Saliva sampling at up to 10 time points throughout the testing session to assess levels of stress-sensitive compounds, including cortisol, alpha amylase.
- Children chew on an absorbent sponge for 1-2 minutes to acquire each saliva sample.

- 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, pulse oximeter and/or 3 EKG leads, MRI-safe skin conductance electrodes on the fingers or palm of one hand or foot).
- Performance of two tasks during MRI scanning: one emotional faces matching task and one emotionally charged go-no go task.
- Completion of a post-scan survey to assess perceived stress at various points throughout the session.
- Optional: Wearing of an actigraphy device on the wrist for several days to record activity and sleep patterns.

Child's Friend: [*Applicable only if the child is randomized to the friend-present buffering condition*]

- MRI safety screening questionnaire to rule out any contraindications for sitting in the MRI area
- Completion of the Network of Relationships questionnaire
- Sit next to scanner bed and listen to music, radio, audio book, podcast, etc. or read a book for duration of MRI scan (approx. 1 hour) OR join the session online via video conference call to research staff in the MRI console room for duration of MRI scan , chatting with the first child periodically.
- *Note: Friend's parent will complete a full consent procedure over the telephone or video conference call prior to the session and will return a signed consent and child safety-screening form via REDCap or by mail or with the participating family.*

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, Parent, or Close Friend conditions. Definition of Close Friend. We will ask the parent to have the child select the close same-sex friend they want to have accompany them. The parent then has to contact that child's family and get their permission for us to contact them. We have successfully conducted studies in which the child brings their best friend.

Affective Paradigms: ~~Aversive Learning Paradigms:~~ Consent, assent, and questionnaires will occur during an online session (to reduce in-person requirements; minimize the impact of COVID on study feasibility.) At the session, after a reminder of procedures, youth will be separated from their parents. Youth will be taken to a private changing room to change into

hospital scrubs prior to moving to the MRI scanner. An MRI-safe respiration belt, pulse oximeter and EKG electrodes, and SCR electrodes will be positioned for physiological assessment and the child will be given ear plugs, a button response box for behavioral ratings, and an emergency call button. Youth will rest and/or watch a video during structural MRI sequences (6-10 minutes), then lie quietly with eyes open for a 5-10 minute scan with no video or audio material. The experimenter will then remind the child of the instructions for the affective task, and scanning will begin with the start of the task. (1) In the first task, one target face or shape is presented at the top of the screen and two option faces or shapes are presented at the bottom of the screen. One of the bottom faces shows the same emotion, or is the same shape, as the top face or shape and the participant is instructed to quickly identify which face or shape matches and select that option using the button box in their hand. The task includes five blocks of shape matching and four blocks of face matching, with each block containing six trials. Each trial presents the stimuli for 4.5 seconds and has an interstimulus interval of 0.5 seconds. At the end of the task, the child will be given a chance to move their bodies and rest their eyes before moving on to the second task. (2) In the second task, a target letter is presented in the center of the screen with a large image in the background. Participants are instructed to press a button on the button box if the letter is not an 'X' and refrain from pressing when they see the letter 'X.' The images presented behind the letters are sourced from the International Affective Picture System (IAPS) and are presented in blocks of positive, negative, neutral, negative, or scrambled (masked) images. The task includes two blocks of each affective category (positive, negative, neutral, and scrambled) in addition to two all-go blocks which present no 'X' and two rest blocks which present only a fixation cross. Each block contains six trials which present stimuli for 0.85 seconds with a variable interstimulus interval of 0.5-0.8 seconds. Following the second fMRI task, children will be removed from the scanner and will complete a stress questionnaire. Saliva samples will be collected every 10-20 minutes throughout the scanning session, including between scan segments, to assess dynamic changes in stress-related hormones. In the scanner, this involves moving the child partway out of the scanner bore and using a long collection sponge that 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 the stress questionnaire, the child will change back into their own clothing and return to the lobby to complete additional questionnaires and wait for up to 30 min while being sampled for stress hormones every 10 min.

Pubertal Development: We will create a summary pubertal development index from parent & child report. A planned nurse exam was dropped due

to COVID and funding considerations. Parents & youth will complete the Morris and Udry (1980) pubertal development questionnaire, which allows placement in Tanner stages for hair, testicles, and breast development.

Questionnaires.

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: Youth and their friend (when assigned to friend buffer condition) will complete the Network of Relationships Inventory (NRI, Furman & Buhrmester, 2009) for parents 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 include the full set of scales from the NRI,: 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.

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.

State-Trait Anxiety Index: A brief scale to assess current and general anxiety, which might be an important covariate.

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

Morris and Udry Pubertal Development Questionnaire: The parent and youth will both report on hair, testes, and breast development.

Responses to Stress Questionnaire: Includes a checklist of stressors and how often they have occurred recently, then asks how respondents coped with each stressor listed and what coping methods were used more often.

Child Life Events Inventory: Parent reports on past significant events in the child's life that may have caused trauma, stress, or other relevant outcomes.

Sleep Diary: Child reports on daily sleep habits over several days including timing of falling asleep and waking, perceived sleep quality, and sleep disruptions. This sleep diary would be completed once a day for up to 14 days.

Perceived Stress Scale: Child reports on perceptions of life stress over the past month.

Child Life Challenges: Parent reports on their child's global lifetime adversity exposure.

Child Life Challenges: Child reports on their global lifetime adversity exposure.

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. 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.

Hair Hormones: Hair will be assayed for a full panel of stress, puberty, and sleep hormones. Hair cortisol provides a measure of the stress sensitive hormone cortisol indexed over approximately 3 months. We will take the ~ 3 cm of hair next to the scalp on the posterior vertex. Based on an average hair growth rate of 1 cm/month, this represents hair grown over the 3-month period prior to sampling. Wash and steroid extraction procedures will be conducted in Kirschbaum's ISO lab, following their published protocol (Stalder & Kirschbaum, 2012, study II) with 7.5 mg of hair used for analysis. Participants will also complete a questionnaire about hair products and treatment that can be included as covariates. We anticipate that we will not be able to collect hair cortisol for some participants whose hair is too short or who refuse this sampling. In previous work, we have

had missing samples for ~ 10% of participants. We will deal with this using multiple imputation by chained equations (MICE) to impute missing data.

Physiological Recording: Participants will wear a combination of standard Siemens physiological recording equipment for the MRI environment (respiration belt, pulse oximeter, EKG leads). In addition, they will wear BioPac MRI-safe skin conductance electrodes. Samples will be collected throughout the entire MRI scan. Response measures will be computed by regressing aversive learning condition values on comparison values during the resting state scan and saving the residual as the response measure. If the parent and child opt-in, the child will wear an actigraphy device for several days. The actigraphy device uses accelerometers and gyroscopes to measure body movement. After completion of actigraphy data collection, the parent will return the device back to the researchers.

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. Birthdate will be collected from parent at the session so that a precise age of child can be obtained.

5.3 Study Duration: Participants will participate in two sessions, one online, and the other in-person. Each session will last approximately 2 hours.

- All participants will be enrolled and assessed within approximately 42-months.
- Primary data analysis endpoints will be available within 6-12 months of the end of data collection (i.e., end of 5-year grant funding cycle).

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 managed by the CMRR.

6.0 Data and Specimen Banking

6.1 Storage and Access: Saliva samples will be stored in a -20 degree freezer and hair samples will be stored in a dry dark secure location 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 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:

Regarding 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 10- to 15-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 research during a stressful situation such as emergency room setting, childbirth (labor), etc.	Excluded from Participation
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 10-15 years old. This study focuses on adolescents because our goal is an analysis of the impact of social buffering and puberty on aversive learning 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 at either the Institute of Child Development or the Center for Magnetic Resonance Research, centers specifically designed to be child-friendly. In addition to testing rooms, each site includes a playroom/waiting room. While many of our participants will be "too old" for the playroom, they will likely find this a very welcoming environment.

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 a social stress paradigm; therefore, we are confident that we are well positioned to properly inform children about the procedures and to ensure that we are not stressing children beyond normal everyday levels.

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.

Individuals or groups with a fear of negative consequences for not participating in research are included/allowed to participate in this research study. These individuals are neither screened for nor excluded, but will be protected through our careful consent/assent procedures. During these procedures, as well as throughout the study sessions, participants are reminded that they are allowed to stop their participation at any time with no consequence. Our staff members are trained and experienced at noticing participants' apprehension so that they may further discuss an individual's participation in the study or stop the study procedures all together if necessary.

10.0 Local Number of Participants

10.1 Local Number of Participants to be Consented: We plan to analyze data from at least 150 target participants. Of these, 50 will bring friends as social buffers. Thus, we plan to enroll at least 200 children. 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 the IRB-approved recruitment flyer. 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. Souvenir brain images may be shared with families to increase interest. These images will only show a single cross-section of the brain.

Regarding recruitment of a friend: The family of the participant will contact the family of the friend make sure that they have permission to give us their contact information. Parents of the friend will then be contacted by telephone and/or email to provide an overview of the study and the role of the friend. The friend's parent will then be emailed a link to a consent & assent forms and MRI Safety Screening form (University of Minnesota REDcap) that they can complete and sign online before the session. If they do not respond to email in ~3 days, our recruiter will call them for a reminder. There will be phone and email contact information so that parents can ask any questions they have before their child comes to the lab. The assent form provided to the friend at the time of the study visit will also have a line that the parent of the main child participant can sign as a witness to the consent process. We are currently using this process successfully in STUDY00006288.

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. Paper and electronic flyers will be posted to supplement the list, and these materials may be physically posted as flyers, or electronically distributed via social media or list-servs such as CEHD. The Community Engagement and Education (CEEd) hub at the Masonic Institute for the Developing Brain (MIDB) will be used for consultation services to improve community engagement and recruitment

efforts. No protected records are involved. Study staff will make initial contact.

The Institute of Child Development Participant Pool or IPP does not contain data that generates new knowledge. Indeed, maintaining the contact information of parents who have voluntarily expressed interest in child development research has been classified by the IRB as "Not Human Research" as defined by DHHS and FDA regulations and informed by HRP-310.

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, other social media outlets, local organizations' newsletters, and share via listserv so that families can contact us. 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; $30 for optional actigraphy device
- Parent of Child Participant: $25 in debit or gift card for Visit 1; $25 in debit or gift card for Visit 2
- Friend of Child Participant: $40 in debit or gift card for Visit 2

Cards will be given at the session to parent & child as specified. Where appropriate, due to remote buffering for example, we will mail cards. 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 affect paradigm or general 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:

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.

Amygdala-activating Tasks. The tasks are designed to be uncomfortable, but not painful or distressing. Children encounter many types of affective stimuli in their everyday lives. Thus, we do not consider these stimuli to be out of the ordinary. To reduce risk, youth are told that they can always stop without prejudice if they choose. In addition, we carefully expose youth and their parents to example stimuli to ensure that all are comfortable with continuing.

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 and/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 identically 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, 10-15 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 (including youth serving as the friend social buffer) who have started menstruating for possible pregnancy. In addition, if buffers are in-person, they will be screened for possible pregnancy and exclude women who think they could be pregnant from serving as the social buffer (i.e., sitting in the MRI scanner room with the child).

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 6.0.1) 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: In first level analyses (single subject), brain activity will be modeled for each participant using a voxel-wise multiple regression analysis with a task predictor (aversive image vs. safe image), 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 1 mm (1/2 voxel), as well as the immediately preceding and following data points. Data points with DVARS values exceeding the individual's 75^{th} 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. This analysis will be used to identify brain regions differentially activated by aversive learning. 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 aversive face/shape and the safe face/shape 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.

Although these affect paradigms have 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 literature. Therefore, we will also conduct an exploratory group-level analysis to identify primary regions of activation for the same aversive vs. safe stimuli (aversive image vs. safe image, cluster corrected p<.005). Because we expect that social buffering will alter the activity difference between aversive and safe 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.

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, and physiological responses, 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 physiological measures, then we will examine whether brain activity differences mediate the neuroendocrine or physiological 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 150 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: 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 child 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 on REDCap 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, and will be digitized/verified/destroyed.

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 or brain activity is being measured.

There is no risk associated with the assignment to conditions (No Social Buffer, Close Friend, 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 other potential risks of this study to which all of the participants are exposed regardless of condition are low, including, 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: 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.

The following information will be collected to monitor risk:

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

How often will risks be assessed? 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.

The individuals responsible for trial monitoring and advising the appointing entity. 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 links from study ID to participant name will be maintained in a password-protected spreadsheet on RedCap and/or University Box Storage. 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 given 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.

- The consent process will be conducted in an online secure Zoom session using RedCap econsent and assent forms. In the case of friends who act as buffers links to the forms will be sent to parents and verified prior to the session.
- 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 written assent.
- Only those individuals listed as personnel on the study will be allowed to obtain consent. All listed researchers have completed HIPAA and CITI training. 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.
- In January of 2024 we created a consent addendum to expand on the allowed assays that could be performed on the child's hair samples. This addendum would be sent to all participants that have completed participation in the study and provided a hair sample through the date of this modification's approval (MODCR00004974). This expansion on allowed assays is also included in the consent form submitted for approval within this modification.

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.
- All participating youth will complete a written assent form to accompany the written 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 provided 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 as described above.
- Research will be conducted at the Institute of Child Development and 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 Institute of Child Development (ICD) and the Center for Magnetic Resonance Research (CMRR) are ideal facilities to collect these data, with appropriate rooms, parking, private consent areas, and sibling/family-friendly waiting areas. The ICD and CMRR have 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 the32caners to ensure high-quality data acquisition, as well as linen services to provide necessary sheets, blankets, and hospital scrubs for participant safety and comfort.
- We do not anticipate the need for either medical or psychological resources as risk is minimal in the current study. However, the ICD and CMRR all have First Aid and AED kits on-site, and the CMRR facilities are located 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 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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