

Mind, Brain, Body

By: Bridget Callaghan, Emily Towner, Kristen Chu

Updated on: 2020-08-11

Contents

1	5
2 Information	7
2.1 Summary	7
2.2 Procedure	9
2.3 Measures	10
3 Wave 1	45
3.1 Checklists	45
3.2 Protocols - Pre-Session	51
3.3 Protocols - Parent	58
3.4 Protocols - Child/Teen	67
3.5 Protocols - Post-Session	87
4 Wave 1 Online	99
4.1 Checklists	99
4.2 Protocols - Pre-Session 1	105
4.3 Protocols - Session 1	119
4.4 Protocols - Post-Session 1	138
4.5 Protocols - Session 2	141
4.6 Protocols - Final	143
5 Wave 1 SONA	155
5.1 Summary	155
5.2 Measures	155

Chapter 1



Figure 1.1:

The Mind, Brain, Body study looks at how early caregiving experiences influence emotional, cognitive, and brain development, as well as physical health and wellness.

The study also explores how the bacteria that live inside us (the microbiome) are connected to the development of our brains and bodies.

Chapter 2

Information

2.1 Summary

2.1.1 Abstract

The Mind, Brain, Body (MBB) study examines links between early life adversity (ELA), the gastrointestinal microbiome, memory and affective neurodevelopment, across three years in middle childhood through adolescence. The specific aims of this study are to (1) determine how adversity affects developmental change in the microbiome in middle childhood and adolescence, (2) establish associations between the microbiome, memory development, and the hippocampus, and (3) identify independent and joint influences of adversity exposure, the microbiome, brain and memory performance on the emergence of anxiety symptoms across development. We are recruiting N = 75 ELA exposed, and N = 75 not adversity exposed (Comparison) children and adolescents to take part in 3 waves of data collection. Children in the ELA group must be 6-16 years of age, while children in the comparison group must be 6-9 or 13-16 years of age. Participants complete a laboratory session (in-person or online) in Wave 1-3, and also complete a neuroimaging session in Wave 2. Study procedures for the laboratory session in Wave 1-3 remain similar. For the ELA group, we are recruiting youth who were adopted from institutional or foster care: previously institutionalized and who are now adopted internationally (PI), adopted domestically from foster care (DA). Comparison youth have not experienced these forms of early life caregiving adversities (i.e., they have always been with their biological parents). In addition to the primary aims of the MBB study, described above, we are also collecting a range of supplemental biological, questionnaire, and MRI measures aimed at assessing cognitive and emotional functioning, family functioning, as well as immune, biomarkers, and physical health assessments to aid in understanding the mechanisms behind the specific aims under investigation. The MBB study will test for links between the microbiome, memory, hippocam-

pal function, and anxiety across a three-year time frame in middle childhood – adolescence (6-16 years of age). Participants may opt out of the study at any point in time but will be asked to participate in testing every year until funding ceases (current grant funding this research – R00 NIMH – ends in 2022).

2.1.2 Aims

The study will test four primary and two supplemental hypotheses. The primary hypotheses are: (H1) that there will be greater stability in microbiome composition across 3 years in the ELA than Comparison group, (H2) ELA will be associated with higher expression of anxiety symptoms across time, (H3) the microbiome in Wave 1 will predict developmental change in memory performance between Wave 1-3, and that hippocampal activity during learning will mediate that association, (H4) that the microbiome, hippocampal encoding patterns, and memory behavior longitudinally mediate the adversity and anxiety association across development. The supplemental hypotheses are: (SH1) that ELA will be associated with a higher incidence of physical health (particularly gastrointestinal) problems, as well as dysregulation in stress response and immune systems, (SH2) that physical symptoms, stress response and immune system will mediate the link between adversity exposure, microbiome changes, hippocampal neurodevelopment, and memory.

2.1.3 Background

An individual's early rearing history has a significant effect on their emotional functioning across the lifespan. In particular, access to consistent/stable caregivers is strongly associated with mental health (Repetti et al., 2002), and parent-related early adversities (e.g., abuse and neglect) contribute to more than a third of mental illnesses (anxiety being the most common diagnosis; Kessler et al., 2005). Clinical, epidemiological and basic science suggests that two systems affected by early adversity – the gastrointestinal microbiome, and the hippocampal memory system, are also consistently associated with the emergence and maintenance of anxiety symptoms. Rodent and monkey models have established that adversity alters microbial communities in the gut (Bailey and Coe, 1999; Bailey et al., 2010; O'Mahony et al., 2009) and that microbial populations are causally related to the expression of anxious behaviors (Collins et al., 2013). In humans too, microbiome-related gastrointestinal disturbances (e.g., irritable bowel syndrome) and anxiety are highly comorbid (Kennedy et al., 2014; Callaghan et al., 2019). In terms of the memory system, rodent studies show that typically weak memories in childhood get stronger following adversity (Callaghan and Richardson, 2012; callaghan_2012b; Cowan et al., 2013), and exposure to early stress promotes hippocampal synaptic development (Huang et al., 2005), which aids long-term memory persistence. Memory dysfunction is

key to maintaining anxiety disorders, which are characterized by persistent and intrusive memories of threat(Acheson et al., 2012; Zlomuzica et al., 2014), and hippocampal dysfunction is common in anxiety disorders (Godsil et al., 2013). Importantly, hippocampal development is causally tied to the microbiome in early life (Callaghan et al., 2016; Clarke et al., 2013; Cowan et al., 2016; Gareau et al., 2011), suggesting that interactions between these two systems could contribute to anxiety emergence. Although microbial and hippocampal memory systems exert their effects on anxiety in the context of one another, until now, they have always been studied independently.

Using a completely novel longitudinal design, we will test the hypothesis that changes in the gastrointestinal microbiome are associated with early adverse caregiving and underlie altered hippocampal development and memory functioning in middle childhood – adolescence. Moreover, we will examine whether those microbial, and neurodevelopmental changes mediate the progression towards anxiety symptoms in early adversity exposed individuals. To answer these questions, we will compare longitudinal data from a group of adolescents who have experienced early caregiving adversity, to a group of comparison youth who have not experienced those same events. The study will involve examining the gastrointestinal microbiome (measured through stool samples), and functional magnetic resonance imaging (fMRI) to understand hippocampal functional development. In addition, the study will collect saliva and blood spot samples to assess saliva biomarkers (e.g., cortisol, testosterone) and blood immune markers (e.g., CRP, interleukins), as well as hair samples to assess chronic cortisol exposure, which can be used to understand the mechanisms behind microbiome-brain associations in youth.

2.2 Procedure

All youth will be recruited through community settings, clinics (e.g., pediatricians office), specialized services (e.g., adoption services), and through the internet (community boards, targeted advertisements).

Three assessments (spaced 12 months apart – Wave 1-3) will occur with youth who fall within the period of development in the hippocampal and microbiome: ‘children’, and ‘adolescents’ aged 6-16 years. Biological (stool, saliva, blood spots, hair), questionnaire, and behavioral data will be collected in each wave of the study, and an fMRI scan will be collected in Wave 2. Participants will be recruited who have either experienced early life adversity (ELA) through adverse caregiving (previously institutionalized, domestically adopted), or who have not been exposed to those caregiving experiences (Comparison). A sample size of N = 75 youth will be recruited into each of those groups, resulting in a final target of N = 150 youth in the study (N = 37-38 in each of the age groups = children versus adolescents, for the ELA and Comparison groups at Wave

1). That target sample size takes attrition into account across the longitudinal study (expected at 20%, to arrive at a final sample size of N = 120). Study procedures for each wave of data collection will be similar, except for Wave 2 where an fMRI scan will occur in a second session. In addition, Wave 1 of data collection was transitioned online due to the outbreak of the global COVID-19 pandemic.

Participants will therefore range in age from 6-16 years at Wave 1, 7-17 years at Wave 2, and 8-20 years at Wave 3. Participants will be recruited through targeted mailout (birth records), online advertising (e.g., Craigslist), school and community organization partnerships, street-fairs and community gathering events, and flyers. After contacting the lab to express interest in study participation, parents of child and adolescent participants are contacted by telephone or email to be screened for study eligibility. An experienced research associate will conduct a scripted telephone interview to assess whether the participant/s meet criteria for inclusion/exclusion. If participants prefer to communicate via email, they will be sent a detailed infographic which contains the same key information communicated in the telephone script. In addition to this informational infographic, in the body of the email, participants will be sent a few brief questions to determine eligibility and basic demographics.

During this initial contact, if eligible and interested, participants will be scheduled for their Wave 1 session and sent the consent and assent forms. They will also be told about Wave 2-3 and that scheduling for those visits will take place approximately 12 and 24 months after Wave 1. Participants involved in any wave will be told that they will be invited to participate in future waves, but are not required to do so. When the study transitions to Wave 2, parents of children and adolescents who participated in Wave 1 will be re-contacted and screened for eligibility in Wave 2 using the same procedure. When the study transitions to Wave 3, parents of children and adolescents who participated in Wave 1 and/or Wave 2 will be re-contacted and screened for eligibility for Wave 3.

2.3 Measures

2.3.1 Observations

2.3.1.1 Parent-Child Interaction

2.3.1.1.1 Description

Parents and children participated in a discussion where they were filmed having a conversation. They were presented with a laminated sheet which contained a list of pleasant events on one side and a list of issues on the other side. These lists contained topics and events that children and parents might experience

(for example, pleasant events included talking about sports, going to a concert, camping, etc., while issues included cleaning, homework, cleanliness, etc.). Participants were instructed to take 1-minute to choose something on the list and then were given 5-minutes to discuss what they had chosen. They were instructed to try to resolve the conflict and to try to plan the pleasant event. Participants were permitted to expand beyond topics on the list. Participants were filmed during the interaction. The conflict interaction was completed first, and the pleasant event was discussed second to ensure that parents were not thinking about the negative interaction upon completing the clinical interview and questionnaires about their child immediately after the observation. Videos were coded using the Family Interaction Macrocoding System (FIMS)(Holmbeck et al., 1995).

2.3.1.1.2 Details

2.3.1.1.3 Pleasant Events & Issues

Participants choose events from these two lists (MacPhillamy and Lewinsohn, 1982):

Pleasant Events Checklist	Issues Checklist
Being in the country	Telephone calls
Talking about sports	Bedtime
Going to a concert	Cleaning bedroom
Planning trips or vacations	Doing homework
Being at the beach	Putting away clothes
Doing art work (painting, sculpture, drawing, movie-making)	Using the television
Rock climbing or mountaineering	Cleanliness (washing, showers, brushing teeth)
Playing golf	Which clothes to wear
Re-arranging or redecorating my room or house	How neat clothes look
Going to a sports event	Making too much noise at home
Reading stories, novels, poems, or plays	Table manners
Making music together	Fighting with siblings (brothers and sisters)
Boating (canoeing, kyaking, motorboating, sailing, etc	Cursing
Watching TV	How money is spent
Camping	Picking books or movies
Playing cards	Allowance
Completing a difficult task	Going places without parents (shopping, movies, etc)
Laughing	Playing stereo or radio too loudly

Pleasant Events Checklist	Issues Checklist
Solving a problem, puzzle, crossword	Turning off lights in house
Playing tennis	Taking care of records, games
Driving long distances	Buying records, games, toys, and other things
Woodworking, carpentry	Going on dates
Writing stones, novels, plays or poetry	Who friends should be
Being with animals	Selecting new clothes
Riding in an airplane	Coming home on time
Exploring (hiking away from known routes)	Getting to school on time
Going to a party	Getting low grades in school
Playing a musical instrument	Getting in trouble at school
Making snacks	Lying
Snow skiing	Helping out around the house
Doing craft work (pottery, jewelry, leather, beads, weaving, etc)	Talking back to parents
	Getting up in the morning
	Bothering parents when they want to be left alone
	Bothering child/adolescent when they want to be left alone
	Putting feet on furniture
	Messing up the house
	What time to have meals
	How to spend free time
	Earning money away from the house
	What child/adolescent eats

2.3.1.1.4 Coding the Interaction

- After the observation are collected, videos will be coded by two observers blind to the caregiving group of the child (adversity or comparison).
- Videos will be coded using the Family Interaction Macrocoding Schedule (FIMS)(Holmbeck et al., 1995).
- We ultimately decided to go with FIMS for several reasons:
 - Expense - Approximately \$1000 USD for a 10 hour skype training session with one of Holmbeck's team
 - Validation in age range - FIMS was designed for older children and adolescents, and Sarah Whittle has validated it in a community sample of 8 year olds and their mothers.

- FIMS is a less intensive coding schedule, producing global codes, rather than micro coded (i.e., minute to minute) scales - which makes more intuitive sense in the age range for MBB.
 - FIMS has a peer version that we might branch out to in the future (but likely not needing further training)(Holbein et al., 2014).
 - Sarah Whittle’s group looked at the component structure for the FIMS and found components that seemed close to what they were finding with the Hops LIFE system - namely: negative maternal affect during pleasant event, negative maternal during conflict discussion, and pleasant maternal affect across both tasks (warmth). The negative maternal during pleasant event was the most predictive of child behavior problems. Overall, the correlations they report in their paper are all very sensible and convinced me that we should use the FIMS (Richmond et al., 2018)
-

2.3.2 Interviews

2.3.2.1 KSADS

2.3.3 Physiology

2.3.3.0.1 ECG - Electrocardiogram

Child and adolescent participants will have their heart rate recorded using a Biopac recording device. Two small stickers containing a recording electrode are placed on the front of participant’s bodies (underneath their collarbone on the left and right side). A third sticker, also containing a recording electrode, will be placed on participant’s left lower rib. The electrodes are attached to recording wires, which lead to the Biopac machine, which is itself hooked up to a computer. Participants will have their heart rate measured during all of the computer tasks (which include playing computer games and watching movie clips).

2.3.3.0.2 GSR - Galvanic Skin Response

Child and adolescent participants will have their GSR (sweat) response measured while they are undergoing the computer tasks. GSR is measured by small stickers with electrodes that are placed on the participants hand to measure very small variations in sweating (which are a marker of attention). Two stickers are placed on the participants non-dominant hand, with wires leading to the Biopac machine and computer.

2.3.3.0.3 EGG - Electrogastrogram

Child and adolescent participants will have their gastric activity monitored through an EGG. Similar to measures of heart rate, the EGG is collected through small stickers containing recording electrodes that are stuck on the abdomen, and are connected through recording wires to the Biopac machine and then computer. EGG will be measured at the same time as participants heart rate and sweat response (during the computer tasks).

2.3.4 Tasks

2.3.4.1 Memory Intrusion

2.3.4.1.1 Description

Child and adolescent participants will listen to a list of words that surround a theme (e.g., for the theme sleep, they might hear – ‘pillow’, ‘bed’, ‘night’, ‘tired’, ‘cosy’). There will be a series of words that are conceptually related to the theme that they do not hear (e.g., they will not hear ‘sleeping’). Then participants are asked to recall the words they heard from the list, and the number of memory intrusions from related but not presented words (i.e., ‘sleeping’) is recorded. Participants will do two versions of this task, one preceded by a relaxing task (neutral movie clips described above), and another preceded by a mild stressor (sad/scary movie clips).

Note that the movie clips are age appropriate and follow film classification guidelines for the child’s/adolescent’s age (i.e., G-rated movies for the child age group). Such movies might involve scenes like the stampede scene from ‘The Lion King’.

2.3.4.1.2 Details

Physiology Marks - We have inserted start and stop times for the physiology for the movie component of the task, which will be used for one of the analyses. We have also inserted physiology marks for the start and stop times for each of the word lists, and finally for the recall phases.

Note: Be careful using physiology during the recall phase, as the participant is talking during that phase.

2.3.4.2 Halloween

2.3.4.2.1 Description

The Halloween task is a memory task comprised of both recognition and associative memory components. Children are told that they are going to play a Halloween game. In one block, they see bright and cheery indoor and outdoor

scenes, and in the next block the scenes are dark and haunted looking. In addition to these scenes, an item (toy or candy) is overlaid somewhere in the scene. They are told that their job is to collect the toys and sweets and remember what house the toys/candy came from. Each block has 20 trials (10 are the house + toy, 10 are the house + candy). The toys/candy that are paired with the houses are not counterbalanced, but the order of the blocks will be counterbalanced between participants (some will have day then night, and some will have night then day). The context is presented for 500ms before the item is overlaid on the context image for an additional 2500ms (3000ms total). There is a 500ms interstimulus interval between trials. There is a self-advancing break between the blocks.

2.3.4.2.2 Background

In prior work, across typical development, the research team found that during an associative learning task (contexts paired with objects or faces) there were different levels of granularity in the anterior third versus posterior third of the hippocampus - where representations were more granular in the posterior than anterior and that this granularity increased across age. Although brain data from previously institutionalized youth have not yet been analyzed, we have seen interesting behavior associations with adversity. Specifically, we see an age by adversity interaction on memory retention, where previously institutionalized children have better long-term memory retention, which becomes normalized by adolescence.

To further this line of research, we will continue looking at the association between granularity in the anterior versus posterior hippocampus and long-term memory retention. In this task, we also include a component looking at the role of emotion in processing. To incorporate this, we have one block of the task being emotional or threatening in some way – the contexts (scenes) will be either scary or not scary. The idea for this task comes from several papers (Brunec et al., 2018; Lambert et al., 2019; Tambini et al., 2010).

2.3.4.2.3 Details

The idea from this task comes from four papers:

1. Tambini et al. (2010) - enhanced brain correlations at rest are associated with long term memory. Based on the results of this paper, Callaghan, Tottenham and Davachi developed the task that Bridget subsequently used for the K99 grant. See details below.
2. Brunec et al. (2018) - Multiple scales of representation along the hippocampal anteroposterior axis in humans. The analysis used in this paper - representational granularity - we adopted for my K99 project.
3. Results of the K99 project - in the K99, across typical development, we found that during an associative learning task (contexts paired with objects or faces) there were different levels of granularity in the anterior third

vs. posterior third of the hippocampus - where representations were more granular in the posterior than anterior and that this granularity increased across age. Although we have not yet analyzed the brain in PI youth, we have seen interesting behavior associations with adversity. Specifically, we see an age x adversity interaction on memory retention, where PI children have better long term memory retention, which becomes normalized by adolescence.

4. Lambert et al. (2019) - altered development of hippocampus dependent associative learning following early life adversity. In this paper they found that adversity (violence exposed youth) were impaired in hippocampus associative memory only when the item (faces) were angry. That is, when threat cues were present, they suggested that is hijacked encoding and made more elemental and less context integrated.

In the R00, I wanted to continue looking at the association between granularity in the anterior vs. posterior hippocampus and long term memory retention. However, rather than do the exact same task as the in the K99, I wanted to include a component that looked at the role of emotion in processing. One way to do this is doing the same item + context task as I did during the K99, but having one of the runs of the task being emotional or threatening in some way.

Idea for the halloween game:

Items will be toys and sweets, and the contexts will either be scary or not scary. This is different than the Lambert study in that her contexts were neutral and the items (i.e., faces) were angry or not. So therefore the scary item prevented the processing of the context. In this version, the context will be scary and the item will be neutral - so will the context hijack the processing and make the scary run harder for adversity exposed kids to learn the association? Or will it be the opposite, heightening attention and improving learning?

Structure of the game: Children will be told that they are going to play a halloween game. In one run, they will see bright and cheery indoor and outdoor scenes, and in the next run the scenes will be dark and haunted looking. They will be told that their job is to collect the toys and sweets and remember what house the toys/sweets came from.

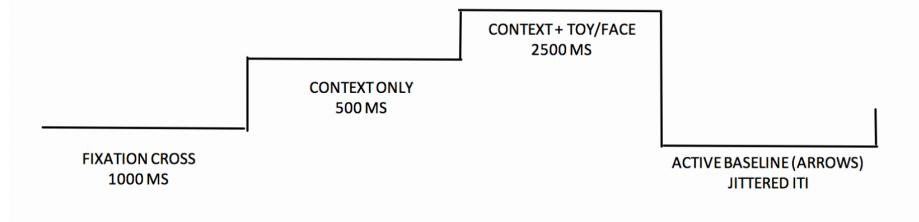


Figure 2.1:

This is the basic structure of each block. First there is a fixation cross for 1 second, then a house will appear on the screen for 500ms, then the house+toy/sweets will appear on the screen for an addition 2.5 seconds (total of 3 seconds for the house and the house+toy/sweet, then there will be a baseline period that is jittered in length. The baseline is jittered in the MRI but it will be a fixed length of 1 seconds in the lab-based version of the task. In the MRI the active baseline will involve pressing buttons to indicate the direction of arrows on the screen, in the lab-based version of the task, it involves simply looking at a fixation dot.

There will be two blocks (day, and night). The day block is the emotionally neutral block, whereas the night block is the scary block. Each block has 20 trials (10 are the house + toy, 10 are the house + sweets). The toys/sweets that are paired with the houses are not counterbalanced, but the order of the blocks will be counterbalanced between participants (some will have day then night, and the rest will have night then day).

As an added layer to this task, we will not only pair an item with a house, but we will place it on top of the house in one quadrant. To encode a detailed associative memory, the individual will need to remember what item was paired with the house and then what quadrant it was in. For this test, a picture of a house will be shown along with 3 choices for which item matches with the house, one being correct and 2 serving as filler “foils.” The two “foil” options are items from other houses, so the child will have seen them before. Each item will both be a correct answer for the house it was initially paired with and a foil answer for two other houses throughout the trial. Additionally, the child will have to recall which quadrant the selected item was in on the house. The quadrants appear

For the recognition test there should be an equal number of foils as target items.

2.3.4.3 Characters (monsters/aliens)

2.3.4.3.1 Description

Participants are instructed that their job in this game is to learn which “character” is right and which is wrong through trial-and-error. Participants are presented two stimuli side-by-side for 3500 milliseconds and indicate which character they choose by using the left and right arrow keys on the keyboard. Stimuli are presented in pairs AB, CD, EF (and their counterbalanced version BA, DC, FE). Stimuli A is correct in the AB/BA trials 80% of the time. Stimuli C is correct in the CD/DC trials 70% of the time. Stimuli E is correct in the EF/FE trials 60% of the time. Stimuli are randomly shuffled and assigned a letter at the beginning of the task. For Mind, Brain, Body, the stimuli consisted of colorful monsters/aliens.

If participants choose “correctly” they will see a green check mark and hear

“ding” sound. If they choose “incorrectly” they will see a red x mark and hear a “horn” sound. If they take longer than 3500 milliseconds to respond, they are shown a screen that says “Too Slow”. All feedback is presented for 2000 milliseconds before moving on to the next trial. Stimuli pairs are presented 10 times each in random order in each block (total of 60 trials). PsychoPy tallies the correct and incorrect responses on a block-by-block basis. Participants move onto the test phase once they have reached a performance criterion (65% accuracy for AB/BA trials, 60% accuracy for CD/DC trials, 50% accuracy for EF/FE trials). If participants do not reach criterion, they are automatically directed to the test after reaching a certain number of blocks (3 for children, 5 for adolescents).

During the test, participants are presented with all training pairs (AB/BA, CD/DC, EF/FE), and novel pairs including A and B (AC/CA, AD/DA, AE/EA, AF/FA, BC/CB, BD/DB, BE/EB, BF/FB). Test pairs are each presented 6 times. This section is untimed, and no feedback is provided.

2.3.4.3.2 Background

The characters task assesses learning. It is a cognitive reinforcement learning task in which participants must learn to choose one stimulus over another through reinforcement. Participants can employ two strategies to learn the correct response—either they can learn to choose stimulus ‘A’ or to avoid stimulus ‘B’. At test, these stimuli are paired with novel stimuli. If participants choose stimulus ‘A’ over the novel stimulus, that is evidence of positive feedback learning. If participants choose the novel stimulus over stimulus ‘B’, that is evidence of negative feedback learning. As a result, this task allows for direct comparison of sensitivity to these two types of learning (Frank et al., 2004).

2.3.4.3.3 Details

PsychoPy parameters should be set to:

- 10 trial_loop nreps
- 3 test_loop nreps
- Maximum 5 block_loops nreps (advances after criterion is met)

Physiology markers are set to:

- Channel 1 (28) - Train stimulus onset
- Channel 2 (29) - Check
- Channel 3 (30) - X
- Channel 4 (31) - Miss
- Channel 5 (32) - Test stimulus onset

Task based on original task by Frank et al. (2004), re-programmed and adapted by Emily Towner and Ryan Burnell.

2.3.4.4 Discrimination / Conditioning / Extinction

2.3.4.4.1 Description

Children and adolescent participants will take part in a visual perceptual threshold task. Participants will be shown visual stimuli (e.g., two black stripes at different orientations, or two Gabors with different levels of contrast) and asked to discriminate between them: “which stripe is rotated more clockwise 1 or 2?” for the stripes, or “which picture is darker/clearer 1 or 2?” for the Gabors. The magnitude of the difference between the two choices will be decreased after two correct choices, and increased after one correct choice, and will be continued until 6 wrong choices are made. The smallest magnitude where participants were able to correctly identify the stripe or Gabor in question is their Just Noticeable Difference (JND) threshold. The JND will be calculated before and after the threat learning task, and after the extinction task.

2.3.4.4.2 Details

This task has two purposes.

1. The first is to simply look at threat learning and extinction in children and adolescents across typical development and after adversity exposure. This has been done many times before, but we will also have children attached to an electrogastrogram (EGG - which is completely novel), GSR (sweat - which is the common measure), and heart rate (which is somewhat common but less so in the conditioning literature) while they are learning and extinguishing.
2. The second purpose of the task is to look at how conditioning and extinction affect perceptual thresholds. This is built from a large literature in adults showing that threat learning can lead to a broadening or narrowing of tuning curves following threat conditioning, depending on the specific parameters in place (more narrowing - i.e., better, occurs when a discriminatory conditioning procedure is employed and where the CS- and the CS+ are really different; more broadening occurs when the CS- and the CS+ are similar and when there is not explicit discriminative conditioning. We are going to employ a form of conditioning that has been shown to lead to perceptual broadening in adults.

Past literature: This paper by Shalev et al. (2018) demonstrates the effect we are trying to test in children/adolescents. They use an across sensory modality procedure (CSs = auditory, USs = visual) and between subjects design (control group = CS+ are positive/neutral, and experiments group where CS+ are negative) with pre/post perceptual tuning curves to determine the effect of threat learning on sensory discrimination.

The basic task structure:

The task and results:

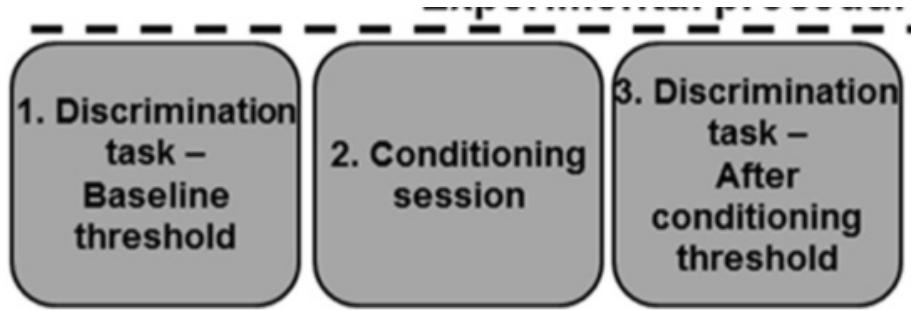
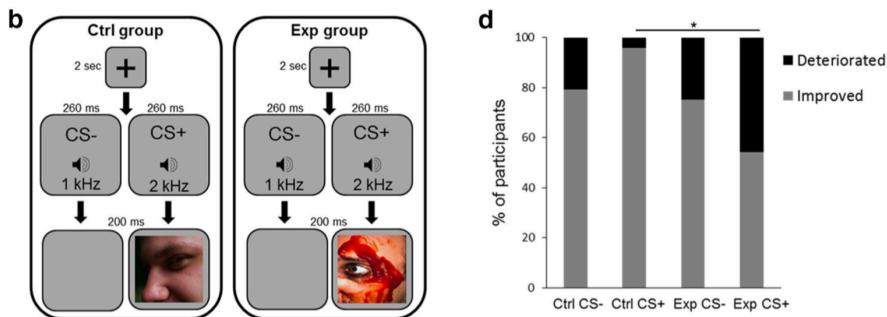


Figure 2.2:

They find that when the CS+ is paired with the aversive picture, people show a deterioration in their ability to discriminate the CS+ from tones that are similar to it, whereas they show very little change in their ability to discriminate the CS- from tones that are similar to it. They saw the same thing with gabor that differed in contrast (right) and lines that differed in orientation (left).





Current Task Structure:

We will use an approach where we pair aversive/pleasant noises (USs) with lines at different orientations (CSs). In the control group the noise will be pleasant or neutral. In the experimental group the noise will be aversive.



Figure 2.3:

The timeline of the task will be as follows:

- Number of trials was based on review of five papers (Norholm et al., 2011, (56 trials); Norholm et al., 2006, (72 trials); Schiller et al., 2013, (32 trials); Phelps et al., 2004, (34 trials); Jovanovic et al., 2014).



Figure 2.4:

The experimental protocol consisted of two phases: fear acquisition and extinction. The sessions were separated by 10 minutes. The acquisition phase consisted of 3 blocks, each with 3 CS+ trials, 3 CS– trials, and 3 noise alone (NA, no CS presented during startle probe) trials, for a total of 27 startle trials. Both CSs were colored shapes presented on a computer monitor for 6000 ms prior to the delivery of the startle probe, and co-terminated with the US 500 ms after the presentation of the startle stimulus. The CS+ was reinforced with the airblast 100% of the time. The extinction phase consisted of 4 blocks with 3 trials of each type. The CSs were same as above, except that the CS+ was no longer paired with the airblast. In all phases of the experiment, inter-trial intervals will be randomized between 9 and 22 seconds.

Person	Role
Bridget	Developed concept
Psychopy tutorial	The structure of the staircase
Paul Bloom	Helped to adapt the staircase (changed from sequential to serial, centered stimuli, randomized order, added mask)
Emily Towner	Building the task and completing the staircase

2.3.4.5 Memory Generalization

2.3.5 Tests

2.3.5.1 WASI

2.3.5.2 WIAT

Wechsler Individual Achievement Test (WIAT) – 4-85 years. Child/adolescent self-report. Domain assessed cognitive function. The WIAT is a comprehensive yet flexible measurement tool useful for achievement skills assessment, learning disability diagnosis, special education placement, and clinical appraisal for preschool children through adults. Norms allow for assessment of those from ages 4 to 85.

2.3.6 Measurements

2.3.6.1 Height & Weight

Tri-ponderal mass (TPM) and body mass index (BMI) will be calculated using height and weight measurements. TPM/BMI has been related to the micro-

biome in past studies and is important to consider as a confounding variable in those analyses, as well as an outcome variable related to early adversity.

2.3.6.2 Waist Circumference

Waist circumference will be taken using measuring tape around the child and adolescent participant's waist. This will be an outcome measure related to effects of early adversity exposure on physical development.

2.3.7 Biological Samples

2.3.7.1 Hair sample

Child and adolescent participants will donate a head hair sample for the purpose of measuring average cortisol levels during the past month. To collect the sample, a few strands of hair behind the crown of the head will be cut close to the root. This method of collecting hair is not invasive and has been performed in babies (32 weeks of age; Staufenbiel et al., 2013). Hair samples will never be used for genetic analysis.

2.3.7.2 Saliva sample

Saliva samples will be collected from child and adolescent participants in the lab using omnigene oral tubes (dnagenotek). Participants spit into a tube using a saliva collection kit. If participants are unable to spit, they will place small sterilized sponges in their mouths to collect the saliva. The sponges are then placed into the tube. After the cap of the tube is closed, it will break open a seal inside that releases a stabilizing solution into the tube which stabilizes the saliva. The saliva will be used to analyze bacteria in the oral cavity. The stabilization technique we use allows the saliva samples to remain at ambient temperature for several months. In batches, the saliva will sent to the processing facility. The samples will be labelled with participant ID codes only – no personally identifying information. Only microbial DNA, not human DNA, is analyzed in these samples.

2.3.7.3 Blood sample

Dried Blood Spot Collection: Participants can opt into or out of participating in the dried blood spot collection (identified during the consent process). Those who opt into the blood spot collection will have one finger on their non-dominant hand pricked in order to provide approximately ten drops of blood that will be placed on special filter paper cards and examined for circulating and molecular markers of inflammation. We will not collect blood from participants who are feeling ill or participants who take anticoagulants or blood thinners (e.g. Heparin, Warfarin – verified through medication checklist). Participants will be offered a Virtual Reality (VR) immersive headset to watch

a video during the blood spot procedure. Previous studies have empirically demonstrated that VR can significantly reduce child/adolescent anxiety about blood spot/draw procedures. The trained researcher will massage the finger to be pricked to draw the blood circulation to that area, and will use BD Microtainer contact-activated lancets to make one prick the middle or ring finger on the non-dominant hand, disposing of the lancet in a bio-hazard sharps container immediately. This procedure is similar to what children/adolescent may experience at a pediatrician visit. Five drops of blood are placed onto each of two Whatman 903 Proteinsaver cards (GE Healthcare Bio-Sciences). After collection, the finger will be cleaned (with an alcohol swab), dried with gauze, and bandaged. Blood spot samples will be dried overnight, then closed, labeled with ID number and date, and stored in a small plastic bag with a desiccant pack. Bags are stored in -80 Celsius freezer until shipped for further processing. When shipped for further processing, the bags are placed in an insulated box with dry ice before being transported for analysis. Blood test results are not diagnostic and will not be shared with study participants. Risks of the procedure include temporary soreness at the site of the finger prick, or in very rare circumstances, infection. To minimize the risk of infection, we will follow sterile procedures, including the use of sterile, one-time-use lancets, sterile gauze/bandage, and alcohol wipes of the site. Blood will be collected in the Health Psychology Lab in Franz or in the SAND lab in Franz, which has a drape to maintain cleanliness of the collection area, a sharps container, as well as antibacterial cleaning materials to maintain cleanliness of the collection room. Experimenters performing the needle prick will wear gloves throughout the procedure and wear covered clothing as a safety precaution. The non-dominant hand will be used and bandaged to avoid soreness from overuse at the site of the finger prick. If there is no blood, or not sufficient blood on the first finger prick, a second prick will be attempted on a different finger to avoid unnecessary discomfort. Experimenters performing the finger prick will be trained and certified in dried blood spot collection.

2.3.7.4 Stool sample

A stool sample will be collected from the child and adolescent participants in their home with the assistance of their parent using omnigene gut tubes (dnagenotek). Using a regular toilet, participants use the paper toilet hat to catch the stool. Participants will use a small sterile spatula to collect a pea sized amount of stool from the toilet hat and place it in the tube. After the lid is sealed, with the sample inside, the participant will shake the tube. A homogenization bead inside will break up the sample and cover all of the sample with a stabilizing liquid. The participant will place the stool sample into a biohazard bag, into a padded mailer, which will be returned to the lab through the registered post. The stabilization technique we use allows the stool samples to remain at ambient temperature for several weeks. Once the sample arrives at the lab, it will be placed into the locked -80 Celcius freezer until processing. Once all samples are collected we will pack the samples in dry ice and send to

the processing facility. The samples will be labelled with participant ID codes only – no personally identifying information. Only microbial DNA, not human DNA, is analyzed in these samples.

2.3.8 Questionnaires

2.3.8.1 Child

Title	Description	Reference	Respondent	Wave	Version
Alexithymia	Child/adolescent(Rieffe, self-report. Domain assessed: mental health/affective function. This questionnaire asks youth to endorse a number of items falling within three factors (1) Difficulty identifying feelings, (2) difficulty describing feelings, (3) externally oriented thinking.	Rieffe, Oosterveld, & Terwogt, 2006)	Wave 1, Wave 1 Online		

Title	Description	Reference	Respondent	Wave	Version
Children's Perception of Interparental Conflict Scale (cpic)– 6-18 years	Child/adolescent(ABC, self-report. Domains assessed: parenting and family structure. The CPIC assesses children's/adolescent's experience of parental conflict, including subscales (Conflict Properties, Threat, Self-Blame).	XXXX)	Wave 1, Wave 1 Online		
Child Somatization Symptom Inventory (cssi)– 6-17 years	Child/adolescent(ABC, self-report. Parent report for children under 8 years. Domain assessed: physical symptoms. The CSSI assesses a variety of nonspecific somatic symptoms.	XXXX)	Wave 1, Wave 1 Online		

Title	Description	Reference	Respondent	Wave	Version
Security Scale (ss) – 8-18 years	Child/adolescent(ABC, self-report. XXXX) Domain assessed: attachment. This measure asks chil- dren/adolescents to endorse statements about their feelings towards their parents (in the positive or negative) and how much each endorsed statement is characteristic of them. Statements assess domains of being able to rely on parents in times of need, feelings of closeness with parent etc.		Wave 1, Wave 1 Online		

Title	Description	Reference	Respondent	Wave	Version
Benevolent Childhood Experiences Scale-Revised (bce)	Child/adolescent(ABC, self-report. Domain assessed: benevolent childhood experiences. This child/adolescent self-report questionnaire consists of 10 items used to identify favorable childhood experiences, with regards to potential child adversity.	XXXX	Wave 1 Online		

Notes:

Attention checks were embedded in several child questionnaires beginning with MBB online.

- attention_check_1 (ss)
- attention_check_2 (cpic)
- attention_check_3 (alexithymia)
- attention_check_4 (bce)
- attention_check_5 (cssi)

2.3.8.2 Parent

2.3.8.2.1 Parent Self

Title	Description	Reference	Respondent	Wave	Version
Beck Depression Inventory – II (bdi_ii)	Mental health/affective functioning. Developed for the assessment of symptoms corresponding to criteria for diagnosing depressive disorders listed in the DSM IV.	(ABC, XXXX)	Parents (self report)	Wave 1, Wave 1 Online	
COVID-19 Objective Questionnaire (covid_objective)	COVID-19 objective measures. This questionnaire consists of 12 items to identify health changes and lifestyle changes made From the impacts of the COVID-19 outbreak.	(ABC, XXXX)	Parents (self report)	Wave 1	Online

2.3.8.2.2 Parent Proxy

Title	Description	Reference	Respondent	Wave	Version
Demographic Questionnaire	Demographics. The project developed questionnaire asks parents about their household income, their own and their child's/adolescent's race/ethnicity, the parent age, education, and marital status, and contact details.	(ABC, XXXX)	Parents (proxy)	Wave 1, Wave 1	Online
Pediatric Quality of Life - Gastrointestinal (pedsql_gi)	Physical symptoms. The PedsQL Gastrointestinal Symptoms Scale will be administered. These questionnaires are designed to assess the incidence of gastrointestinal symptoms in youth.	(ABC, XXXX)	Parents (proxy)	Wave 1, Wave 1	Online

Title	Description	Reference	Respondent	Wave	Version
Pediatric Quality of Life - Well Being (pedsql_wb)	Physical symptoms. The PedsQL General Wellbeing Scale will be administered. These questionnaires are designed to assess general feelings of wellbeing in youth.	(ABC, XXXX)	Parents (proxy)	Wave 1, Wave 1	Online
Pediatric Quality of Life (pedsql_f)	Physical symptoms. The PedsQL Multidimensional Fatigue Scale will be administered. These questionnaires are designed to assess the incidence of fatigue symptoms in youth.	(ABC, XXXX)	Parents (proxy)	Wave 1, Wave 1	Online
Revised Evaluation of Activity Survey in Youth (easy)	Physical symptoms. The EASY asks parents to rate how physically active their child/adolescent has been during COVID-19.	(ABC, XXXX)	Parents (proxy)	Wave 1, Wave 1	Online

Title	Description	Reference	Respondent	Wave	Version
Revised Traumatic Events Screening Inventory (tesi)	Caregiving adversity. The TESI-C assesses a child's/adolescent's experience of a variety of potential traumatic events including physi- cal/sexual abuse and neglect.	(ABC, XXXX)	Parents (proxy)	Wave 1, Wave 1	Online

Title	Description	Reference	Respondent	Wave	Version
Child Behavior Checklist (cbcl)	Mental health/affective function. Assesses behavioral competency and behavioral problems in children and adolescents within the past six months. The following syndrome scales are assessed: anxious/depressed, withdrawn/depressed, somatic complains, social problems, thought problems, rule-breaking behavior, and aggressive behavior.	(ABC, XXXX)	Parents (proxy)	Wave 1, Wave 1	Online
Child Sleep Habits Questionnaire (csqh)	Sleep. Multi-dimensional sleep assessment including sleeping difficulties, behavioral problems around sleep etc.	(ABC, XXXX)	Parents (proxy)	Wave 1, Wave 1	Online

Title	Description	Reference	Respondent	Wave	Version
Microbiome metadata questionnaire (mb_metadata)	Microbiome metadata. This study developed questionnaire asks parents to report on a number of variables known to influence the microbiome, including whether their child/adolescent was born prematurely, mode of birth, pre- or post-natal antibiotic usage, pets in the home, country of birth, breast or bottle feeding, special diets (e.g., vegetarianism or dairy free).	(ABC, XXXX)	Parents (proxy)	Wave 1, Wave 1	Online

Title	Description	Reference	Respondent	Wave	Version
Medication Checklist (med_check)	Parents are asked to list all medications that their children/adolescents are on. This information is used as a covariate in analyses of brain, microbiome, and biomarker data, as different medications can affect the readouts from these assays/analyses.	(ABC, XXXX)	Parents (proxy)	Wave 1, Wave 1 Online	

Title	Description	Reference	Respondent	Wave	Version
Petersen Physical Development Scales (pds)	The Peterson Physical Development Scales (pds) is an adaptation of an interview-based puberty-rating scale by Petersen, and includes scores for each of five items rating physical development, an overall maturation measure, and a categorical maturation score. It is designed to be non-invasive, not requiring the use of pictures.	(ABC, XXXX)	Parents (proxy)	Wave 1, Wave 1 Online	Male and female
Digestive Health and Wellbeing Survey (dhws)	This survey asks parents to endorse whether a doctor has ever diagnosed their child/adolescent with a range of allergic and autoimmune conditions.	(ABC, XXXX)	Parents (proxy)	Wave 1, Wave 1 Online	

Title	Description	Reference	Respondent	Wave	Version
Hair-Care Practice Questionnaire (hpq).	This questionnaire consists of 9 items for parents to indicate their child/adolescent's hair care practices for information relevant to the hair sample.	(ABC, XXXX)	Parents (proxy)[for Wave 1 children under 10, for Wave 1 online all children]	Wave 1, Wave 1	Online
Child Somatization Symptom Inventory (cssi)	The CSSI assesses a variety of nonspecific somatic symptoms.	(ABC, XXXX)	Parents (proxy)[children< 8 years]	Wave 1	Online
Foster Care Inventory (fcii)	This instrument was made for the current study and assesses the number of foster care placements and care history.	(ABC, XXXX)	Parents (proxy)[adopted, only]	Wave 1	Online
International Adoption Inventory (iai)	This instrument was made for the study and asks parents of internationally adopted youth about the quality of institution and details of the adoption process.	(ABC, XXXX)	Parents (proxy)[adopted only]		

Title	Description	Reference	Respondent	Wave	Version
Financial Support Questionnaire (financial)	The project developed financial support questionnaire assesses public assistance received, and health insurance information. All questions on this assessment (as with all assessments) will have an option not to disclose this information.	(ABC, XXXX)	Parents (proxy)	Wave 1, Wave 1 Online	

Title	Description	Reference	Respondent	Wave	Version
Bristol Stool Scale (bss)	<p>Stool sample short questionnaire:</p> <p>After collecting the stool sample, participants will be asked to indicate on a short questionnaire whether they were feeling ill on the day the sample was collected, what time the sample was collected, and consistency of stool. They will also be asked if their diet on the day of sample collection was typical.</p>	(ABC, XXXX)		Parent (proxy)	Wave 1, Wave 1 Online
COVID-19 Objective Questionnaire (covid_objective)	<p>COVID-19 objective measures.</p> <p>This questionnaire consists of 12 items to identify health changes and lifestyle changes made From the impacts of the COVID-19 outbreak.</p>	(ABC, XXXX)	Parents (proxy)		

Title	Description	Reference	Respondent	Wave	Version
Parenting Stress (parenting_stress)	Designed to evaluate the magnitude of stress in the parent-child system, focusing on three major domains of stress: 1) child/adolescent characteristics, 2) parent characteristics, and 3) situational/demographic life stress. Revised questionnaire focuses on stress in the context of COVID-19.	(ABC, XXXX)	Parents (proxy)	Wave 1 online	

2.3.8.3 Qualitative

2.3.8.3.1 COVID-19 Written Responses

Title	Description	Reference	Respondent	Wave	Version
COVID-19 Written Response (written_responseimpactsself)	Domain assessed: Emotional COVID-19. This self-report measure consists of one long-form qualitative response, prompting a parent to write continuously for five minutes about the impacts of COVID-19 on their life and family. This qualitative response was adapted from previous prompts in writing about emotional experiences, and seeks to assess the emotional and behavioral impacts of the Pandemic on children and families.	(ABC, XXXX)	Parents (self report)	Wave 1 On-line [optional]	

Title	Description	Reference	Respondent	Wave	Version
COVID-19 Written Response (written_response)	Domain assessed: Emotional impacts of COVID-19. This self-report measure consists of one long-form qualitative response, prompting a child to write continuously for five minutes about the impacts of COVID-19 on their life and family (for children who cannot write or do not feel comfortable writing, children can dictate and parent can write). This qualitative response was adapted from previous prompts in writing about emotional experiences, and seeks to assess the emotional and behavioral impacts of the Pandemic on children and families.	(ABC, XXXX)	Children	Wave 1 On-line	[optional]

Title	Description	Reference	Respondent	Wave	Version
-------	-------------	-----------	------------	------	---------

Chapter 3

Wave 1

3.1 Checklists

3.1.1 Checklist - Initial

Scheduling and Confirmation

- Schedule lab session
- Send confirmation email (in templates)
 - Attach Next Steps

Enrollment

- Create participant Box folder using MBB_template (delete blank README from newly created folder)
- Enroll participant in Wave 1 on REDCap
- Fill participant instrument on REDCap
- Fill counterbalance order on REDCap (Checklist - Lab Session Child Instrument)

Calendar

- Create MBB calendar event *Lab Session* and invite researchers
- Create DBS calendar event *DBS Session* (SAND calendar or HPL calendar)
- Create MBB calendar event *Lab Reminder 1* (email) (1 week prior)
- Create MBB calendar event *Lab Reminder 2* (email and call) (3 days prior)
- Create MBB calendar event *Home Reminder 1* (email) (1 week after lab session)
- Create MBB calendar event *Home Reminder 1* (call) (8 days after lab session)

- Create MBB calendar event *Home Reminder 2* (email) (10 days after lab session)
- Create MBB calendar event *Home Reminder 3* (email) (14 days after lab session)

Reminders

- Send *Lab Reminder 1* email (in templates - attach next steps, consent/assent)
 - Send *Lab Reminder 2* email (in templates - attach previous and parking info)
 - Confirm participant
 - Preferably by phone
 - Update *Lab Session* calendar status
-

3.1.2 Checklist - Pre-Lab-Session

3.1.2.1 Checklist - Lab-Session Setup - 1 Day Prior

- Create participant manila folder
- Print assent/consent forms (Check IRB expiration)
 - Parent consent
 - Assent - Child or Teen (None if under 7 years)
 - Referral consent
 - Contact list
 - DBS consent
- Print MBB Lab-Session Checklist-Child (Enter counterbalance order)
- Print MBB Lab-Session Checklist-Parent
- Print KSADS Summary Diagnostic Checklists (Write participant ID on all pages)
- Print and prepare WASI Form (Enter starting point; write participant ID on all pages)
- Print and prepare WIAT Form & Booklet (Enter starting point; Write participant ID on all pages)
- Print Memory Intrusion Scratch Paper
- Print and insert Bristol Stool Scale (MBB Specific Version)
- Print and fill in codes on Participant Info Brochure
- File participant manila folder in front section of file cabinet (Upcoming)
- Charge
 - iPads
 - iPad pencils
 - Biopac transmitters
 - VR headset (Check remote battery)
 - Audio recorders
- Make sure audio recorder batteries have enough charge
- Label electrodes with color stickers

- Blue=EGG
 - Yellow=ECG
 - Make participant name tags
 - Print Payment Receipt Template
 - Assemble home kit (white paper gift bag with BABLab sticker)
 - Insert brochure
 - Insert gut kit
 - Insert toilet hat
 - Insert oral kit
 - Insert biohazard bag
 - Insert Bristol Stool Scale
 - Label all items with participant ID (in sharpie)
 - Insert MBB info cards (adopted and bio)
 - Attach FedEx slip to mailer
 - Label padded mailer with “Exempt human specimen” (in sharpie)
-

3.1.2.2 Checklist - Lab-Session Setup - 1 Hour Prior

- Place in Rainbow Room
 - Consent/assent/DBS/contact on clipboard with pens
 - Consent protocol
 - Pleasant Events Checklist and Issues Checklist
 - Place WASI & books (2)/WIAT & card/protocol in testing room
 - Place audio recorders in testing rooms
 - Attach researcher documents to clipboards
 - Child - Checklist, Memory Intrusion notes
 - Parent - Checklist, KSADS summary
 - Turn iPads on airplane mode and WiFi off
 - Clear and setup KSADS on iPad (duplicate blanks)
 - Photograph FedEx slip
 - Pre-load questionnaires on computers
 - (Parent and Child; under 8-laminated faces)
 - Pre-load physiology data templates (8)
 - Move physiology station near Rainbow Room
 - Move iPad and iPad stand near Rainbow Room
 - Insert Participant Info Brochure in home kit
 - Assemble hair sample materials
 - Prep blood spot kit
-

3.1.3 Checklist - Lab-Session

3.1.3.1 Child

- Assent

- Physiology setup
- Parent-child observation (video record)
- Drink bottle of water
- Memory intrusion (audio record)
- Halloween training
- Characters (monsters/aliens)
- Halloween test
- Discrimination (run 1 of 3) *no physio
- Conditioning (sound)
- Discrimination (run 2 of 3) *no physio
- Height
- Hair sample
- Weight
- Saliva sample
- Memory generalization training (audio record)
- Extinction
- Discrimination (run 3 of 3) *no physio
- Memory generalization test
- Waist circumference
- Snack and water break
- WASI (audio record)
- WIAT (audio record)
- Blood sample
- Questionnaires
- Prize

3.1.3.2 Parent

- Consent
 - Parent-child observation (video record)
 - KSADS (audio record)
 - Transfer observation video/KSADS audio recording
 - Questionnaires
 - Parent Proxy and Parent Self
 - Home kit issues and explained
 - Take photo of Fedex label
 - Payment issued and signed
 - Take photo of receipt
-

3.1.4 Checklist - Post-Lab-Session

3.1.4.1 Clean Up

- Tidy lab
- Disinfectant spray

- Disinfectant wipe

3.1.4.2 Notes

- Make note in Boxnote (core meeting) of issues to discuss (if needed)

3.1.4.3 Sample Storage

- Label and leave blood sample to dry
- Store blood sample
- Label and store hair sample
- Label and store saliva sample
- Create and assign Trello reminder to store blood sample
- Update sample storage log on Box (after lab session)

3.1.4.4 Filing

- File consent and assent forms in filing cabinet (consent manila folder)
- File contact list in filing cabinet (contact list manila folder)
- Log participant payment in reimbursement log book
- File payment receipt photo in Box payment folder
- File FedEx tracking photo in Box folder

3.1.4.5 Data Entry

- Transfer and rename video recordings to external hard drive (delete originals)
 - Transfer and rename audio recordings to external hard drive (delete originals)
 - Copy behavioral task data to participant folder (raw)
 - Copy physiology task data to participant folder
 - Save and upload KSADS screen from iPad to participant Box folder
 - Save and upload any KSADS supplements from iPad to participant Box folder
-

3.1.5 Checklist - Final

3.1.5.1 Scoring

- Fill out KSADS Summary Diagnostic Checklists
- Score WASI
- Score WIAT

3.1.5.2 Filing

- Scan DBS consent and file in participant Box folder

- Scan Memory Intrusion Notes and file in participant Box folder
- Scan KSADS Summary Diagnostic Checklist and file in participant Box folder
- Scan lab session checklists (parent & child) and file in participant Box folder
- Scan WASI/WIAT (once scored) and file in participant Box folder
- Make low-res parent-child interaction video and save on BABLab Drive & Box (under secondary ID)
- Copy audio files to Box (under secondary ID)
- Burn all audio and video (low res) files to CD and label/store CD in binder
- Check video transfer and delete original
- Check audio transfers and delete originals

3.1.5.3 Data Entry - Lab-Session

- Enter contact list information into recruitment database
- Enter KSADS Summary Diagnostic Checklist data to REDCap
- Enter height, weight, waist to REDCap
- Score and enter WASI data to REDCap
- Score and enter WIAT data to REDCap
- Enter Memory Intrusion Notes to REDCap
- Enter lab session checklist - Child data to REDCap
- Enter lab session checklist - Parent data to REDCap

3.1.5.4 Reminders

- *Home Reminder 1* email sent
- *Home Reminder 1* phone call made
- *Home Reminder 2* email sent
- *Home Reminder 3* email sent

3.1.5.5 Home Session

- Halloween test delay
- Memory Generalization test delay
- Stool kit received
- Bristol Stool Scale data received
- ASA

3.1.5.6 Data Entry - Home-Session

- Enter home session checklist child data to REDCap
- Download and upload ASA data to participant Box folder
- Scan and upload Bristol Stool Scale to Box
- Enter Bristol Stool Scale data to REDCap

3.1.5.7 Sample Storage

- Label and store stool sample (add data quality to REDCap)
- Update sample storage log on Box (once all received)
- Upload sample photo to Box

3.1.5.8 Reimbursement

- Prep report card
- Send thank you email (in templates)
 - Attach letter, certificate, and report card
- Mail gift card
 - Include thank you letter, certificates, and any additional stool kits if needed

3.1.5.9 Data Quality

- Data quality check 1
- Data quality check 2
- Data audit

3.1.5.10 Retention

- Update participant Wave 2 status
-

3.2 Protocols - Pre-Session

3.2.1 Protocol - Recruitment

3.2.1.1 Pre-Screening

1. Check if participant is in Recruitment Database
 - If not, add them to the Recruitment Database
2. Check if participant is in ID Drive
 - If yes, check if they have a Screener ID
 - If not, assign them a Screener ID once contact has been established based on the next available Screener ID # in REDCap and proceed with screening
 - If yes, proceed with screening under existing Screener ID in REDCap

3.2.1.2 Screening

1. To screen a new participant click “Add / Edit Records”
2. Click to enter a new Subject ID
 - Make sure Arm 1: Recruitment is selected
3. Type “SMBB#” (Screener ID) to create a record and hit “Enter”
 - Make sure to link the participants Screener ID and their name on the **ID Drive ONLY**
 - Before creating a new record, be sure to check the ID Drive to see if the participant already has an existing Screener ID
 - If a record exists, add a new instance of the screen instead of creating

The screenshot shows the REDCap project home interface. At the top, it displays the user is logged in as emilytowner@ucla.edu. Below this, there are sections for 'My Projects' (Project Home or Project Setup), 'REDCap Messenger' (Project status: Development), and 'Data Collection' (Survey Distribution Tools, Scheduling, Record Status Dashboard, Add / Edit Records). On the right, there are several panels:

- Mind, Brain, Body**: A panel for managing project settings like survey use and longitudinal data collection.
- Project status**: Shows development status with 'Not started' and 'I'm done!' buttons.
- Main project settings**: Buttons for disabling survey use or longitudinal data collection.
- Design your data collection instruments**: A panel for managing fields and using the Online Designer.
- Define your events and designate**: A panel for creating events and using the Online Designer.
- Enable optional modules and customize**: A panel for enabling optional modules like Repeatable Instruments, Auto-numbering, Scheduling module, Randomization module, and Designate an email field.
- Set up project bookmarks (optional)**: A panel for managing project bookmarks.

Mind, Brain, Body

Add / Edit Records

You may view an existing record/response by selecting it from the drop-down lists below. To create a new record/response, new value in the text box below and hit Tab or Enter. To quickly find a record without using the drop-downs, the text box will populate with existing record names as you begin to type in it, allowing you to select it.

NOTICE: This project is currently in Development status. Real data should NOT be entered until the project has been moved to Production status.

Total records: 24	
Choose an existing Subject ID	Arm 1: screening <input type="button" value="-- select record --"/>
Enter a new or existing Subject ID	Arm 1: screening <input type="text"/>

Data Search

Choose a field to search (excludes multiple choice fields)	All fields <input type="button" value=""/>
Search query Begin typing to search the project data, then click on an item in the list to navigate to that record.	<input type="text"/>

a new record

4. The screening arm contains two parts
 - The screen
 - The wave1_status
 - The wave1_status is to be updated after the first and each subsequent contact

Record Home Page

Record "PP6" is a new Subject ID. To create the record and begin entering data for it, click any gray status icon below.

The grid below displays the form-by-form progress of data entered for the currently selected record. You may click on the colored status icons to access that form/event. If you wish, you may modify the events below by navigating to the [Define My Events](#) page.

Legend for status icons:	
● Incomplete	○ Incomplete (no data saved) ?
○ Unverified	○ Partial Survey Response
● Complete	✓ Completed Survey Response
● Many statuses (mixed)	● ○ ● Many statuses (all same)

NEW Subject ID PP6
Arm 1: screening

Data Collection Instrument	screener
screen	○ <input type="radio"/>
screen_status	○ <input type="radio"/>

quent contact

5. Click on the radio button in the “screen” row to screen the participant

The screenshot shows a REDCap form titled "screen". At the top, it says "Adding new Subject ID PP6" and "Event Name: screener (Arm 1: screening)". The "Subject ID" field is populated with "PP6". The "Date and time of screening" field has a date picker set to "Now". Below this, there's a section for "Starting the phone call" with three radio button options: "Answering a call" (selected), "Returning a call", and "Leaving a message". A message to the researcher is displayed: "Hello, this is [researcher name] calling from the Brain and Body Lab at UCLA. Can I speak to [name of potential participant]?". Below this, there are two radio button options: "Yes" and "No". The "Notes" section is empty. In the "Form Status" section, there's a "Complete?" field with a radio button set to "Incomplete". A "Lock" option is available with a checked checkbox. At the bottom, there are three buttons: "Save & Exit Form" (blue), "Save & Stay" (grey), and a "Cancel" button.

6. Click “Now” to enter today’s date and time
7. Select the appropriate choice to start the phone call and follow the skip logic
8. Follow the skip logic to the end
 - For items without a text field, write the information down in the Recruitment database (This identifying information cannot be on REDCap)
9. Once done, select “Complete” and “Save & Exit Form”
 - The screen can be entered multiple times - for instance if there are multiple phone calls or contacts
 - It is important to keep a record of all instances of contact

Record Home Page

The grid below displays the form-by-form progress of data entered for the currently selected record. You may click on the colored status icons to access that form/event. If you wish, you may modify the events below by navigating to the [Define My Events](#) page.

Choose action for record ▾

Subject ID PP5
Arm 1: screening

Data Collection Instrument	screener
screen	
screen_status	←

Repeating Instruments

screen	screener (Arm 1: screening)
1	
+ Add new	

screen_status

Editing existing Subject ID PP5

Event Name: **screener (Arm 1: screening)**

Subject ID	PP5
Status	✓
Form Status	Enrolled
Complete?	Contact
Lock this record for this form? <small>If locked, no user will be able to edit this record on this form until someone with Lock/Unlock privileges unlocks it.</small>	
<input type="checkbox"/> Lock	
Save & Exit Form Save & Stay ▾	
-- Cancel --	

10. Click the screen_status radio button
11. Select the appropriate option
 - Contact - Participant needs to be re-contacted (add Recruitment Database & ID Drive)
 - Ineligible - Participant not eligible for study
 - To Enroll - Participant to enroll (need to create subject ID, enter subject info, schedule participant, add to Recruitment Database, add to ID Drive)
 - Enrolled - Participant has been enrolled (all above have been completed)
 - To Remove - Participant wants to be removed
12. Be sure to update the screen status after each contact
 - After 3 contacts (with no response) - review (time of day, contact method, etc.)
13. If enrolled, proceed to pre-session checklist in the participant log

3.2.1.3 Other Screening Information

Accessing Lists

To find out where participants are in the recruitment process, there are several lists.

The screenshot shows the REDCap dashboard with the following details:

- Left Sidebar:** Shows 'My Projects' (Project Home, Project Setup, REDCap Messenger), 'Data Collection' (Survey Distribution Tools, Scheduling, Record Status Dashboard, Add / Edit Records), and 'Log out'.
- Project Status:** Development.
- Current View:** Recruitment - To Contact.
- Displaying:** Instrument status only | Lock status only | All status types.
- Table Headers:** Subject ID, screener, screen, screen status.
- Message:** No records were returned.

1. Click on “Record Status Dashboard”
2. Participants who have been enrolled will be listed in the Enrollment - Wave 1 list
3. Participants in the process of recruitment will be listed in one of the 4 Recruitment lists for the appropriate wave - *These lists are populated based on the individuals “Screen Status” so be sure to update after each contact!

List Types

- Contact - List of individuals who need to be contacted or re-contacted (also includes waitlist)
- Ineligible - Participants are ineligible but interested
- To Enroll - Participants who have been screened and are eligible to enroll
- To Remove - Participants who were not interested in being contacted for this or future research

3.2.1.4 Addressing Concerns

If a parent has a concern about the study before the session, send the email template:

- [MBB - CONCERNS]
-

3.2.2 Protocol - Calendar

- Lab session events format
 - W1 MBBXXX - Lab Session
 - * MBBXXX - Sex, Age #, Group
 - * Status: Scheduled / Confirmed / Completed
 - * Arrival: X AM
- Lab session reminders format
 - W1 MBBXXX - Lab Reminder 1 (email)

- * Status: Incomplete / Complete
 - W1 MBBXXX - Lab Reminder 2 (Email & Call)
 - * Status: Incomplete / Complete
 - Home session reminders format
 - W1 MBBXXX - Home Reminder 1 (Call)
 - * Status: Incomplete / Complete
 - W1 MBBXXX - Home Reminder 1 (Email)
 - * Status: Incomplete / Complete
 - W1 MBBXXX - Home Reminder 2 (Email)
 - * Status: Incomplete / Complete
 - W1 MBBXXX - Home Reminder 3 (Email)
 - * Status: Incomplete / Complete
-

3.2.3 Protocol - Home Kit Assembly

Please refer to the diagram below for the complete list of items in a home kit:



Figure 3.1:

3.3 Protocols - Parent

3.3.1 Protocol - Consent & Assent

Once the parent and child/teen come into the lab, seat them in the Rainbow Room on the couch for consenting (parent) and assenting (child aged 7+ or teen).

Make some small talk - Ask the participant how they got here. If they have participated before in research. Offer them a bottle of water. Thank them for coming and for giving up their weekend to help science.

Tell the parent and child that the first thing you are going to do is go over all of the things they will do today, and have them sign the consent and assent forms.

Speak to them and direct them through the whole process.

3.3.1.1 Things you will do in the lab

- Stick stickers on you to measure heart rate, sweat, stomach muscles.
- Sit with parent and talk about fun things and hard things (filming).
- Parent stays in room and answers more questions.
- Child goes next door to play computer games (look at pictures, watch movies). Some of the movies and pictures will be a little bit scary, others sad, others boring.
- One of the games involves a loud annoying noise, we will adjust it for you.
- You will also do some other games on paper and pencil - like puzzle and word games
- You will answer some questionnaires
- We will also measure your height, weight, and waist circumference.
- We will take three biological samples:
 - Hair - stress hormones
 - Saliva - microbiome
 - Blood - immune - wear goggles
- Do you get sick or dizzy when you see blood or hurt yourself?
- If we need to, can we prick two fingers?
- When you are done with all of that, you will get a big prize, then we will pay you and you will go home.
- You will get \$45 for the work you put in today.

3.3.1.2 Things you will do at home

Child

- Poop sample - microbiome
- Stool scale
- Memory game - to see what you remember from lab.

Parent

- 24 hour food recall

When you complete the poop sample and the games at home, we will pay you another \$20 in the form of a giftcard.

3.3.1.3 Things to know

You are a volunteer, which means that you do not have to do anything, or say anything that makes you uncomfortable. We would like you to try everything you can, and to do your best, but if there are things you absolutely do not want to do, just tell us, that is o.k.

We keep your participation confidential - ID number.

We want you to come in again in the future, so we will ask for some information so we can contact you in the future.

Sign consent/assent forms including DBS form and Contact Sheet

3.3.2 Protocol - Parent/Child Observation

The parent and child will be in the Rainbow Room for 15 minutes. During that time they will be filmed while planning a conflict event, and then again while discussing a pleasant event. The conflict event will always go first, followed by the pleasant event. We did this to ensure that the parents were not thinking of the negative interaction upon answering the questionnaires about their child, which they did immediately after the observation interaction. If participants indicate that they prefer to speak in a language other than English, they may do so.

Step 1:

Parent and child will be situated on the grey couch in the Rainbow Room. The iPad video camera will be placed about 4 feet away from the dyad, on a tripod stand. The screen of the iPad will be facing away from the parent and child.

Step 2:

The researcher will give the parent and child the Pleasant Events Checklist (PEC) on a piece of paper.

Researcher: Next we are going to take some film of you while you discuss a source of conflict (or something you disagree on) and try to resolve it. On this piece of paper is a list of things that parents and children sometimes have disagreements about. Please take a moment to read the list and think about some that you would like to discuss together. When I knock on the door, please start discussing the things you have selected from the list and try to resolve the areas of conflict you have chosen from the list. You do not need to tell us what you chose to discuss, and it does not matter if you chose something from the



Figure 3.2:

list, or decide to choose something else not included on the list. I will give you five minutes to discuss the event, then I will come back and give you further instructions.

Step 3:

Researcher press record on the iPad and leave the room. Start timer for 1 minute, then knock on the door and ask the dyad to begin discussing their event. Start timer for 5 minutes. At the end of 5 minutes, again knock on the door and enter the Rainbow Room.

Researcher: Thank you for taking the time to discuss the source of conflict and try to resolve it. Next we are going to take some film of you while you discuss a pleasant event you could do together. On this piece of paper is a list of events that parents and children sometimes find pleasant to do together. Please take a moment to read the list of events and think about what you would like to plan to do together. When I knock on the door, please start discussing the event you would like to do together and make a plan for how you could do it. You do not need to tell us what you chose to discuss, and it does not matter if you chose something from the list, or decide to choose something else not included on the list. I will give you five minutes to discuss the event, then I will come back and give you further instructions.

Step 4:

Researcher press record on the iPad and leave the room. Start timer for 1 minute, then knock on the door and ask the dyad to begin discussing their event. Start timer for 5 minutes. At the end of 5 minutes, again knock on the door and enter the Rainbow Room.

Step 5:

Researcher reenter the room, switch the iPad off and move the child/adolescent to their next session.

3.3.3 Protocol - KSADS

3.3.3.1 Audio Recording

- Make a separate recording for each KSADS administered if there is more than one child in one session.

Step-by-step guide on how to use recorder:

Step 1: Press and hold highlighted Power button to turn recorder.

Step 2: Press highlighted button until “TALK” appears on the screen. Now you are on the “Talk” setting.



Figure 3.3:



Figure 3.4:

Step 3: Push highlighted button up to start recording. Push down to stop recording.



Figure 3.5:

3.3.3.2 Using the iPad for KSADS Summary Checklist

- Before the start of every session, be sure to duplicate and rename all the KSADS documents in Acrobat (25 documents per participant) and rename them (MBBXXX_KSADS_suppX_XXX).
 - *Note 1:* This may take a while, especially if there are more than one participant, so be sure to do it ahead of time.
 - *Note 2:* With multiple participants in one session, keep them all on the same iPad as the same iPad will be used to administer all KSADS.
- Follow instructions below on how to duplicate and rename the documents:
 - Turn on iPad and go to the “Acrobat” app.
 - Your screen should look like this. If it does not, tap on “Files” at the bottom, and ensure that the Locations is set to “On This iPad”.



- For each document, tap the three horizontal dot to the right, and se-



lect “Duplicate”.

- The duplicated document should appear right below the original document.
- Tape the three horizontal dot to the right of the duplicated document,



and select “Rename”.

- Replace the word “Blank” with the participant ID, and remove the “(1) at the end of the name. For example, if the participant ID is MBB001, the name of the duplicated document should be: MBB001_KSADS_screen
- Do the same for all 25 documents (Duplicate, Rename).

3.3.4 Protocol - Questionnaires

3.3.4.1 Parent Proxy

Parents will complete REDCap questionnaires for each child.

3.3.4.2 Parent Self

Parents will complete REDCap questionnaires about themselves only once (under eldest child's [lower number] ID)

3.3.5 Protocol - Home Kit

Explain to the parent what is included in the home kit / home session and how to collect the stool sample.

3.4 Protocols - Child/Teen

3.4.1 Protocol - BioPac Electrode Hookup

3.4.1.1 Electrode Placement/Preparation

- Wheel the cart into the Rainbow Room
- Prep 2 electrodes with Gel 101. Stick to the participant's ring and middle fingers on their non-dominant hand (we want to keep the pointer finger free so they can use it for tasks)
- Wrap medical tape around these to secure them, but ensure that the metal poles are still accessible
- Look at the skeleton diagram and use the EL-PREP Gel to abrade the skin around the remaining electrode sites (below the collarbones, below the sternum, on the left lower ribs, and in the remaining two positions on the stomach and left ribs)
- Clean the remaining EL-PREP off with a tissue or baby wipe
- Prep 8 electrodes with Gel 100. Stick to the locations indicated on the skeleton diagram
- Let all electrodes sit for the duration of the parent/child observation

3.4.1.2 GSR

- Put on gloves
- Ensure that the finger electrodes are properly adhered and have had time to rest
- Make sure the lead wire module is connected to the transmitter (PPGED, green sticker) in the "EDA" channel
- Take the transmitter and secure it around the participant's wrist as shown below
- Hook up the lead wires so that the black wire connects to the middle finger and the red wire connects to the ring finger



Figure 3.6:

- Ask the participant to wear a glove over the whole setup to secure it throughout the tasks
- To check if the GSR is working properly, ask the participant to briefly hold his/her breath - you should see a rise in the signal on the graph

3.4.1.3 ECG

- Ensure that the chest electrodes are properly adhered and have had time to rest.
- Make sure the lead wire module is connected to the transmitter (RSPEC-R, yellow sticker) in the ECG channel.
- Take the transmitter and secure it around the participant's stomach as shown below.
- Hook up the lead wires so that the white lead connects to the Right Collarbone electrode, the black lead connects to the Left Collarbone electrode, and the red lead connects to the lowest Left Rib electrode.

3.4.1.4 EGG

- Ensure that the chest electrodes are properly adhered and have had time to rest.
- Make sure the lead wires are connected to the transmitter (EGG2-R, blue sticker). The lead module labelled "A" (3 short leads) should be in the EGG A channel, while the lead module labelled "B" (2 long leads) should be in the EGG B channel.
- Take the transmitter and secure it around the participant's stomach as show below.
- Hook up the lead wires so that the "A" and "B" channel white leads connect to the sternum electrodes (which goes to which does not matter), the "A" and "B" channel red leads connects to the upper left rib electrode and stomach electrode, and the "B" channel black lead connects to the remaining lower left rib electrode.

3.4.1.5 AcqKnowledge

- Turn off the wifi on the Mac Mini and turn on the BioPac/transmitters
- Open AcqKnowledge
- Choose the graph template file
- Once it loads, make sure all of the transmitters are connected
- Press run and click through all of the dialog boxes that are generated

3.4.2 Protocol - Memory Intrusion/Movie Watching

- There are 4 counterbalanced versions of this task:
 - DRM_A_incongruent_first.psyexp

- DRM_A_congruent_first.psyexp
- DRM_B_congruent_first.psyexp
- DRM_B_incongruent_first.psyexp
- Choose the counterbalanced version that is correct for the participant
- First open the task, add the participant ID number and press start
- Read the instructions to the participant:

Welcome to the movie game. First you will watch some movies. Then you will hear a list of words. Try to remember the words on the list.

- Press the space bar to progress to the next screen and read the instructions:
Get ready, to watch the movie. Turn to the laptop to watch.
- Open up the first movie for the participant. It will depend on the counterbalancing condition what movie goes first.
- For the two files that start with “DRM_A”:
 - DRM_A_congruent_first.psyexp
 - DRM_A_incongruent_first.psyexp

The order will be: Sad → Neutral → Scary

- For the two files that start with “DRM_B”:
 - DRM_B_congruent_first.psyexp
 - DRM_B_incongruent_first.psyexp

The order will be: Scary → Neutral → Sad

- Simultaneously press play on the movie on the laptop while also pressing the spacebar on the psychopy task. This will start the physiology and the movie at about the same time. While the movie is playing there will be a box of popcorn on the computer screen.
- When the movie is done, press spacebar on the psychopy computer to progress to the next screen. Read the instructions for the participant:
- Click on the face that shows how you feel after watching the movies.
- Participants can use a mouse to click on the face that matches the way they feel after watching the movie. The faces range in valence (negative to positive) across the X axis, and arousal (low to high) up the Y axis.
- After the participant has selected a face, you will be taken to the next screen where you can read the instructions for the participants:

Listen to the list of words and try to remember all of them.

- When the participant indicates that they have understood the instructions, press the spacebar and progress to the next screen. Make sure that the computer volume is up and the participant can hear the words being

pronounced on the computer screen. After the participant hears all the words the distractor task will start.

- Read the distractor task instructions to the participant:

Count backwards from the number 25 out loud for the researcher.

Note: Whether the participant completes the distractor task correctly or not doesn't matter. The only purpose of the distractor task is to distract the participant for a brief period of time. Listen while they count backwards. If they can't count backwards, ask them to count forwards.

- When the participant is finished counting backwards from 25, or after approximately 30s has passed (whatever comes first), progress to the next screen by pressing the spacebar.

- Read the instructions out loud to the participant:

Recall 1. Please tell the researcher all the words that you can remember from the list.

- Before the participant starts to tell you what they remember, start a new voice recording and then tell them to start while you record what they say (on the talk setting). Also write what they say on a piece of paper. Make sure to note that this is 'Recall 1' (as there is a second recall later on). When the participant has told you all the words they can remember, or after 60s, progress to the next screen (instructions for the next word list).

- Make one recording for all of the memory intrusion task
- Read the instructions aloud for the participant.

Now you will hear another word list. Get ready!

- Press the spacebar to hear the word list. After the word list, move to the distractor task (counting backwards from 25). After the distractor task, move to the second recall task and record the participant like the first. When the second recall task is complete, a new loop will begin. Read the instructions to the participant then start the next movie (which will always be neutral). Repeat everything again for the final loop after they watch the final movie, which will either be sad or scary.

3.4.3 Protocol - Halloween

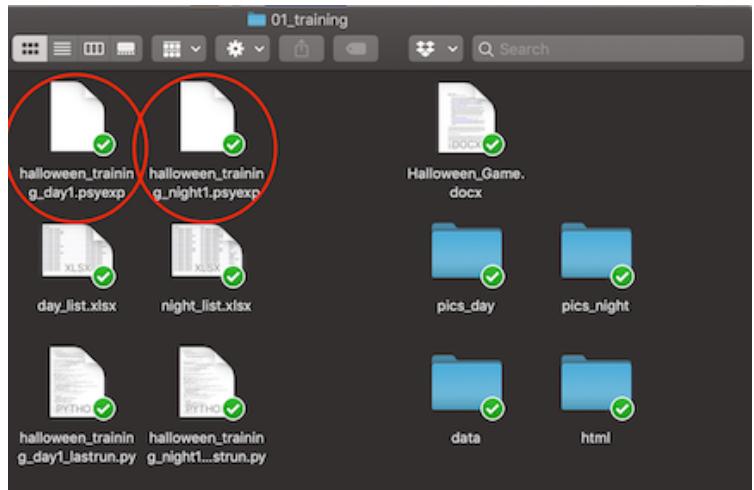
- Check master counterbalance sheet and fill out the participant's ID # in the group which you assigned them.
- Seat the participant at the computer desk in the Bear's Den. Close the door to ensure privacy and freedom from distractions.

- Set up the task:

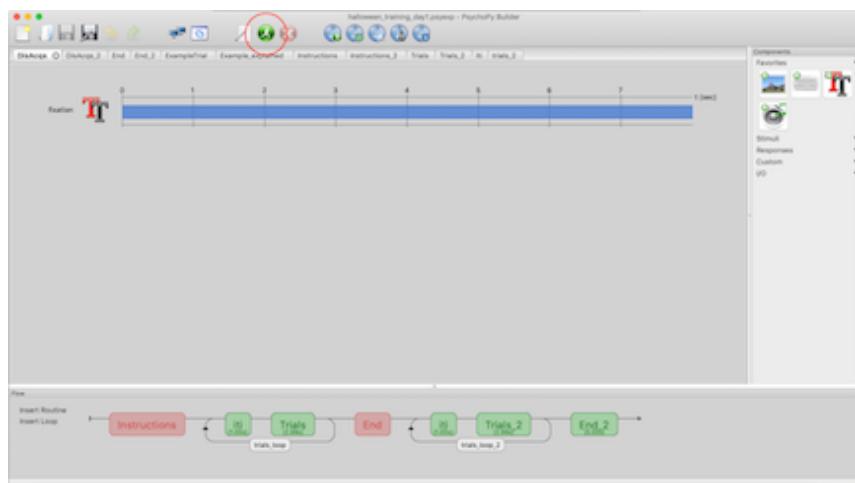
- a. Navigate through the Finder to get to the task following this path: Dropbox/BAB/Studies/Mind_Brain_Body/Tasks/Wave1/04_halloween_pilot



- b. Start by going into the folder titled “01_training” and selecting “Halloween_training_day1.psyexp” if the participant is in the “day first” group or “Halloween_training_night1.psyexp” if the participant is in the “night first” group.



- c. When the task pulls up and the participant is situated and ready, select the run button indicated below:



- When prompted, fill in the participant's ID # in the "participant" field:



- Guide the participant through the instructional slides by pressing the space bar every time [s] shows up on the screen. Make sure to remind the participant to read all of the instructions carefully.
- Once the training task starts, sit quietly and do not disturb the participant. It is important for them to pay their undivided attention to the images on screen during the training.
- When the break slide appears, ask them to let you know when they are ready to continue. Press the space bar to proceed on to the next set of images.
- Once the task is complete, you can exit out by pressing any key and then closing the the PsychoPy file.
- Prompt the testing phase of the exercise by saying something along the lines of: "*And now we want to see how much of your trick-or-treating adventure you remember.*"
- Go into the "02_test" file and select the "Halloween_day1.psyexp" file if the participant is in the "day first" group or "Halloween_night1.psyexp" if



the participant is in the “night first” group.

- When the task pulls up and the participant is situated and ready, select the run button indicated below:



- Guide the participant through the instructional slides by pressing the space bar every time [s] shows up on the screen. Make sure to remind the participant to read all of the instructions carefully. Remind them that they need to click the words for the answer they want to provide.
- When the break slide appears, ask them to let you know when they are ready to continue. Press the space bar to proceed on to the next tests.
- Guide the participant through the instructional slides by pressing the

space bar every time [s] shows up on the screen. Make sure to remind the participant to read all of the instructions carefully. Remind them that they need to click the item for the answer they want to provide, and then click which quadrant of the scene image they want to pick.

- Once the task is complete, you can exit out by pressing any key and then closing the PsychoPy file.
- Data is saved automatically in the data folder. You do not need to save anything before exiting out of the psychopy folder

Troubleshooting:

If the task exits due to an error, take a screenshot of the error screen and message Emily, Kristen, or Bridget for assistance. Move onto the next task in the meantime.

3.4.4 Protocol - Disc/Cond/Ext

3.4.4.1 Discrimination

- Click into the discrimination folder
- Right click on either discrimination_horiz.py or discrimination_vert.py (depending on counterbalancing) and open in PsychoPy
 - This task was not created in Builder view, so does not have a .psyexp file.
- Click the green running man and enter the Participant ID
 - Make sure to enter the correct run number
 - Discrimination will be repeated 3 times (run 1, run 2, and run 3)
- Move the keyboard over so that the you, the researcher, can control it
 - You will press the buttons for the participant in this task (it is difficult to do and pay attention to on one's own)
- Read the instructions for the participant
- Ask the participant to tell you which line was more tilted, the first or second and press the corresponding button for them

3.4.4.2 Conditioning

- Click into the conditioning folder
 - Test the sound file on the laptop - screech.ogg
 - Set so that the sound is loud and uncomfortable, but not hurting
 - Record the volume setting on the session checklist
- Open the .psyexp file for the appropriate counterbalance by right clicking and opening in PsychoPy
- Click the green running man icon and enter the Participant ID

3.4.4.3 Extinction

- Click into the extinction folder
 - Open the .psyexp file
 - Click the green running man icon and enter the Participant ID
-

3.4.5 Protocol - Height

- Place participant directly against wall/frame
 - Advise participant to stand up straight
 - Make sure heels of participant are up against the wall/frame
 - Use a flat object (booklet, ruler, sheet of paper, etc.) to accurately measure height in centimeters
 - Record height on Lab Session Checklist
-

3.4.6 Protocol - Hair Sample

3.4.6.1 Training video

3.4.6.2 Set Up Hair Sample Station

- Ensure the hair-sample station is set up accordingly:
 - 1 sheet of aluminum foil
 - 1 small ziplock bag with participant ID
 - 1 salon grade scissor
 - 1 wide and narrow tooth parting comb
 - 1 alcohol swab
 - Painter-tape
 - 1 permanent marker
 - 1 pair of gloves
 - 2 alligator curl clips
 - 1 hair claw clip (for long hair)
 - Sample hair amount taken from wig

3.4.6.3 Prior to Getting Hair Sample

- With the parent present in the room, explain to both the child and parent that we will be collecting 30-50 strands of hair. The amount of hair to be collected is less hair than is lost in normal everyday-brushing from the back of the head.
- Inform them how the site for the sampling is hidden by the surrounding hair, therefore not visible after collection.
- Explain how the sample is used to measure a hormone called cortisol that is present in the hair.

- Show the hair sample taken from the wig to illustrate the amount of hair that will be collected (30-50 strands).
- Complete the Hair-Care Practice Questionnaire.

3.4.6.4 Hair Sample Prep

- Ask the parent to be present in the room when we collect the child's hair sample.
- Put on a pair of gloves.
- Wipe down the hair scissor/comb/clips with an alcohol swab.

3.4.6.5 Hair Length

- For short hair (less than 3cm), follow the Short-Hair Protocol below.
- For medium-length hair (3-6cm), follow the Medium-Hair Protocol below.
- For long hair (more than 6cm), follow the Long-Hair Protocol below.
- Ideally, all hair sample should be at least 3cm long. If the hair is less than 1cm long, the sample cannot be used.

Short-Hair Protocol (1-3cm)

- Take the comb and part the hair horizontally between the tips of the ears.
- After parting, ask the participant to hold the parted hair close to the scalp.
- Hold the loose hair tightly with index finger and thumb, and cut the hair along the part.
- Place loose hairs in foil and fold it securely. Do not tape the hair to the foil.
- Fold the foil without bending the hair, and ensure that the hair does not fall out of the foil.
- Label the root-end on the aluminum foil and place it in the ziplock bag.
- Label the ziplock bag with the participant's ID.
- Store the sample in a dry area at room temperature (in the plastic folder under the participant ID in cabinet 1).

Medium-Hair Protocol (3-6cm)

- Take the comb and part the hair horizontally between the tips of the ears.
- Take a clip to clip away the hair from the top of the parting.
- Place another clip at the bottom to expose a 5x10cm rectangle of loose hair between the two clips.
- Ask if the child prefers the wide or narrow tooth comb to comb through the loose hair.
- Ask if it is ok to discard any loose hair from the comb.
- Grasp approx. 30-50 strands of hair to the right of the rectangle.
- Gently pull and twist the hair away from the scalp in a rolling motion between the fingers.

- Collect the sample as close to scalp as possible, but be careful to not cut the scalp.
- Attach the hair to the center of the aluminum foil by taping with painter's tape - do not cover the root end.
- Label the root end on the tape.
- Fold the foil without bending the hair, and ensure that the hair does not fall out of the foil.
- Label the root-end on the aluminum foil and place it in the ziplock bag.
- Label the ziplock bag with the participant's ID.
- Store the sample in a dry area at room temperature (in the plastic folder under the participant ID in cabinet 1).

Long-Hair Protocol (>6cm)

- Part the hair left to right at the posterior vertex.
 - Clip away any extra hair, then create a twist of hair and hold tightly with index finger and thumb.
 - Make a clean cut as close to scalp as possible.
 - If the hair is thin, cut 2-3 small areas (1cm apart) across the posterior vertex to conceal the site of the cut.
 - Attach the hair to the center of the aluminum foil by taping with painter's tape - do not cover the root end.
 - Label the root end on the tape.
 - Fold the foil without bending the hair, and ensure that the hair does not fall out of the foil.
 - Label the root-end on the aluminum foil and place it in the ziplock bag.
 - Label the ziplock bag with the participant's ID.
 - Store the sample in a dry area at room temperature (in the plastic folder under the participant ID in cabinet 1).
-

3.4.7 Protocol - Weight

- Instruct participant to step on weight scale
 - Measure weight (in kg)
 - Record weight on Lab Session Checklist
-

3.4.8 Protocol - Saliva Sample

Sample Storage:

- Screw lids on very tight (to prevent evaporation)
 - Log the location (grid) on the sample storage log
-

3.4.9 Protocol - Memory Generalization

3.4.9.1 Training

- There are two versions of this task (They differ in the pictures that are used for training):
 - memory_generalization_beta.psyexp
 - memory_generalization_beta_B.psyexp
- Run the task on PsychoPy.
- Read the instructions out loud to the participant.
- When you see “[s]” it means that you can progress to the next screen.
- There will be 60 photographs the participant has to see. They are presented in random order.
- There are 10 red triangles. The participant is asked to press a button when they see the red triangles so that we can later on gauge their attention in the task.
- After the 60 photographs are shown, the participant is asked to recall all of the photos they just saw. Press record on the recorder (on talk setting). Make one recording for the whole memory generalization task.
- They will go through this photo viewing and recall phase another 2 times.
- When the task is complete, save the PsychoPy output file, as well as the recorded responses to the participant folder on the Dropbox.

3.4.9.2 Test

- Immediately after the memory generalization training, administer the memory generalization test.
- There is only one version of the memory generalization test: memory_test.psyexp
- Read the instructions to the participant, emphasizing that we only want them to respond YES if the picture is EXACTLY the same as the one they just saw in the training task.
- If the participant responds “Yes” or “No” they will progress to a confidence rating screen, asking them how sure they are in their response.
- If the participant responds “I Don’t Know” they will skip the confidence rating screen.
- When the task is complete, save the participants data output from PsychoPy into their participant folder on the server.

3.4.9.3 Physiology Marks

Markers for physiology have been included for each trial type (object neutral, object negative, scene neutral, scene negative). For the test, physiology markers are entered for every trial. That way, we might be able to go back and look at GSR for the times they got the item correct.

3.4.10 Protocol - Waist Measurement

- Stand and hold tape measure at the participant's belly button and bring it around their waist, over their t-shirt
 - Make sure measuring tape is horizontal around the waist and even in the front and back
 - Keep the tape snug around the waist, but not compressing the skin
 - Have participant breathe in
 - Measure the participant's waist just after they breathe out (in cm)
-

3.4.11 Protocol - WASI & WIAT

- Ensure that you have all of the following materials in the testing room:
 - WASI Stimulus Book
 - WASI Manual
 - WASI Score Sheet (should be in participant folder)
 - WIAT Word Reading List
 - WIAT Math Booklet (should be in participant folder)
 - WIAT Score Sheet (should be in participant folder)
 - Pens and Pencils
 - Recording device
- Sit the child diagonally from you at the table
- Start your recording device (using the talk setting)
- Make one recording for the WASI and one for the WIAT
- Say the following:

"We're going to be doing a few things today, like playing some word games and answering some math questions. Some of these things might be really easy for you, but some might be hard. Most people do not answer every question correctly or finish every item, but please try your best. Do you have any questions?"

3.4.11.1 WASI

- Open the WASI Stimulus Book to Vocabulary
- Say the following:

"First, I am going to say some words. Tell me what each word means. If there's one you don't know, we can skip it. Are you ready?"

- For ALL of our participants, we will skip the visual stimuli and go straight to the words (they are all ages 6+). Point to the words and say them aloud to the participant, asking

"What does _____ mean?" or "What is _____?"

- Record answers in the WASI Score Sheet. Score by comparing their response with the Manual's response criteria. If

- Once the end criteria are met (3 consecutive 0's) OR the participant hits the max score for their age group (for age 6, item 22; for ages 7-11, item 25; for ages 12-14, after item 28), say:

"Okay, we are going to stop there and move on to the next task."

- Open the WASI Stimulus Book to Matrix Reasoning
- Say the following:

"Now we're going to look at some patterns, and I want you to tell me which picture completes the pattern. If there's one you don't know, we can skip it."

- Flip to Sample Item A and ask "Which one of these items here (motion to the bottom row) goes here (motion to the blank space)?" Correct and teach if the participant gets the question wrong.
- Repeat for Sample Item B.
- If the child is 6-8 years old, start at Item 1. If the child is 9+, start at item 4. For each item, ask the same question as above, but do not give feedback or teach if they got the question wrong. Record answers in the WASI Score Sheet.
- Once the end criteria (3 consecutive 0's) are met OR the participant hits the max score for their age group (for ages 6-8, item 24), say:

"Okay, we are going to stop there and move on to the next task."

3.4.11.2 WIAT

- Next, get the WIAT Word Reading List and the WIAT Score Sheet
- Say the following:

"Now you're going to read some words out loud for me. Please read off of this list left to right, top to bottom just like a book (motion along with the directions as you say them). If you read all of the words on the front, flip over to the back and continue the same way. Go at your own pace, and say the words as clearly as you can. If there's one you don't know, we can skip it. Any questions?"

- Hand the word card to the participant and begin recording their answers in the WIAT Score Sheet. Keep track of self-corrections, responses taking longer than 3 seconds, and ask for repeat pronunciations if they are sounding out the word or ambiguous.
- Once the end criteria (4 consecutive 0's) are met, say:

"Okay, we are going to stop there and move on to the next task."

- Lastly, get the WIAT Math Booklet
- Ask the participant what grade they are in in school
- Say the following:

"Now, I want you to solve some math problems. Start here (motion to the appropriate item, item 1 for Grade 1, Item 14 for Grades 2-4, Item 18 for Grades 5+) and work left to right, top to bottom. If you get to a problem you

don't know, just skip it. Continue on and let me know when you're finished. Any questions?"

- When they have indicated they're complete, take all of their materials and put them back in their folder. Congratulate them and let them know they did well.
-

3.4.12 Protocol - Blood Sample (DBS)

3.4.12.1 DBS Prep

- Label Whatman Protein cards with subject ID, date and time, and card number.
 - Use cards in the order you have numbered them.
- Check DBS Collection Consent
 - Only proceed if no illness, phobia, bleeding disorder, blood thinners taken.
- Experimenter must wash hands.
- Participant must wash hands.
 - Use water as hot as participant can stand and interlace fingers and rub together while washing as shown in photo to increase circulation in the hands.



Palm to palm with fingers interlaced

Figure 3.7:

- Prepare collection area. It should look like this:

3.4.12.2 VR Headset Setup

- Put VR goggles on for participant.



Figure 3.8:

- Ensure the headset has been charged before it needs to be used.
- Bring the headset and the remote to the DBS Collection room.
- Ask the subject if they are familiar with VR headsets, if they make them feel motion sick, and if they want to use the headset during the DBS protocol.
- One of the RA's should pre-load the BABLab Youtube page:
 - Power on the headset (top center button on headset)
 - Go to Library
 - Select Youtube VR
 - Go to the “Account” tab
 - Go to the “Liked Videos” tab
- Show the child how the controller works:
 - moving your hand acts as the pointer/cursor
 - to make selections, use the large bumper button on the back or press down the touchpad at the top of the controller



Touchpad



Back bumper

- to scroll, move your finger up and down on the touch pad
- to go back to the movie selection list, press the upper round button below the touchpad



Back button

- To put on the headset, loosen the side velcro straps and ask the child to hold the goggles in a comfortable position on their face. If the child wears glasses, they should be fine to use the headset while wearing them.
- Tighten the straps so that the headset stays on its own but isn't uncomfortable for the child to wear.
- Tell them they can watch any of the videos on the playlist.
- Periodically check in with the child to ensure they aren't feeling motion sick or uncomfortable in any way.
- After the DBS is completed, take the headset off the child and power it down.

3.4.12.3 Blood Sample Administration

- Place heating pad on participant's hand, making sure to cover fingers. Set to low or medium heat.
 - Check to make sure it does not get too hot.
 - Set timer for 10 minutes.
- When 10 minutes are up, put on gloves. Have participant pull up heating pad and hold it with their free hand on the upper arm. Make sure the heating pad cable is not in the way of collection.
- Clean middle or ring finger with alcohol and wipe with gauze.
- Prick finger pad slightly off-center toward the side closest to the pinky finger and immediately dispose of lancet in sharps container.
- Wipe first drop with gauze then start collecting on Whatman Protein cards in numbered order.
- When finished, wipe with gauze, put pressure to stop bleeding and apply bandage.

- Remove gloves, use hand sanitizer immediately, then wash hands ASAP.

3.4.12.4 Precautions

- Before, during and after the procedure, ask if the participant is feeling lightheaded.
- Check the participant's complexion - turning pale is a warning sign for impeding faintness.
- If participant feels faint/lightheaded, terminate the procedure, ask them to bend forward, and place their head between their knees. You may apply a cold compression to the back of the neck to speed up recovery.
- Stay with them for at least 15 minutes until they feel completely fine.
- Report the incident to Bridget.

3.4.12.5 Fainting Emergency

- In case of fainting or any warning symptoms, lay the participant down flat on a surface on their back and elevate their feet if possible (to a level higher than their heart, about 30cm).
- Loosen any constrictive clothing/belts etc.
- Symptoms usually disappear after a short rest.
- If the participant does not regain consciousness within 1 minute, call 911.
- If the participant regains consciousness, avoid having him/her get up too quickly.
- Have them sit for at least 15 minutes until they feel completely fine.
- Offer water or warm sweet drinks.
- Report the incident to Bridget.

3.4.12.6 Biohazard Spill Emergency

- Equipments:
 - Disinfectant (Sodium Hypochlorite (Bleach))
 - Absorbent materials sufficient to completely cover spilled liquid and can be disposed (e.g. paper towels)
 - Physical tools that allow safe handling of sharp materials (e.g. tongs, forceps, broom/dustpan)
 - Warning signs to notify others that a spill occurred in the area
- Check self for contamination and change PPE if necessary.
- Put on new PPE to proceed with clean up.
- Pick up broken glass/sharps with available physical tools and dispose as biohazardous sharps.
- Place absorbent materials on and around spill.
- Put disinfectant on paper towels and let it sit for at least 5 minutes.
- Dispose of absorbent materials as biohazardous waste.
- Repeat step 4-6 as necessary.
- Remove PPE and wash hands with soap and water.
- Report all spills to Bridget.

Note: Only proceed with biohazardous spill cleanup if you feel comfortable; Always use physical tools for handling sharps.

3.4.12.7 Incident Response and Reporting

An exposure incident is specific contact with hazardous agents. Exposure incidents at UCLA must be reported, investigated, and documented by UCLA Insurance & Risk Management; Environment, Health & Safety; and/or the supervisor of the facility.

- Notify all personnel in the room of the incident.
- Move exposed individual(s) to a safe location, taking care to not spread biohazardous materials.
- Remove contaminated clothing, turn exposed areas inward, and place in a leak-proof bag or container for future decontamination.
- Wash skin with soap and water for 15 minutes.
- Go directly to the Occupational Health Facility at 67-120 CHS (M-F, 7am-4pm) or the RRM ER.
- Notify Bridget ASAP.
- Report the incident to EH&S within 8 hours (24-hour hotline: 310-825-9797).
- Record the incident in the Incident and Near Miss Log in the Biosafety Manual.

Note: Keep an extra set of clothes or shoes available to replace contaminated items.

3.4.13 Protocol - Child Questionnaires

- Ask the participant how comfortable they are reading and comprehending in English
- If not fully comfortable, read the questionnaires for the participant
- Read the first questionnaire - the SS - to all participants

3.4.13.1 Children 8 & Under

- The researcher will need to read all questions to child
 - PEDSQL GI & PEDSQL F need the laminated face sheet
-

3.5 Protocols - Post-Session

3.5.1 Protocol - Data Entry

3.5.2 Protocol - Data Quality Check

3.5.3 Protocol - Stool Sample Storage

3.5.3.1 Training Video

3.5.3.2 Sample Quality

- Put on gloves.
- Open the mailer to ensure that it contains both the stool sample (in biohazard bag) and the Bristol Stool Scale.
- Check for quality of the stool sample by shaking it up and down vigorously (keep the sample in the biohazard bag), then check for its consistency and color - It should be a dark-brown liquid.
- If stool sample does not meet requirement (e.g. sample is in solid form or amount collected is too little), contact the family to see if they would be willing to send another sample with compensation.
- Contact family if the Bristol Stool Scale is missing in the mailer.

3.5.3.3 Sample Transfer

- Wear appropriate PPE:
 - Gloves
 - Lab coat
 - Safety glasses
 - Surgical Mask
 - Closed-toe shoes
 - Long pants
 - Hair tied back
- Prepare your station and ensure that you have the following:
 - Drape
 - 2.0mL cryogenic vials
 - Stool samples in biohazard bag
 - Test tube racks
 - Transport box with divider
 - Sharpie for labeling

Steps:

- Lay a new drape on the work station and keep all equipments and sample on the drape throughout the transfer process.
- With the stool sample collection vial still in the biohazard bag, shake it up and down vigorously.
- Take the stool sample out of the bag and put it on the test tube rack.
- Untwist two 2.0mL vials and place them on the test tube rack.

- Untwist the stool sample collection vial, and carefully pour the sample into the first 2.0mL vial. (It's okay if the ball does or does not get transferred)
 - Stop pouring when solution reached the 1.8mL line to prevent overflow, and pour the remaining sample (if any) in a second 2.0mL vial.
 - Cap the 2.0mL vials tightly to prevent spills.
 - Label the 2.0mL vials with a sharpie, ensure it has the participant ID and vial number.
 - Place the labeled 2.0mL vials in the transport box with divider.
 - Close the now-empty stool sample collection vial, put it back in the biohazard bag, and dispose it in the biohazard waste bin.
 - Clean up work station, dispose the drape, and wipe down the table top with disinfectant wipe.
 - Remove PPE and wash hands with soap and water thoroughly.
 - Bring the transport box to C454 where the -80 °C freezer is located (key in BABLab Lock Box).
 - Place the 2.0mL vials in their designated space in the freezer box (in accordance to the Sample Storage Log Diagram).
 - Log the sample in the Sample Storage Log.
-

3.5.4 Protocol - DBS Sample Storage

- Using a new drape, place the protein cards along the long horizontal midline of the drape.
 - Lightly fold the drape in half along the midline, covering the protein cards but ensure no contact between the drape and the blood spots (i.e. ensure complete exposure of the blood spots).
 - Leave the protein cards to dry for 8-24 hours.
 - After drying, close the protein cards with the flap (make sure each one is labeled with wave #, participant ID, date and card number), and place each card in a separate ziplock bag with a silica gel pack.
 - Place the bags in the BABLab freezer box in the -80 °C freezer in room C454 (key in BABLab lockbox).
-

3.5.5 Protocol - Report Card Generation

1. Open a participant data folder
2. Navigate to the report card folder and rename the template file - MBB999 to the relevant participant - and open the file
3. If an ASA nutrition report has been generated for this participant, delete page 4 of the pdf. If no ASA nutrition report has been generated, delete page 3 of the pdf.



Figure 3.9:



Figure 3.10:

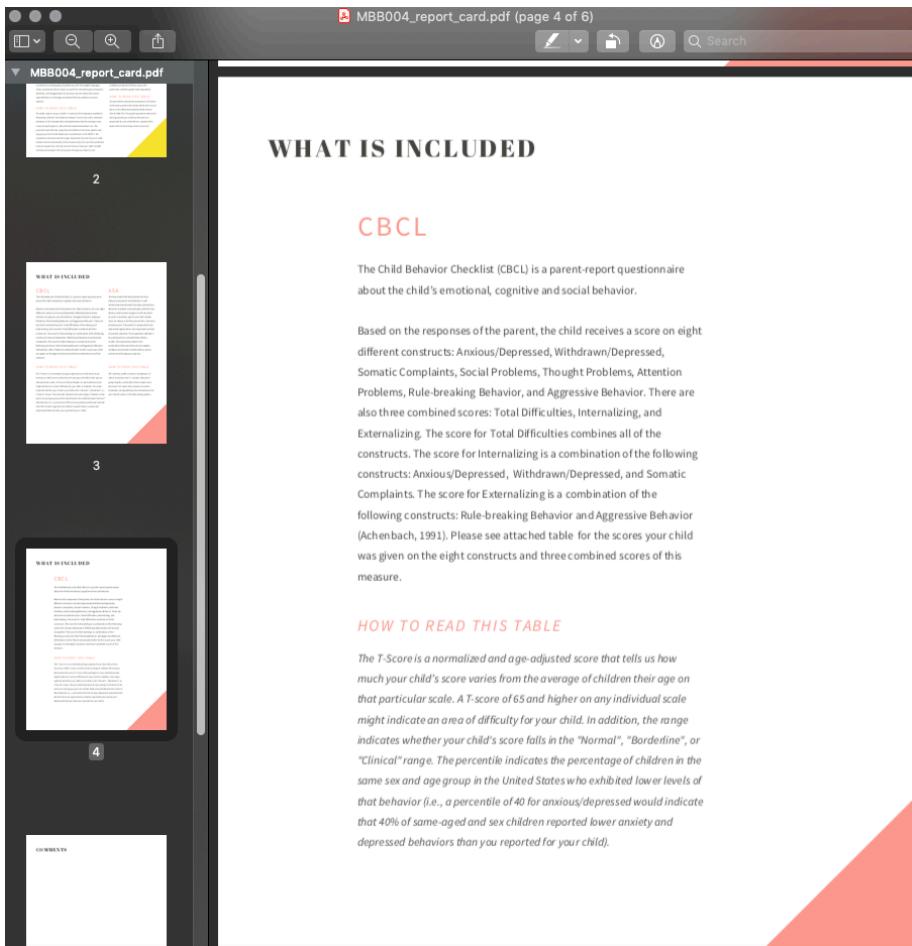


Figure 3.11:

4. Navigate to the last page of the pdf, and fill in the scores for this participant. You can type directly on the page - it is a fillable form.



Figure 3.12:

5. After you have entered the data, it should look like this
6. If there are any comments, enter them on the comments page.
 - For example, if any NA's are present due to less than 70% of data for that subset being available to calculate a score - note that here. Or, for example if the child was too young to receive a grade based score, you could note the aged based reading of the table here.
 - If there are no comments, delete this page.
7. **Important** - Once you have completed the edits to the pdf, you must follow these steps to "lock" the data so that it is no longer editable before sending to the participant. To do so, click file/print/PDF/Save as PDF.

CHILD REPORT					
WAVE 1 - MIND, BRAIN, BODY STUDY					
WECHSLER ABBREVIATED SCALE OF INTELLIGENCE - II					
Vocabulary T-Score	56				
Matrix Reasoning T-Score	32				
Estimated IQ	89				
IQ Confidence Interval	83-97				
Percentile	23%				
WECHSLER INDIVIDUAL ACHIEVEMENT TEST-III		WORD READING	NUMERICAL OPERATIONS		
Standard Score	106				
Standard Score Confidence Interval	101-111		81-101		
Percentile	66%		27%		
Grade Equivalent	5.2		3.7		
CHILD BEHAVIOR CHECKLIST		T-SCORE	PERCENTILE		
Anxious/Depressed	50	50%	Normal		
Withdrawn/Depressed	52	58%	Normal		
Somatic Complaints	50	50%	Normal		
Social Problems	52	58%	Normal		
Thought Problems	50	50%	Normal		
Attention Problems	51	50%	Normal		
Rule-breaking Behavior	55	69%	Normal		
Aggressive Behavior	50	50%	Normal		
Internalizing	39	14%	Normal		
Externalizing	44	27%	Normal		
Total Difficulties	40	16%	Normal		

Figure 3.13:



Figure 3.14:

Save the PDF to your desktop, then replace the original PDF with the desktop version.



Figure 3.15:

8. The report card is now ready to be sent to the participant.

3.5.6 Protocol - Data Review & Audit

3.5.6.1 Follow-Up (completed by Scheduling Coordinator)

- Before sending Home Reminder 3, make sure RA's have completed Data Entry, Data Quality Check 1, and Data Quality Check 2.
- After sending Home Reminder 3 - create blank Trello card for participant on *In Data Review* list.

3.5.6.2 Data Review (completed by Lab Manager #1)

- Once card has been created, do Data Review.
- After completing Data Review, move card to *Good Sample*, *Bad Sample*, or *No Sample* list based on the stool sample.

3.5.6.3 Data Audit (completed by Lab Manager #2)

If Good Sample:

- Send payment, thank you letter, and certificate via mail.
- Send [MBB - PAID] email and attach thank you letter, certificate, and parent report (including outstanding items).
- Move to *Paid* list.
- One week after payment is sent, check outstanding items do Audit Call #1.
- One week following Audit Call #1, check outstanding items do Audit Call #2.
- Within 2 days, check outstanding items and do Audit Call #3.
- After Audit Call #3 (or all items completed), send [MBB - DONE] email and move to *Done* list.
- If participant completes item on list, check card on Trello, mark off on participation log and data check sheets, and note in participant's README in data folder.

If Bad Sample:

- Send payment, thank you letter, and certificate via mail. Include a new stool sample kit.
- Send [MBB - PAID] email and attach thank you letter, certificate, and parent report (including outstanding items - emphasize stool sample kit).
- Move to *Paid* list in Trello.
- One week after payment is sent, check outstanding items do Audit Call #1.
- One week following Audit Call #1, check outstanding items do Audit Call #2.
- Within 2 days, check outstanding items and do Audit Call #3.
- After Audit Call #3 (or all items completed), send [MBB - DONE] email and move to *Done* list.
- If participant completes item on list, check card on Trello, mark off on participation log and data check sheets, and note in participant's README in data folder.

If No Sample:

- Send [MBB - UNPAID] email and attach thank you letter, certificate, and parent report (including outstanding items - emphasize stool sample kit).
- Leave participant on Unpaid list.

- If stool sample received - send payment, thank you letter, and certificate via mail and move to *Paid* list.
 - If stool sample not received, one week after email is sent, check outstanding items do Audit Call #1.
 - One week following Audit Call #1, check outstanding items do Audit Call #2.
 - Within 2 days of Audit Call #2, check outstanding items and do Audit Call #3.
 - After Audit Call #3 (or all items completed), send [MBB - DONE] email and move to *Done* list.
 - If participant completes item on list, check card on Trello, mark off on participang log and data check sheets, and note in partipant's README in data folder.
-

Chapter 4

Wave 1 Online

4.1 Checklists

4.1.1 Checklist - Initial

Scheduling and Confirmation

- Schedule session 1 two weeks in advance from “package mailing day” (see package preparation in pre-session checklist)
- Schedule session 2 ~one week after session 1
- Make Zoom link with scheduled session times and save to google calendar
- Send session 1 confirmation email (in templates)
 - Attach Next Steps, Computer Zoom Download Instructions, and Phone Zoom Download Instructions

Enrollment

- Create participant Box folder using MBB_template (delete blank README from newly created folder)
- Enroll participant in Wave 1 on REDCap
- Fill participant instrument on REDCap
- Fill counterbalance order on REDCap (Checklist - Lab Session Child Instrument)

Calendar

- Create MBB session calendar events (and invite researcher)
 - *MBBXXX - Online Session 1*
 - * Add *Status: Incomplete* to the description
 - * Add *Session 1 Links Email Sent: Incomplete* to the description
 - * Add *Status Of Package: Not Received* to the description

- *MBBXXX - Online Session 2*
 - * Add *Status: Incomplete* to the description
 - * Add *Session 2 Confirmation Email Sent: Incomplete* to the description
 - * Add *Session 2 Links Email Sent: Incomplete* to the description
- Create MBB mailing calendar event
 - *MBBXXX - Mail Package*
- Create MBB reminder calendar events
 - *MBBXXX - Session 1 Reminder 1 (email)* - 1 week prior
 - *MBBXXX - Session 1 Reminder 2 (email and call)* - 3 days prior
 - *MBBXXX - Session 2 Reminder 1 (email)* - 3 days before second session
 - *MBBXXX - Session 2 Reminder 2 (call)* - 2 days before second session

Reminders

- Send *Session 1 Reminder 1* email
 - In templates - attach next steps, consent/assent, zoom instructions, researcher information, “What is involved in this study?” video
 - Confirm package is received
 - Send *Session 1 Reminder 2* email
 - In templates - attach previous
 - Confirm package is received
 - Confirm package is delivered and received
 - Confirm participant
 - Preferably by phone
 - Update *Session 1* calendar status
 - Send *Session 2 Reminder 1* email
 - Confirm participant
 - Preferably by phone
 - Update *Session 2* calendar status
-

4.1.2 Checklist - Pre-Session 1

4.1.2.1 Package preparation: “magic box”

(prepare and send from all scheduled participants in the last week, to be mailed 2 weeks prior to session)

NOTE: Printing can be done in black and white.

- Print [What is in this magic box and what goes back to the lab?]
- Print Reward Board (plus gold star stickers)
- Print/Staple Parent Questionnaire Booklet (in this order)
 1. Parent Questionnaire Cover Page / Parent Proxy Intro
 2. demographics
 3. financial

4. covid_objective (parentproxy version)
 5. pedsql_gi_parentproxy
 6. pedsql_wb_parentproxy
 7. pedsql_f_parentproxy
 8. easy (revised)
 9. tesi (revised)
 10. cbcl (revised)
 11. cshq (revised)
 12. mb_metadata
 13. med_check
 14. pds
 15. dhws
 16. hpq
 17. parent_stress
 18. cssi (for children under 8)
 19. fci (only adopted)
 20. iai (only internationally adopted)
 21. Parent Self Intro
 22. bdi
 23. covid_objective (parentself version)
- Print/Staple Session 1 Booklet (in this order)
 1. Session 1 Cover page
 2. Fill in codes on Participant Info Brochure
 3. Pleasant/Unpleasant Events Checklist
 4. Child Measurement Sheet
 5. Height Measurement Instruction
 6. Weight Measurement Instruction
 7. Waist Measurement Instruction
 8. Saliva Sample Instructions Sheet
 9. Hair Sample Instructions Sheet
 - Print/Staple Session 2 Booklet (in this order)
 1. Session 2 Cover Page
 2. Contact List and label with participant ID
 3. Stool Sample Instructions Sheet
 4. Bristol Stool Scale and label with participant ID (MBB Specific Version)
 - Prepare 2 sharpened pencils
 - Prepare paper measuring tape (for waist and height measurements)
 - Label 2 biohazard bags (with 2 cotton balls in each bag)
 - Label 1 cardboard box (for samples)
 - Label hair sample kit (aluminum foil 7"x7", painter's tape with "root end" labeled, 1 ziplock bag pre-labeled with participant ID)
 - Label stool sample collection kit (paper clip collection tube and toilet hat together)
 - Label saliva sample collection kit (collection tube)
 - Insert MBB info card

- Attach FedEx slip to return mailer
 - Label return mailer with “exempt human specimen” (in sharpie)
 - Take picture of prepaid blue return mailer (marked with MBB number) and file in participant data folder on Box
 - Insert all labeled items and forms for post-session in blue return mailer
 - Insert all labeled items and forms for session itself in magic box
 - Insert blue return mailer into study package
 - Tape package closed and put BABLAB sticker on (if enough supplies)
 - Take a picture of study package with tracking information to file on Box
 - Mail package to participant
-

4.1.2.2 Setup - 1 Hour Prior

- Pull up session scripts/protocol
 - Prepare Session 1 checklist with counterbalance order
 - Have the Participant’s MBB and secondary MBB number on hand
 - Preload the following tabs on researcher’s computer:
 - Consent/Assent picture slideshow
 - Hair Sample Collection video
 - REDCap with child questionnaire codes ready
 - Prepare biological sample kits for demonstration during session
 - hair sample, saliva sample, stool sample
 - Ensure researcher’s Zoom security settings are set for study session
 - Fill out the “[MBB_online - LINKS EMAIL] UCLA Mind, Brain, Body Study (online)” email with the following links:
 - link to Consent on REDCap
 - link to Child’s Gorilla Game
 - link to COVID-19 Qualitative Responses on REDCap
 - Zoom link for Session 1
 - Send the “[MBB_online - LINKS EMAIL] UCLA Mind, Brain, Body Study (online)” to the participant
 - Activate the participant’s ID on Gorilla
-

4.1.3 Checklist - Session 1

- Session walk-through/package explanation
- Consent/Assent
- Parent-child observation (note recording via Zoom or pre-recording)
- If pre-recorded, instruct participant how to upload to Box
- Explain Questionnaires Parent Proxy or Parent self on second device if available (for parent to complete during Halloween training, Halloween test, and Child Questionnaires)
- Halloween training

- Height
 - Weight
 - Waist circumference
 - Halloween test
 - Saliva sample
 - Hair sample
 - Child Questionnaires
 - Stool Sample explanation
 - Contact list explanation
 - Confirm mailing address for payment
 - Schedule time to complete post-session tasks ~1 week post-session
 - Qualitative parent and child free responses (optional)
-

4.1.4 Checklist - Post-Session 1

4.1.4.1 Notes

- Make note of issues to discuss (if needed) in Boxnote for next core meeting

4.1.4.2 Filing

- “Scan” Session 1 checklist and file in participant folder
- Transfer and rename Zoom recording to Box

4.1.4.3 Reminders

- Session 2 Confirmation Email sent with Zoom link, researcher info (right after Session 1)
 - Session 2 reminder 1 phone call made (day before Session 2)
 - Session 2 reminder 1 email sent with Zoom link (right before Session 2)
-

4.1.5 Checklist - Session 2

- Halloween test delay completed
 - Stool sample questions answered
 - Bristol Stool Scale reminder
 - Contact information sheet reminder
 - Walk through package to send back (check “mbb_online_package_checklists”) for checklist of items participant needs to send back to the lab
-

4.1.6 Checklist - Final

4.1.6.1 Filing

- Make low-res parent child interaction video and save on BABLab External Hard Drive
- Burn all audio and video (low res) files to CD and label/store CD in binder
- Make manila folder for participants to file all hard copies

4.1.6.2 Data Entry

- Enter online session checklist data to REDCap
- Enter height, weight, waist to REDCap

After package has been received...

4.1.6.3 Package confirmation

- Halloween test delay completed
- Hair sample received
- Saliva sample received
- Stool sample received
- Bristol Stool Scale data received
- Questionnaires received (if paper versions were sent)
- Consent/Assent forms received (if paper versions were sent)
- Contact information sheet received

4.1.6.4 Data Entry

- Enter contact list information into recruitment database
- Enter questionnaires data (if paper versions were sent)
- Scan and upload Bristol Stool Scale to Box
- Enter Bristol Stool Scale data to REDCap

4.1.6.5 Filing

- File Consent/Assent forms in filing cabinet (consent manila folder)
- File contact list in filing cabinet (contact list manila folder)
- File Bristol Stool Scale in filing cabinet (participant folder)
- File questionnaires in filing cabinet if paper versions were sent (participant folder)

4.1.6.6 Sample Storage

- Label and store stool sample (add data quality to REDCap)
- Label and store saliva sample
- Label and store hair sample
- Update sample storage log on Box (once all received)
- Upload all sample photos to Box

4.1.6.7 Data Quality

- Data quality check 1
- Data quality check 2
- Data review
- Data audit

4.1.6.8 Retention

- Prep report card
- Send report card email (in templates - attach report card)
- Update participant Wave 2 status

4.1.6.9 Reimbursement

- Mail payment with science kits
 - Take a picture of tracking information and upload to Box
 - Log participant payment in reimbursement log book
 - Log participant payment in reimbursement spreadsheet
 - Send payment confirmation email to participant
-

4.2 Protocols - Pre-Session 1

4.2.1 Protocol - Recruitment

4.2.1.1 Pre-Screening

1. Check if participant is in Recruitment Database
 - If not, add them to the Recruitment Database
2. Check if participant is in ID Drive
 - If yes, check if they have a Screener ID
 - If not, assign them a Screener ID once contact has been established based on the next available Screener ID # in REDCap and proceed with screening
 - If yes, proceed with screening under existing Screener ID in REDCap

4.2.1.2 Screening

1. To screen a new participant click “Add / Edit Records”
2. Click to enter a new Subject ID
 - Make sure Arm 1: Recruitment is selected
3. Type “SMBB#” (Screener ID) to create a record and hit “Enter”
 - Make sure to link the participants Screener ID and their name on the **ID Drive ONLY**
 - Before creating a new record, be sure to check the ID Drive to see if the participant already has an existing Screener ID
 - If a record exists, add a new instance of the screen instead of creating

The screenshot shows the REDCap project home interface. At the top, it displays the user is logged in as emilytown@ucla.edu and has a project status of Development. The main menu includes Project Home, Project Setup, REDCap Messenger, and Data Collection. The Data Collection section lists Survey Distribution Tools, Scheduling, Record Status Dashboard, and Add / Edit Records. The Applications section lists Calendar, Data Exports, Reports, and Stats, Data Import Tool, Data Comparison Tool, Logging, Field Comment Log, File Repository, DAGs, Record Locking Customization, E-signature and Locking Mgmt, Data Quality, API and API Playground, Reports, and run_sheet. The Help & Information section includes Help & FAQ, Video Tutorials, and Suggest a New Feature. On the right side, there are several panels: Mind, Brain, Body; Main project settings; Design your data collection instru...; Define your events and designate...; Enable optional modules and custo...; and Set up project bookmarks (option...). Each panel contains various configuration options and status indicators like 'Not started' or 'In progress'.

Mind, Brain, Body

Add / Edit Records

You may view an existing record/response by selecting it from the drop-down lists below. To create a new record/response, new value in the text box below and hit Tab or Enter. To quickly find a record without using the drop-downs, the text box will populate with existing record names as you begin to type in it, allowing you to select it.

NOTICE: This project is currently in Development status. Real data should NOT be entered until the project has been moved to Production status.

Total records: 24	
Choose an existing Subject ID	Arm 1: screening <input type="button" value="-- select record --"/>
Enter a new or existing Subject ID	Arm 1: screening <input type="text"/>

Data Search

Choose a field to search (excludes multiple choice fields)	All fields <input type="button"/>
Search query Begin typing to search the project data, then click an item in the list to navigate to that record.	<input type="text"/>

a new record

4. The screening arm contains two parts
 - The screen
 - The wave1_status
 - The wave1_status is to be updated after the first and each subsequent contact

Record Home Page

Record "PP6" is a new Subject ID. To create the record and begin entering data for it, click any gray status icon below.

The grid below displays the form-by-form progress of data entered for the currently selected record. You may click on the colored status icons to access that form/event. If you wish, you may modify the events below by navigating to the [Define My Events](#) page.

Legend for status icons:	
● Incomplete	○ Incomplete (no data saved) ?
○ Unverified	○ Partial Survey Response
● Complete	✓ Completed Survey Response
● Many statuses (mixed)	● ○ ● Many statuses (all same)

NEW Subject ID PP6
Arm 1: screening

Data Collection Instrument	screener
screen	○
screen_status	○

quent contact

5. Click on the radio button in the “screen” row to screen the participant

The screenshot shows a REDCap form titled "screen". At the top, it says "Adding new Subject ID PP6" and "Event Name: screener (Arm 1: screening)". The Subject ID is listed as "PP6". The Date and time of screening field contains a placeholder "Now" with a calendar icon and a "M-D-Y H:M" button. Below this, there's a section for "Starting the phone call" with three radio button options: "Answering a call" (selected), "Returning a call", and "Leaving a message". A note below says "Hello, this is [researcher name] calling from the Brain and Body Lab at UCLA. Can I speak to [name of potential participant]?" and asks if the user would like to tell more about the research. There are "Yes" and "No" radio buttons. The "Notes" section has a text area and an "Expand" link. In the "Form Status" section, there's a "Complete?" field with a radio button set to "Incomplete" and a "Lock" button. At the bottom, there are "Save & Exit Form", "Save & Stay", and a "Cancel" button.

6. Click "Now" to enter today's date and time
7. Select the appropriate choice to start the phone call and follow the skip logic
8. Follow the skip logic to the end
 - For items without a text field, write the information down in the Recruitment database (This identifying information cannot be on REDCap)
9. Once done, select "Complete" and "Save & Exit Form"
 - The screen can be entered multiple times - for instance if there are multiple phone calls or contacts
 - It is important to keep a record of all instances of contact

Record Home Page

The grid below displays the form-by-form progress of data entered for the currently selected record. You may click on the colored status icons to access that form/event. If you wish, you may modify the events below by navigating to the [Define My Events](#) page.

Choose action for record ▾

Subject ID PP5
Arm 1: screening

Data Collection Instrument	screener
screen	 
screen_status	 

Repeating Instruments

screen	screener (Arm 1: screening)
1	
+ Add new	

screen_status

 [Editing existing Subject ID PP5](#)

Event Name: **screener (Arm 1: screening)**

Subject ID	PP5
Status	 Enrolled Enroll Contact Waitlist Remove
Form Status	Enrolled
Complete?	
Lock this record for this form? <small>If locked, no user will be able to edit this record on this form until someone with Lock/Unlock privileges unlocks it.</small>	
<input type="checkbox"/>  Lock	
Save & Exit Form Save & Stay ▾	
-- Cancel --	

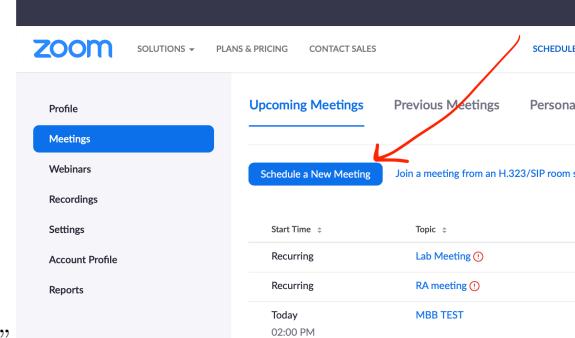
10. Click the screen_status radio button
11. Select the appropriate option
 - Contact - Participant needs to be re-contacted (add Recruitment Database & ID Drive)
 - Ineligible - Participant not eligible for study
 - To Enroll - Participant to enroll (need to create subject ID, enter subject info, schedule participant, add to Recruitment Database, add to ID Drive)
 - Enrolled - Participant has been enrolled (all above have been completed)
 - To Remove - Participant wants to be removed
12. Be sure to update the screen status after each contact
 - After 3 contacts (with no response) - review (time of day, contact method, etc.)
13. If enrolled, proceed to pre-session checklist in the participant log

4.2.1.3 Scheduling

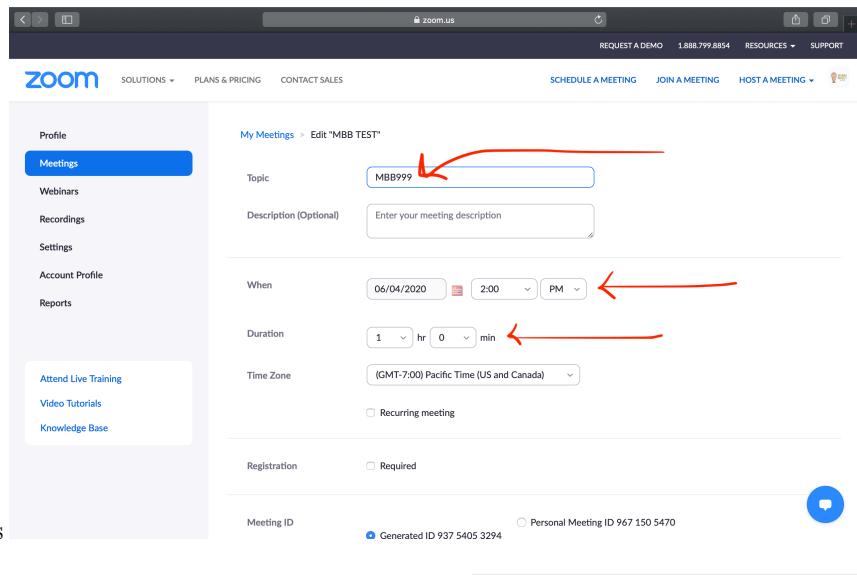
1. Open BabLab google calendar and note availability for designated data collection research team.
2. Check-in with the Lab Manager to see what the designated “package mailing day” of the week is. Participants must be scheduled 2 weeks or more in advance from the “package mailing day”, to ensure appropriate time for the package to be received by the participant.
3. Create event on google calendar for 2 hours. Notify the participant that sessions may not last the full indicated time, however, we like to designate additional time just in case.
4. As soon as the participant has been scheduled, create/add to a google calendar event for the designated “package mailing day” of the week the participant ID (MBB number).
5. This will notify the Lab Manager to create a package for this participant with session and post-session materials when they go into the lab for “package mailing day.”

Making a Zoom link

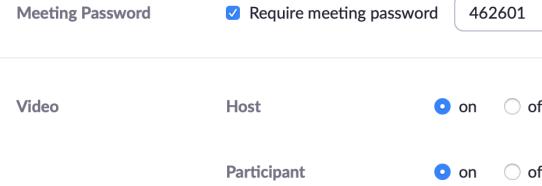
1. Log onto <https://zoom.us>



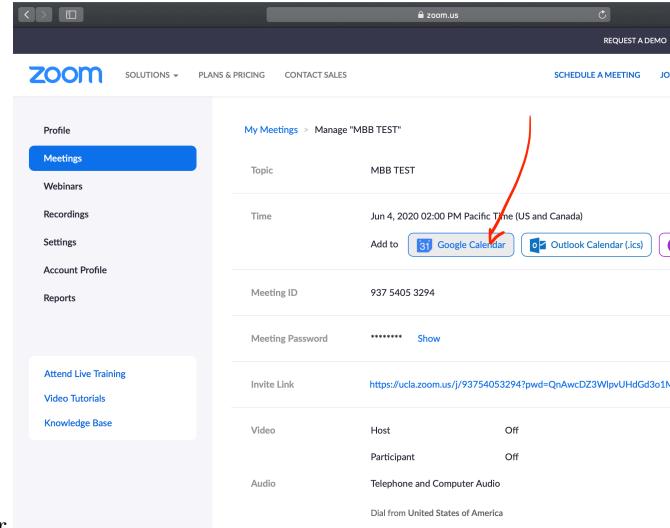
2. Click to “Meetings” and “Schedule a new meeting”
3. Title the meeting with the Participant’s MBB number, set scheduled time,



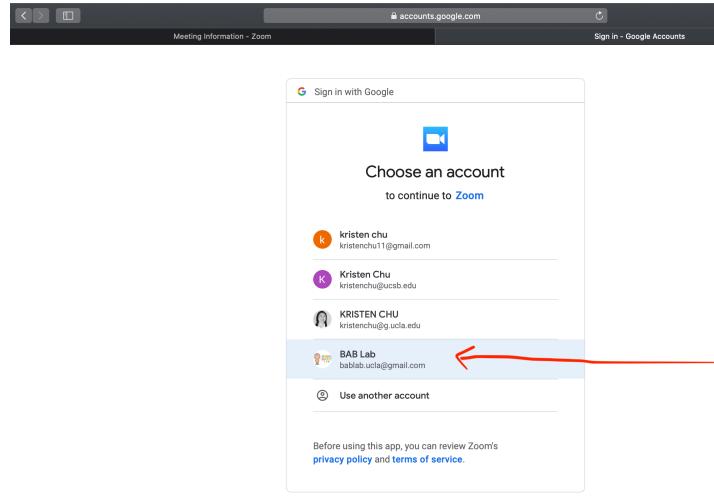
indicate 3 hours



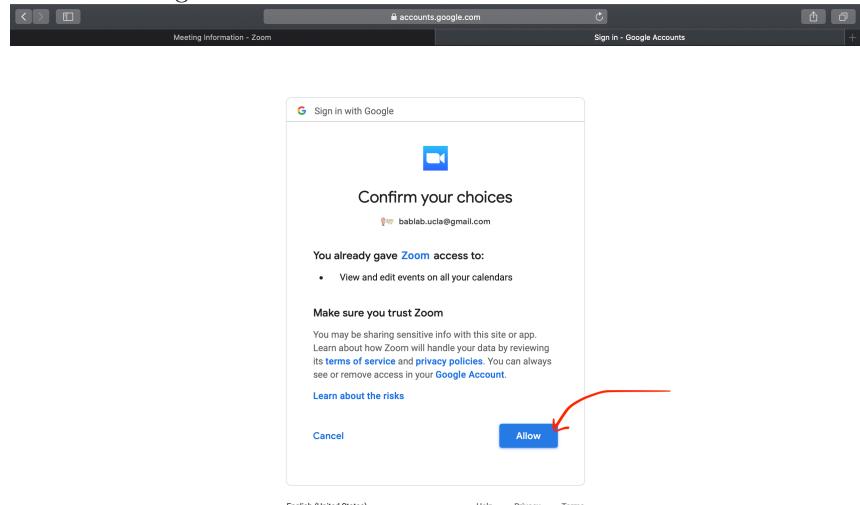
- Set setting with password and turn host/participant on



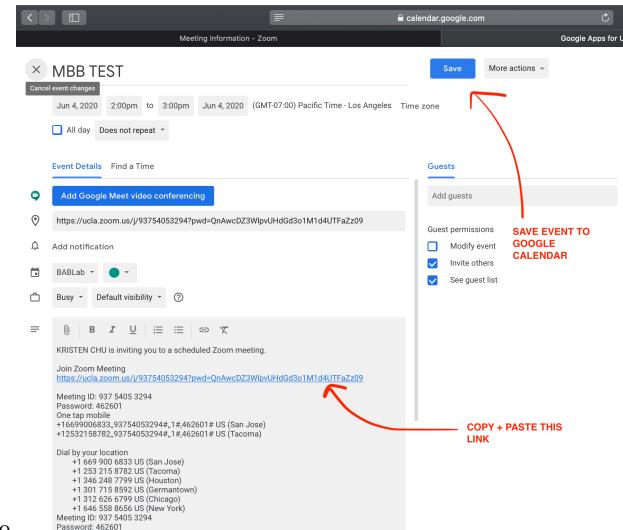
- Save and click to add Zoom meeting to google calendar



6. Click on the bablab.ucla@gmail.com



7. Click allow



8. Copy the Zoom link from the Description section and save
9. Paste Zoom link into the “confirmation email” you send to the participant with their session confirmation, Zoom instruction sheet, and Next Steps sheet

4.2.1.4 Other Screening Information

Accessing Lists

To find out where participants are in the recruitment process, there are several

lists.

Recruitment - To Contact

Displaying: Instrument status only | Lock status only | All status types

Subject ID	screener	screen	screen.status
No records were returned			

Enrollment - Wave 1

Displaying: Instrument status only | Lock status only | All status types

Subject ID	screen	screen.status
No records were returned		

Legend for status icons:

- Incomplete (Incomplete)
- Unverified (Unverified)
- Complete (Complete)
- Many statuses (mixed) (Many statuses)
- Many statuses (all same) (Many statuses)

1. Click on “Record Status Dashboard”
2. Participants who have been enrolled will be listed in the Enrollment - Wave 1 list
3. Participants in the process of recruitment will be listed in one of the 4

Recruitment lists - *These lists are populated based on the individuals “Screen Status” so be sure to update after each contact!

List Types

- Contact - List of individuals who need to be contacted or re-contacted (also includes waitlist)
- Ineligible - Participants are ineligible but interested
- To Enroll - Participants who have been screened and are eligible to enroll
- To Remove - Participants who were not interested in being contacted for this or future research

4.2.1.5 Concerns

If a parent has a concern about the study before the session, send the email template:

- [MBB_online - CONCERNS]
-

4.2.2 Protocol - Session Preparation

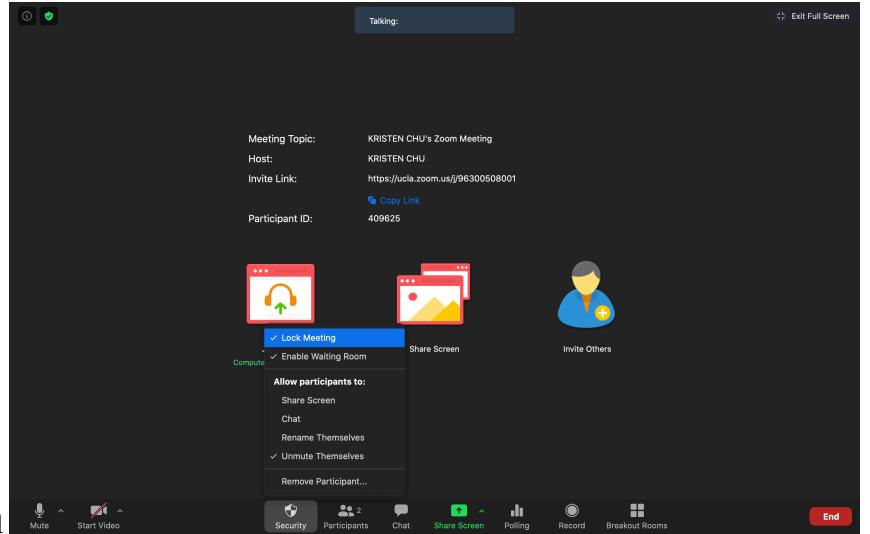
4.2.2.1 Package creation

- There will be a designated “package mailing day” one day a week in which the Lab Manager will go into the lab to prepare necessary materials and send out packages from scheduled participants in the last week, on the same package mailing day.
- Once the package has been put together, it is time to bring the package down to Tyler’s office in the Psychology building OR to the UCLA MDDS.
- To mail the package to the participant, you will need the following information:
 - Recharge ID
 - Participant name
 - Participant mailing address
- From Tyler’s office, you will receive a FedEx label in which you can write this information
- At MDDS, they will package your materials for you.
- Take a picture of the FedEx label and upload to Box
- Leave the package in Tyler’s office for FedEx pickup/MDDS to mail out

4.2.2.2 Zoom security Settings

1. Require Encryption for 3rd Party Endpoints*
2. Prevent participants from saving chat

3. Click on the “security” button and ensure the following items are checked



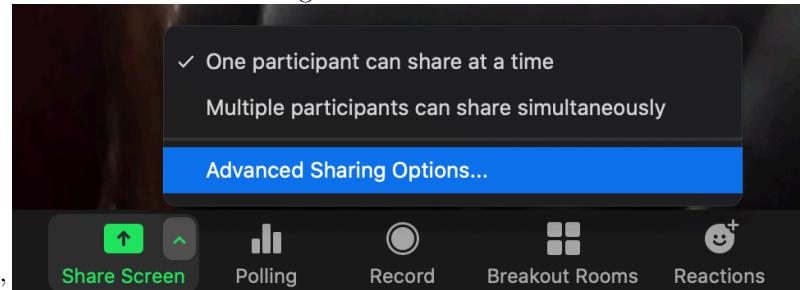
and all other items unchecked

- a. “Enable Waiting room”
- b. “Lock Meeting” after participant has entered
- c. Allow participant to “Unmute Themselves”

4. Disable Cloud recording*

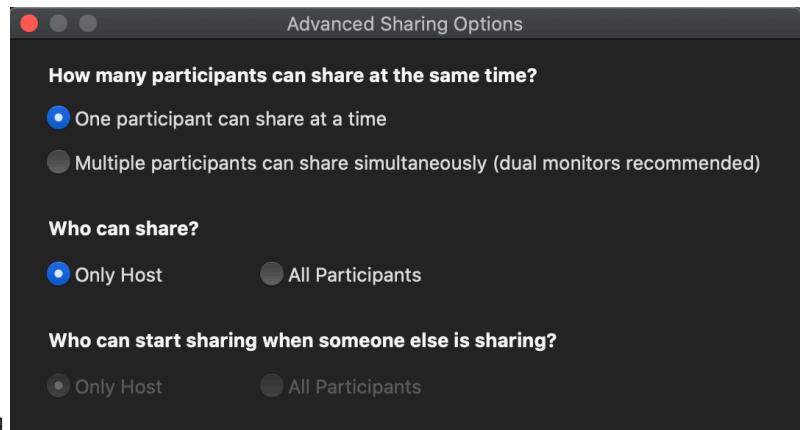
5. Host-only screen-sharing

- a. click on the arrow next to “screen sharing” and click on “Advanced”



sharing options”

- b. Ensure “one participant can share at a time” and “only host” options



*Note that #1 and #4 are the default settings (so those don't have to be changed).

4.2.2.3 Activating participant on Gorilla

1. Log in to Gorilla
2. Navigate to Projects/MBB/MBB_wave_1_online
3. Navigate to the participants tab
4. Click "Activate" for the designated participant

Figure 4.1:

Projects

Show Archived Projects

My Projects

Name	Description
MBB	
Pregnancy_memory	

Projects I'm collaborating on

Name	Description

Figure 4.2:

The screenshot shows the MBB project interface. At the top right are 'Settings' and '+ Create' buttons. Below is a section titled 'Experiments' with a 'Complete Participants' table:

Name	Description	Complete Participants
MBB_sona		0
MBB_sona_2		0
MBB_wave_1_online		2

A red arrow points to the 'MBB_wave_1_online' row.

Below is a 'Tasks & Questionnaires' section with a table:

Name	Description	Type
halloween_test		Box
halloween_training		Box
introduction SONA		List

Finally, there is an 'Open Materials' section with a table:

Name	Description	Type

Figure 4.3:

The screenshot shows the GORILLA™ software interface. At the top right is the user 'Bridget Callaghan'. Below is a navigation bar with 'Home > Projects > MBB > MBB_wave_1_online' and a search bar. A red arrow points to the 'Participants' tab, which is highlighted in red.

The 'Participants' tab shows the following details:

- Found 160 participants
- Show page 1 of 16
- Buttons: Download All, Force Include All, Activate All
- Table headers: PublicID, Name, Email Address, Recruitment Policy, Group, Version, Checkpoint, Progress, Status, Included, Activated, Actions
- Table rows:
 - MBB001: Supervised, night, View Progress, Pending, Activate, Actions
 - MBB003: Supervised, day, View Progress, Pending, Activate, Actions

Figure 4.4:

MBB157	Supervised	day	<button>View Progress</button>	Pending	<button>Activate</button>	▼ Actions
MBB158	Supervised	night	<button>View Progress</button>	Activated	Activated	▼ Actions
MBB160	Supervised	day	13	<button>View Progress</button>	Complete ✓	09/08/2020 ▼ Actions
MBB159	Supervised	day	13	<button>View Progress</button>	Complete ✓	11/08/2020 ▼ Actions

Figure 4.5:

4.3 Protocols - Session 1

4.3.1 Protocol - Consent & Assent

[ONCE ZOOM IS CONNECTED]

Hi! Thank you so much for joining us today! We are so looking forward to today's session with you. Usually, when we conduct a study such as this, we would do it our lab at UCLA. However, with COVID-19 we've decided it would be safer to carry out this study online for the time being- social distancing and all!

Our session today should take around 1 hour long. In addition to what we do here today, there will be a follow up zoom appointment with us one week from now. At that appointment, we will reconnect on a second Zoom call in which your child will log on for 10 minutes and complete a computer game.

Sounds good? Let's get started!

[Researcher to open up the MBB_Consent_Script_Presentation]

First thing we are going to do is go over what is on the consent and assent forms. (These are the attached documents we sent you in the emails leading up to this session.) We will walk through, in a little bit more detail, all of the things we will be doing during today's session.

During today's online session we are going to be doing some interactive things. First, we are going to have you and your parent sit and talk about some fun things and some not so fun things while on ZOOM. This conversation will be recorded but we will not be watching or listening in, you and your parent will be "virtually alone."

Next, we are going to have you play a game on the computer. In this game, you will be looking at pictures. Some of the pictures will be a bit scary, some sad, others a bit boring. While you are playing this game, your parent will stay with you in the room but will be working on some surveys to fill out.

After the game is over, your parent [NAME] will help measure your height, weight, and waist circumference.

You will also be answering some surveys (for children) with the researcher OR (for teens) on your own.

Lastly for today, your parent will help take two biological samples during this session.

1. One is the hair sample which helps measure hormones that everyone has in their hair
2. Two is the saliva sample which helps tell us learn a little bit more about your microbiome
 - Do you know what a microbiome is?
 - A microbiome is all the little bacteria that live inside your mouth. Everyone has these, they are healthy! We just want to know what kind and how many of each there are.

For helping us out in today's session, you will be getting \$45 for the work you put in! After this online session is over, we'll ask you to do three more things at home:

1. One is the Child poop sample – this helps us learn a bit more about your microbiome
 - There are also little bacteria that live in your tummy! Everyone has these and we want to know more about them.
2. Two is filling out the stool scale – this is a short scale that gives a description of your sample
3. Three is the computer memory game – this is when you will log back on with us via ZOOM in a week's time to see what you remember from today's session

Great! Do you have any questions for us about any of the samples

When you complete the poop sample and the computer game at home, we will pay you another \$20!

We will send the full payment of \$65 (\$45 for today's session and \$20 for completing the home session) as soon as we receive the samples back through the mail.

Here are some things to keep in mind:

You are a volunteer in this study, which means you do not have to do anything, or say anything, that makes you uncomfortable. We would like you to try everything you can, and to do your best, but if there are things you absolutely do not want to do, just tells us, that is o.k.

We will keep your participation confidential. You are given an ID number in order to keep your data confidential and separated from your name. Therefore, any identifying information (like your name, email, address, etc.) will be kept private and not paired with your data. We will also use a secondary ID number to save the videos with (since your faces are in it), which will help separate this from your name and the other data we collect form you. Only members of our research team will have access to your name and ID numbers.

As this is a longitudinal study, and we want you to come again in the future if you are interested, we will ask for some information so that we may reach out to you in the future. As I mentioned earlier, we sent you an email with a bunch of links that would be used in the session today. Please go there and click on the first one, which will allow you to virtually indicate your consent to participate in the study. If you'd like, you can share your screen with us now and we can walk you through it. Once you get that pulled up, you will see the consent/assent documents attached there as well- please feel free to take your time reading through the consent and assent forms now if you'd like.

[Then once they are done reading through]

All set and any questions?

[IF NO,] Please click to indicate your consent/assent and submit the REDCap form. You may press submit once you are finished to "send in the form."

Researcher to grab magic box.

Once you are all set with that, we'd like you for to grab the magic box we sent you in the mail. This contains everything you will need for today's session and what you will do at home after today's session.

The magic box should contain a sheet of paper at the top which details all of the contents, which we will unpack now!

For today's session here are the materials we will use:

1. Reward board with gold star stickers
2. 2 pencils
3. A session booklet
4. Parent surveys booklet
5. Paper measuring tape
6. Hair sample kit
7. Saliva sample collection kit
8. Cardboard box with a bag and cotton balls inside

It will also contain post-session materials. You will use these after today's session is complete:

1. Home session booklet
2. Bristol Stool Scale sheet
3. Stool sample collection kit
4. Cardboard box with bag and cotton balls inside
5. Mind Brain Body information card
6. Return mailer with FedEx slip attached on the top

Please set aside the magic box for now but keep the materials near you as we may need to access materials from the magic box throughout today's session!

What you can pull out now and use throughout the session is the Reward Board and Gold Star Stickers. Use this reward board to track all the steps you accom-

plish today! Each space is like each task for the session. Once you complete the task you can put a gold star on the space!

4.3.2 Protocol - Parent Child Observation

The parent and child will be seated together in view on the Zoom camera. During that time they will be filmed while solving a conflict, and then again while discussing a pleasant event. The conflict event will always go first, followed by the pleasant event. We did this to ensure that the parents were not thinking of the negative interaction upon answering the questionnaires about their child, which they did immediately after the observation interaction.

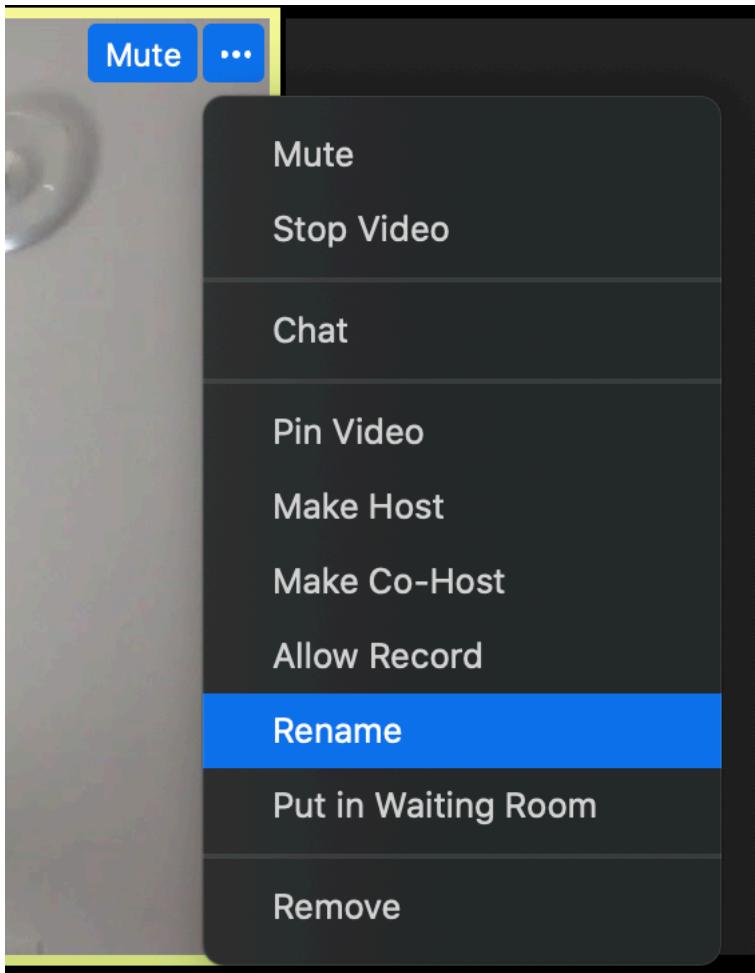
Step 1: The researcher will ask the parent to find the Pleasant/Unpleasant Events Checklist piece of paper from their session package.

Researcher: So the first thing we will have you pull out of the magic box is the paper packet titled “Online Session Booklet.” You can flip to the page that says “Pleasant and Unpleasant Events Checklist.”

Researcher will wait for the participant to find the Online Session Booklet and flip to the correct page.

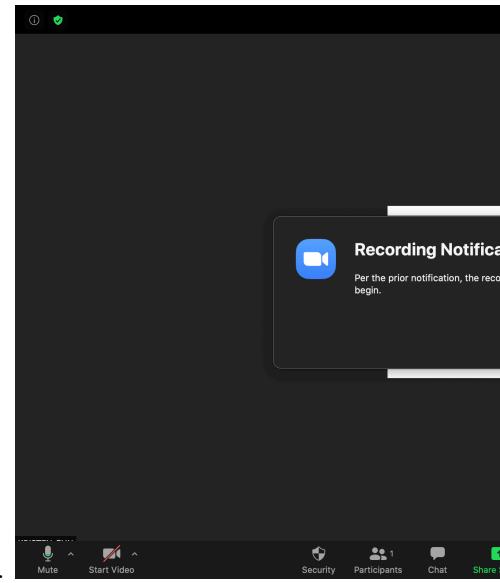
Researcher: Next we are going to take some film of you while you discuss something that’s hard and try to resolve it. On this piece of paper is a list of things that parents and children sometimes have disagreements about. We will give you a moment to read the list and think about some that you would like to discuss together. Then after about one minute, you will start discussing the things you have selected and try to resolve them. You do not need to tell us what you chose to discuss, and it does not matter if you chose something from this list, or decide to choose something else. I’ll be there while you choose, but once we start recording I am going to step out of the room to be away from the camera view and lower the volume on my computer so I won’t be watching or listening during your conversation. I am not going to respond to you during this time. I will give you a total of five minutes once the recording begins. Take 1 minute now to choose.

Step 2: - Researcher set timer for 1 minute (mute self). - Researcher will ensure Zoom security settings are set up for the video. - Parent and child will be situated side-by-side in view on the Zoom camera. - Researcher will rename the participant’s name to the participant Secondary MBB ID number.

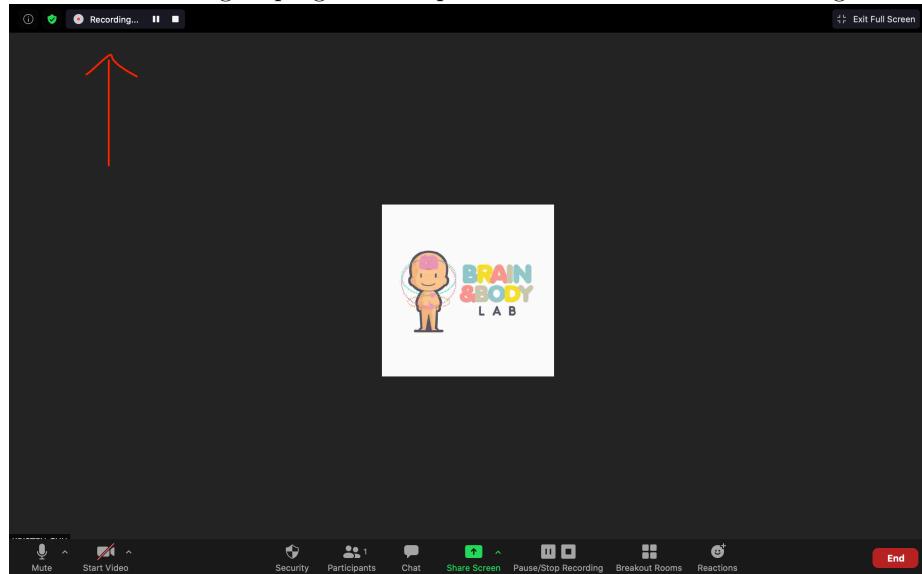


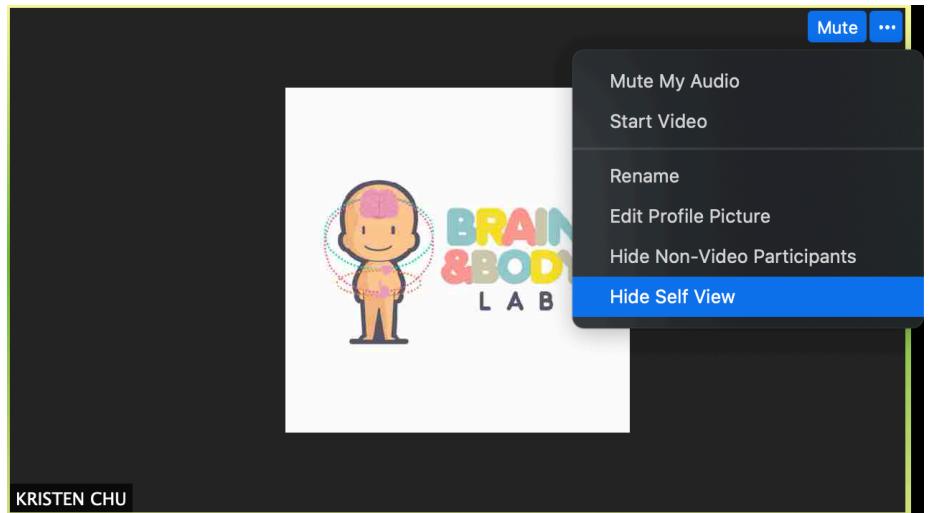
Ok, now it's time to get started. Remember, try to solve whatever problem or disagreement you talk about for the next five minutes. After, I will come back into the video call and give you further instructions. Do you have any questions?

If no questions, proceed.



Step 3: - Researcher will press record on the Zoom application.
Wait to hear the audio Zoom confirmation "*this meeting is now being recorded*"
and view recording in progress at top left of screen to ensure recording is live.





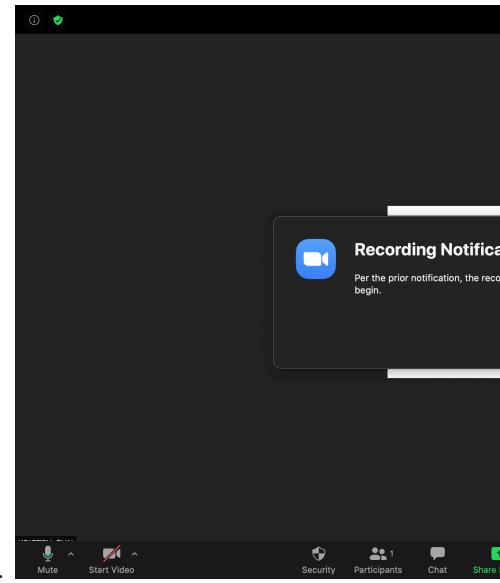
- Researcher will “hide self view”
- Researcher will lower the volume on their computer (but do not mute computer volume entirely), and mute their audio on Zoom.
- Researcher will start timer for 5 minutes. At the end of 5 minutes, reenter camera view and turn up volume on researcher’s computer.

Researcher: Thank you for taking the time to discuss something difficult. Next we are going to take some film of you talking about something nice. On your piece of paper is a list of fun things that parents and children sometimes do together. I will give you a moment to read the list and pick something that you would like to plan to do together. Then after about one minute, you will start discussing the things you have selected and try to plan them. You do not need to tell us what you chose to discuss, and it does not matter if you choose something from this list, or decide to choose something else. I'll be there while you choose, but once we start recording I am going to step out of the room to be away from the camera view and lower the volume on my computer so I won't be watching or listening during your conversation. I am not going to respond to you during this time. I will give you a total of five minutes once the recording begins. Take 1 minute now to choose.

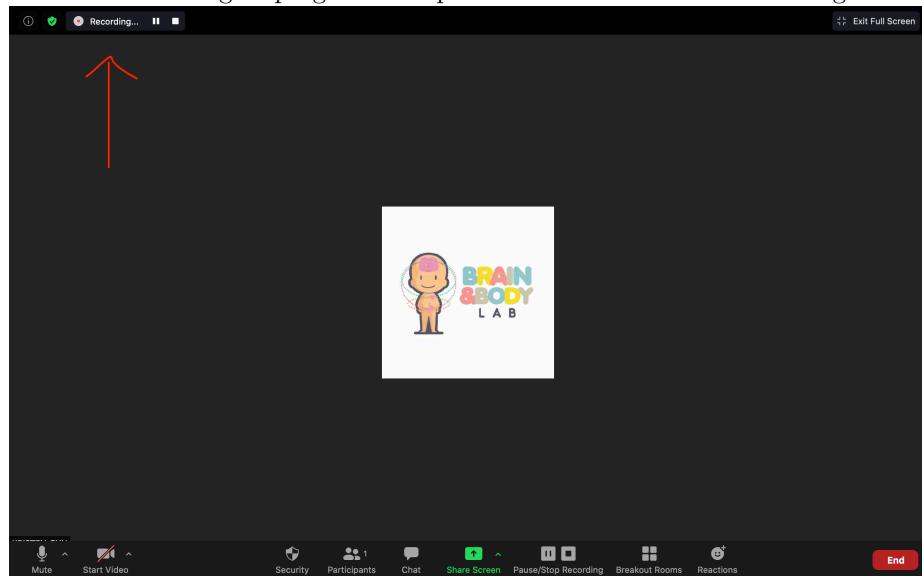
Step 4: - Researcher set timer for 1 minute (mute self).

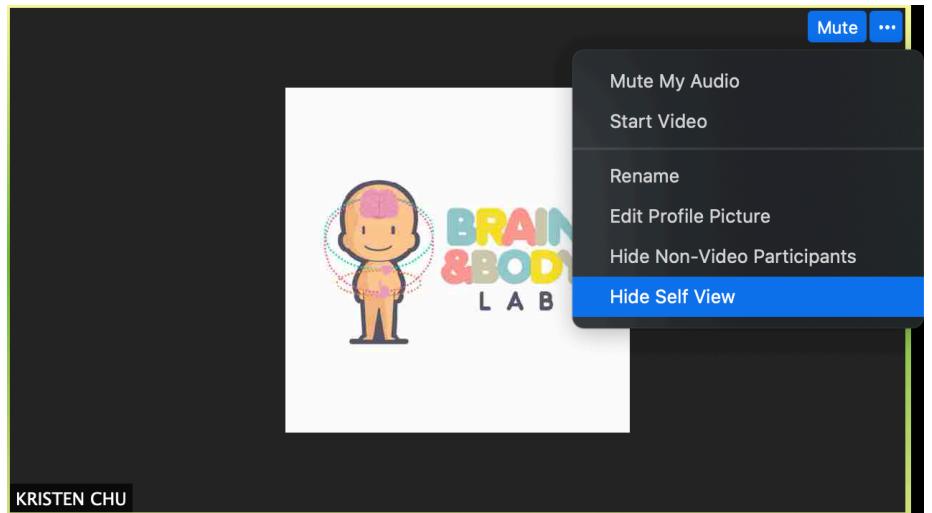
Ok, now it's time to get started. Remember, try to plan whatever fun thing you talk about for the next five minutes. After, I will come back into the video call and give you further instructions. Do you have any questions?

If no questions, proceed.



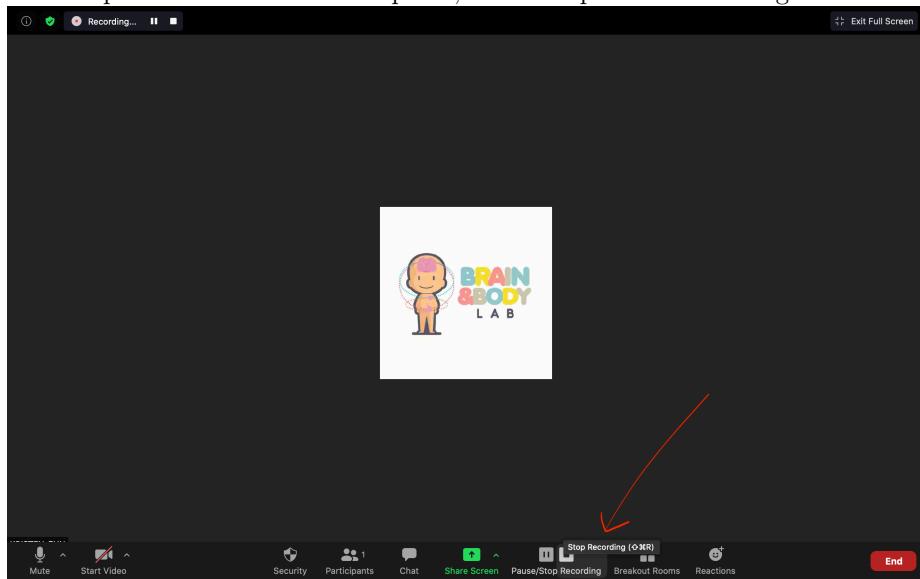
Step 5: - Researcher will press record on the Zoom application.
Wait to hear the audio Zoom confirmation "*this meeting is now being recorded*"
and view recording in progress at top left of screen to ensure recording is live.



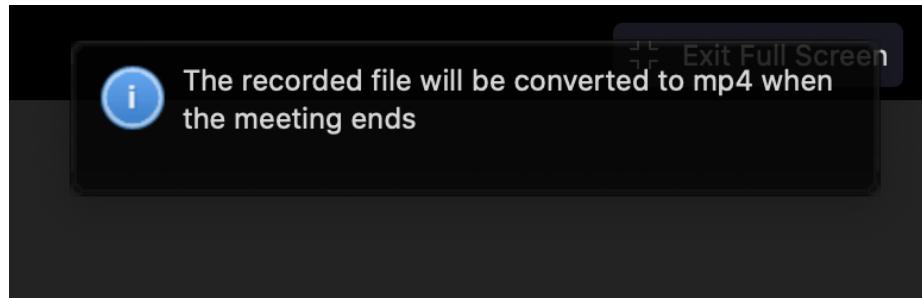


- Researcher will “hide self view”
- Researcher will lower the volume on their computer (but do not mute computer volume entirely), and mute their audio on Zoom.
- Researcher will start timer for 5 minutes. At the end of 5 minutes, reenter camera view and turn up volume on researcher’s computer.

Step 6: Researcher reenters the room and back into camera view, turns up volume on the computer, and stops the recording on Zoom.



You will view this notification in the upper right hand corner that states the recorded file will be converted to mp4 once the meeting ends.



Move the child/adolescent and parent onto the next task in the session, as the video will not be saved until after the session is complete.

Please visit "Post-Online Session Protocols to view instructions on how to save the video recording.

4.3.2.1 Plan B

If Zoom recording is not possible, parents will be given same instructions - but told to record on their cell phone and upload to a secure private link.

Participants can access the link here. Parents can access this link from their mobile phone or computer.

When they click on the link, they will see the screen below.

Parents can drag their video file directly from their phone/computer into the folder.

The video will appear to the researchers in our Box under BABLAB/Studies/Mind_Brain_Body/Data/W

Videos can then be renamed and organized by the researcher.

4.3.3 Protocol - Halloween Training

Now we are done with the group activity. Next we will move on to some individual activities, where you the parent will complete some surveys while your child plays a computer game. The computer game is about your child's learning and memory so it is important they don't get help from you! We actually prefer you don't watch the screen so you don't know which pictures they see. If you recall the example from our powerpoint at the beginning of this session, this will be the time that your child will see some pictures on the screen

So to get your child setup for the computer game, we will have you log go back to that "LINKS EMAIL" we sent right before the session to access a link to the activity. Once you find the email, please click on the link under "Child Learning and Memory" and let me know once you have done this.

[Wait for them to pull up the Gorilla task]

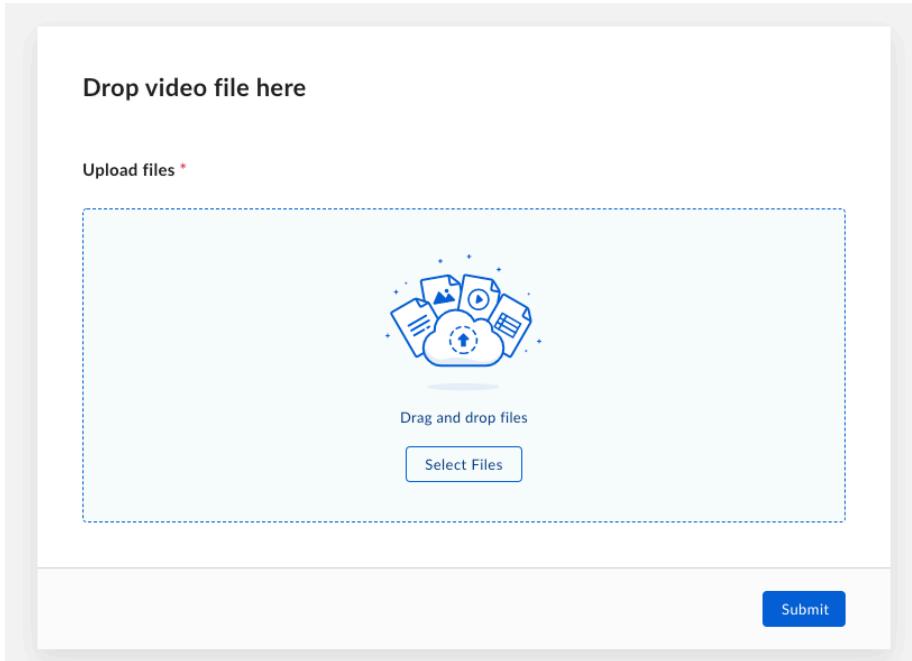


Figure 4.6:

If necessary - the link can be found here.

[Then, confirm they are on the right page] You should be seeing a login screen that asks you to enter your participant ID screen.

You can now enter your MBB number on the screen, but don't click start yet.

4.3.4 Protocol - Parent Questionnaires

While your child is doing the game, you can get started on some parent surveys. So now I will ask you to reach into your Session Package and pull out the booklet titled "Parent Survey Booklet" and a pencil to fill these out.

[Wait for parent to pull the parent survey booklet out]

We ask that you please fill the surveys out in the order that they are presented. Some of these surveys are about your child and some of these surveys are about you. The game will take around 15-20 minutes and I will notify you when we are finished. Don't worry - you will have time to return to the surveys during another part of this session! You can go ahead and work on the surveys now and your child can start the game. It will tell you once the game is over, and we can regroup!

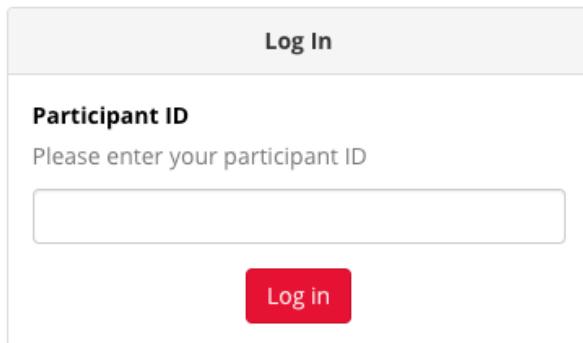


Figure 4.7:

[If participants do not seem to check-in with researcher after ~15 minutes, ask if they have any questions] *Hi, just wanted to check-in and see if everything was going alright. Do you have any questions?*

[Once the Halloween training is complete, you may proceed to Height]

4.3.5 Protocol - Height

Ok, for the next part of this session, we will have you (parent) pause on your surveys so you can help take some measurements from your child (height, weight, and waist). I will ask you to reach into your session package and pull out the "Session Booklet", a pencil, and the paper measuring tape.

[Wait for participant to retrieve session booklet, pencil, and paper measuring tape]

This booklet contains all the instructions for our measurements and sample collections during today's session. Please flip the booklet to the first page, titled "child measurement sheet." This is the page we will use to fill out your child's measurements. As you can see, the following pages are instructions sheets for each measurement so feel free to flip through these for reference as we go through each measurement.

We will start with Height! Do you have a measuring tape? (Explain that sometimes measurements are easier done with real measuring tapes).

[If yes, wait for them to retrieve. If no, proceed with the paper measurement.]

- Researcher will be walking the parent through how to measure their child's height via Zoom video call. Ask parent if they have a full length measurement tape. If not, we will proceed with the paper measurement tape.
 - Ask parent to place child/adolescent directly against wall/frame
 - Advise child/adolescent to stand up straight
 - Have parent ensure heels of child/adolescent are up against the wall/frame
 - Use a flat object (booklet, ruler, sheet of paper, etc.) to accurately mark the height on the wall/frame
 - Use the paper measuring tape to scale up the wall and measure the height marking
 - Record height on Online Session Checklist
-

4.3.6 Protocol - Weight

Next, we will do weight! You can flip to the instructions on weight measurement in your session booklet. Do you have a weight scale?

[If yes, say] *Please go and weigh your child, and return to the screen so we may record the number. [Once they return, say] Please record your child's weight on the child measurement sheet in the space next to "Measured Weight"*

[If no, say] *Can you please record an estimate of your child's weight on the child measurement sheet in the space next to "approximate weight"?*

- Instruct parent to have child/adolescent to step on weight scale
 - Measure weight
 - Record weight on Online Session Checklist
 - Specify on Online Session Checklist if weight was estimated or not
-

4.3.7 Protocol - Waist Measurement

Next, we will do the Waist measurement! You can flip to the instructions on Waist measurement in your session booklet.

- Advise parent to hold tape measure at the child/adolescent's belly button and bring it around their waist, over their t-shirt
 - Make sure measuring tape is horizontal around the waist and even in the front and back
 - Keep the tape snug around the waist, but not compressing the skin
 - Have participant breathe in
 - Measure the participant's waist just after they breathe out
 - Record waist measurement on Online Session Checklist
-

4.3.8 Protocol - Halloween Test

OK! It is time to go back to our learning and memory section. Can you please log back onto the “Gorilla” website. If you still have the browser up that’s great - please refresh the screen to continue. If not, you can go back to the same link as before and enter your MBB ID. Here you will be completing the second part of the computer game?

[Wait for child to log back on]

If necessary - the link can be found here.

[Confirm that they are on the correct page] *If you left your browser up, you should see Part 2 of the game, if you had to click on the link again, you should see the samee logon screen where you can enter your MBB ID.*

[Once confirmed, let the parent know they can resume on their parent surveys during this time] *So just to reiterate, this next part is about your child’s learning and memory so it is important they dont get help from you! We prefer you don’t watch the screen so you don’t know which pictures they see. (If you recall the example from our powerpoint at the beginning of this session, this will be the time that your child will see some pictures on the screen). While you wait for your child to complete their task for the next 15 minutes, you can get continue on your parent surveys. Once your child is finished, we can regroup!*

[If participants do not seem to check-in with researcher after ~15 minutes, ask if they have any questions] *Hi, just wanted to check-in and see if everything was going alright. Do you have any questions?*

[Once the Halloween test is complete, you may proceed to Saliva Sample Collection]

4.3.9 Protocol - Saliva Sample

Now, we will do some sample collections! The first one is the Saliva Sample. You can flip to the instructions on Saliva Sample Collection in your session booklet. Feel free to take a quick look through these instructions, then grab your “spit tube” from the Session Package. When you are ready, I will walk you through it step-by-step!

- Researcher has saliva “spit tube” example for explanation to participants
- Advise parents to have child/adolescent fill spit tube to indicated line
- Do not count the bubbles at the top, ensure that the saliva reaches the line
- Close the cap on the saliva tube, to release the stabilizing solution and seal the sample
- Put the sample in the biohazard bag with the two cotton balls inside

- Put the biohazard bag with sample inside the rigid box and set aside for now
-

4.3.10 Protocol - Hair Sample

Next up is the Hair Sample. You can flip to the instructions on Hair Sample Collection in your session booklet. Feel free to take a quick look through these instructions, then grab the Ziploc bag with the foil inside from the Session Package. When you are ready, I will walk you through it step-by-step!

Participants can also watch the video below:

4.3.10.1 Set Up Hair Sample Station

- Ask parent to gather the following materials for their “hair-sample station”:
 - 1 sheet of aluminum foil (provided)
 - 1 small ziplock bag with participant ID (provided)
 - Painter-tape (provided)
 - 1 scissor (salon grade if they have)
 - 1 parting comb (optional)
 - 2 alligator curl clips (optional)
 - 1 hair claw clip (optional- for long hair)
- Researcher set up the following materials (to help explain hair sample collection):
 - 1 sheet of aluminum foil
 - 1 small ziplock bag with participant ID
 - 1 salon grade scissor
 - 1 wide and narrow tooth parting comb
 - 1 alcohol swab
 - Painter-tape
 - 1 pair of gloves
 - 2 alligator curl clips
 - 1 hair claw clip (for long hair)
 - Sample hair amount taken from wig

4.3.10.2 Explanation

- Explain to both the child and parent that they will be collecting 30-50 strands of hair. The amount of hair to be collected is less hair than is lost in normal everyday-brushing from the back of the head.
- Inform them how the site for the sampling is hidden by the surrounding hair, therefore not visible after collection.
- Explain how the sample is used to measure a hormone called cortisol that is present in the hair.

- Show on the hair sample picture directions sheet the hair sample taken from the wig to illustrate the amount of hair that will be collected (30-50 strands).
- Offer to show our hair sample collection video

4.3.10.3 Hair Sample Prep

- Have parent use provided pair of gloves.
- Wipe down the hair scissor/comb/clips with an alcohol swab.

4.3.10.4 Hair Length

- For short hair (less than 3cm), follow the Short-Hair Protocol below.
- For medium-length hair (3-6cm), follow the Medium-Hair Protocol below.
- For long hair (more than 6cm), follow the Long-Hair Protocol below.
- Ideally, all hair sample should be at least 3cm long. If the hair is less than 1cm long, the sample cannot be used.

Short-Hair Protocol (1-3cm)- advise parent to:

- Take the comb and part the hair horizontally between the tips of the ears.
- After parting, ask the participant to hold the parted hair close to the scalp.
- Hold the loose hair tightly with index finger and thumb, and cut the hair along the part.
- Place loose hairs in foil and fold it securely. Do NOT tape the hair to the foil.
- Fold the foil without bending the hair, and ensure that the hair does not fall out of the foil.
- Ensure the root-end on the aluminum foil is labeled and place it in the ziplock bag.
- Ensure the ziplock bag is labeled with the participant's ID.

Medium-Hair Protocol (3-6cm)- advise parent to:

- Take the comb and part the hair horizontally between the tips of the ears.
- Take a clip to clip away the hair from the top of the parting.
- Place another clip at the bottom to expose a 5x10cm rectangle of loose hair between the two clips.
- Ask if the child prefers the wide or narrow tooth comb to comb through the loose hair.
- Ask if it is ok to discard any loose hair from the comb.
- Grasp approx. 30-50 strands of hair to the right of the rectangle.
- Gently pull and twist the hair away from the scalp in a rolling motion between the fingers.
- Collect the sample as close to scalp as possible, but be careful to not cut the scalp.

- Attach the hair to the center of the aluminum foil by taping with painter's tape - do not cover the root end.
- Label the root end on the tape.
- Fold the foil without bending the hair, and ensure that the hair does not fall out of the foil.
- Ensure the root-end on the aluminum foil is labeled and place it in the ziplock bag.
- Ensure the ziplock bag is labeled with the participant's ID.

Long-Hair Protocol (> 6cm)- advise parent to:

- Part the hair left to right at the posterior vertex.
 - Clip away any extra hair, then create a twist of hair and hold tightly with index finger and thumb.
 - Make a clean cut as close to scalp as possible.
 - If the hair is thin, cut 2-3 small areas (1cm apart) across the posterior vertex to conceal the site of the cut.
 - Attach the hair to the center of the aluminum foil by taping with painter's tape - do not cover the root end.
 - Label the root end on the tape.
 - Fold the foil without bending the hair, and ensure that the hair does not fall out of the foil.
 - Ensure the root-end on the aluminum foil is labeled and place it in the ziplock bag.
 - Ensure the ziplock bag is labeled with the participant's ID.
-

4.3.11 Protocol - Child Questionnaires**4.3.11.1 Ages 8+**

Next, we will move on to some Child Surveys. I will ask you to now to pull up the link to the child surveys from your email.

[Wait for participant to pull up REDCap]

[To the child, say] Please fill the surveys and the questions out in order. Let me know if anything is confusing, or if you have any questions! Let us know when you are all done!

[To the parent, say] At this point, you may continue on your parent survey booklet.

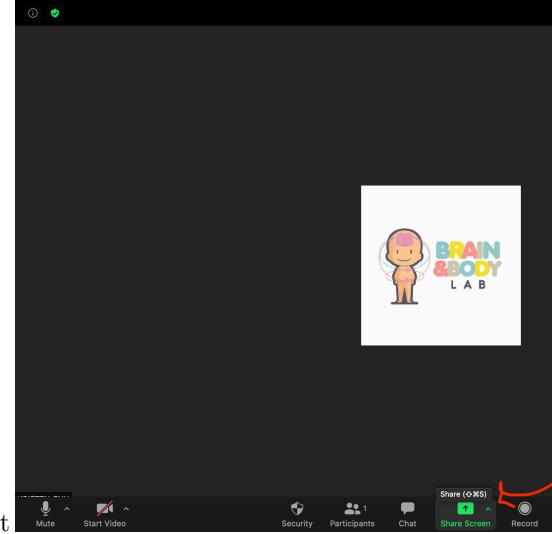
4.3.11.2 Ages 6-7

Next, we will move on to some Child Surveys. In a moment, I am going to share my screen with you, so you can see the survey questions. I will then read out each question and answer choice and [child's name] can tell me your answer. Does that sound okay?

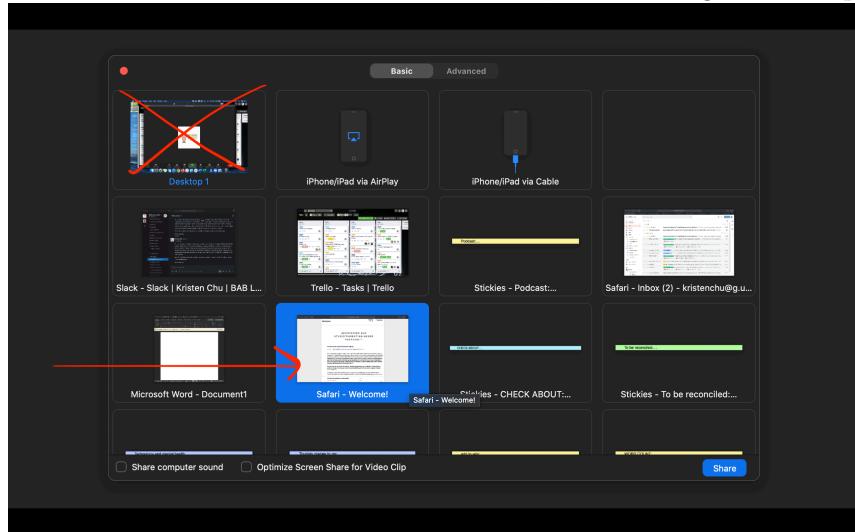
[Researcher to share screen]

4.3.11.3 Sharing Your Screen

- Researcher will open REDCap on their computer and enter in child code



- On Zoom, click “share screen” with the participant
- Be sure to indicate the correct screen to share, NOT sharing full desktop



- Researcher will read through all questionnaires with children and indicate their responses on REDCap

4.3.12 Protocol - Multiple Child Sessions

If there are multiple participants in one session, refer back to top of protocols for MBB Online Session 1 from this point on. Repeat all protocols for each child, then proceed to Protocol - Home Session once the last child has finished their questionnaires.

4.3.13 Protocol - Home Session

At this time, I will now ask you to reach into your Session Package and pull out your “Home Session Booklet” so I can walk through its contents.

[Wait for participant to retrieve Home Session Booklet]

- Stool Sample explanation & BSS sheet
 - *In the “Home session booklet” there are instructions for the Stool Sample Collection and a short survey that should be filled out after the stool sample collection. There is a toilet hat and a gut kit in the session package, which are the two major materials you will need for this collection. If you would like, we encourage you to try the stool sample collection this week so if you run into any trouble before the second session, we can answer any questions you might have at that time.*
 - Contact list explanation
 - *In a longitudinal study, information may change in time and we want to ensure we have a way to recontact you for the next wave if you are still interested in joining. The contact list is also in your Home Session Booklet for you to fill out.*
 - Confirm pre-scheduled time to complete post-session tasks ~1 week post-session
 - Payment
 - Confirm mailing address
 - Explain that once the return mailer has been received to the lab after the second session, we will send payment through the mail along with a few educational science kits
-

4.3.14 Protocol - Qualitative

The last part of this session is optional to you and your child! It is a five minute writing activity that allows you to logon and type for five minutes about you and your family’s experiences with COVID-19. These written responses are meant to capture the impacts of COVID-19 on families, caregivers, and children. Are you interested in doing this?

[If they are interested, direct the participant to their LINKS EMAIL and have them click on the REDCap link for Written Responses.]

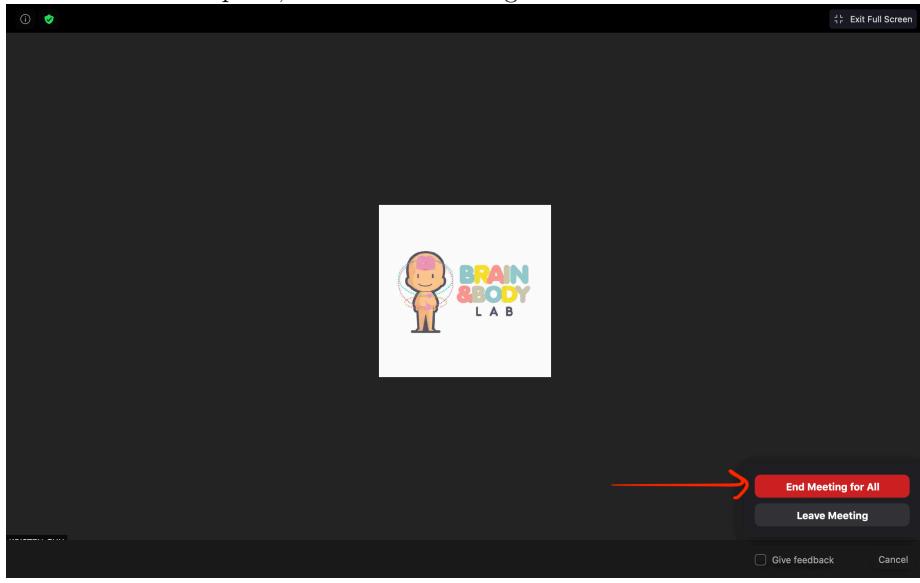
[If not interested, say] *No problem at all! We thank you so much for your participation today and look forward to seeing you next week for our second session. We will send you a confirmation email right after we hang up today with the next session time and Zoom link. Please feel free to give us a call or email if you have any follow-up questions. Thanks so much!*

- Two qualitative free responses on REDCap are optional, and should be offered to parents and children to complete if they are interested
 - These qualitative responses require five minutes of continued writing to capture the impacts of COVID-19 on families, caregivers, and children
 - If interested, researcher will advise parents to click on the REDCap link for parent and child to complete
-

4.4 Protocols - Post-Session 1

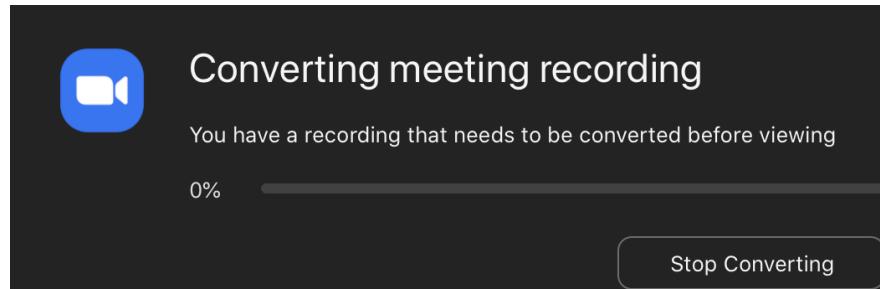
4.4.1 Protocol - Saving the Video

Step 1: When the session is complete, click the bottom right hand button to end



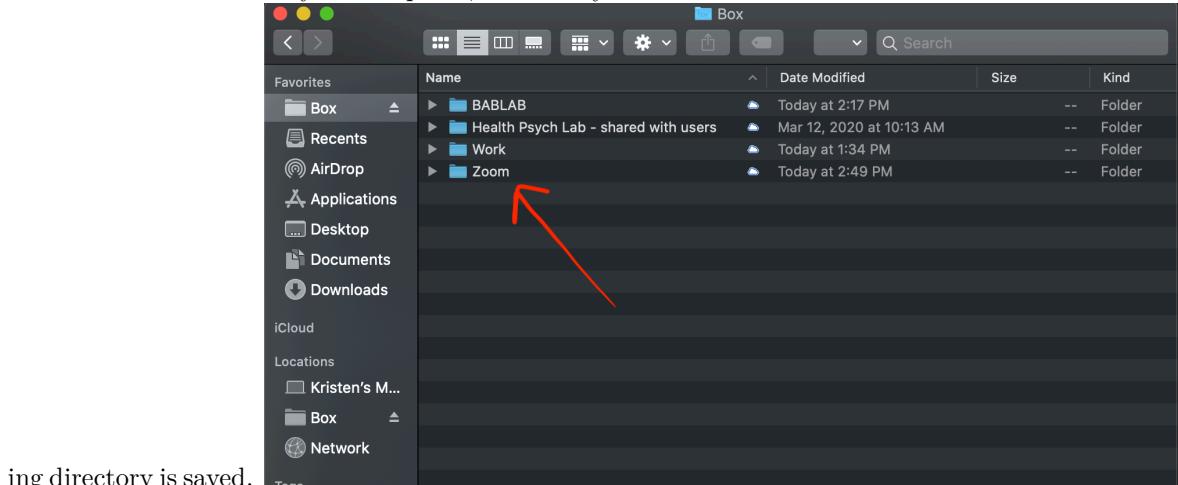
the meeting.

You will immediately see this window pop up to indicate the recording is being



converted and saving to your computer.

Step 2: When the video conversion is complete, the video files will be saved in a folder titled “Zoom” on your computer, wherever your current automatic work-



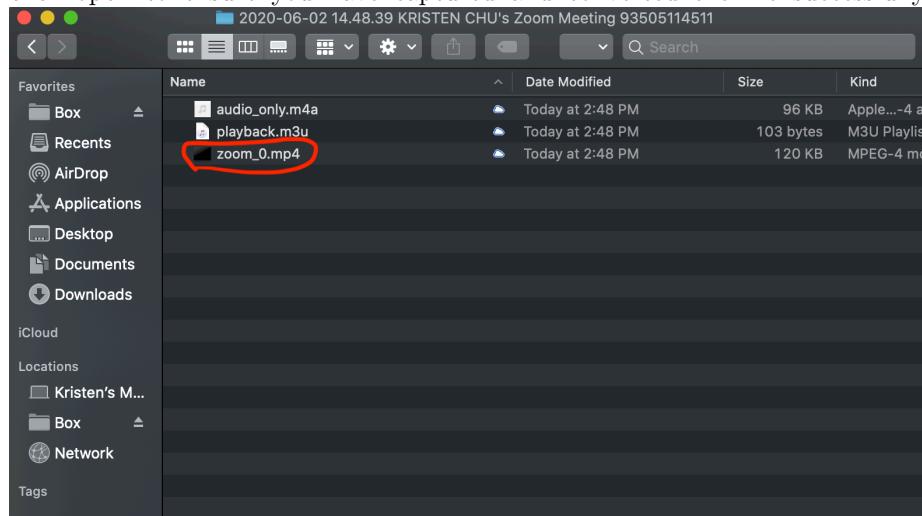
To check where your automatic working directory is saved, login to Zoom and click on “Recordings” on the left menu column. Then switch to “Local Recordings” and view the Location for correct Meeting Recording you have just camp-

The Local recordings listed below are accessible only from the computer on which they were recorded.

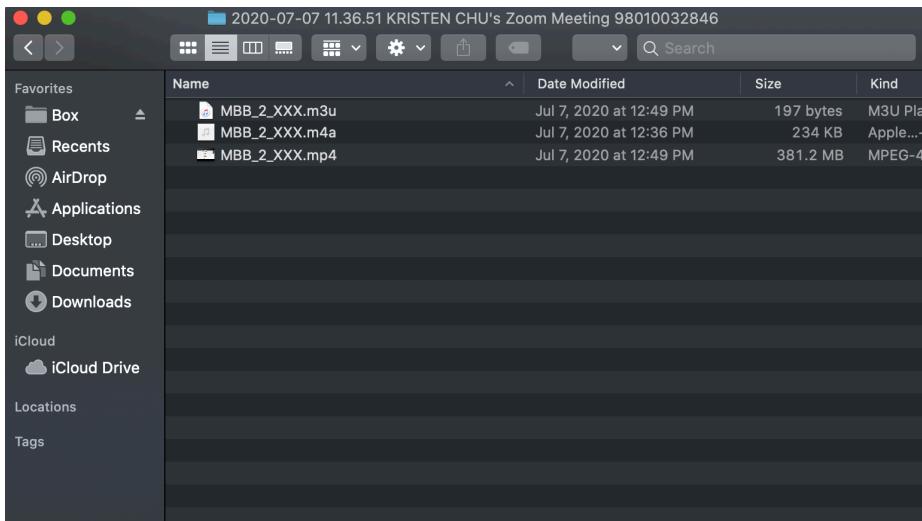
<input type="checkbox"/>	Topic	ID	Start Time	Computer Name	Location	<input type="button" value="Delete"/>
<input type="checkbox"/>	KRISTEN CHU's Zoom Meeting	980 1003 2846	Jul 7, 2020 11:36 AM	Kristen's MacBook Pro	/Users/kristen/Documents/Zoom/2020-07-07 11.36.51 KRISTEN CHU's Zoom Meeting 98010032846/	<input type="button" value="Delete"/>
<input type="checkbox"/>	KRISTEN CHU's Zoom Meeting	997 2501 9066	Jun 11, 2020 04:09 PM	Kristen's MacBook Pro	/Users/kristen/Documents/Zoom/2020-06-11 16.09.42 KRISTEN CHU's Zoom Meeting 99725019066/	<input type="button" value="Delete"/>

tured.

Step 3: There will be three files in the folder- find the mp4 file and click open to ensure you have captured and converted the file successfully.



Step 4: Check the External drive for the participant's secondary ID number, and rename all 3 files with their secondary ID (MBB_2_XXX). Then upload to Box.



4.4.2 Protocol - Session 2 Confirmation Email

- If session scheduling has not changed, copy Zoom link and Session 2 time information into Session 2 Confirmation Email and send
- If session scheduling has changed, update google calendar. Then, copy Zoom link and updated Session 2 time information into Session 2 Confirmation Email and send.

4.5 Protocols - Session 2

4.5.1 Protocol - Halloween Test Delay

[Once Zoom is Connected]

Hello! Welcome back to Session 2! [Ask how they are doing, and if they were able to try the Stool Sample collection. Answer any questions they have about their Home Session tasks (Stool sample, BSS sheet, Contact list)]

So today we are just going to do one quick task and then I will walk you through how to close up package and send it back to us, then answer any questions you might have

[Log into REDCap, pull up the instrument titled “halloween_test_delay” for this participant in the home_child arm and click into the survey options. You can either send them the general link and unique password, or give them a shortcode that expires within 1-hour]

4.5.2 Protocol - Mailing Package

Next, I just want to check-in with you about mailing the package back to the lab.

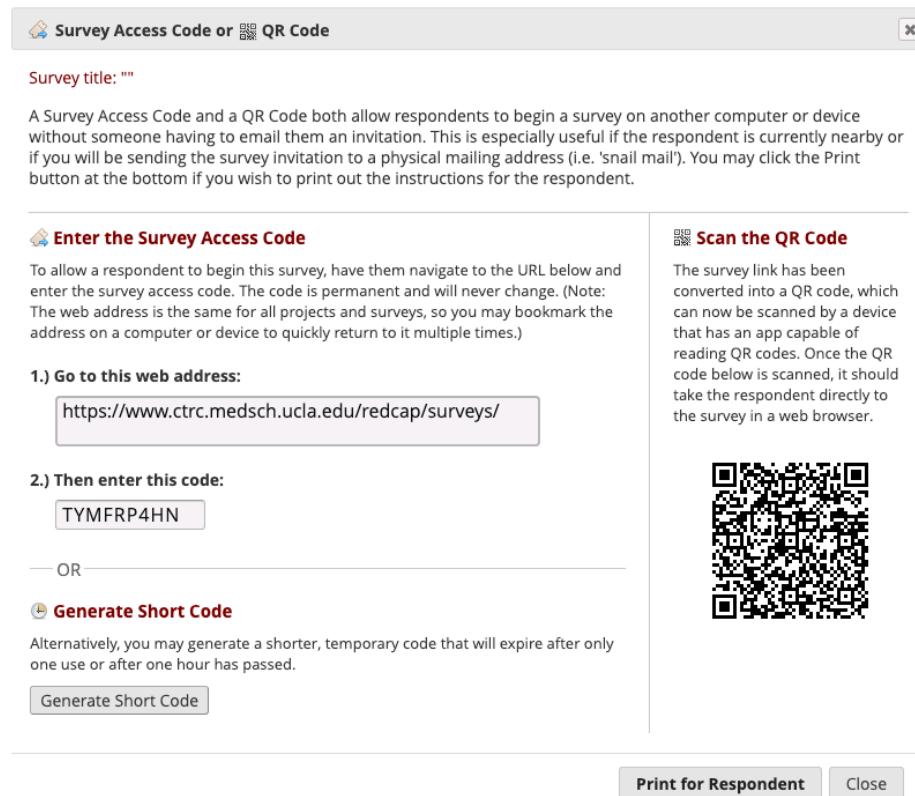


Figure 4.8:

- Reference the Package Checklist which has a checklist for every item they need to send back to the lab
- Everything goes into the mailer with the FedEx sheet on top
- Double check that stool and saliva sample are correctly packed (tube in biohazard bag with cotton balls, inside rigid white box)
- Drop off at any FedEx location or post box

4.5.3 Protocol - Payment

- Confirm mailing address
- Explain that once the return mailer has been received to the lab after the second session, we will send payment through the mail along with a few educational science kits
- Explain that they should expect an email from us when we send the package, and if they haven't heard from us one week after we have sent the package, call to check-in about the payment

4.6 Protocols - Final

4.6.1 Protocol - Saliva Sample Storage

- Screw lids on very tight (to prevent evaporation)
 - Log the location (grid) on the sample storage log
-

4.6.2 Protocol - Hair Sample Storage

- Store the sample in a dry area at room temperature
-

4.6.3 Protocol - Stool Sample Storage

4.6.3.1 Sample Quality

- Put on gloves.
- Open the mailer to ensure that it contains both the stool sample (in biohazard bag) and the Bristol Stool Scale.
- Check for quality of the stool sample by shaking it up and down vigorously (keep the sample in the biohazard bag), then check for its consistency and color - It should be a dark-brown liquid.
- If stool sample does not meet requirement (e.g. sample is in solid form or amount collected is too little), contact the family to see if they would be willing to send another sample with compensation.
- Contact family if the Bristol Stool Scale is missing in the mailer.

4.6.3.2 Sample Transfer

- Wear appropriate PPE:
 - Gloves
 - Lab coat
 - Safety glasses
 - Surgical Mask
 - Closed-toe shoes
 - Long pants
 - Hair tied back
- Prepare your station and ensure that you have the following:
 - Drape
 - 2.0mL cryogenic vials
 - Stool samples in biohazard bag
 - Test tube racks
 - Transport box with divider
 - Sharpie for labeling

Steps:

- Lay a new drape on the work station and keep all equipments and sample on the drape throughout the transfer process.
- With the stool sample collection vial still in the biohazard bag, shake it up and down vigorously.
- Take the stool sample out of the bag and put it on the test tube rack.
- Untwist two 2.0mL vials and place them on the test tube rack.
- Untwist the stool sample collection vial, and carefully pour the sample into the first 2.0mL vial. (It's okay if the ball does or does not get transferred)
- Stop pouring when solution reached the 1.8mL line to prevent overflow, and pour the remaining sample (if any) in a second 2.0mL vial.
- Cap the 2.0mL vials tightly to prevent spills.
- Label the 2.0mL vials with a sharpie, ensure it has the participant ID and vial number.
- Place the labeled 2.0mL vials in the transport box with divider.
- Close the now-empty stool sample collection vial, put it back in the biohazard bag, and dispose it in the biohazard waste bin.
- Clean up work station, dispose the drape, and wipe down the table top with disinfectant wipe.
- Remove PPE and wash hands with soap and water thoroughly.
- Bring the transport box to C454 where the -80 °C freezer is located (key in BABLab Lock Box).
- Place the 2.0mL vials in their designated space in the freezer box (in accordance to the Sample Storage Log Diagram).
- Log the sample in the Sample Storage Log.

4.6.4 Protocol - Data Entry & Quality

4.6.4.1 Data Entry

4.6.4.2 Data Quality

4.6.5 Protocol - Data Review & Audit

4.6.5.1 Follow-Up (completed by Scheduling Coordinator)

- Before sending Home Reminder 3, make sure RA's have completed Data Entry, Data Quality Check 1, and Data Quality Check 2.
- After sending Home Reminder 3 - create blank Trello card for participant on "In Data Review" list of Data Audit Board.

4.6.5.2 Data Review

- Once card has been created, do Data Review.
- Checking for completion of:
 - child questionnaires (see child questionnaire table)
 - parent proxy questionnaires (see parentproxy questionnaire table)
 - parent self questionnaires (see parentself questionnaire table)
 - hair sample
 - saliva sample
 - stool sample
 - bss sheet
 - contact sheet
 - halloween delay test
 - height, weight, waist
 - PC interaction video
 - halloween training and test data captured
- After completing Data Review, move card to Good Sample, Bad Sample, or No Sample list based on the stool sample.

4.6.5.3 Data Audit

If Good Sample:

- Send payment, thank you letter, certificate, and science kits via mail
- Send [MBB - PAID] email and attach thank you letter, certificate, and parent report (including outstanding items).
- Move to Paid list.
- One week after payment is sent, check outstanding items and do Audit Call #1.
- One week following Audit Call #1, check outstanding items do Audit Call #2.
- Within 2 days, check outstanding items and do Audit Call #3.

- After Audit Call #3 (or all items completed), send [MBB - DONE] email and move to Done list.
- If participant completes item on list, check card on Trello, mark off on participation log and data check sheets, and note in participant's README in data folder.

If Bad Sample:

- Send payment, thank you letter, certificate, and science kits via mail. Include a new stool sample kit.
- Send [MBB - PAID] email and attach thank you letter, certificate, and parent report (including outstanding items - emphasize stool sample kit).
- Move to Paid list in Trello.
- One week after payment is sent, check outstanding items do Audit Call #1.
- One week following Audit Call #1, check outstanding items do Audit Call #2.
- Within 2 days, check outstanding items and do Audit Call #3.
- After Audit Call #3 (or all items completed), send [MBB - DONE] email and move to Done list.
- If participant completes item on list, check card on Trello, mark off on participation log and data check sheets, and note in participant's README in data folder.

If No Sample:

- Send [MBB - UNPAID] email and attach thank you letter, certificate, and parent report (including outstanding items - emphasize stool sample kit).
- Leave participant on Unpaid list.
- If stool sample received - send payment, thank you letter, certificate, and science kits via mail and move to Paid list.
- If stool sample not received, one week after email is sent, check outstanding items do Audit Call #1.
- One week following Audit Call #1, check outstanding items do Audit Call #2.
- Within 2 days of Audit Call #2, check outstanding items and do Audit Call #3.
- After Audit Call #3 (or all items completed), send [MBB - DONE] email and move to Done list.
- If participant completes item on list, check card on Trello, mark off on participation log and data check sheets, and note in participant's README in data folder.

4.6.5.3.1 Getting A Code From REDCap

1. Log onto REDCap and click on "record status dashboard"

Since this project is currently in **PRODUCTION**, changes will not be made in real time. [Tell me more](#)

Instrument name	Fields	View PDF	Enabled as survey	Instrument actions
participant	11			Choose action
screen	51			Choose action
wave1_status	10			Choose action
wave2_status	3			Choose action
wave3_status	3			Choose action
checklist_lab_session_child	85			Choose action
checklist_lab_session_parent	14			Choose action

2. Click on designated participant

Participant ID	info			
	participant	checklist_lab_session_child	checklist_lab_session_parent	checklist_home_session_cl
MBB011				
MBB012				
MBB013				
MBB014				
MBB015				
MBB016				
MBB017				
MBB018				

3. Click on the first incomplete questionnaire for the parentself, parentproxy, or child questionnaire sets

Data Collection Instrument	consent	info	lab_child	lab_parentproxy	lab_parentself	home_child
memory_invasion			green	orange		
wasi			green	green		
wiat			green	green		
intro_parentproxy (survey)				green	orange	
acuteillness_parentproxy (survey)				red	red	
pedsql_gi_parentproxy (survey)				red	red	
pedsql_wb_parentproxy (survey)				red	red	
pedsql_f_parentproxy (survey)				red	red	
easy_parentproxy (survey)				red	red	
radc_parentproxy (survey)				red	red	
leq_parentproxy (survey)				red	red	
tesl_parentproxy (survey)				red	red	
scared_parentproxy (survey)				red	red	
rcads_parentproxy (survey)				red	red	
cbcl_parentproxy (survey)				red	red	
cshq_parentproxy (survey)				red	red	
bmcmriscreen_parentproxy (survey)				red	red	
mb_metadata_parentproxy (survey)				red	red	
med_check_parentproxy (survey)				red	red	
pds_parentproxy (survey)				red	red	
dhws_parentproxy (survey)				red	red	
hpq_parentproxy (survey)				red	red	
cssl_parentproxy (survey)				green	red	
fri_parentproxy (survey)						

4. Click on Survey Options

ALLIOTS: [Download PDF of instrument\(s\)](#) [Share instrument in the Library](#) [VIDEO: Basic Data Entry](#) [-- Cancel --](#)

acuteillness_parentproxy

Invitation status: [Email](#)

Editing existing Participant ID MBB014

Event Name: **lab_parentproxy (Arm 2: wave_1)**

Participant ID MBB014

Has your child been sick in the past two weeks? Yes No

Form Status

Complete? [Incomplete](#)

Lock this record for this form?
If locked, no user will be able to edit this record on this form until someone with Lock/Unlock privileges unlocks it. [Lock](#)

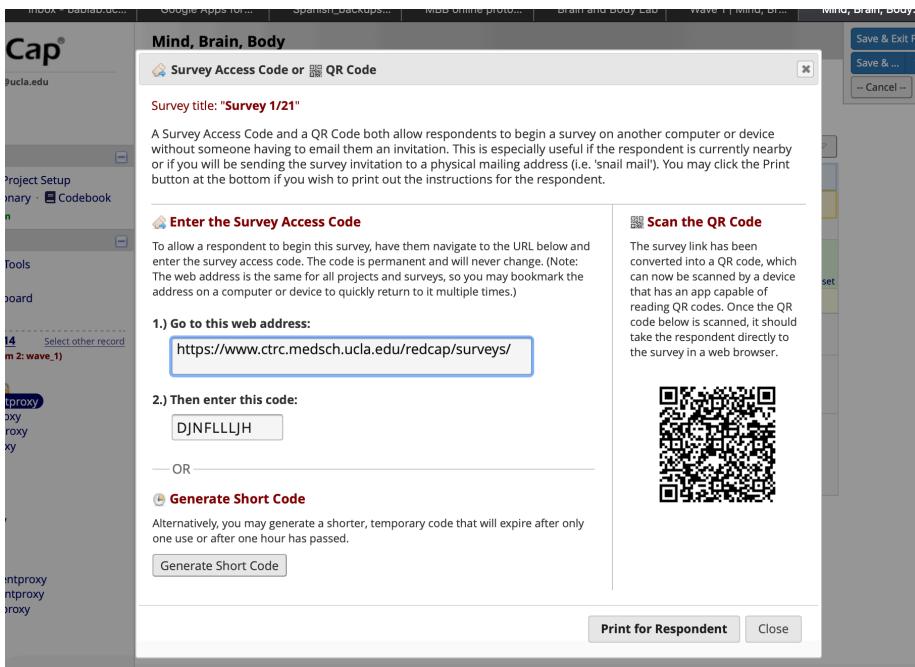
[Save & Exit Form](#) [Save & ...](#) [-- Cancel --](#)

Survey options

- [Open survey](#)
- [Log out + Open survey](#)
- [Compose survey invitation](#)
- [Survey Access Code and QR Code](#)

[reset](#)

5. Click Survey Access Code and QR Code



6. Copy and paste web address and code + send to email to participant

4.6.6 Protocol - Report Card

1. Open a participant data folder

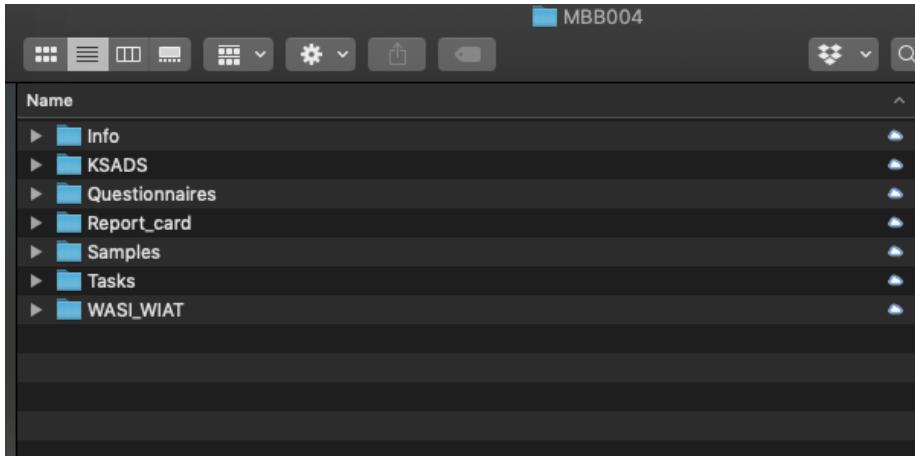


Figure 4.9:

2. Navigate to the report card folder and rename the template file - MBB999 to the relevant participant - and open the file

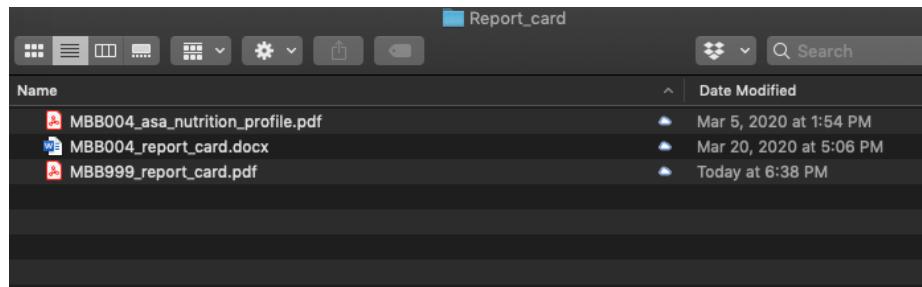


Figure 4.10:

3. Navigate to the last pge of the pdf and fill in the scores for this participant.
You can type directly on the page- it is a fillable form.

4. After you have entered teh data, it should look like this:

photo coming soon

5. If there are any comments, enter them on the comments page.

- For example, if any NA's are present due to less than 70% of data for that subset being available to calculate a score - note that here.

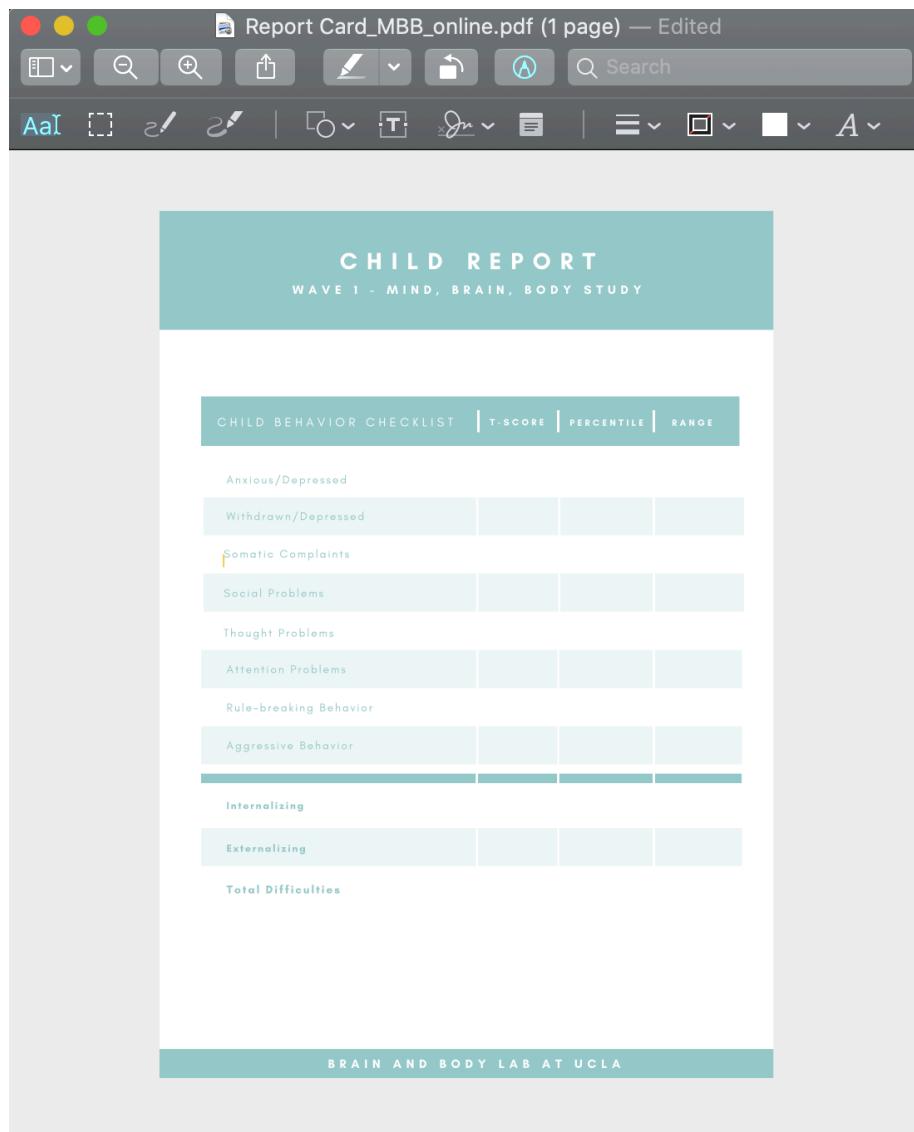


Figure 4.11:

COMMENTS

- If there are no comments, delete this page.
6. **Important-** Once you have completed the edits to the pdf, you must follow these steps to “lock” the data so that it is no longer editable before sending to the participant. To do so, click file/print/PDF/Save as PDF. Save the PDF to your desktop, then replace the original PDF with the desktop version.
7. The report card is now ready to be sent to the participant.

4.6.7 Protocol - Payment

Payment package contents:

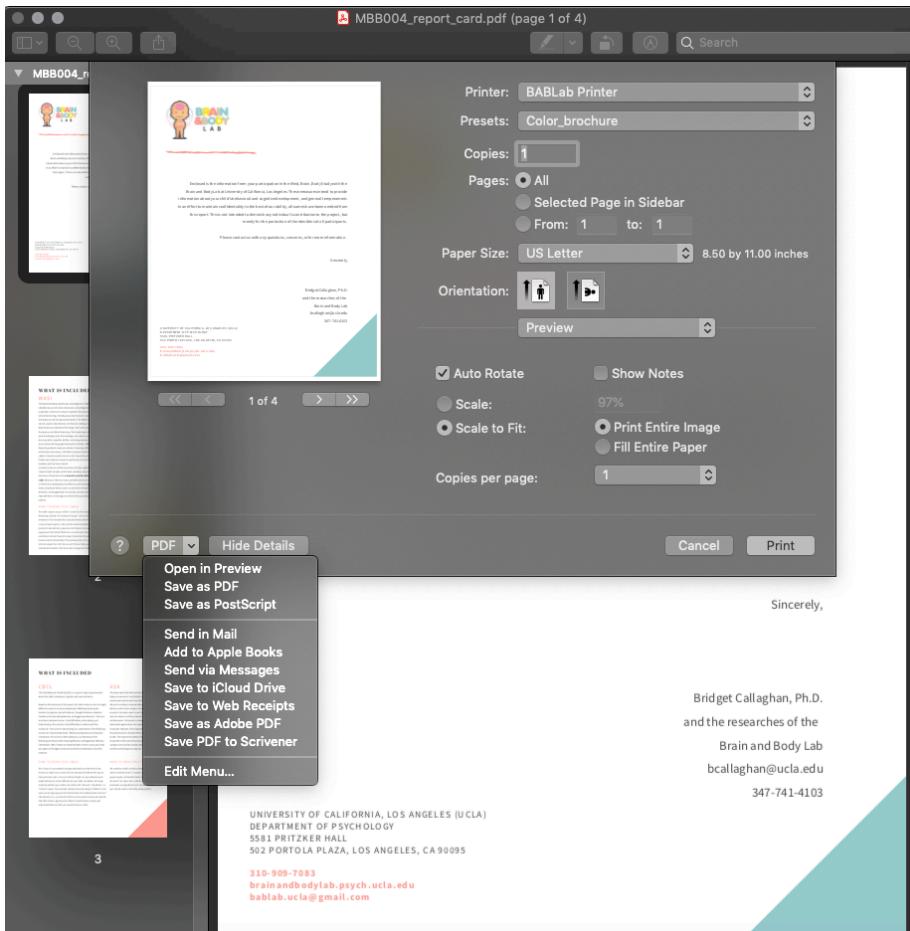


Figure 4.12:

- Payment box
- Type in participant's name and print copy of certificate
- Print thank you letter
- Include \$65 Amazon gift card
- Check stool sample quality- if poor, send another stool kit
- Science kit- Neuron
 - 4 pipe cleaners
 - Pipe Cleaner Neuron photo directions quarter sheets
 - goodie bag + tie
- Science kit- Brain hat
 - left + right side brain hat sheet
 - Brain hat photo directions quarter sheets
 - plastic sheet cover
- Science kit- Petri Dish
 - petri dish sheet
 - Microbiome photo directions quarter sheets
 - plastic sheet cover
 - virus stickers

Mailing payment package

- Once the package has been created and sealed, it is time to bring the package down to Tyler's office in the Psychology building.
- To mail the package to the participant, you will need the following information:
 - Recharge ID
 - Participant name
 - Participant mailing address
- From Tyler's office, you will receive a FedEx label in which you can write this information
- Take a picture of the FedEx label and upload to Box
- Leave the package in Tyler's office for FedEx pickup
- Send payment confirmation email to participants

Recording Payment

- Log participant payment in reimbursement log book
 - Log participant payment in reimbursement spreadsheet
-

Chapter 5

Wave 1 SONA

5.1 Summary

Additional data collection of the 2 part Halloween task (for immediate and delayed testing of the memory task) will be taken with participants aged 18+. We project collecting from 250 participants (due to potential attrition of the 2 part task) and will compensate participants with school credit on the SONA system.

5.2 Measures

5.2.1 Questionnaires

Title	Description	Reference
Demographic Questionnaire	Adult self-report. Domain assessed: demographics. The project developed questionnaire asks participants about their household income, their race/ethnicity, the parent age, education, and marital status, and contact details.	(ABC, XXXX)

Title	Description	Reference
Revised Childhood Traumatic Experiences Survey (cte_revised) – 18+ years of age.	Adult report. Domain assessed: Traumatic Experiences. The Childhood Trauma Questionnaire is a brief 6 item survey of retrospectively-reported traumatic experiences (death, divorce, violence, sexual abuse, illness, and upheaval).	(ABC, XXXX)

Bibliography

- Acheson, D. T., Gresack, J. E., and Risbrough, V. B. (2012). Hippocampal dysfunction effects on context memory: Possible etiology for posttraumatic stress disorder. *Neuropharmacology*, 62(2):674–685.
- Bailey, M. T. and Coe, C. L. (1999). Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. *35(2)*:146–155.
- Bailey, M. T., Dowd, S. E., Parry, N. M. A., Galley, J. D., Schauer, D. B., and Lyte, M. (2010). Stressor Exposure Disrupts Commensal Microbial Populations in the Intestines and Leads to Increased Colonization by *Citrobacter rodentium*. *Infection and Immunity*, 78(4):1509–1519.
- Brumec, I. K., Bellana, B., Ozubko, J. D., Man, V., Robin, J., Liu, Z.-X., Grady, C., Rosenbaum, R. S., Winocur, G., Barense, M. D., and Moscovitch, M. (2018). Multiple Scales of Representation along the Hippocampal Anteroposterior Axis in Humans. *Current Biology*, 28(13):2129–2135.e6.
- Callaghan, B. L., Cowan, C. S. M., and Richardson, R. (2016). Treating Generational Stress: Effect of Paternal Stress on Development of Memory and Extinction in Offspring Is Reversed by Probiotic Treatment. *Psychological Science*, 27(9):1171–1180.
- Callaghan, B. L., Fields, A., Gee, D. G., Gabard-Durnam, L., Caldera, C., Humphreys, K. L., Goff, B., Flannery, J., Telzer, E. H., Shapiro, M., and Tottenham, N. (2019). Mind and gut: Associations between mood and gastrointestinal distress in children exposed to adversity. *Development and Psychopathology*, pages 1–20.
- Callaghan, B. L. and Richardson, R. (2012). The effect of adverse rearing environments on persistent memories in young rats: Removing the brakes on infant fear memories. *Translational Psychiatry*, 2(7):e138.
- Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., Dinan, T. G., and Cryan, J. F. (2013). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry*, 18(6):666–673.
- Collins, S. M., Kassam, Z., and Bercik, P. (2013). The adoptive transfer of

- behavioral phenotype via the intestinal microbiota: Experimental evidence and clinical implications. 16(3):240–245.
- Cowan, C. S. M., Callaghan, B. L., and Richardson, R. (2013). Acute early-life stress results in premature emergence of adult-like fear retention and extinction relapse in infant rats. 127(5):703–711.
- Cowan, C. S. M., Callaghan, B. L., and Richardson, R. (2016). The effects of a probiotic formulation (*Lactobacillus rhamnosus* and *L. helveticus*) on developmental trajectories of emotional learning in stressed infant rats. *Translational Psychiatry*, 6(5):e823.
- Frank, M. J., Seeberger, L. C., and O'Reilly, R. C. (2004). By Carrot or by Stick: Cognitive Reinforcement Learning in Parkinsonism. *Science*, 306(5703):1940–1943.
- Gareau, M. G., Wine, E., Rodrigues, D. M., Cho, J. H., Whary, M. T., Philpott, D. J., Macqueen, G., and Sherman, P. M. (2011). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut*, 60(3):307–317.
- Godsil, B. P., Kiss, J. P., Spedding, M., and Jay, T. M. (2013). The hippocampal-prefrontal pathway: The weak link in psychiatric disorders? *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology*, 23(10):1165–1181.
- Holbein, C. E., Zebracki, K., and Holmbeck, G. N. (2014). Development and validation of the Peer Interaction Macro-Coding System Scales (PIMS): A new tool for observational measurement of social competence in youth with spina bifida. *Psychological Assessment*, 26(4):1235–1246.
- Holmbeck, G., Belvedere, M., Gorey-Ferguson, L., and Schneider, J. (1995). Manual for family macro-coding. *Unpublished manual*.
- Huang, C.-C., Chou, P.-H., Yang, C.-H., and Hsu, K.-S. (2005). Neonatal isolation accelerates the developmental switch in the signalling cascades for long-term potentiation induction. *The Journal of Physiology*, 569(Pt 3):789–799.
- Jovanovic, T., Nylocks, K. M., Gamwell, K. L., Smith, A., Davis, T. A., Norrholm, S. D., and Bradley, B. (2014). Development of fear acquisition and extinction in children: Effects of age and anxiety. *Neurobiology of Learning and Memory*, 113:135–142.
- Kennedy, P. J., Cryan, J. F., Dinan, T. G., and Clarke, G. (2014). Irritable bowel syndrome: A microbiome-gut-brain axis disorder? *World Journal of Gastroenterology : WJG*, 20(39):14105–14125.
- Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., and Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. 62(6):593–602.

- Lambert, H. K., Peverill, M., Sambrook, K. A., Rosen, M. L., Sheridan, M. A., and McLaughlin, K. A. (2019). Altered development of hippocampus-dependent associative learning following early-life adversity. *Developmental Cognitive Neuroscience*, 38:100666.
- MacPhillamy, D. J. and Lewinsohn, P. M. (1982). The pleasant events schedule: Studies on reliability, validity, and scale intercorrelation. *Journal of Consulting and Clinical Psychology*, 50(3):363–380.
- Norholm, S. D., Jovanovic, T., Olin, I. W., Sands, L. A., Karapanou, I., Bradley, B., and Ressler, K. J. (2011). Fear Extinction in Traumatized Civilians with Posttraumatic Stress Disorder: Relation to Symptom Severity. *Biological Psychiatry*, 69(6):556–563.
- Norholm, S. D., Jovanovic, T., Vervliet, B., Myers, K. M., Davis, M., Rothbaum, B. O., and Duncan, E. J. (2006). Conditioned fear extinction and reinstatement in a human fear-potentiated startle paradigm. *Learning & Memory*, 13(6):681–685.
- O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A.-M., Quigley, E. M., Cryan, J. F., and Dinan, T. G. (2009). Early Life Stress Alters Behavior, Immunity, and Microbiota in Rats: Implications for Irritable Bowel Syndrome and Psychiatric Illnesses. *Biological Psychiatry*, 65(3):263–267.
- Phelps, E. A., Delgado, M. R., Nearing, K. I., and LeDoux, J. E. (2004). Extinction Learning in Humans: Role of the Amygdala and vmPFC. *Neuron*, 43(6):897–905.
- Repetti, R. L., Taylor, S. E., and Seeman, T. E. (2002). Risky Families: Family Social Environments and the Mental and Physical Health of Offspring. page 37.
- Richmond, S., Schwartz, O., Johnson, K. A., Seal, M. L., Bray, K., Deane, C., Sheeber, L. B., Allen, N. B., and Whittle, S. (2018). Exploratory Factor Analysis of Observational Parent–Child Interaction Data. *Assessment*, page 1073191118796557.
- Schiller, D., Kanen, J. W., LeDoux, J. E., Monfils, M.-H., and Phelps, E. A. (2013). Extinction during reconsolidation of threat memory diminishes prefrontal cortex involvement. *Proceedings of the National Academy of Sciences*, 110(50):20040–20045.
- Shalev, L., Paz, R., and Avidan, G. (2018). Visual Aversive Learning Compromises Sensory Discrimination. *The Journal of Neuroscience*, 38(11):2766–2779.
- Staufenbiel, S. M., Penninx, B. W. J. H., Spijker, A. T., Elzinga, B. M., and van Rossum, E. F. C. (2013). Hair cortisol, stress exposure, and mental health in humans: A systematic review. *Psychoneuroendocrinology*, 38(8):1220–1235.

- Tambini, A., Ketz, N., and Davachi, L. (2010). Enhanced Brain Correlations during Rest Are Related to Memory for Recent Experiences. *Neuron*, 65(2):280–290.
- Zlomuzica, A., Dere, D., Machulska, A., Adolph, D., Dere, E., and Margraf, J. (2014). Episodic Memories in Anxiety Disorders: Clinical Implications. *Frontiers in Behavioral Neuroscience*, 8.