Mind, Brain, Body

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Chapter 1

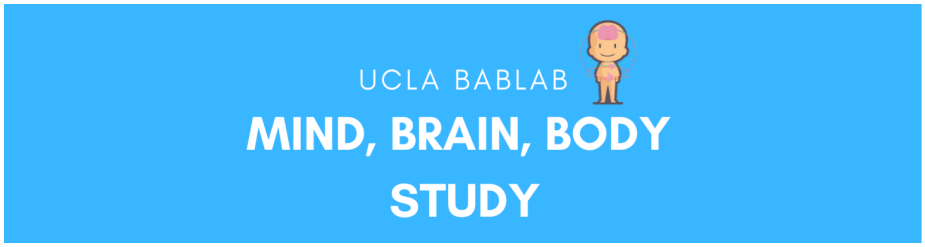


Figure 1.1:

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, hippocampal 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 individuals 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.

2.1.4 Contributions

Study was designed by Dr. Bridget Callaghan.

Database formation, REDCap creation, task development, and setup was carried out by Dr. Bridget Callaghan, Emily Towner, Kristen Chu, Eason Taylor, Francesca Querdasi, Aileen Gozali, and Danielle Ladensack, with assistance from Alyssa Wieand, Reese Wix, and Nicole Fonacier.

Wiki Protocol development and modification was conducted by Dr. Bridget Callaghan, Kristen Chu, Emily Towner, Francesca Querdasi, Maria Calderon, and Chloe Schwartz.

Recruitment was carried out by Dr. Bridget Callaghan, Kristen Chu, Emily Towner, Sienna Osadon, Deborah Banner, Francesca Querdasi, Eason Taylor, Aileen Gozali, Danielle Ladensack, Maria Calderon, Charis Stanek, Megan Ngai, Ananya Eeraveni, Alyssa Wieand, Reese Wix, Nicole Fonacier, Leti Herrera, Grant Grech, Keegan Buch, Genesis Flores, Daisy Ramirez, Alyssa Ortega, Tiffany Nassirian, Yash Mehta, Lorena Gonzalez, and Andre Chan.

Scheduling/contacting participants/maintaining participant databases was carried out by Danielle Ladensack, Aileen Gozali, Maria Calderon, Charis Stanek, Tiffany Nassirian, Sienna Osadon, Deborah Banner, Yash Mehta, Madelyn Robinson, Andrew Hanna, Lorena Gonzalez, Kristen Chu, Emily Towner, Eason Taylor, and Anna Bretz.

Primary Data Collection for Wave 1 (in person) was carried out by Dr. Bridget Callaghan, Emily Towner, Eason Tayler, Aileen Gozali, Danielle Ladensack, and Kristen Chu with assistance from Alyssa Wieand, Reese Wix, and Nicole Fonacier.

Primary Data Collection for Wave 1 (online) was carried out by Francesca Querdasi, Kristen Chu, Maria Calderon, Charis Stanek, Deborah Banner, Sienna Osadon, Yash Mehta, and Andrew Hanna.

Primary Data Collection for Wave 2 (online) was carried out by Dr. Bridget Callaghan, Francesca Querdasi, Naomi Gancz, Kristen Chu, Yash Mehta, Andrew Hanna, Deborah Banner, Sienna Osadon, Madelyn Robinson, Lorena Gonzalez, and Anna Bretz.

Data Entry and Data Quality checks were carried out by Kristen Chu, Emily Towner, Francesca Querdasi, Eason Taylor, Alyssa Ortega, Megan Ngai, Daniel Huang, Aileen Gozali, Danielle Ladensack, Maria Calderon, Charis Stanek, Deborah Banner, Sienna Osadon, Andre Chan, Keegan Buch, Yash Mehta, Lorena Gonzalez, Madelyn Robinson, Genesis Flores, Rory Simpson, Ananya Eeraveni, Alyssa Wieand, Reese Wix, Nicole Fonacier, Leti Herrera, Grant Grech, Daisy Ramirez, Elizabeth Harty, and Tiffany Nassirian.

Data Reviews and Data Audits were conducted by Kristen Chu, Emily Towner, Deborah Banner, Yash Mehta, Madelyn Robinson, Danielle Ladensack, Chloe Schwartz, and Tiffany Nassirian.

Behavioral coding was carried out by Rory Simpson, Elizabeth Harty, Danielle Ladensack, Grant Grech, Genesis Flores, Daniel Huang, and Nicole Fonacier. Dr. Bridget Callaghan and Kristen Chu are also validated behavioral coders using the Family Interaction Macro-Coding System (FIMS).

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

Pleasant Events Checklist	Issues Checklist
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
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

Pleasant Events Checklist	Issues Checklist
	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 sensical 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.

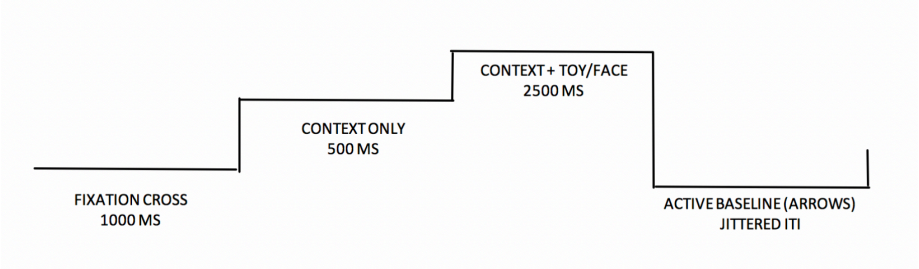


Figure 2.1:

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 and 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+ and 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:

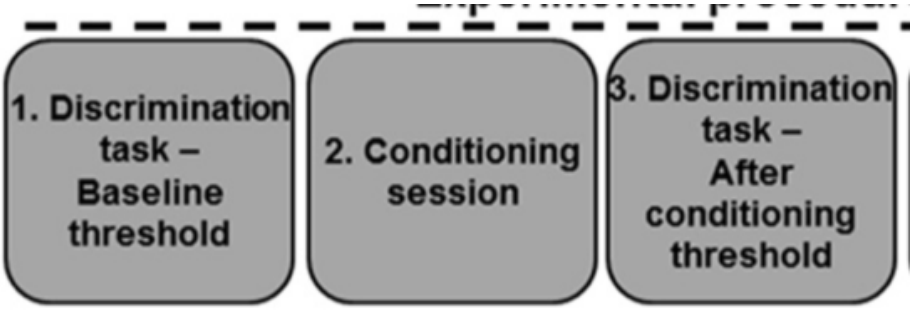
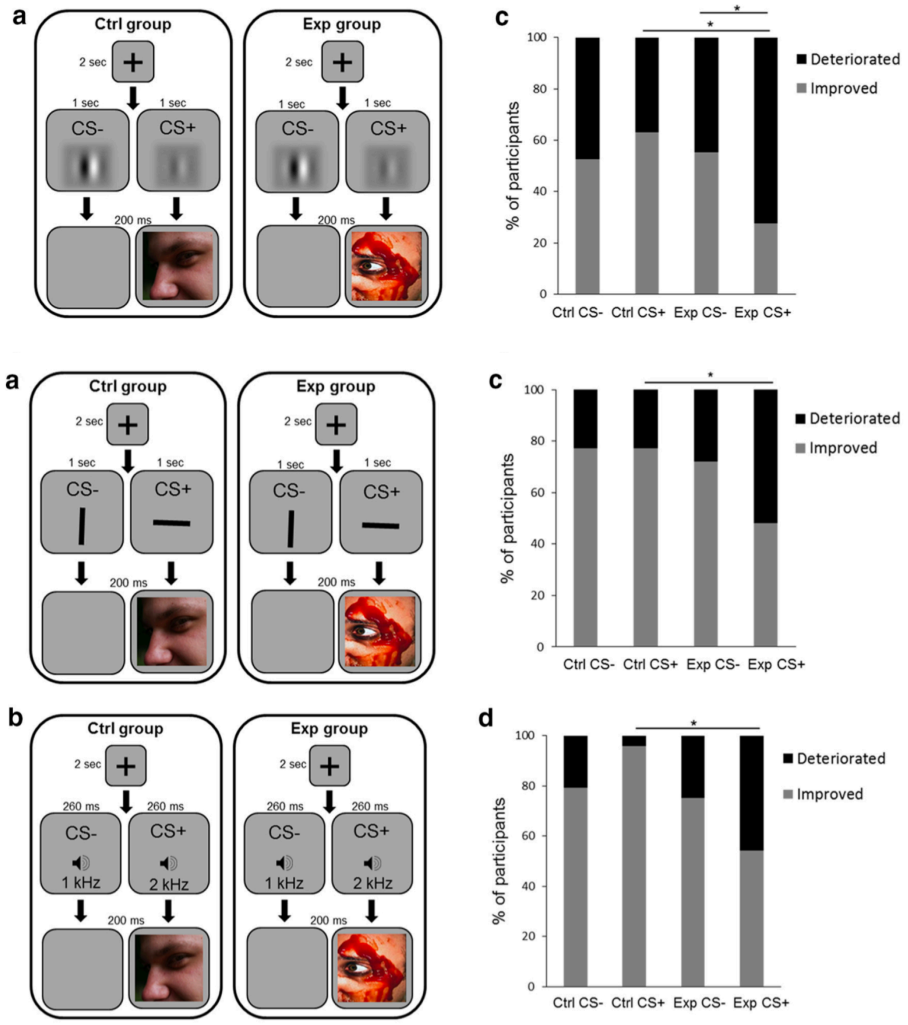


Figure 2.2:

The task and results:

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 gabors 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.

The timeline of the task will be as follows:

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

The experimental protocol consisted of two phases: fear acquisition and extinction. The sessions were separated by 10 minutes. The acquisition phase

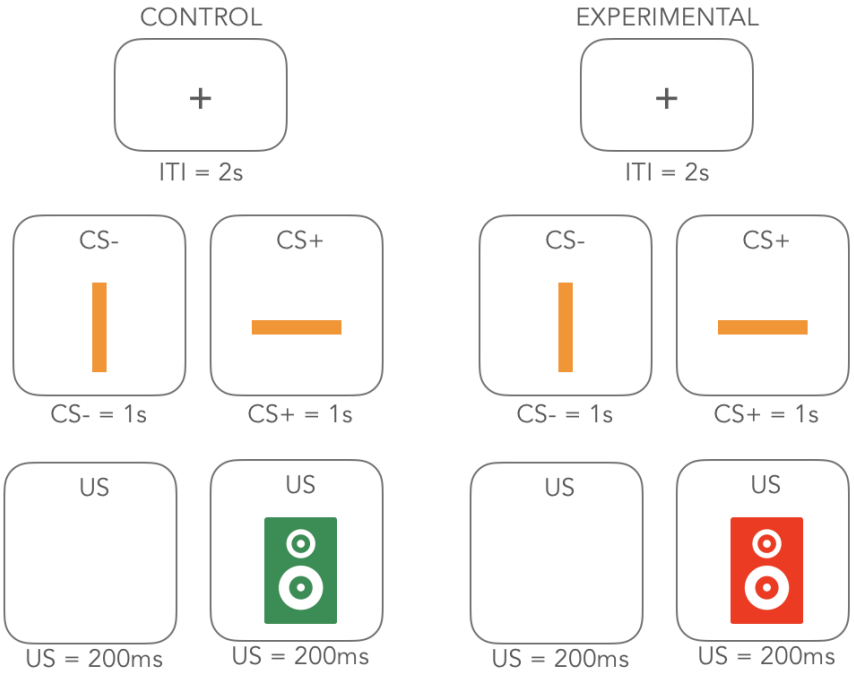


Figure 2.3:

Figure 2.4:

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 microbiome 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.

Hair samples were processed and analyzed according to the methods described in Meyer et al. (2014) with minor modifications. Briefly, each sample was weighed, washed twice with isopropanol to remove external contaminants, and then air-dried. Washed samples were ground to a fine powder using a bead mill, extracted overnight into methanol, and centrifuged to spin down the beads and the powdered hair. An aliquot of the methanol extract was transferred to a clean tube, dried using a vacuum evaporator, and then reconstituted in assay buffer. Reconstituted extracts were spin-filtered to remove any residual particulate material, then assayed in duplicate along with standards and quality controls using the Arbor Assays DetectX Cortisol ELISA kit. Intra- and inter-assay coefficients of variation for this assay are both <10%

2.3.7.3 Blood sample

Dried Blood Spot Collection (in-person): 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.

Tasso DBS collection (over zoom): Tasso-M20 kits were used during Wave 2 to collect dried blood spots from consenting children remotely over Zoom. Kits are shipped to participants with their magic box, families are instructed on how to use them to collect DBS during session 2, and then the samples are shipped back to our lab within 24 hours of collection (see Wave 2 protcol for more information on participant collection). Drying the blood spots promptly and fully is important for maintaining integrity of the RNA and cytokines contained within it. The Tasso kits contain several features that facilitate quick drying of the blood spots, including a tab the participant pulls to release a drying accelerant, and a dessicant pack inside the bag the participant sends the sample back in that dries out the air inside; thus, leaving the sample out to dry is not needed. In terms of measures of circulating cytokines from the DBS, Tasso said that how long we could store the samples at room temperature depends on the analyte. Not much has been published on the stability of inflammatory cytokines in DBS at room temperature; most studies have been with serum blood collected by venipuncture. Some cytokines (e.g., TNF-alpha) are unstable in serum stored at room temperature, but all are stable in serum stored in a refrigerator. The single study that investigated stability of DBS CRP found it to be stable at ambient temperature (21 degrees C) for up to 1 week (Brindle et al., 2019). However, research does show that refrigerating or freezing samples promptly after drying is always advisable to minimize the chances of degradation, even though the stability of most analytes in DBS provides flexibility in the collection of samples in field settings (McDade et al., 2007). That is why our protocol instructs participants to send back the Tasso kits within 24 hours of sample 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.

Note: On Nov 16, 2020 there was a product change notification sent by DNA Genotek regarding the OMNIgene • GUT (OM/R-200, OM/R-200.100) and PERFORMAbiome • GUT (PB-200) devices. In brief, DNA Genotek removed "Made in Canada" from their packaging and updated the lot information (reflected in the barcode), to allow for an increase in manufacturing sites sand production lots sizes. This is to note that different sites may have made the omnigene packages we use in different study waves. Please see here for more information: product_change_notification

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2.3.8 Questionnaires

2.3.8.1 Child

Title	Description	Reference	Respondent	Wave	Version
Alexithymia	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)	Child/adolescent (self report)	Wave 1, Wave 1 On-line	
Children's Perception of Interparental Conflict Scale (cpic)– 6-18 years	Domains assessed: parenting and family structure. The CPIC assesses children's/adolescent's experience of parental conflict, including subscales (Conflict Properties, Threat, Self-Blame).	(Grych, Seid, & Fincham, 1992)	Child/adolescent (self report)	Wave 1, Wave 1 On-line	

Title	Description	Reference	Respondent	Wave	Version
Child Somatization Symptom Inventory (cssi)– 6-17 years	Parent report for children under 8 years. Domain assessed: physical symptoms. The CSSI assesses a variety of nonspecific somatic symptoms.	(Walker et al., 2009)	Child/adolescent (self report)	Wave 1, Wave 1 On-line	
Security Scale (ss) – 8-18 years	Domain assessed: attachment. This measure asks children/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.	(Kerns et al., 2001)	Child/adolescent (self report)	Wave 1, Wave 1 On-line	

Title	Description	Reference	Respondent	Wave	Version
Benevolent Childhood Experiences Scale-Revised (bce)	Child/adolescent 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.	(Narayan et al., 2018)	Child/adolescent (self report)	Wave 1 Online	
Visceral Sensitivity Index (VSI)					

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.	(Beck, Steer, & Brown, 1996)	Parents (self report)	Wave 1, Wave 1 On-line	
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.	(Made by BABLab)	Parents (self report)	Wave 1 On-line	

Title	Description	Reference	Respondent	Wave	Version
Financial Hardship	This 2-item questionnaire asks how often in the past 12 months the participant was worried or stressed about having enough money to pay rent/mortage and to buy nutritious meals.	(Jachimowicz et al., 2020)	Parents (self report)	Wave 1 online	
Community Financial Support	This 3-item questionnaire assesses the degree that participants feel they can rely on others in their community for financial support if needed.	(Jachimowicz et al., 2020)	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.	(Made by BABLab)	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.	(Varni et al., 2015)	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.	(Varni et al., 2015)	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.	(Varni et al., 2015)	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.	(Pate et al., 2018)	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 physical/sexual abuse and neglect.	(Ippen et al., 2002)	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.	(Felitti et al.,)	Parents (proxy)	Wave 1, Wave 1 Online	
Child Sleep Habits Questionnaire (csqh)	Sleep. Multidimensional sleep assessment including sleeping difficulties, behavioral problems around sleep etc.	(Owens, Spirito, & McQuinn, 2000)	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).	(Made by BABLab)	Parents (proxy)	Wave 1, Wave 1 On-line	

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.	(Made by BABLab)	Parents (proxy)	Wave 1, Wave 1 Online	

Title	Description	Reference	Respondent	Wave	Version
Petersen Physical Development Scales (pds)	The Peterson Puberty Scale 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.	(Petersen et al., 1988)	Parents (proxy)	Wave 1, Wave 1 On-line	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.	(Holoski et al., 2019)	Parents (proxy)	Wave 1, Wave 1 On-line	

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.	(Made by BABLab)	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.	(Walker et al., 2009)	Parents (proxy)[children < 8 years]	Wave 1, Wave 1 Online	
Foster Care Inventory (fci)	This instrument was made for the current study and assesses the number of foster care placements and care history.	(Made by Tottenham Lab)	Parents (proxy)[adopted only]	Wave 1, 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.	(Made by Tottenham Lab)	Parents (proxy)[adopted only]	Wave 1, Wave 1 Online	

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.	(Made by BABLab)	Parents (proxy)	Wave 1, Wave 1 On-line	

Title	Description	Reference	Respondent	Wave	Version
Bristol Stool Scale (bss)	Stool sample short questionnaire: 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.	(Developed by Bristol Royal Infirmary)	Parent (proxy)	Wave 1, Wave 1 On-line	
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.	(Made by BABLab)	Parents (proxy)	Wave 1 on-line	

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.	(Made by BABLab)	Parents (proxy)	Wave 1 online	

2.3.8.3 Qualitative

2.3.8.3.1 COVID-19 Written Responses

Title	Reference	Respondent	Wave	Version
COVID-19 Written Response (written_response_parentself)	(Pennebaker, 1997)	Parents (self report)	Wave 1 Online [optional]	

Domain assessed: Emotional impacts of 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.

Title	Reference	Respondent	Wave	Version
COVID-19 Written Response (written_response)	(Pennebaker, 1997)	Children (self report)	Wave 1 Online [optional]	

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.

Chapter 3

Wave 1

3.1 W1 Checklists

3.1.1 W1 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 W1 Checklist - Pre-Lab-Session

3.1.2.1 W1 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 and Wave (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 W1 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 W1 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 W1 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

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3.1.5 W1 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

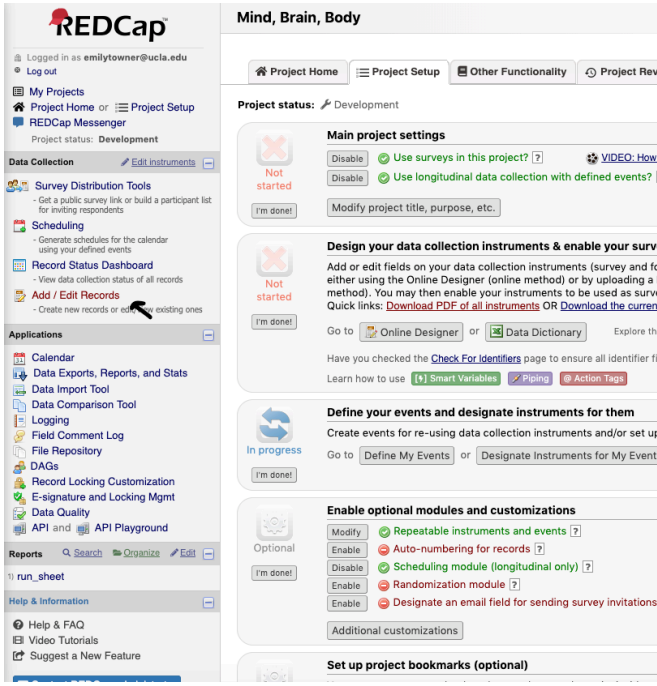
3.2 W1 Protocols - Pre-Session

3.2.1 W1 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

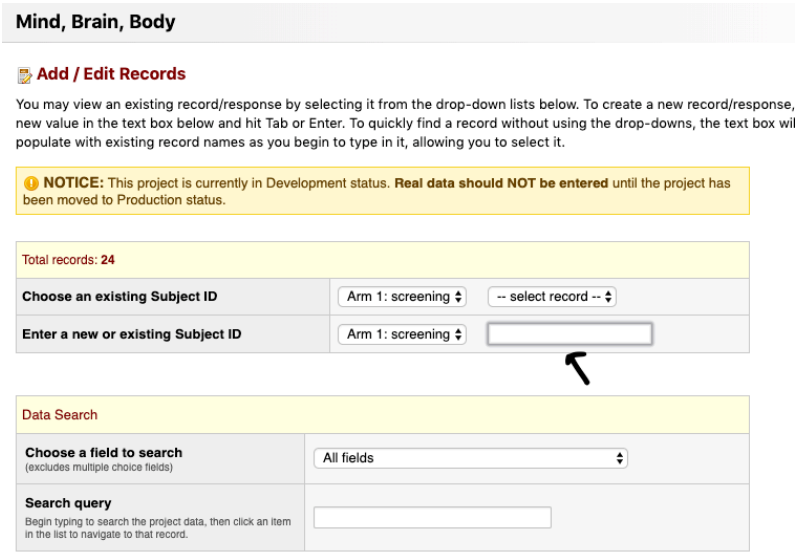


1. To screen a new participant click “Add / Edit Records”
2. Click to enter a new Subject ID

   - 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

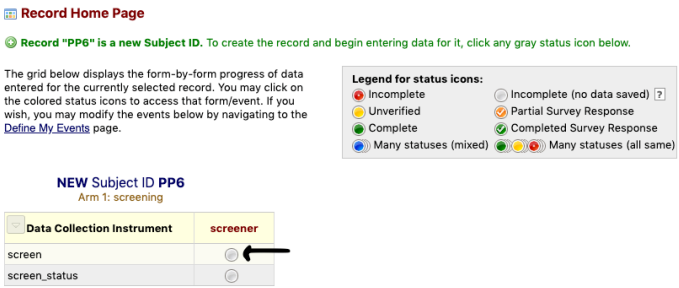


a new record

4. The screening arm contains two parts

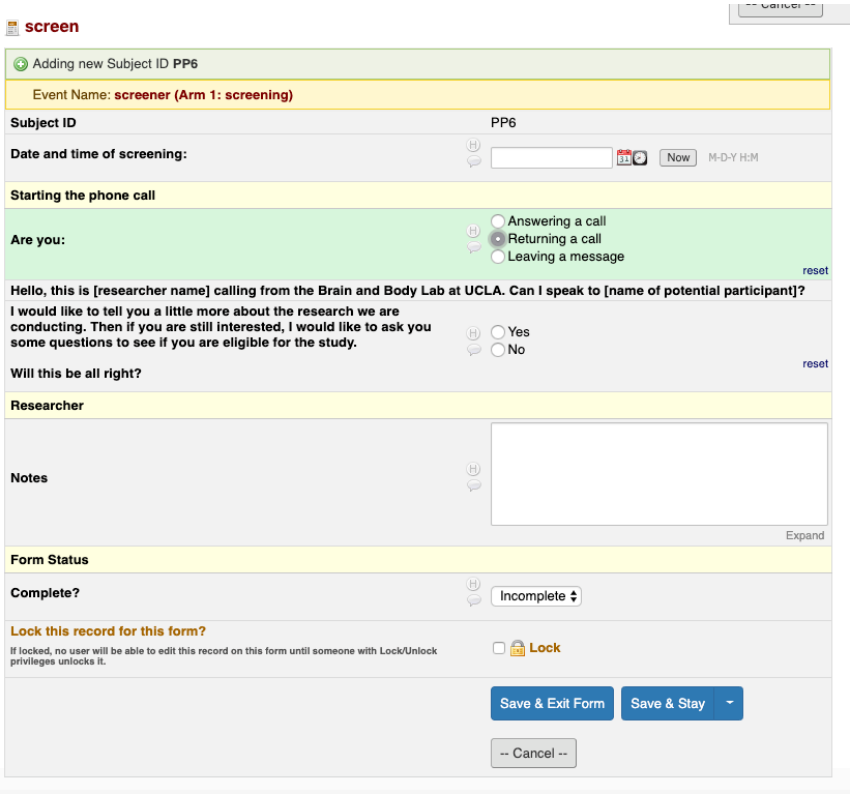
   - The screen
   - The wave1_status

     – The wave1_status is to be updated after the first and each subse-



quent contact

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

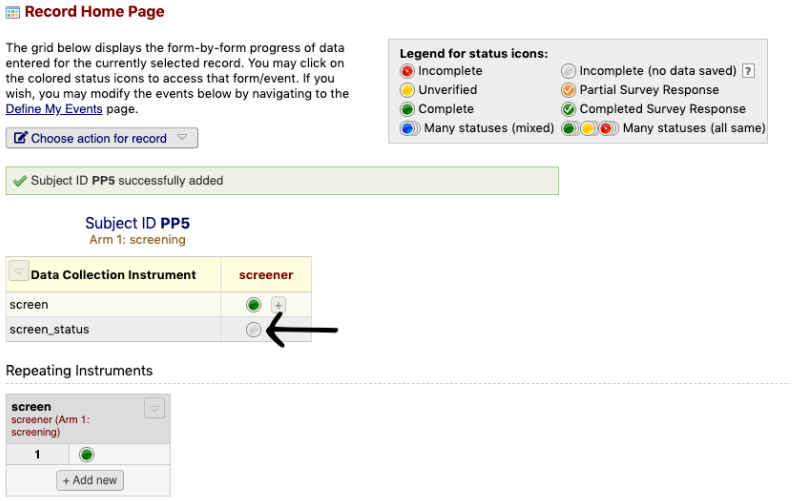


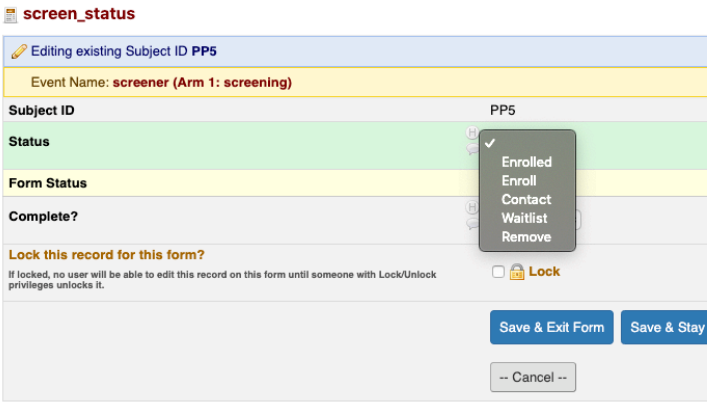
6. Click “Now” to enter today’s date and time
7. Select the appropriate choice to start the phone call and follow the skip logic
8. Follow the skip logic to the end

    - 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



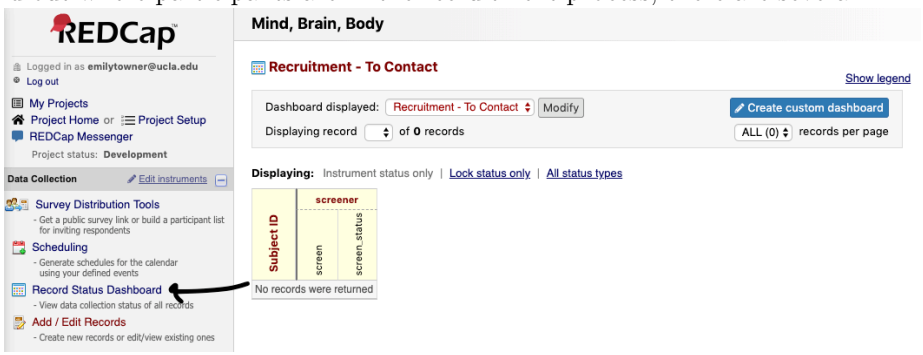


10. Click the screen_status radio button
11. Select the appropriate option
    - Contact - Participant needs to be re-contacted (add Recruitment Database & ID Drive)
    - Ineligible - Participant not eligible for study
    - To Enroll - Participant to enroll (need to create subject ID, enter subject 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.



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 W1 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 W1 Protocol - Home Kit Assembly

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

3.3 W1 Protocols - Parent

3.3.1 W1 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.

Figure 3.1:

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 W1 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. Participants should complete this activity in English.

Step 1:

Figure 3.2:

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 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 W1 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.

Figure 3.3:

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

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

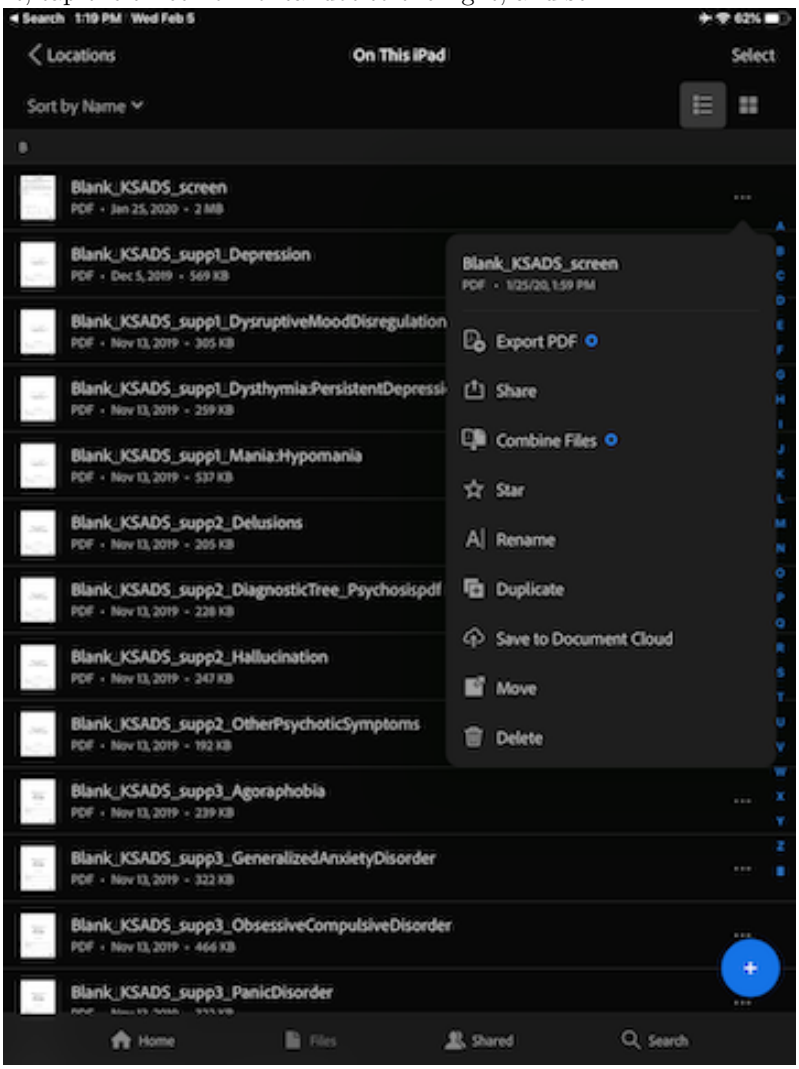
3.3.3.2 Using the iPad for KSADS Summary Checklist

- 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.

Figure 3.4:

Figure 3.5:

- 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 select "Duplicate".



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

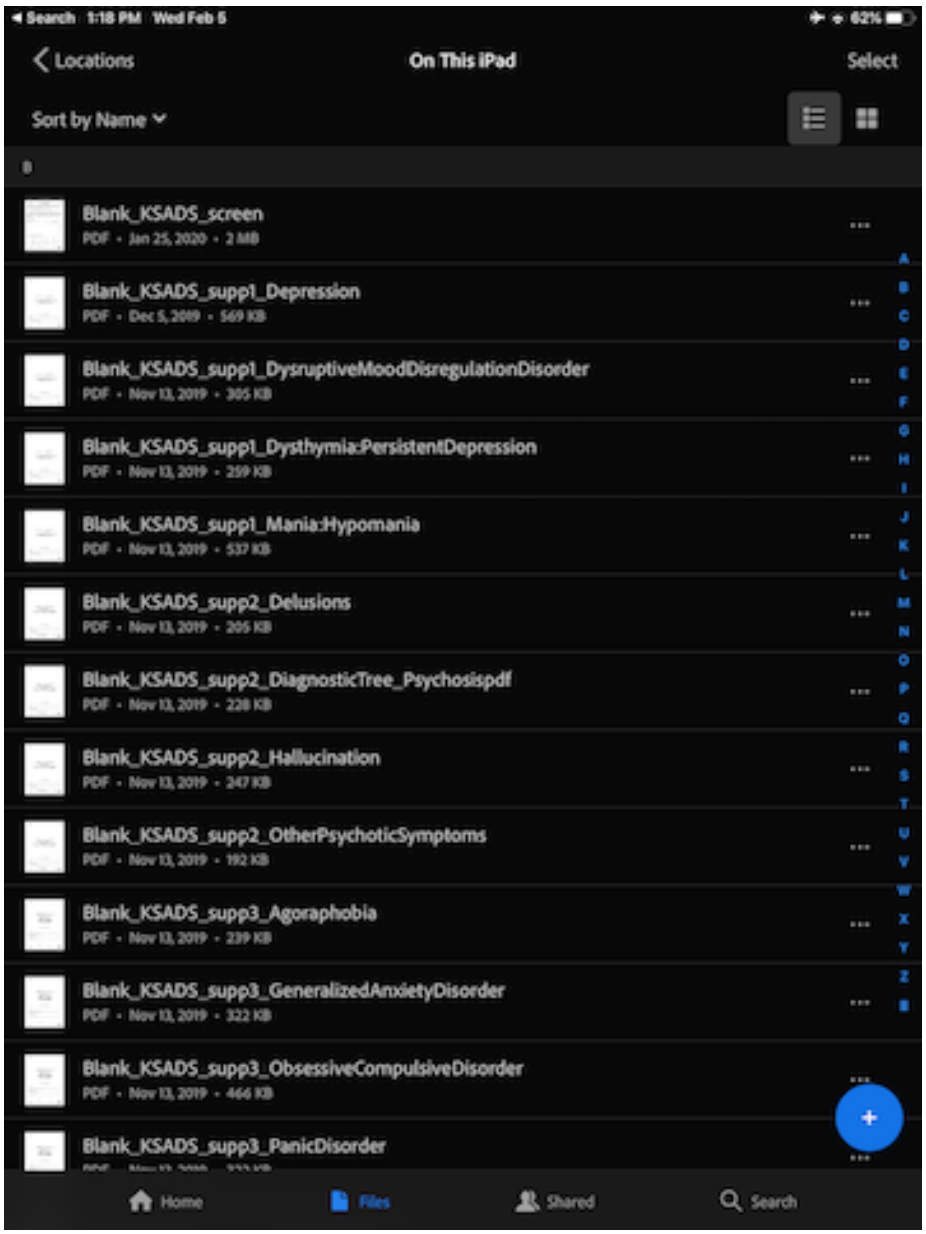
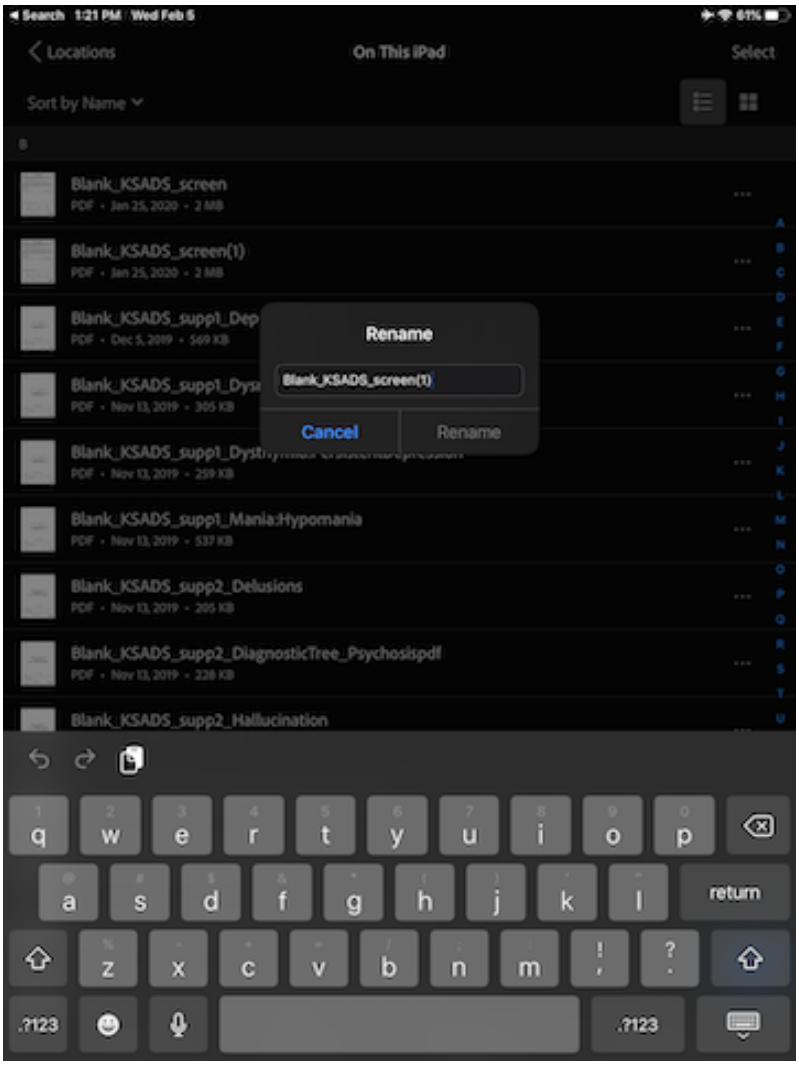

Search 1:18 PM Wed Feb 5
62%
Locations
On This iPad
Select
Sort by Name
Blank_KSADS_screen
PDF • Jan 25, 2020 • 2 MB
Blank_KSADS_supp1_Depression
PDF • Dec 5, 2019 • 569 KB
Blank_KSADS_supp1_DysruptiveMoodDisregulationDisorder
PDF • Nov 13, 2019 • 305 KB
Blank_KSADS_supp1_Dysthymia:PersistentDepression
PDF • Nov 13, 2019 • 259 KB
Blank_KSADS_supp1_Mania:Hypomania
PDF • Nov 13, 2019 • 537 KB
Blank_KSADS_supp2_Delusions
PDF • Nov 13, 2019 • 205 KB
Blank_KSADS_supp2_DiagnosticTree_Psychosispdf
PDF • Nov 13, 2019 • 228 KB
Blank_KSADS_supp2_Hallucination
PDF • Nov 13, 2019 • 247 KB
Blank_KSADS_supp2_OtherPsychoticSymptoms
PDF • Nov 13, 2019 • 192 KB
Blank_KSADS_supp3_Agoraphobia
PDF • Nov 13, 2019 • 239 KB
Blank_KSADS_supp3_GeneralizedAnxietyDisorder
PDF • Nov 13, 2019 • 322 KB
Blank_KSADS_supp3_ObsessiveCompulsiveDisorder
PDF • Nov 13, 2019 • 466 KB
Blank_KSADS_supp3_PanicDisorder
Home
Files
Shared
Search

Figure 3.6:



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 W1 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 W1 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 W1 Protocols - Child/Teen

3.4.1 W1 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

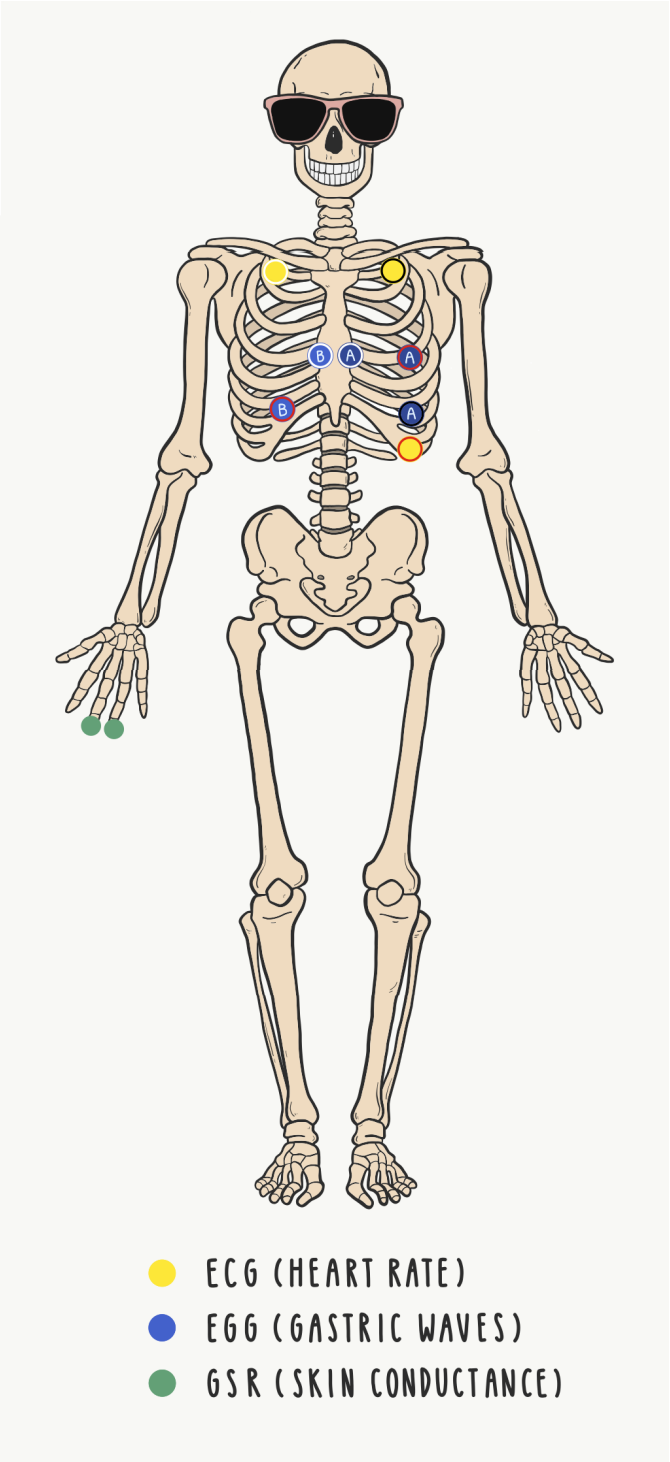

B
A
A
B
A
ECG (HEART RATE)
EGG (GASTRIC WAVES)
GSR (SKIN CONDUCTANCE)

Figure 3.7:

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
- 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 W1 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 W1 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.

- 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:

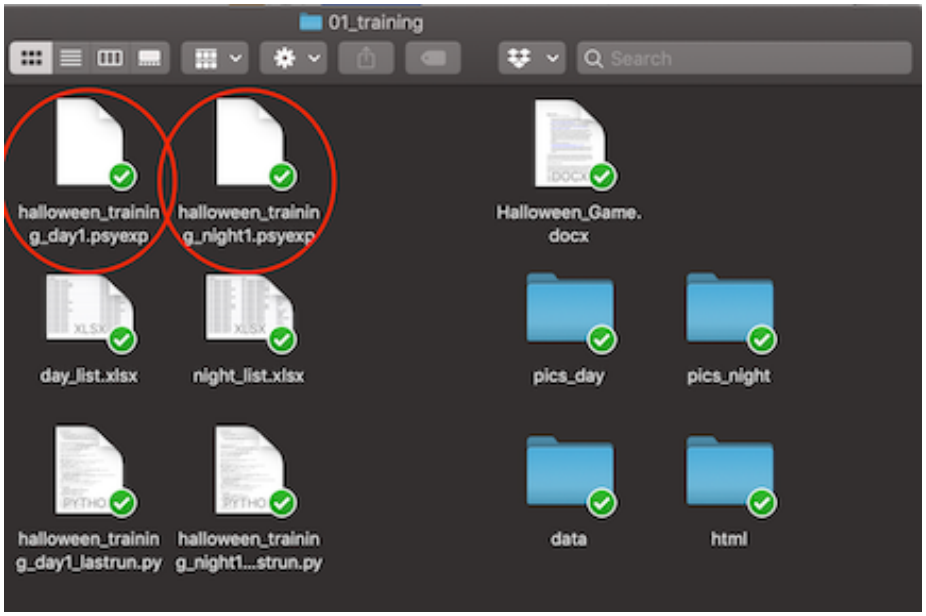


Figure 3.8:

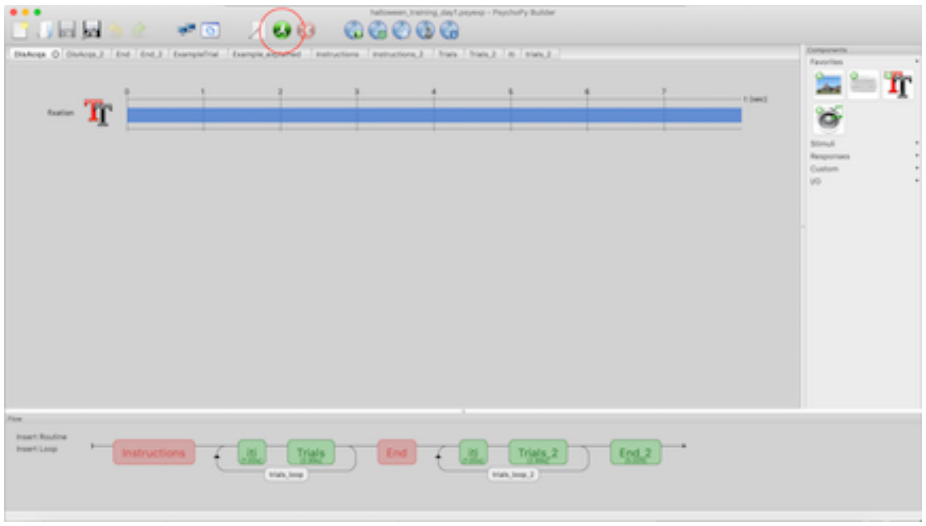
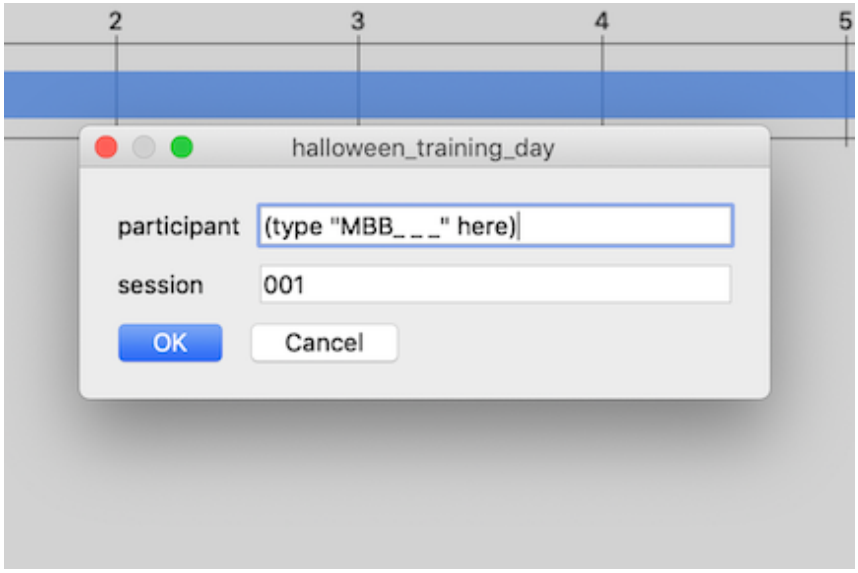


Figure 3.9:



- 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.

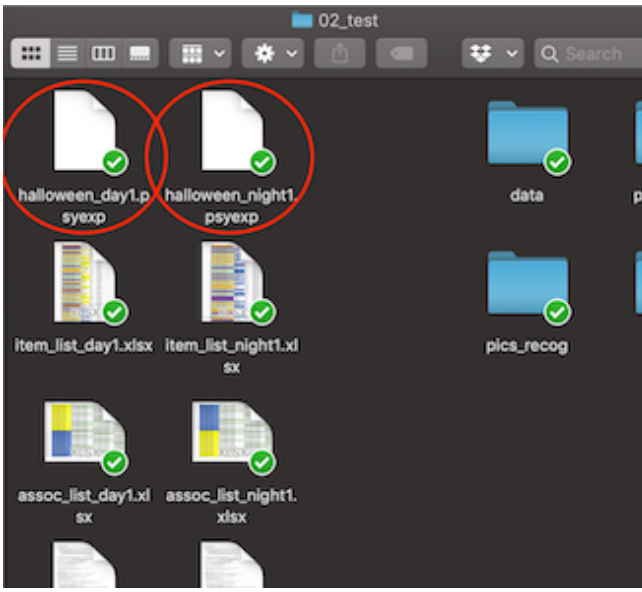
- Once the training task starts, sit quietly and do not disturb the participant. It is important for them to pay their undivided attention to the images on screen during the training.

- 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:

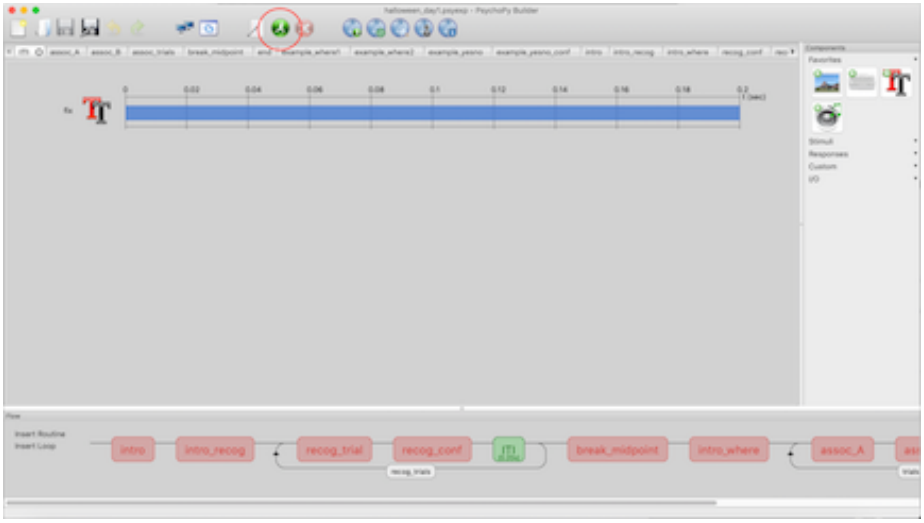


Figure 3.10:

- 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 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 W1 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 W1 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 W1 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 and Wave.
- Store the sample in a dry area at room temperature (in the plastic folder under the participant IDin 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 and Wave.
- 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 and Wave.
- Store the sample in a dry area at room temperature (in the plastic folder under the participant ID in cabinet 1).

3.4.7 W1 Protocol - Weight

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

3.4.8 W1 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 W1 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 W1 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 W1 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 W1 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.

Figure 3.11:

- Prepare collection area. It should look like this:

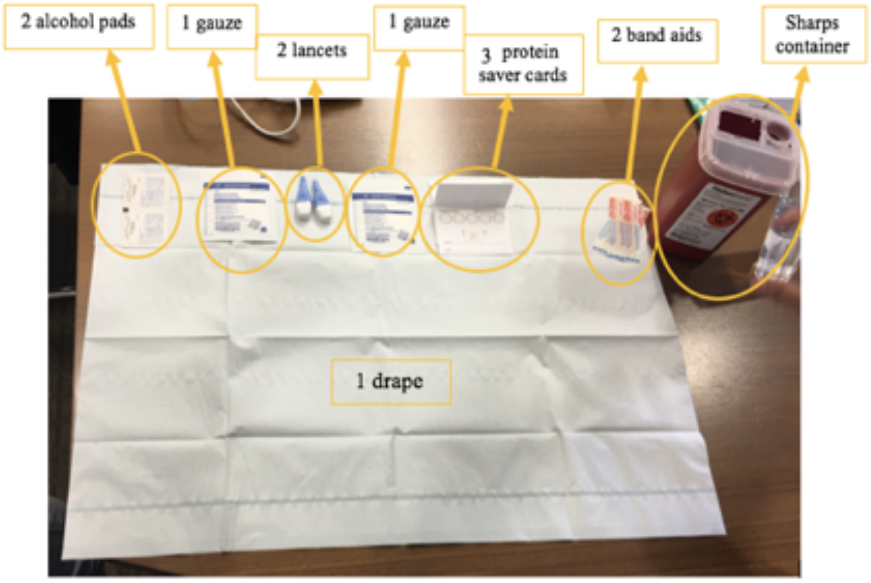


Figure 3.12:

3.4.12.2 VR Headset Setup

- Put VR goggles on for participant.
- 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 RRMC 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 W1 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 W1 Protocols - Post-Session

3.5.1 W1 Protocol - Data Entry

3.5.2 W1 Protocol - Data Quality Check

3.5.3 W1 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, Wave, 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 $^\circ$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 W1 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 W1 Protocol - Downloading the ASA data

1. Navigate to the ASA website - Slack Lab Manager or check internal for login
2. Check whether participant has completed their ASA surveys by clicking on "Track Recall/Record"

- User IDs for Wave 1 should take the format of MBB999
- Scroll to the bottom of the list for the most recent entries

3. If the participant has completed the ASA, download their nutrition report

- Scroll to the right and click on the "View" button under the Nutrition Report column
- Click File>Print then Save as PDF under the naming convention "MBB999_asa_nutrition_profile.pdf"
- Save to the participant's Wave 1 MBB data folder in the Report_card folder and delete the 999 template

4. If there is no nutrition report, it is because the particiant neglected to fill out crucial information (e.g. age, sex, pregnancy questions) that ASA requires in order to build the report. We cannot get the report unless the participant were to redo the entire survey, so move to step 5 if this is the case.
5. If the participant has completed the ASA, we also need to get their data outputs

- Click on "Analytic Files" and select "One Respondent"
- Type in the participant's MBB999 ID in the User Name
- Select "Download analysis files" and you should get 6 csv files in your downloads folder

- Rename the files according to the templates in the participant's MBB data folder in questionnaires>asa and save

6. Mark off as complete on the Wave 1 data entry sheet of the participant log

---

3.5.6 W1 Protocol - Report Card Generation

1. Open a participant data folder

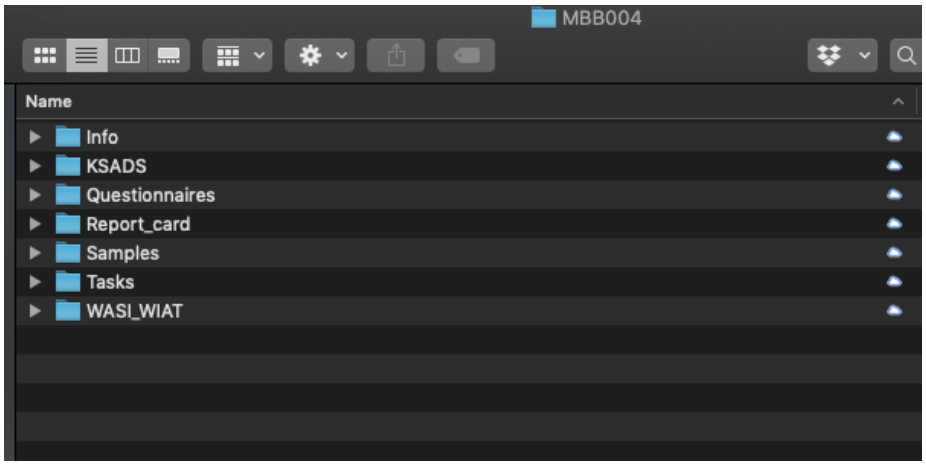


Figure 3.13:

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.

4. 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.

5. After you have entered the data, it should look like this

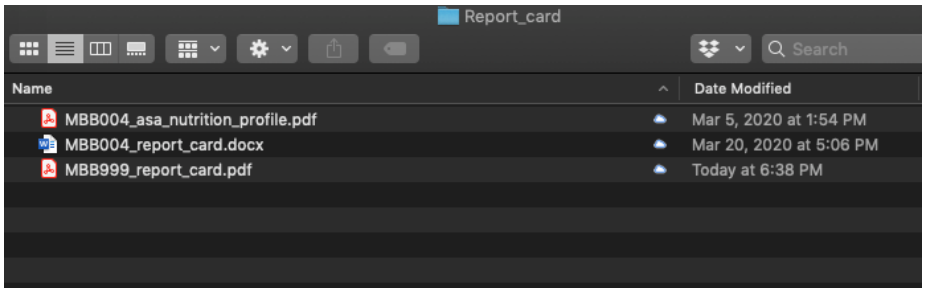


Figure 3.14:

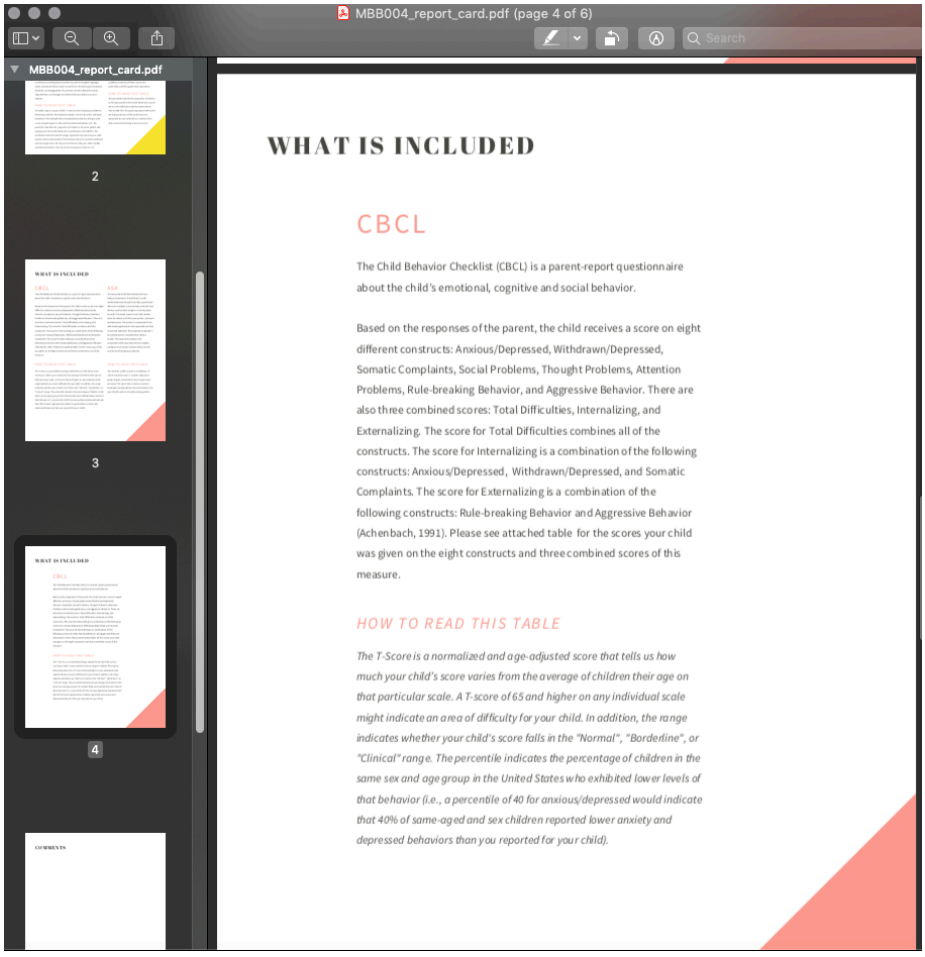


Figure 3.15:

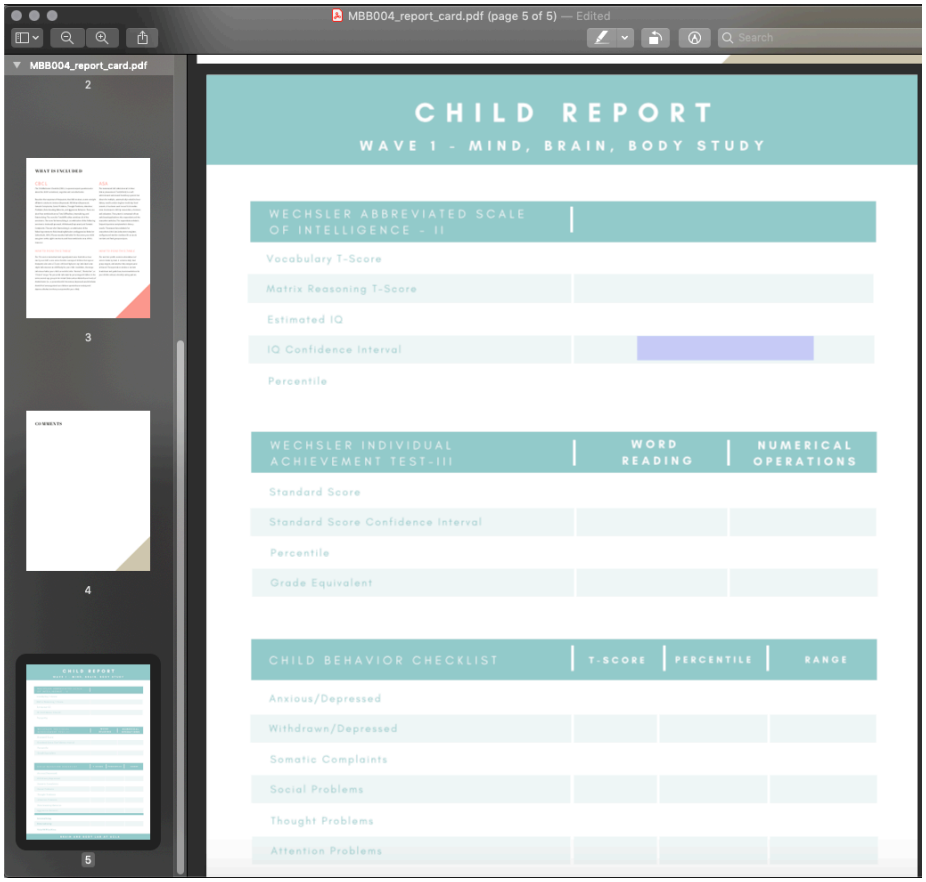

MBB004_report_card.pdf (page 5 of 5) — Edited
MBB004_report_card.pdf
2
3
4
5
CHILD REPORT
WAVE 1 - MIND, BRAIN, BODY STUDY
WECHSLER ABBREVIATED SCALE OF INTELLIGENCE - II
Vocabulary T-Score
Matrix Reasoning T-Score
Estimated IQ
IQ Confidence Interval
Percentile
WECHSLER INDIVIDUAL ACHIEVEMENT TEST-III
WORD READING
NUMERICAL OPERATIONS
Standard Score
Standard Score Confidence Interval
Percentile
Grade Equivalent
CHILD BEHAVIOR CHECKLIST
T-SCORE
PERCENTILE
RANGE
Anxious/Depressed
Withdrawn/Depressed
Somatic Complaints
Social Problems
Thought Problems
Attention Problems

Figure 3.16:

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	91
Standard Score Confidence Interval	101-111	81-101
Percentile	66%	27%
Grade Equivalent	5.2	3.7

CHILD BEHAVIOR CHECKLIST	T-SCORE	PERCENTILE	RANGE
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 3.17:

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

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

3.5.7 W1 Protocol - Data Review & Audit

3.5.7.1 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.

3.5.7.2 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.

3.5.7.3 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 report card (including outstanding items).

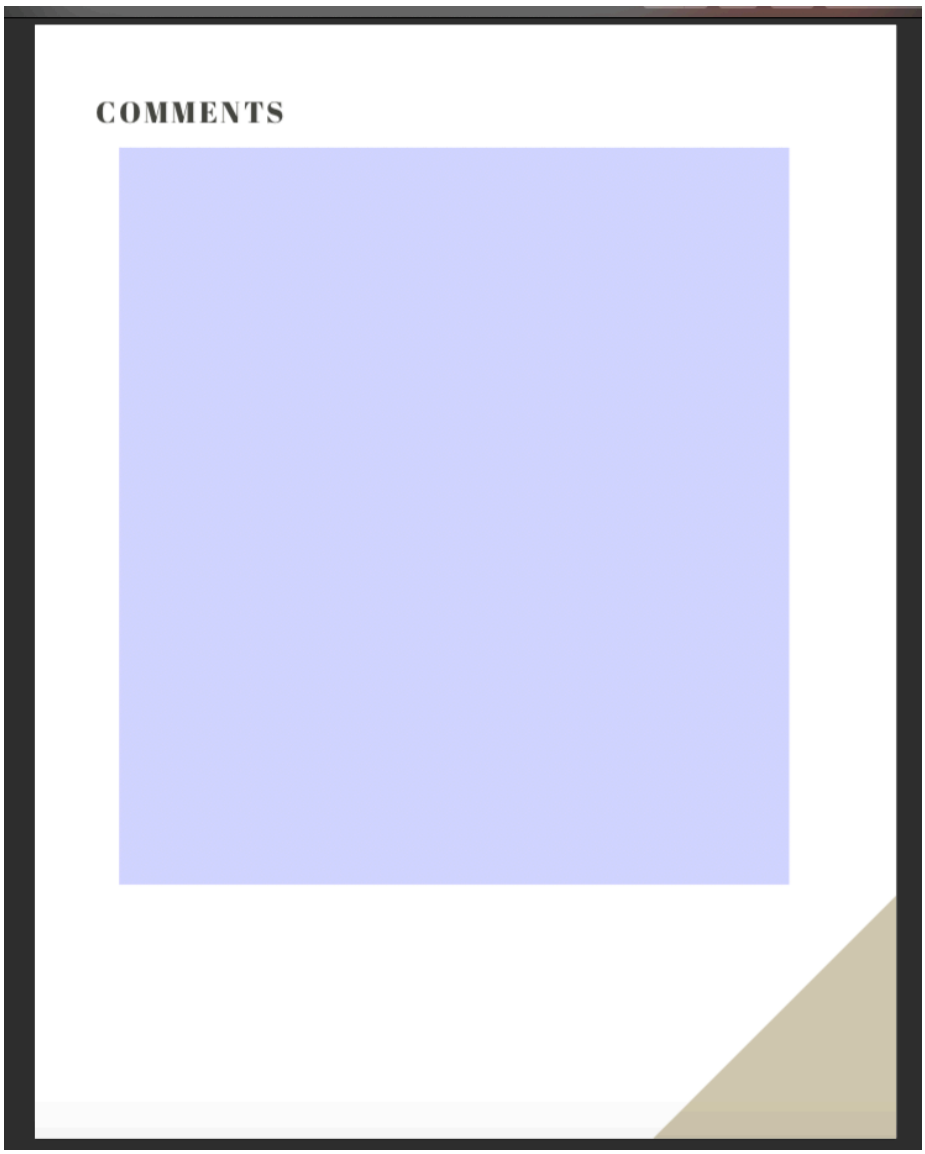


Figure 3.18:

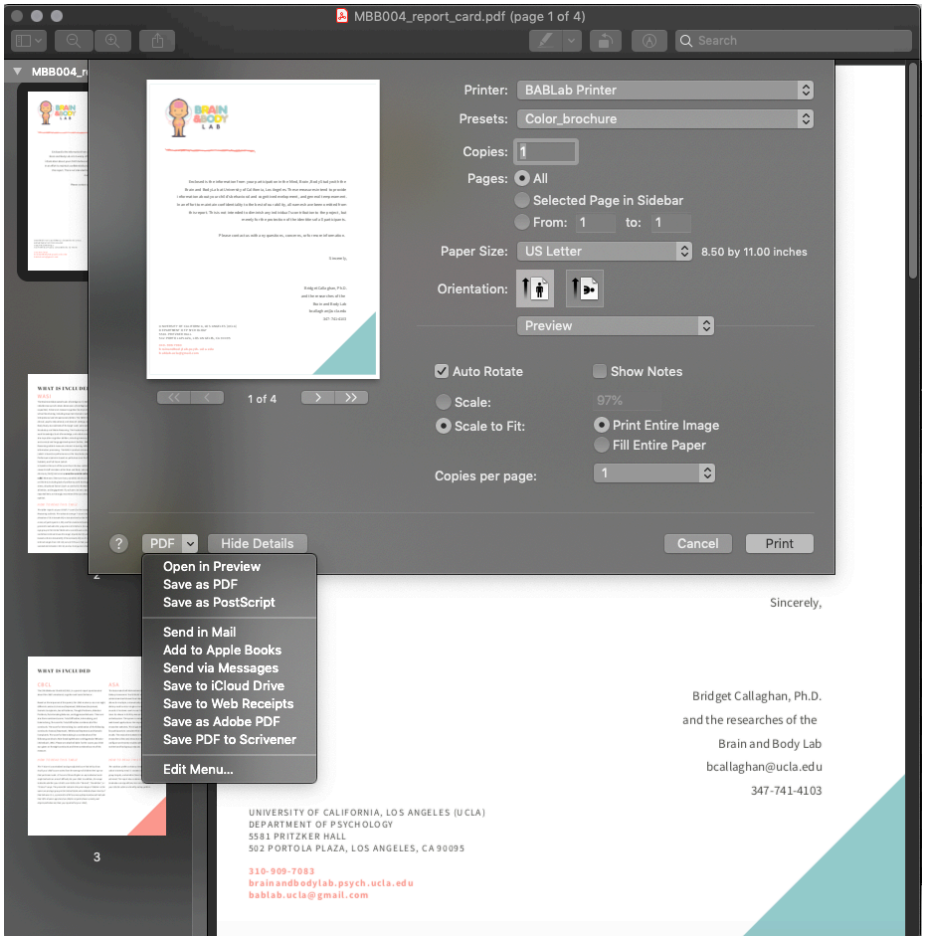

MBB004_report_card.pdf (page 1 of 4)
Search
Printer: BABLab Printer
Presets: Color_brochure
Copies: 1
Pages: All
Selected Page in Sidebar
From: 1 to: 1
Paper Size: US Letter 8.50 by 11.00 inches
Orientation:
Preview
Auto Rotate
Show Notes
Scale:
97%
Scale to Fit:
Print Entire Image
Fill Entire Paper
Copies per page: 1
1 of 4
PDF
Hide Details
Cancel
Print
Open in Preview
Save as PDF
Save as PostScript
Send in Mail
Add to Apple Books
Send via Messages
Save to iCloud Drive
Save to Web Receipts
Save as Adobe PDF
Save PDF to Scrivener
Edit Menu...
Sincerely,
Bridget Callaghan, Ph.D.
and the researchers of the
Brain and Body Lab
bcallaghan@ucla.edu
347-741-4103
UNIVERSITY OF CALIFORNIA, LOS ANGELES (UCLA)
DEPARTMENT OF PSYCHOLOGY
5581 PRITZKER HALL
502 PORTOLA PLAZA, LOS ANGELES, CA 90095
310-909-7083
brainandbodylab.psych.ucla.edu
bablab.ucla@gmail.com

Figure 3.19:

- 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 participang log and data check sheets, and note in partipant'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 report card (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 participang log and data check sheets, and note in partipant's README in data folder.

If No Sample:

- Send [MBB - UNPAID] email and attach thank you letter, certificate, and report card (including outstanding items - emphasize stool sample kit).
- Leave participant on Unpaid list.
- If stool sample recieved - 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 particpang log and data check sheets, and note in partipant's README in data folder.

Chapter 4

Wave 1 Online

4.1 W1-O Checklists Online

4.1.1 W1-O Checklist - Scheduling Stage

Calling participants from REDCap

- Participants can be considered "lost to follow up" if they meet all of the following criteria:
  - Researcher has tried to contact individual at different times of day.
  - Researcher has used at least 2 different approaches (e.g. email and phone), UNLESS only 1 form of contact has been given, in which case participant should fully meet all other criteria.
  - Person has been contact at least 4 times.
  - Situation is NOT considered "phone tag" (a participant calls and lab member returns call but keeps missing one another- this shows participant may still be interested, therefore number of contact and lack of response may not be due to lack of interest. Participants in "phone tag" situation should stay on contact list).

Waitlist criteria

- This regards the "out of town" option on the wave1_Status on REDCap
- Should include:
  - any participants here live outside of LA, and won't be coming back to LA (e.g. did not relocate just for COVID-19 pandemic purposes)

  - participants whose age tally exceed 10, the target collection number for each age group in our age range. Refer to the Participant Tally for reference.

PLEASE NOTE: Participants who have not aged out yet, or who are not free at the moment yet, OR live outside the US but plan to come back as soon as the pandemic is over should all still be kept on the contact log and just have a future recontact date. We only want to put people who are going on our "out of town" waitlist (like a plan B, only contact if necessary)!

We will only refer to the waitlist if (1) we will be behind on recruitment for Wave 1 and will need to just schedule people who we know cannot come back for future waves to complete data collection or (2) we will be behind on recruitment for Wave 1 and will need to just schedule people who are anywhere within our age range, rather than targeted numbers by age to complete data collection (3) we have to stay in the pandemic and all future waves will be remote.

Important Reminders

Screening

- Check participant tally before screening to know if we would be scheduling or waitlisting if they qualify
- When answering the call ask who you are speaking with (so we know if it's a parent or teen)
- If a potential participant is not of age then put their recontact to future around the time they would qualify
- If screening a family with multiple children each child has to be screened with their own smbb
  - Oldest child gets next available smbb number just like the mbb number protocol
  - Only exception is if we only knew about younger child then during screening mentions they have another child that wants to participate.
- Triple check you write down the address correctly for participants (repeat it back to them / spell it out)
- Do not run session until you are 100% sure they are eligible. If unsure of a criteria verify it before session.

Calendar

- Put the W1 for wave one (e.g. S1 W1) on session events
- For reminder emails put Status: Incomplete in description
- For Session 1 Reminder 1 call event put "Only if no email response" in the description and then status: Incomplete

- Whenever you complete a reminder event mark it as complete and update the MBB participant log

Emails to participant

- Move potential participant email to "Added" tab of MBB once you add them to the participant database
- All emails to a participant should be in the same thread
  - The thread we initially contacted them at should be the one we send the session confirmation to
    - **Remember that email templates need to be edited**(e.g. there are no written responses in wave 2 so that chunk should be deleted, there is no ASA in wave 1 so that should be deleted in wave 1, etc.)
- The easiest way to not forget steps is to do all " session has just been scheduled" tasks based on the MBB log
- Make sure you are sending the correct reminder email - S1 R2 is the one with the researcher card

Confirming sessions

- Do not put as " confirmed session" on MBB log unless we got verbal confirmation / email confirmation
- If called and no answer leave a note on the calendar event saying was not able to verbally confirm & also put into SRA chat

REDCap

- Log into REDCap with your own account to avoid confusion and for easier identification of who screened each smbb participant
- After contacting someone change the Wave status "date to recontact" to one week from the current date (can be two weeks if it's someone that hasn't responded in long time)
- When you schedule a new MBB participant change their SMBB "Wave 1 status" to enrolled

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
- Ask Lab Manager to 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

Enrollment

- Create participant Box folder using MBB_template (delete blank README from newly created folder)
- Enroll participant in Wave 1 on REDCap
  - Go to "Add / Edit Records" of MBB
  - Make sure the tab is "Arm 2: wave 1"
  - Type in the next available MBB
  - * Be careful because any premature clicking makes a new ID*
- Fill participant instrument on REDCap
  - **Age should be the age of participant at the time of *Session 1***
  - In notes section add the date and time of session
- Update Participant Tally

Calendar

- Create MBB Session 1/2 calendar events (and invite researcher)
  - *MBBXXX WX- Online Session 1*
    - Add Lead and backup reseracher, MBB#, participant age, sex,whether Bio or els, and session time in the event decription
  - *MBBXXX WX- Online Session 2*
    - Add Lead and backup reseracher, MBB#, participant age, sex,whether Bio or els, and session time in the event decription
- Add participant to the weekly MBB mailing calendar event
  - *MBB999 WX (Scheduled for 00/00/00) Sent: Incomplete*
- Create MBB Session 1/2 reminder calendar events
  - *MBBXXX WX- Session 1 Reminder 1 (email)* - 1 week prior
    - Add Status: Incomplete to the description
  - *MBBXXX WX- Session 1 Reminder 1 (call)* - the day after Reminder 1 email
    - Add Status: Incomplete to the description
    - Add "Only if no email response" to description
  - *MBBXXX WX- Session 1 Reminder 2 (email and call)* - 3 days prior

    - Add Status: Incomplete to the description
  - *MBBXXX WX- Session 2 Reminder 1 (email)* - 3 days before second session
    - Add Status: Incomplete to the description
  - *MBBXXX WX- Session 2 Reminder 2 (call)* - 2 days before second session
    - Add Status: Incomplete to the description
- Create MBB Home Session reminder calendar events
  - *MBBXXX WX- Home Session Reminder 1 (email)* - 1 week after Session 2
    - Add Status: Incomplete to the description
  - *MBBXXX WX- Home Session Reminder 1 (call)* - 8 days after Session 2
    - Add Status: Incomplete to the description
  - *MBBXXX WX- Home Session Reminder 2 (email)* - 14 days after Session 2
    - Add Status: Incomplete to the description
  - *MBBXXX WX- Home Session Reminder 2 (call)* - 15 days after Session 2
    - Add Status: Incomplete to the description
    - Add * Only if package missing and have not been in contact with participant* to description

---

4.1.2 W1-O Checklist - Calendar Reminders

NOTE: all template emails are in bablab gmail

- Send *Session 1 Reminder 1* email
  - Update calendar event description to Status: Complete
  - Update MBB participant log
  - Confirm package is received
- *Session 1 Reminder 1* call made
  - Update calendar event description to Status: Complete
  - If no call made put "NA - responded via email"
  - Update MBB participant log - if NA put NA
- Send *Session 1 Reminder 2* email & call
  - Update calendar event description to Status: Complete

  - Update MBB participant log
  - **Make a note on calendar whether they confirmed session**
  - **Send message to SRA chat whether participant confirmed and tag the researcher running the session**
  - Confirm package is received

- *Session 2 Reminder 1* email made
  - Update calendar event description to Status: Complete
  - Update MBB participant log
  - Confirm package is received

- *Session 2 Reminder 2* call made
  - Update calendar event description to Status: Complete
  - Update MBB participant log
  - **Send message to SRA chat whether participant confirmed and tag the researcher running the session**

- Confirm participant
  - Preferably by phone
  - Update calendar event description to Status: Complete
  - Update MBB participant log
  - Update *Session 1* calendar status

- Home Session Reminders
  - Check participant log to see what info is missing before sending home session reminder emails
  - Check with Kristen if unsure what info is missing
  - Make sure the list of "items needed" is tailored to that participant
  - Send written response links if it hasn't been completed yet
  - If package missing after home session email reminder 2:
    * send message to SRA chat that participant is still missing package
    * move on to home sess call 2
    * next steps will be on a case by case basis

General Notes about calendar

Session 1 reminders

- You can differentiate the Session 1 Reminder 1 and Reminder 2 emails by seeing if it says to include Research Profile (reminder 2 is the one with the researcher profile)

- the files you attach to the emails are in BABLAB/Studies/Mind_Brain_Body/Documents/Infograp

- consents are in BABLAB/Studies/Mind_Brain_Body/Documents/Consents/Wave_1_online

- Research profiles are in BABLAB/Studies/Mind_Brain_Body/Documents/Infographic_next_steps and remember to drag the picture in show it shows up in the email body
- When you call you're just asking them to confirm that the session date and time works for them & that they received package/ *Hi this is _____ from the Brain and Body Lab. I am calling to confirm you are still available for your session on _____. Also, have you received the package?*

Session 2 Reminders

- You can check if they consented to stool sample by looking at email thread to see the session @ confirmation email the researcher sent them OR you can go to their Session 1 checklist on REDCap
- Make sure you only include relevant reminders in the email, delete the highlighted parts that don't apply to them

Home Session Reminders

- Check the MBB participant log to see if we haev received their package.
  - If the Package COnfirmation section is filled out as complete then we *have* received package.
  - Lab manager also sends message to SRA to update on packages that are received so can search chat
  - If still unsure if participant has sent package back to us check email thread
- For home session reminders make sure to only send request if they haven't sent package back or are missing something
  - If we *did* receive package but something is missing (contact list, BSS) then you would request it. Lab manager usually tells whoever is in charge of calendar if things were missing from packages that were received

Misc Notes

- If the calendar event is for something else such as checking in with a participapant or rescheduling a session the lab manager or other memmber of team will provide the necessary context
- If unsure about a particicular calendar event you can slack Lab manager or send a message to SRA to see if someone knows answer

- Remember to mark as complete on calendar and on MBB participant log after doing each calendar event
- refer to Wave 2 Checklist for script for the text message if it is not in the description of calendar event

---

4.1.3 W1-O Checklist - Pre-Session 1

4.1.3.1 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. Labeling should be in the notation "MBB____ W_"

- 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/Session 2 Booklet (in this order)
    1. Session 1 Cover page
    2. Pleasant/Unpleasant Events Checklist
    3. Height Measurement Instruction
    4. Weight Measurement Instruction
    5. Waist Measurement Instruction
    6. Saliva Sample Instructions Sheet
    7. Hair Sample Instructions Sheet
    8. Session 2 Cover Page
    9. Contact List and label with participant ID
    10. Stool Sample Instructions Sheet
    11. Bristol Stool Scale and label with participant ID (MBB Specific Version)
- Prepare 1-2 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 & Wave)
- Include a hair comb and alligator clip
- Label stool sample collection kit (paper clip collection tube and toilet hat together)
- Insert purple gloves for stool sample
- 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 & Wave) 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
- Take a picture of study package with tracking information to file offline on researcher computer (NOT Box)
- Mail "Magic Box" package to participant

4.1.3.2 Setup - 1 Hour Prior

- Open up Zoom link for Session 1
- Read "participant" information instrument on REDCap for notes section for any notes from the scheduler about child

- Open Slack and keep open for entirety of session for communication with the research team/Lab Manager
- If Lab Manager, assign all fellow researchers co-host. If SRA, ask Lab Manager to sign on to assign you Host or co-host.
- Pull up session scripts/protocol, Halloween training instructions, and Halloween Test instructions
- Send *Session 1 Links* email
  - NOTE: do not add Gorilla Code to LINKS email- code is their MBB # which cannot be paired with private information (participant's name/email)
- Activate the participant's ID on Gorilla; leave Gorilla open so researcher can track participant progress during behavioral task
- Prepare Session 1 checklist on REDCap
- Have the Participant's MBB and secondary MBB number on hand
- Preload the Consent/Assent picture slideshow on researcher computer
- 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
- Have the following links ready to send to the Participant throughout the session:
  - link to Consent on REDCap with codes ready
  - link to Child's Gorilla Game
  - link to child questionnaires on REDCap with codes ready

4.1.4 W1-O Checklist - Session 1

- Welcome & Introduction to Zoom (important features- chat)
- Session walk-through/package explanation
- Consent/Assent
- Parent-child observation (note recording via Zoom or participant recorded)
- If participant 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
- Qualitative parent and child free responses (optional) explanation
- Confirm mailing address for payment
- Confirm Session 2 time and date

4.1.5 W1-O Checklist - Post-Session 1

4.1.5.1 Notes

- Submit lab session checklist child
- Make note of issues to discuss (if needed) in Boxnote for next core meeting
- Update Participant Tally
- Update Participant Log

4.1.5.2 Filing

- Transfer and rename Zoom recording to Box
- Download and copy behavioral task data (from Gorilla) to Gorilla data folder on Box

4.1.5.3 Reminders

NOTE: all template emails are in bablab gmail

- *Session 2 Confirmation Email* sent with Zoom link, researcher info (right after Session 1)
- *Session 2 reminder 1* email sent with Zoom link (3 days before session 2)
- *Session 2 reminder 1* phone call made (2 days before Session 2)

4.1.6 W1-O Checklist - Pre-Session 2

NOTE: all template emails are in bablab gmail

- Send *Session 2 Links* email

- Open Gorilla to track participant progress
- Have the Participant's MBB number on hand
- Open home session checklist on REDCap

4.1.7 W1-O Checklist - Session 2

- Halloween test delay completed
- Fill in home session checklist on REDCap (halloween test delay information)
- Stool sample questions answered
- Wave 2 Interview
- 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

---

4.1.8 W1-O Checklist - Post-Session 2

- Save and submit home session checklist on REDCap (halloween test delay information)
- Update Participant Log
- Download and copy delayed behavioral task data (from Gorilla) to participant folder (raw)
- Send *Session 2 TO DO List* email from bablab gmail templates

---

4.1.9 W1-O Checklist - Final Online

4.1.9.1 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

4.1.9.2 Data Entry

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

4.1.9.3 Reminders

NOTE: all template emails are in bablab gmail

- *Home Session Reminder 1 Email* made
- *Home Session Reminder 1 phone call* made
- *Home Session Reminder 2 Email* made
- *Home Session Reminder 2 phone call* made

After package has been received...

4.1.9.4 Package confirmation

- Halloween test delay completed
- Hair sample received
- Saliva sample received
- Stool sample received
- Bristol Stool Scale data received
- Questionnaires received
- Contact information sheet received

4.1.9.5 Data Entry

- Enter contact list information into recruitment database
- Scan and upload parentproxy questionnaires to Box
- Scan and upload parentself questionnaires to Box
- Enter questionnaires data to REDCap (parentproxy and parenself)
- Scan and upload Bristol Stool Scale to Box
- Enter Bristol Stool Scale data to REDCap
- Enter height/weight/waist to body measurements on REDCap

4.1.9.6 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)

4.1.9.7 Sample Storage

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

4.1.9.8 Data Quality

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

4.1.9.9 Retention

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

4.1.9.10 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

4.1.10 W1-O Checklist - No Show

- Delete session 2 & reminder calendar events from MBB calendar to avoid confusion
  - Session 2 reminder email
  - Session 2 reminder call
  - Session 2 session event
  - Home session reminder 1 email
  - Home session reminder 1 call
  - Home session reminder 2 email

- Update [Participant Tally] as "rescheduling" (https://ucla.app.box.com/file/724688028024)
- Update MBB_Participant_Log – under "Wave 1- online session date", write "rescheduling"
- Note in REDcap participant instrument "notes" that participant is being rescheduled.
- Rescheduling steps
  - Send No-show rescheduling email same day
  - Add calendar event for reschedule call 1 (following day)
  - Add calendar event for rescheduling email 2 (3 days after original session date)
  - Add calendar event for rescheduling call 2 (4 days after original session date)
  - Add calendar event for rescheduling email 3 (1 week after original session date)
  - Add calendar event for rescheduling call 3 (8 days after original session date)
- IF participant is not reached after 6 previous recontact attempts, make note in the next SRA meeting document, and follow-up with Kristen regarding next steps.
- IF participant gets rescheduled:
  - Update new date of session in:
    - MBB_Participant_Log
    - REDcap Participant Instrument
  - Recreate S1 and S2 calendar events, as well as each reminder calendar event.

4.2 W1-O Protocols - Pre-Session 1

4.2.1 W1-O Protocol - Recruitment Online

4.2.1.1 Adding Participants to Participant Database

For when there is a new email interest form

1. Verify that potential participant is actually a new participant
   - In other words check Participant Database to check that they didn't previously participate / fill out interest form before

2. Add the info provided on interest submission (name, age, email, phone number) 3. For column labeled “Added”:

    - Yes if you’ve added them to REDCap
    - No if they haven’t been added
    - Recommended that you add to REDCap immediately and reach out to them

3. Add participant to REDCap (see below for full instructions)
4. Move interest form email from inbox to “Added” tab of MBB

    - “Move to” button is at top next to labels button, make sure you move not just put “Added” label

4.2.1.2 Pre-Screening

1. Check if participant is in Participant Database

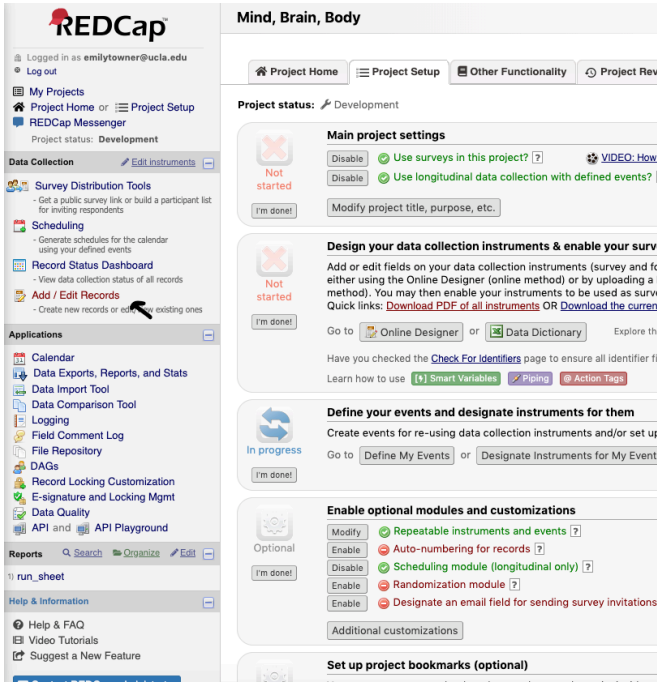
    - If not, add them to the Participant Database

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. Check participant tally before making call to see if they would be scheduled or waitlisted (if we reached 15 participants for that age group) if eligible for study

4.2.1.3 Screening

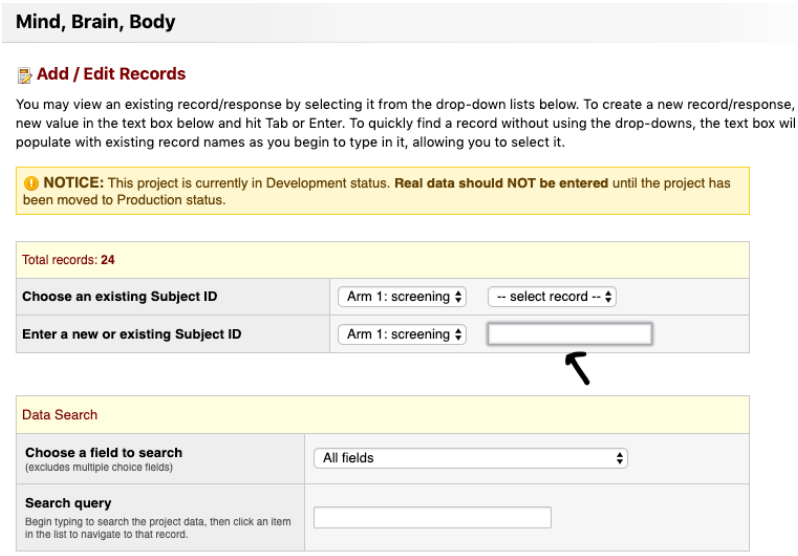


1. To screen a new participant click “Add / Edit Records”
2. Click to enter a new Subject ID

- 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

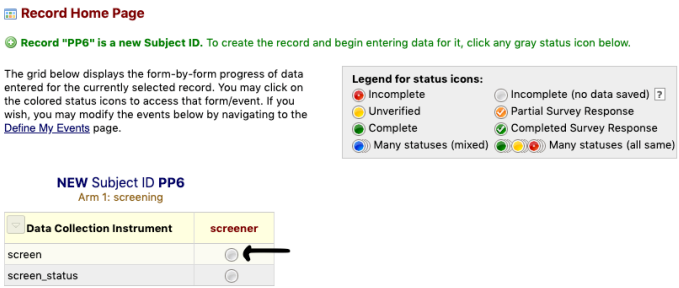


a new record

4. The screening arm contains two parts

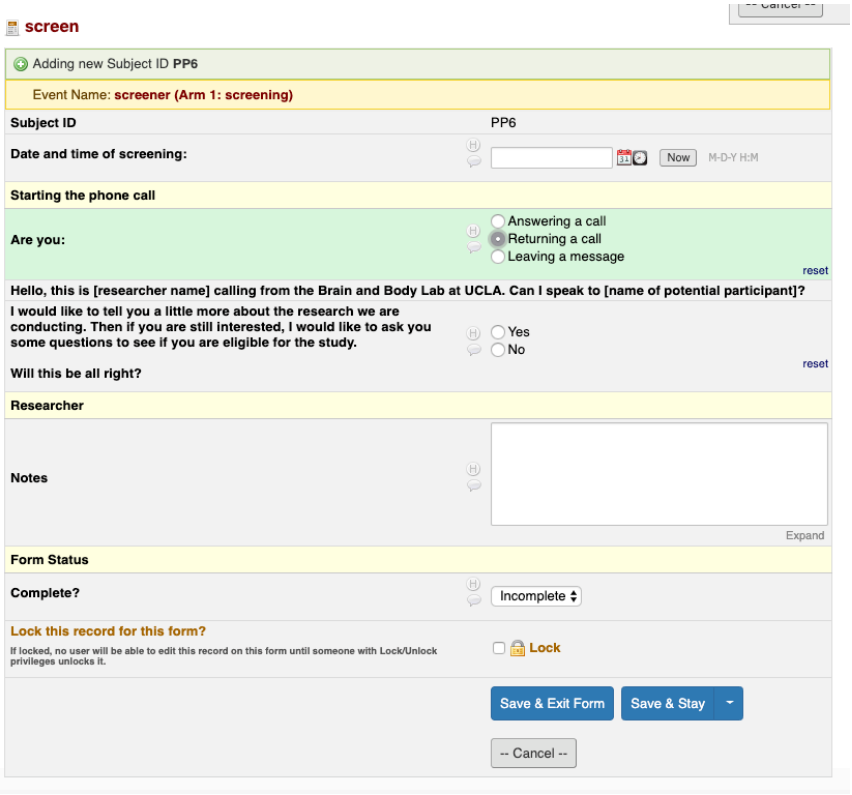
   - The screen
   - The wave1_status

     – The wave1_status is to be updated after the first and each subse-



quent contact

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

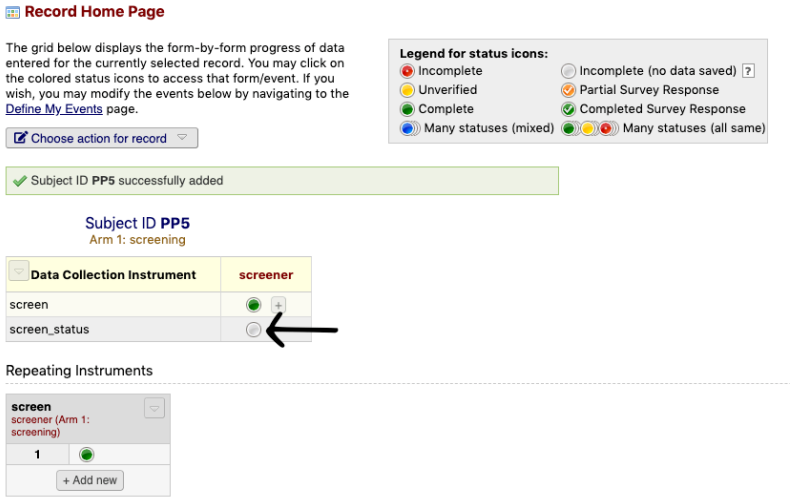


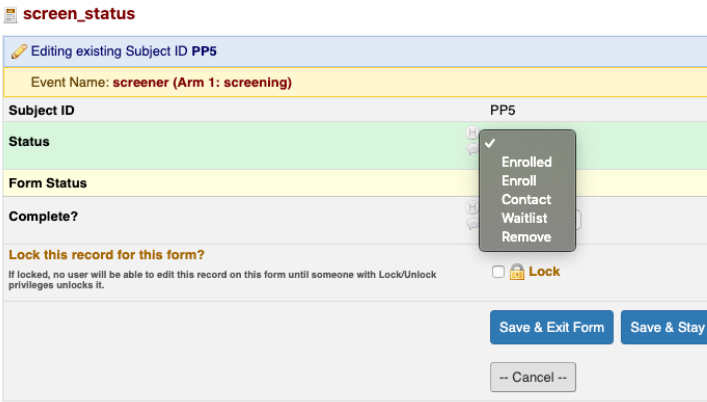
6. Click “Now” to enter today’s date and time
7. Select the appropriate choice to start the phone call and follow the skip logic
8. Follow the skip logic to the end

- For items without a text field, write the information down in the Recruitment database (This identifying information cannot be on REDCap)
- In “Notes” make detailed note of relevant info (eg. Session scheduled for this day, participant has not responded to prior emails, etc)

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





10. Click the screen_status radio button
11. Select the appropriate option

    - Contact - Participant needs to be re-contacted (add Recruitment Database & ID Drive). Participants who are still too young to participate, or are unavailable at the moment should stay on this list but be set for a future recontact time/date.
    - 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
    - Out of Town- Participant lives outside of LA, and will not be returning after the pandemic. This is our “waitlist” of those we will contact if really necessary

12. Be sure to update the screen status after each contact
    - Update the recontact date on wave status
      - One week from today (date you contacted them)
    - 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
    - Highly recommended to do all post-enrollment tasks by following the "Pre-Session Checklist" on MBB participant log

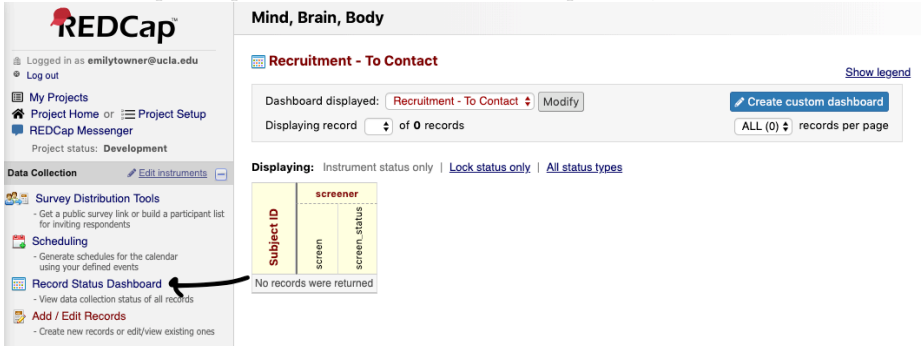
4.2.1.4 Scheduling

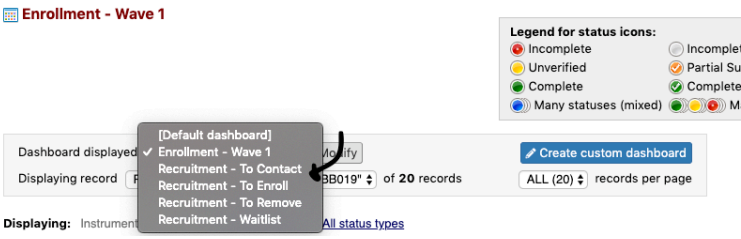
1. Open BabLab google calendar and note availability for designated data collection research team.
2. Check-in with the Lab Manager to see what the designated "package mailing day" of the week is. Participants must be scheduled 2 weeks or more in advance from the "package mailing day", to ensure appropriate 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."

4.2.1.5 Other Screening Information

Accessing Lists

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





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
- Out of Town- Participants who do not live in LA and will not be returning after the pandemic. This is a waitlist of individuals who we may contact if (1) we are behind on recruitment for Wave 1 and will need to just schedule people who we know cannot come back for future waves so we make sure we get data or (2) we have to stay in the pandemic and future waves will be remote.

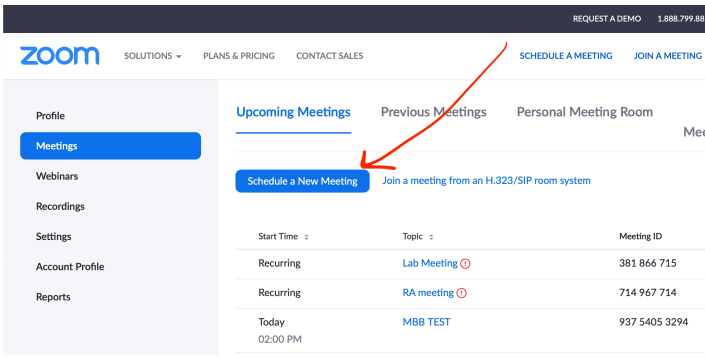
4.2.1.6 Concerns

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

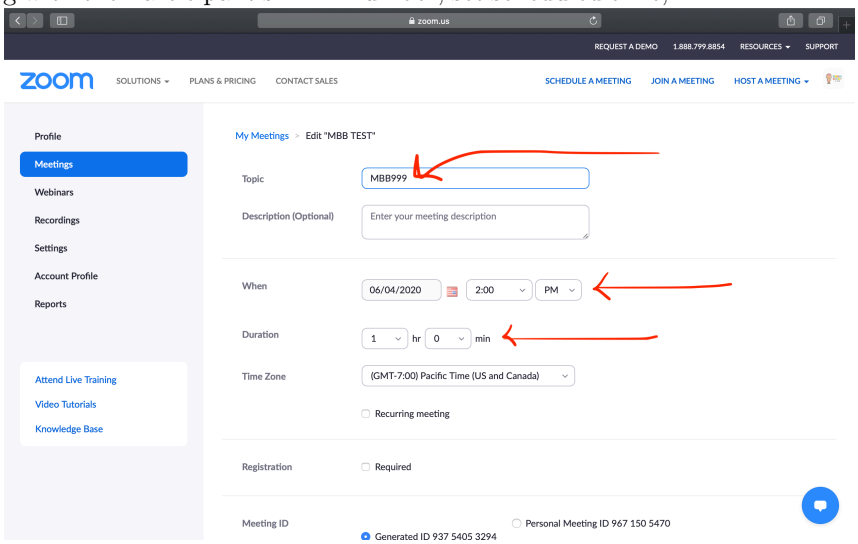
- [MBB_online - CONCERNS]

4.2.1.7 Making a Zoom link

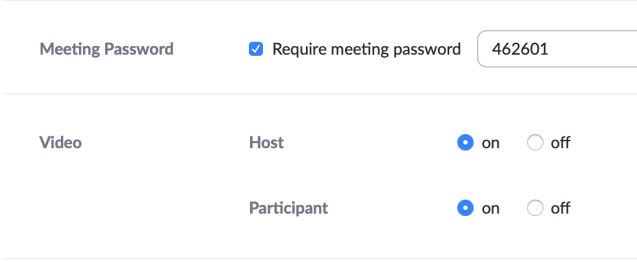
1. Log onto https://zoom.us



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

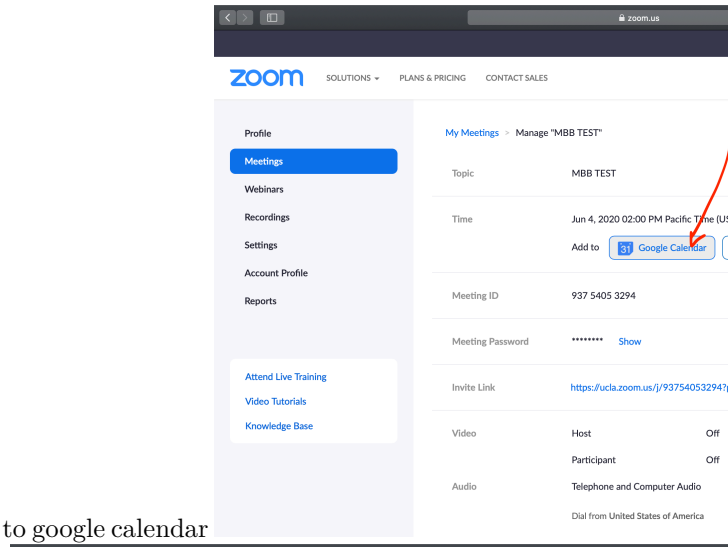


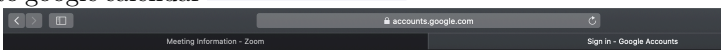
indicate 3 hours



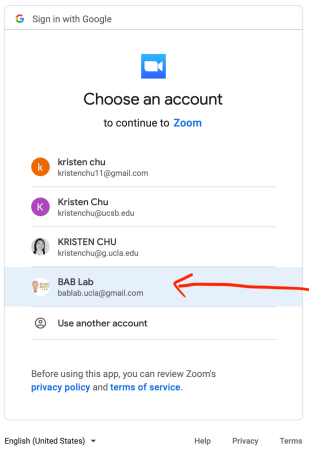
4. Set setting with password and turn host/participant on

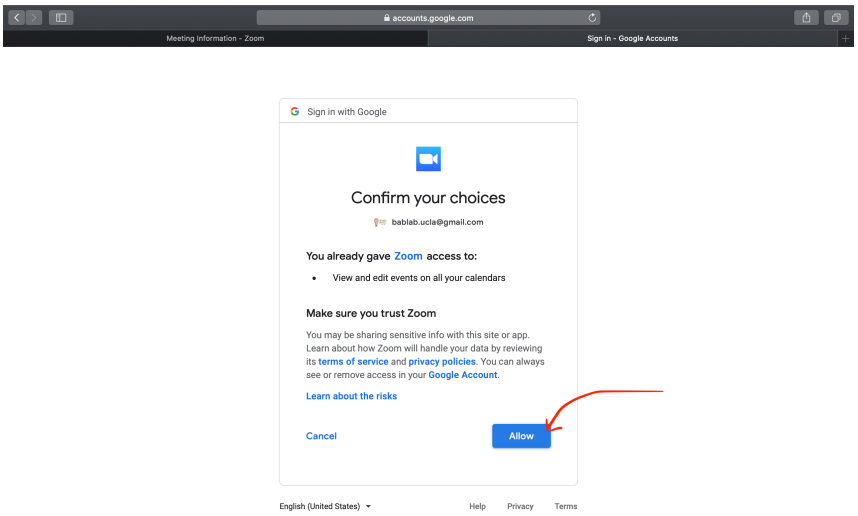
5. Save and click to add Zoom meeting to google calendar



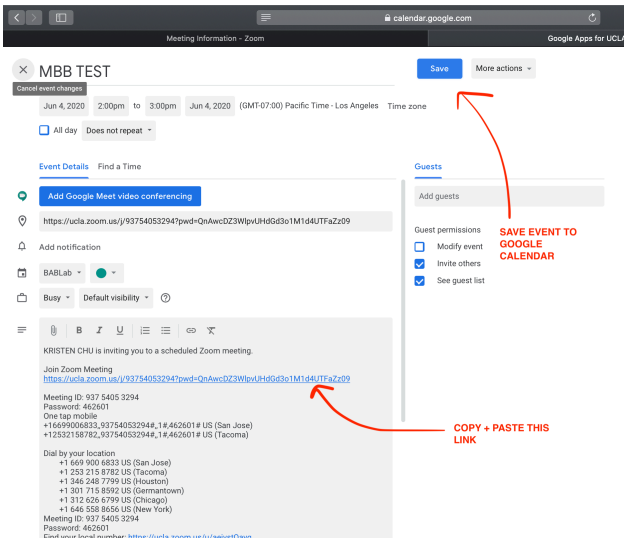


6. Click on the bablab.ucla@gmail.com





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

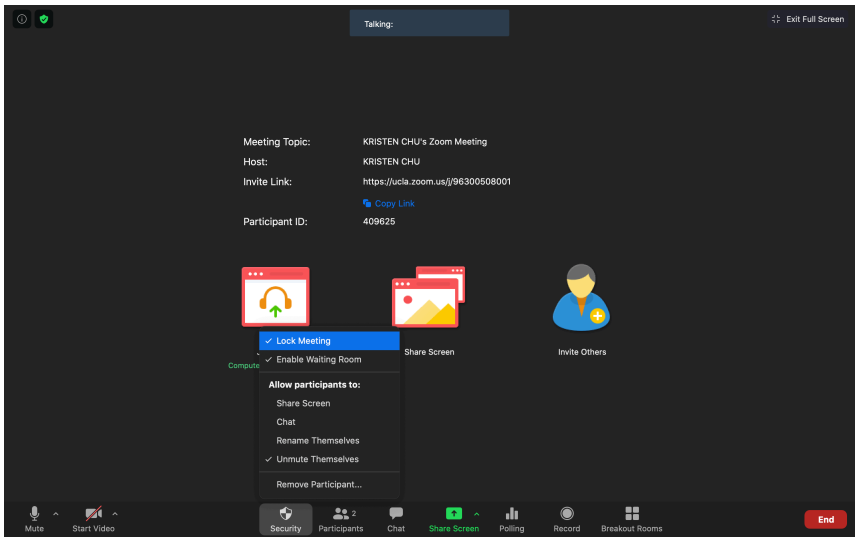
4.2.2 W1-O Protocol - Session Preparation

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 materials have been put together, it is time to bring the package down to the mailroom in the Psychology building, Tyler's Office, OR to the UCLA MDDS.
  - If you go to the mailroom or to Tyler's Office, you need to have your own box. Tyler can tape up the box for you if needed.
  - At MDDS, they provide free mailers (but no boxes), which can fit materials for up to 1 participant
- To mail the package to the participant, you will need the following information:
  - Recharge ID
  - Participant name
  - Participant mailing address
  - Lab mailing address
- From the mailroom: you can write the addresses directly on the box, and circle the recharge ID. Leave the box on the table above the "outgoing mail" sign.
- From Tyler's office: you will receive a FedEx label in which you can write this information. Take a picture of the tracking number and save OFFLINE. Leave the box in Tyler's office for FedEx to pick up.
- From MDDS: they will package your materials for you, and you will write the shipping information on provided labels. You can also request a tracking number, which they attach to the box for you. Take a picture of the tracking number and save OFFLINE Leave the box with MDDS to mail out.

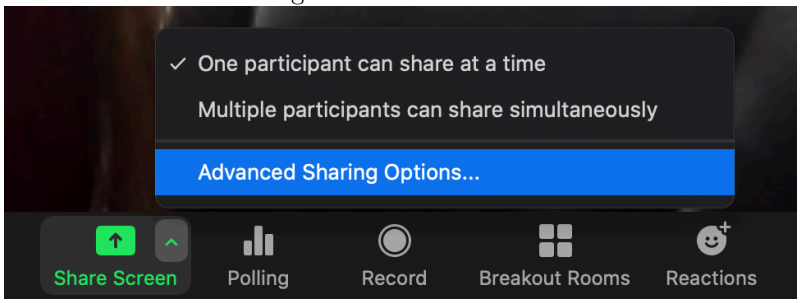
4.2.2.2 Zoom security Settings

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

are selected 

*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

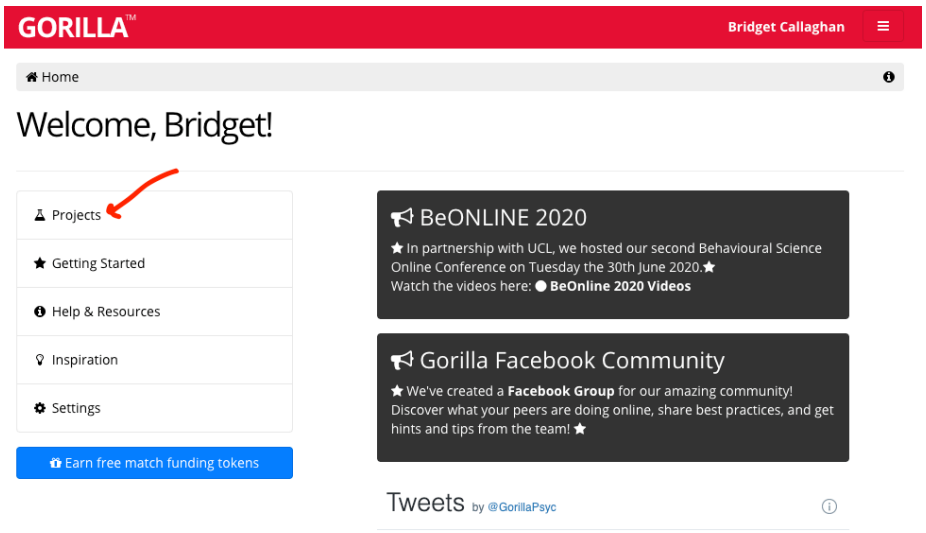


Figure 4.1:

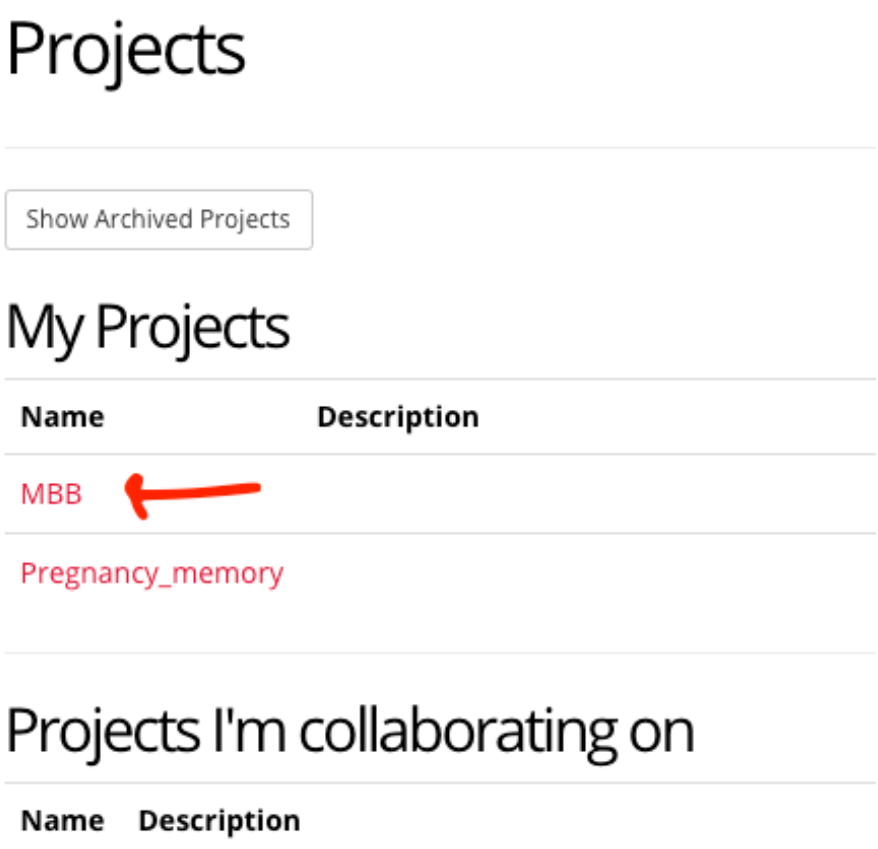

Projects
Show Archived Projects
My Projects
Name
Description
MBB
Pregnancy_memory
Projects I'm collaborating on
Name
Description

Figure 4.2:

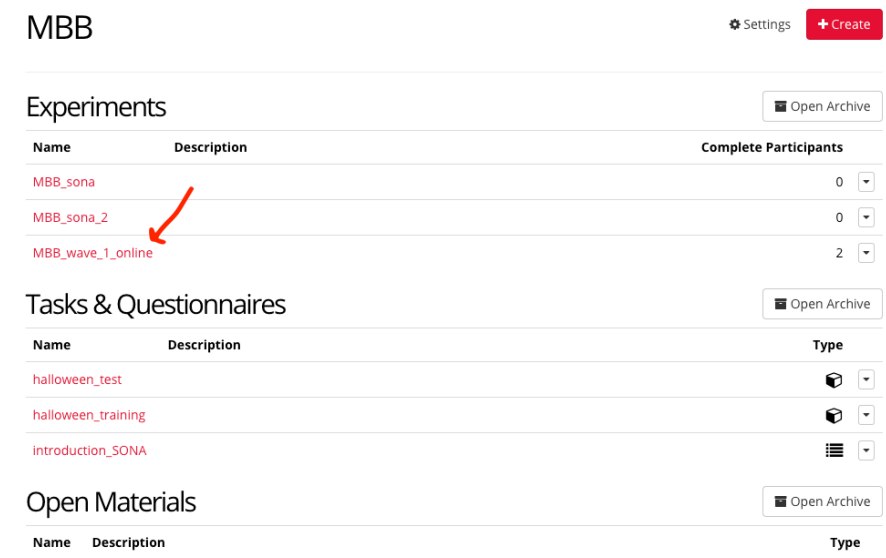


Figure 4.3:

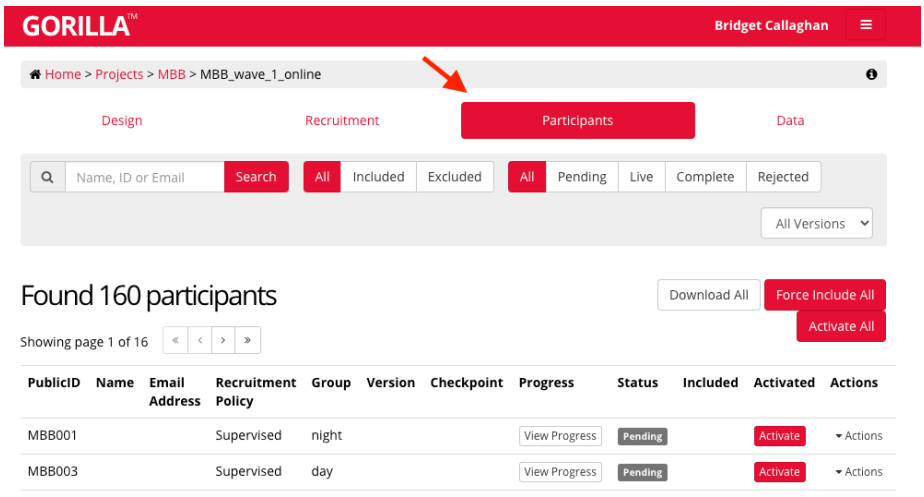


Figure 4.4:

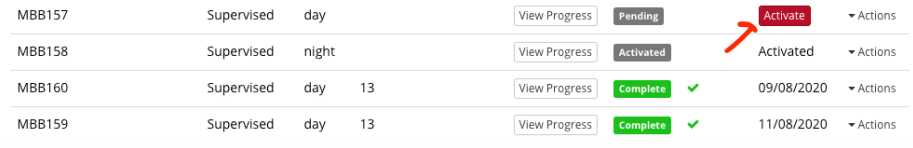


Figure 4.5:

4.3 W1-O Protocols - Session 1

4.3.1 W1-O Protocol - Welcome & Zoom Introduction

[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 2 hours 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 15 minutes and complete a computer game.

So first, I'll just ask- have you ever used Zoom before?

[If yes, say] *Great! So you are probably familiar with the different functions here, but I will just give you a little refresher on some buttons we will need for today. The most important thing you need to be aware of is the "chat" button below. If you click on it, a chat box should open- I will be using this throughuot the session to send you imporant links. You may already know this, but you can also display your camera so that it is in gallery view or speaker view in the top right corner. I think it might be best for you to do speaker view so it will feel more like we are in the room together!*

[If no, say] *No problem! Welcome to Zoom- it is very easy to use! The most important thing you need to be aware of is the "chat" button below. If you click on it, a chat box should open- I will be using this throughuot the session to send you imporant links. You can also display your camera so that it is in gallery view or speaker view in the top right corner. I think it might be best for you to do speaker view so it will feel more like we are in the room together!*

[Troubleshooting...]

Great! If for any reason, we lose each other over Zoom, the connection seems to be bad, or one of us freezes, lets leave the session and try to reconnect on the same link again. If I am frozen- feel free to leave and come back. If you leave and I don;t see you come back, I will try to give you a phone call!

How does that sound? Do you have any questions?

[If no, say] *Great! Lets switch gears and talk a little bit about what we are going to do today. Let me just pull up a little presentation we have got!*

[Researcher to open up the MBB_Consent_Script_Presentation]

4.3.2 W1-O Protocol - Consent & Assent Online

Before we begin, I just wanted to let you know that in this study, we ask a lot of questions about family life and tough things that might have happened to kids. We are going to ask these questions to you and we ask a couple to your children as well, we just want to let you know that it is in our protocol to make sure everyone is safe, so if you or your child reveals to us that you're at current risk of harm or if you're at risk of harming someone else, then we will follow up with you and make sure everything is okay. If it is not, then we will take some steps to make sure that we keep everyone safe.

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 not so fun things and some fun things while on ZOOM. This conversation will be recorded but we will not be watching or listening in

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 split 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 in the second session:

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 as apart of Session 2, we will pay you another $20!

We will send the full payment of $65 ($45 for today's session and $20 for completing the Session 2) as soon as we receive the samples back through the mail.

Here are some things to keep in mind:

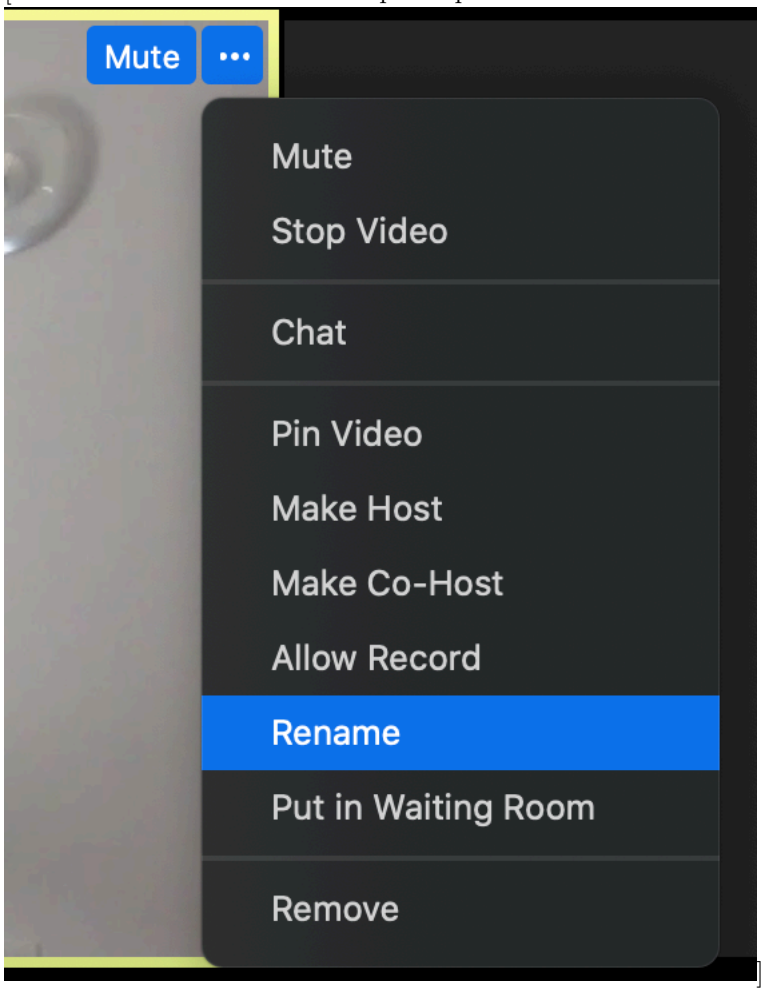
You are a volunteer in this study, which means you do not have to do anything, or say anything, that makes you uncomfortable. We would like you to try everything you can, and to do your best, but if there are things you absolutely do not want to do, just tells us, that is o.k.

Another thing we want to remind you is that nothing you do today is a TEST!! We want you to try your best, but there is no "right" or "wrong" answers in anything you do today. We just want to learn about you!

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.

I will rename your "ZOOM Name" right now with your Secondary ID number, to prepare for the recordings we will take in our session today

[Researcher will rename the participant's name to "P" for participant.



4.3.3 W2 Protocol - Consent & Assent Online

Before we begin, I just wanted to let you know that in this study, we ask a lot of questions about family life and tough things that might have happened to kids. We are going to ask these questions to you and we ask a couple to your children as well, we just want to let you know that it is in our protocol to make sure everyone is safe, so if you or your child reveals to us that you're at current risk of harm or if you're at risk of harming someone else, then we will follow up with you and make sure everything is okay. If it is not, then we will take some steps to make sure that we keep everyone safe.

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 not so fun things and some fun things while on ZOOM. This conversation will be recorded but we will not be watching or listening in

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.

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 split 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.

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

For helping us out in today's session, you will be getting $30 for the work you put in! After this online session is over, we'll ask you to do four more things in the second session:

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

4. Four is the blood sample – this helps us look at immune markers which are very important for interpreting microbiome system because those systems talk to each other

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

Later in the year when things are safer, we would like you to invite you to come in for an optional brain scan using the MRI machine. You don't have to decide whether you want to do that now, but we just wanted to mention it here.

You will receive $20 for doing the poop sample and $15 more for doing the blood sample!

We will send the full payment of $65 ($30 for today's session, $20 for the poop sample, and $15 for the blood sample) 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.

Another thing we want to remind you is that nothing you do today is a TEST!! We want you to try your best, but there is no "right" or "wrong" answers in anything you do today. We just want to learn about you!

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.

I will rename your "ZOOM Name" right now with your Secondary ID number, to prepare for the recordings we will take in our session today

[Researcher will rename the participant's name to "P" for participant.]

Ok- I will now send you a link through the Zoom chat, which will open up a survey that allows you to virtually indicate your consent to participate in the study. Please feel free to take your time reading through the consent and assent forms now if you'd like. As I mentioned earlier, these are the documents we had previously sent you in the emails leading up to today's session.

[Bottom of consent form has the information about the arm poke, make sure to mention that and note whether they consent]

[Then once they are done reading through]

All set and any questions?

[IF NO,] *Please click to indicate yours and your child's consent/assent and verbally let us know each of the items you have consented/not consented for, so we can adjust the session in case there is something you/your child may not feel up for today!*

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 as apart of Session 2 as well.

If you haven't already done so, please open the magic box up. The magic box should contain a sheet of paper at the top which details all of the contents, but we will let you know when you need to take each item out of the box. 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 accomplish today! Each space is like each task for the session. Once you complete the task you can put a gold star on the space!

Are you ready to get started on the session? Great, lets dive in!

4.3.4 W2 Protocol - Parent Child Observation Online

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.

[RESEARCHER SHOULD ASSESS VOLUME. If participant volume is too low, recording will not pick it up. Ensure that volume is at an appropriate frequency before proceeding to PC interaction recordings.]

[RESEARCHER SHOULD CHANGE TO SPEAKER VIEW. Participants should be in speaker view so recording will enlarge the parent and child for behavior analysis.]

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 "Issues 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 (Issues Checklist) 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. Please choose something you can try to resolve (not an emotionally charged topic that would be difficult to discuss together right now). And we want you to remember- this is not a test and there are no right or wrong things to say!

I am going to turn off my camera and mute myself to be out of the recording, and I will also turn my volume down low and will not be listening in closely. I will come back in and let you know when the six minutes are all done!

When you are ready, I will begin the recording and give you a total of 6 minutes- one minute or so to choose, then five minutes to discuss! Are you ready?

Step 2:

- 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 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 set timer for 6 minutes

Step 3:

- Researcher will mute themselves on Zoom and turn off their camera, and step out of the room.
- Researcher will start timer for 6 minutes. At the end of 6 minutes, reenter camera view and unmute themselves.

[After 6 minutes have passed, say] *Thank you for taking the time to discuss something difficult. Next we are going to take some film of you talking about something nice. You can flip to the page that says "Pleasant Events Checklist." On your "Pleasant Events Checklist" 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. Again, 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. When you are ready, I will start the recording and then give you a minute to choose and five minutes to discuss!*

I will be give you six minutes in total, one minute to choose and 5 minutes to discuss- remember, try to plan whatever fun thing you talk about during those 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 start timer for 6 minutes.

Step 6:

- After six minutes, 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 " Wave 1 Post-Online Session Protocols to view instructions on how to save the video recording.

4.3.5 W2 Protocol - Halloween Training/Parent Questionnaires

Now we are done with the group activity. You can go ahead and take out your token board and gold star stickers, and stick one golden star on the first block, where the movie icon is!

Next we will move on to some individual activities, where Mom/Dad will complete some surveys while you [Child's name] 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

[If the parent decided to fill out questionnaires on paper, say] *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 booklets titled "Parent Proxy Surveys" and "Parent Self Surveys" and a pencil to fill these out. We ask that you please fill the parent proxy surveys first and then parent self surveys. Some of these surveys are about your child and some of these surveys are about you. The game will take around 5-10 minutes and I will notify you when we are finished. You can go ahead and work on the surveys now while your child plays the game.*

[If the parent decided to fill out questionnaires online, say] *While your child is doing the game, you can get started on some parent surveys. So now I will ask you to grab your secondary device and log into the "links email" I sent just before the start of our session. In that email, you will find two links and two codes- one for parent proxy surveys and another for parent self surveys. We ask that you please fill the parent proxy surveys first and then parent self surveys. Some of these surveys are about your child and some of these surveys are about you. In addition to these two sets, there is a short packet of paper surveys to complete- these are recollected surveys that you may not have finished from wave 1 of our study. The game will take around 5-10 minutes and I will notify you when we are finished. You can go ahead and work on the surveys now while your child plays the game.*

I will now just give [Child's name] some instructions on the computer game. Let me share my screen so I can show you some examples!

Researcher to open up the halloween_training]

Ok, [Child's name]- so in this computer game, you are going to go on a trick-or-treating adventure! First, you will be shown instructions that look just like this! The places you will be visiting are scenes that either have a toy or food. Your job is to do their best to remember what toy or food is with what scene.

Here is an examples of what you might see

In this picture, you are at a park and you got pancakes!

Here is another example.

In this picture, you are at a dark forest and you got a pogo stick!

Sometimes you will get food while other times you will get a toy, and sometimes you will be visiting a nice place while other times you will be visiting a scary

one. Remember that the type of food or toy will vary and where it is found will differ. You will only be shown the photos for a few seconds, so do your best to memorize what food or toy goes with each place as quickly and as much as possible!

We also want to make sure you are paying attention, so when you see this red triangle, we want you to hit the space bar (or click the square)!

You will be reaching a halfway point where you can just click "continue"

There are TWO PARTS to this game- we are only going to be doing PART 1 right now. So, it is super important that when you see the page that says you're finished with this part, PLEASE LET ME KNOW!!

[Researcher to stop sharing screen.]

So to get setup for the computer game, we will send you a link through the Zoom chat now. Let me know once the link has loaded.

[Researcher to send Gorilla task link through the chat, then wait for them to pull it up.]

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 primary participant ID screen.*

Ok! If you are ready to start, we will just have you or Mom/Dad click "Log in" on [Child's name]'s page, and you can get started!

Researcher to open gorilla to track participant progress. Click on my projects>MBB>MBB_wave_2>participants tab>scroll to their mbb number and click "view progress"]

[RESEARCHER TO TAKE NOTES: PARENT BEHAVIOR DURING THE HALLOWEEN TRAINING]

[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 you confirm on the "view progress" node that Halloween training is complete, say] *Great job! We have completed the second part of this session- you can go ahead and take out your token board and gold star stickers, and put another star on the second block!*

4.3.6 W2 Protocol - Height Online

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 Magic Box and pull out the paper measuring tape.

Please measure your child's height in inches.

[Researcher to note child's height on the session checklist REDCap.]

4.3.7 W2 Protocol - Weight Online

Next, we will do weight! 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. You do not have to bring the scale to the camera!* [Researcher to note child's weight on the session checklist on REDCap.]

[If no, say] *Can you please record an estimate of your child's weight?* [Researcher to note child's weight and check "approximated" on the session checklist on REDCap.]

4.3.8 W2 Protocol - Waist Measurement Online

Next, we will do the Waist measurement! You can go ahead and grab that paper measuring tape once again, and measure your child's waist at the belly button. You can do this over their t-shirt.

- 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

[Researcher to note child's waist on the session checklist on REDCap.]

Great! [Child's name]- We finished the height, weight, and waist measurements you can stick a star on the third block of your token board!

4.3.9 W2 Protocol - Halloween "Test" Online

OK! It is time to go back to our learning and memory section.

[Give child instructions on Halloween Test] [Researcher to open up the halloween_test]

Ok [Child's name]- now we want to see how much of your trick-or-treating adventure you remember. You will see an instructions page like this first!

In part A you will be shown food or a toy and will be asked if you saw that food or toy when you went trick-or-treating. This is where your memory kicks in!

Here are some examples of what you might see.

In this case you are shown a pancake and asked did you see this while you were trick-or-treating

If you answer yes or no you will then be asked how sure are you that you saw that food or toy.

Part B shows the scenes that you visited and asks what food or toy was found there and where it was located on the screen.

Here are examples of what this looks like!

You will be shown a picture like this and asked which of the three choices you saw at that place.

Then you will be asked where on the picture you saw the food or toy.

If you are not sure you remember where the toy or food was,that is completely okay! We just ask that you make your best guess!

Again, when you see the red triangle make sure you hit the space bar or click the square.

When you finish, all you have to do is let me know!

[Researcher to stop sharing screen.]

Can you please go back to 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 with primary participant ID]

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 same logon screen where you can enter your MBB ID. Do not click submit just yet!*

[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 don't get help from you! We prefer you don't watch the screen so you don't know which pictures they see. 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!*

Ok! Now you can get started!

[RESEARCHER TO TAKE NOTES: PARENT BEHAVIOR DURING THE HALLOWEEN TEST]

[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?*

[Child's name] great job! Now that we are done with the computer game, you can go ahead and put another star on your token board!

4.3.10 W2 Protocol - Saliva Sample Online

Now, we will do some sample collections! The first one is the Spit Sample. As I mentioned earlier, you can flip to the instructions in your session booklet if you would like, but I will also walk you through it step-by-step! First, please grab your "spit tube" from the Magic Box. When you are ready, I will let you know what to do next!

First, we just want to check-in to make sure you haven't had any food, water, drink, or gum in the last 30 minutes?

[If yes, say] *Ok- no worries, we just have to wait that time before we can collect the spit sample. Let's move on to the next task and come back to this later!* [Researcher to make note on home session checklist and return to this item later in the session.]

[If no, proceed.]

[Researcher to walk through Spit Sample.]

You can throw away all of the packaging once you have finished with the spit tube. All you need to return to us is the tube inside the biohazard bag with two cotton balls, inside the white cardboard box.

We are now done with our spit collection! You can put another gold star on your token board!

- 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 spit tube, to release the stabilizing solution and seal the sample **Tell parent to close the cap very tightly and to shake the tube for 5 seconds**
- 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

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4.3.11 W2 Protocol - Hair Sample Online

Next up is the Hair Sample. Feel free to flip to the section on Hair Sample Collection in your session booklet, if Magic Box. When you are ready, I will walk you through it step-by-step!

4.3.11.1 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 rat-tail comb to thin out hair (provided)
  - 2 alligator clips (provided)
- 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 rattail comb
  - Painter-tape
  - 2 alligator curl clips

4.3.11.2 Explanation

- Refer to the instructions booklet included in the participant's magic box, found here: hair_sample_collection_instructions
- Tell the parent that you will go over it verbally with them BEFORE they should start collecting the sample.
- 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. Show them the amount on your own 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 if the parent would like a more comprehensive visual.
- After taking the sample, the parent will tape it to the aluminum foil. When doing this, it is VERY important to place the tape at least 3cm below the root end. To show the parent how long 3cm is, you can use the measuring tape provided, or tell them it is 1.5 inches (really closer to 4cm but better longer than shorter).
- If hair kit included painters tape, ensure that at least 3 cm of root is above the painters tape, and that the tape does NOT cover the root. If hair is shorter than 3cm, instruct not to use the tape.
- If hair kit included rubber band, ensure that rubber band is put on hair PRIOR to cutting. The rubber hand then helps secure the bunched hair in the foil after hair sample is taken.
- When they are ready to package the hair sample, ensure that the foil does not fold at the root- this is a very sensitive area in which we are analyzing, and important not to damage!
- When finished, hair sample should be put in the foil, in the ziploc bag, in the white box along with the other samples

4.3.11.3 Hair Length

- For short hair (less than or just above 3cm, i.e., too short to tape and still have 3cm usable), follow the Short-Hair Protocol below.
- For longer hair (>3cm), follow the Longer-Hair Protocol below.
- Ideally, hair samples 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:

- Select a portion of hair from the posterior vertex of the head (in line with ears is a good reference point).
- Use the rattail comb to thin out the hair as much as possible, so that the parent is grasping a horizontal line of hair rather than a chunk.
- Ensure that 30-50 strands are included in the portion being held.
- Hold the loose hair tightly with index finger and thumb, and cut the hair along the part, as close to the scalp and as evenly as possible.
- 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 and Wave.

Longer-Hair Protocol (>3 cm) - 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.
- Instruct the parent to grasp approx. 30-50 strands of hair under the hair that has been clipped up.
- Use the rattail comb to thin out the hair as much as possible, so that the parent is grasping a horizontal line of hair rather than a chunk. After this step, ensure the parent is still grasping 30-50 strands.
- Instruct the parent to cut the hair sample as close to the scalp as possible.

- Attach the hair to the center of the aluminum foil by taping with painter's tape - leave at least 3 cm of hair from the root end; not cover the root end.
- If parent does not know how much 3cm is, you can: say that they can measure it with the cm side of the measuring tape; if they know inches say it is about 1.5 inches (really closer to 4cm but better too long than too short); or offer to demonstrate based on the length of your own finger.
- Place the hair inside the aluminum foil in the orientation indicated by the "root" written on the foil.
- Fold the foil without bending the hair, and ensure that the hair does not fall out of the foil.
- Place the folded foil in the ziplock bag.
- Put the ziploc bag back in the magic box.

Now we are all done with the hair sample! You can go ahead and put another star on your token board!

Participants can also watch the video below:

4.3.12 W2 Protocol - Child Questionnaires Online

4.3.12.1 IF THERE IS NOT ENOUGH TIME

- If parents want to cut time, questionnaires can be done at another time, unless we have to read the surveys to the child through share-screen. (This applies to children who may have trouble reading, or are under the age of 8.)

4.3.12.2 Before Starting Surveys

Next, we will move on to some Child Surveys. [Parent's name], for this next part we will be asking [child's name] some questions. Some of these questions might be about you, like about how supported she feels by mom/dad, or her life. We just want to make sure you don't provide parent help with this part! So if you prefer to step out of the room or if [child's name] wants to wear headphones while they answer that would be fine- if not, no worries!

[If yes, tell parents to work on parent questionnaires in the meantime. If they will leave the room, we will have the child call them when we are ready to regroup.]

[If no, say this is alright and no worries. Parent can stay in room and work on parent questionnaires. Researcher to take note in session 1 checklist.]

4.3.12.3 Recollected Surveys

[If there is a child recollected survey that needs to be administered **complete recollected surveys before doing rest of surveys**]

So these next surveys are ones that we noted were not completed during your first round of sessions with us. We wanted to include them here so that you may complete them if you would like. Of course, like everything else in the study these are optional so if you do not wish to provide this information, you may skip any question.

For recollected surveys we will be sharing screen and reading out the survey to the participant regardless of age.

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]

4.3.12.4 Wave 2 surveys

[**Check how many surveys child has to do before either beginning with child surveys**]

4.3.12.5 Ages 8+

I can either send you the link to the surveys and you fill them out on on your own or I can share my screen and read the questions out loud and you tell me what to respond.

[If the child wants to do it together, refer to instructions under AGES 6-7 below]

[If the child wants to do it on their own, say] *I am going to send you a link through the Zoom chat, with a code you will input to access the survey! We are ALMOST done with the session, and these surveys will not take too long and I will be here if you have any questions!*

[Confirm the child is on the right page before proceeding.]

[To the child, say] *Let me know if anything is confusing, or if you have any questions! Let us know when you are all done!*

[RESEARCHER TO TAKE NOTES: PARENT BEHAVIOR DURING THE CHILD QUESTIONNAIRES]

4.3.12.6 Ages 6-7

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 TAKE NOTES: PARENT BEHAVIOR DURING THE CHILD QUESTIONNAIRES]

END SURVEYS

Now that we have finished all of your surveys, we can take out the token board and put a gold sticker down. Look, we are just about done!

[If parent departed the room or if child is wearing headphones, ask child to get parent back on screen or take headphones out so parent can rejoin the session.]

4.3.13 W2 Protocol – Explaining at home part of Session 2

At this time, I will now ask you to reach into your Magic Box and pull out your "Session 1 Booklet" out and flip to the cover page that says "Session 2 Booklet" so I can walk through the Session 2 instructions

[Wait for participant to retrieve Session 2 Booklet]

- Stool Sample explanation & BSS sheet
  - *In the "Session 2 booklet" there are instructions for the POOP Sample Collection and a short survey that should be filled out after the poop 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.* Please complete the poop sample collection and finish filing out the surveys this week so the package is ready to be sent out by our second session next week.* If you have any questions please reach out to us via email or give us a call.*
  - [RESEARCHER THEN GIVE DETAILED POOP SAMPLE COLLECTION AND PACKAGING INSTRUCTION]
- ASA explanation
  - *You will also be filling out one more survey called the ASA. The survey asks what your child's general diet and what they generally eat which gives us information about their nutrition. If you fill it out we can also send you a nutrition report later if you are interested. It*

is entirely online and we will be sending you the link to the survey in an email shortly. The session 1 packet should have a page that says ASA. That page has the login information which you will use to access the survey.

- Sleep Diary explanation
  - *Your child will be filling out a sleep diary every day for the week between today's session and your session 2. The sleep diary is in the survey packet after the page that says Child self.*
- Confirm Session 2 date and time
  - *I just wanted to confirm that your current session 2 date and time still works for you. If the package is ready by the time of your session 2 are you able to drop it off that day or the next day?*
- Payment
  - Explain that once the return mailer has been received to the lab after the second session, we will send payment through the mail

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4.3.14 W2 Protocol - Reporting

4.3.14.1 If an item on the "reporting" list is activated regarding potential Domestic Violence

- Researcher to chat with parent alone at the end of the session

[Researcher say] *So now that we have reached the end of the session [child's name] is all done! Congratulations and thanks so much for your hard work today [child's name]! Can we just grab [parent's name] for a chat real quick? This last part will just be between mom/dad and me.*

[Researcher to wait for child to leave]

Awesome! So I just wanted to check-in about something with you real quick. Your child may have answered an item in our questionnaires related to [parents pushing or shoving each other/parents throwing things during fights]. It is in our protocol to just check-in and make sure everything is alright at home. UCLA has many resources we can connect you to if necessary. Is everything alright?

If yes, say

Great! I am glad to hear it. Thanks for letting us know! [Then proceed to thanking them for session and what to expect next week, etc.]

If no, say

Are you in any immediate danger at the moment?

[If yes, say] *Thank you for letting me know. We are going to get in touch with our supervisor immediately. Please give me a moment to get in touch with her now.* Researcher will then slack and call Bridget immediately at 347-741-4103.

[If no, say] *Would you like us to connect you with some resources at UCLA?* If yes, *What sort of support would you be looking for? I'll take this to my supervisor and she will give you recommendations and give you a call!*

If no, but you don't feel that everything is alright, say

Thank you for letting me know! I know this might be a bit difficult to talk about, and as we mentioned earlier in the session it is just part of our protocol to check-in, so our supervisor will be giving you a call some time in the next 24 hours to ask you some more questions. Researcher to slack Bridget immediately and call her at 347-741-4103.

4.3.14.2 If Suicidal Ideation arises...

Researcher to slack Bridget and call her immediately at 347-741-4103. Try to keep the person on the phone while Bridget gets in contact with the participant. If something happens and a participant mentions they may harm themselves/dropped off the call before you are able to reach Bridget, let Bridget know immediately and you will proceed together.

4.4 W2 Protocols - Post-Session 1

4.4.1 W2 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 a 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 working directory is saved.

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 captured.

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 in Save files to box in Box/BABLAB/Studies/Mind_Brain_Body/Data/Wave_X/Wave_X_parent_child_interactions/MB

For more detailed instructions with photos see Wave 1 protocol

4.4.2 W2 Protocol - Downloading the Gorilla task data

1. Login to Gorilla and navigate to the experiment's data tab.
2. If data is up to date, you can go ahead and click download.

2b. NOTE: ENSURE you are on the correct VERSION PICKER. You can double check your participant's version number in the "recruitment" tab where the participant lives. Change the version picker to the correct version for that participant.

3. If data is not up to date, scroll down and click the necessary options, then click "Regenerate Data".
4. You will see a wait screen as it generates.
5. After a few minutes click into "Manage experiment data" again and download.
6. Unzip the file to your desktop.
7. Open the correct training and immediate test files and check that the participant's data is all there. NOTE: can check the participant's specific nodes to see the code of their halloween test delay, which will tell you which file their data is located in.
8. Save this file in the Gorilla data folder on Box as "month_day_year.csv"

For more detailed instructions with photos see Wave 1 protocol

NOTE: If the data downloads and the first line of the participant's data is there but the rest is missing, follow this protocol:

1. Go to an earlier version of the task, regenerate, and download data
2. Go back to the version of the task that the participant belongs to, regenerate, and download data
3. Check to see if all the participant's data is there

4.4.3 W2 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 W2 Protocols - Session 2

4.5.1 W2 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 do the Stool Sample collection. Answer any questions they have about their Home Session tasks (Stool sample, BSS sheet, Contact list, ASA)]

So today we are just going to do one quick task, collect the blood sample (if stool sample is done and they consented to blood sample), walk you through how to close up package and send it back to us, and then answer any questions you might have.

OK! It is time to go back to our learning and memory section.

[Give child instructions on Halloween Test] [Researcher to open up the halloween_part_3]

Ok [Child's name]- now we want to see how much of your trick-or-treating adventure you remember. You will see an instructions page like this first!

You will first be shown food or a toy and will be asked if you saw that food or toy when you went trick-or-treating. This is where your memory kicks in!

Here are some examples of what you might see.

The next set of questions show the scenes that you visited and asks what food was found there and where it was located on the screen.

Here are examples of what this looks like!

We also want you to remember that this is not a test! Just try your best!

When you finish, all you have to do is let me know!

[Researcher to stop sharing screen.]

So to get you setup for the computer game, we will send you a link through the Zoom chat now. Let me know once the link has loaded.

[Wait for child to log back on with primary MBB ID]

If necessary - the link can be found here.

[Confirm that they are on the correct page]

[Once confirmed, say] *Ok! Now you can get started!*

[Once finished with game]

[Child's name] great job! Now that we are done with the computer game.

4.5.2 W2 Protocol – Home Session Check In

I wanted to check in if you were able to do the poop sample, fill out the BSS and finish the parent_proxy and parent_self surveys?

If yes: *" Great, thank you for completing all of that before our session today!*

Proceed to doing blood sample.

If no: *When do you think you will be able to finish? We will need to schedule a short 5 minute Zoom call within the next week so we can collect the blood sample. The sooner we are able to do that the better.*

- Schedule the third session within the next week at a time that works for you and the parent so blood sample can be collected.
- Emphasize that poop sample needs to collected before that session.

4.5.3 W2 Protocol – Tasso Blood Sample Online

[Verify they consented to blood sample during session 1]

Now we will be doing the blood sample. The kit comes with detailed instructions you can reference but I will also be going over with you right now.

Tasso Blood Sample Collection Video

1. Rub arm quickly and firmly just below the shoulder. Rub until it's very warm to help your blood flow.

2. Clean arm with alcohol pad.
3. Open device pouch by pulling apart white and clear layers.
4. Remove clear plastic cover over the red button.
5. Peel paper tab behind the red button. Keep sample pod pointing down.
6. Stick device to shoulder. Do not remove once it's on.
7. Press button quickly and firmly until it can't go any farther. Wait two seconds then let go.
8. Start a 5 minute timer. Keep arm at your side and watch dots fill. You won't see blood right away.
9. Peel off the clear film to expose the sample pod vent.
10. Place device in the foil return bag and seal. Leave the moisture packs in the bag.
11. **IMPORTANT: Write collection date and time on the inside flap of the box.**
    - Researcher will also make a note of date and time sample was collected and put it in notes of REDCap checklist
12. Place bag in box and seal with the strip.

- Note that if blood sample needs to be stored overnight (if the package is not sent out on the day it is collected) please store it in a cool dry place not under direct sunlight.
- If the package is ready to be sent back we will do the blood sample now and it needs to be sent out today / tomorrow at the latest
- **It is important that the blood sample be sent back the day it is collected or day after so emphasize that it is important that the whole package get sent back asap after blood sample collection.**
- Check that they write down date and time the blood sample was collected on the box the kit comes with

4.5.4 W2 Protocol - Mailing Package

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

- 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 absorbent, inside rigid white box)
- Double check that blood sample is correctly packed (in biohazard bag with absorbent, inside rigid white box)
- Drop off at any FedEx location or post box

4.5.5 W2 Protocol - Payment

- Explain that once the return mailer has been received to the lab after the second session, we will send payment through the mail
- 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

4.5.6 W2 Protocol - Prize

- Before showing prize options check Prize Log to verify what prizes are available.
- Ask what prize they want. We will share screen and share the prize presentation. Whatever they choose researcher will make a note on checklist and lab manager will reference that when sending payment package. As prizes get claimed those choices will get crossed out.

You get to choose a prize for participating in the study! I am going to share my screen to show you the different prize options we have.

[Researcher will open up Prize Presentation]

- Once participant chooses prize researcher will make a note on the REDCap checklist.

Great, we will send your prize with the payment!

4.5.7 W2 Protocol - Testimonials

[Only ask if participants seem like a good candidate for a testimonial / had a particularly good experience particiapting.]

We wanted to know if you are interested in providing a testimonial for our website about your experience participating in our Mind, Brain, and Body study. If you

choose to provide a testimonial we will post it on our website. We would also include first name and your child's first name and age if you grant us permission to do so. Is that something you would be interested in?

If yes: *Great, thank you for your interest in sharing your experience! Please send us the testimonial via email and indicate whether we can include your names. It can also be anonymous if you would prefer that.*

If no: *No problem at all, thank you for participating in our study!*

4.6 W2 Protocols - Post-Session 2

4.6.1 W2 Protocol - Downloading the Gorilla delay test data

1. Login to Gorilla and navigate to the experiment's data tab.
2. If data is up to date, you can go ahead and click download. NOTE: ENSURE you are on the correct VERSION PICKER. You can double check your participant's version number in the "recruitment" tab where the participant lives. Change the version picker to the correct version for that participant.
3. If data is not up to date, scroll down and click the necessary options, then click "Regenerate Data".
4. You will see a wait screen as it generates.
5. After a few minutes click into "Manage experiment data" again and download.
6. Unzip the file to your desktop.
7. Open the correct delay test file and check that the participant's data is all there. NOTE: can check the participant's specific nodes to see the code of their Halloween test delay, which will tell you which file their data is located in.
8. Save this file in the Gorilla data folder on Box as "month_day_year.csv"

4.6.2 W2 Protocol - Downloading the ASA data

1. Navigate to the ASA website - Slack Lab Manager or check internal for login

2. Check whether participant has completed their ASA surveys by clicking on "Track Recall/Record"

- User IDs for Wave 2 should take the format of MBB2999
- Scroll to the bottom of the list for the most recent entries

3. If the participant has completed the ASA, download their nutrition report

- Scroll to the right and click on the "View" button under the Nutrition Report column
- Click File>Print then Save as PDF under the naming convention "MBB2999_asa_nutrition_profile.pdf"
- Save to the participant's Wave 2 MBB data folder in the Report_card folder and delete the 999 template

4. If there is no nutrition report, it is because the particiant neglected to fill out crucial information (e.g. age, sex, pregnancy questions) that ASA requires in order to build the report. We cannot get the report unless the participant were to redo the entire survey, so move to step 5 if this is the case.
5. If the participant has completed the ASA, we also need to get their data outputs

- Click on "Analytic Files" and select "One Respondent"
- Type in the participant's MBB2999 ID in the User Name
- Select "Download analysis files" and you should get 6 csv files in your downloads folder
- Rename the files according to the templates in the participant's MBB data folder in questionnaires>asa and save

6. Mark off as complete on the Wave 2 data entry sheet of the participant log

4.6.3 W2 Protocol - Session 2 TO DO List Email

- Fill in templated email with all leftover tasks for participant to complete

- This email is designed more for participants who have not done the stool sample yet, or still have outstanding tasks from Session 1 that we'd like to remind them about immediately/are time sensitive.

4.6.4 W2 Protocol – If blood sample not collected during session 2

- Add to calendar the date and time scheduled to meet with participant again to collect blood sample. This is going to be a session 3 of sorts.
- Change home session R1 email 1 calendar event date to one week from blood sample collection (session 3)
- Change home session R1 call calendar event date to 8 days after "session 3"
- Change home session R2 email calendar event date to two weeks after "session 3"
- Change home session R2 call calendar event date to 15 days after "session 3"

4.6.5 W2 Protocol - If participant had feedback to give

- If participant provided feedback on their experience or had comments to give us make a note of it on the "other notes" section of the session 1 checklist on REDCap. Also send a message to lab manager with an overview of feedback provided and whether it is feedback we should consider for the future.

4.7 W2 Protocols - Final

4.7.1 W2 Protocol - Storing Saliva Sample

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

4.7.2 W2 Protocol - Processing Saliva Sample

4.7.2.1 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:

  - Incubator with thermometer
  - Vortex machine
  - Eppendorf pipette (1000uL)
  - Pipette tips (wide bore, sterile, universal fit)
  - 2.0mL cryogenic vials (with O rings, sterile)
  - Saliva samples in room temperature plastic box

  - Test tube rack
  - Transport box with divider
  - Industrial Sharpie for labeling
  - Notebook for sample logs with pen

Steps for incubation:

- Retrieve the box of saliva samples at room temperature in BAB Lab Cabinet 1 and carry them to HPL.
- Plug in and turn on the incubator, turn the dial to level 10 and ensure the red "heating light" is on.
- Continuously check the incubator to see what if temperature has reached 50-55C.
- While waiting for the incubator to hit 50 C, clean workspace using ethynol and paper towels (dispose these in the regular trash bin)
- Open the saliva sample box in HPL and note down the list of samples to be processed today, and ensure the lid on each of these samples is shut very tight. If the lid is slightly opened, the sample may risk evaporation during incubation.
- Ensure you are wearing gloves when grabbing the 2.0mL cryovials. Label two cryovials per sample for those that will be processed today (use the Industrial Sharpie). Place them in the transport box for temporary storage.
- When the incubator has reached 50C, turn down the heat to level 6. The incubator temperature will still rise slowly, but when the door is opened for samples to be put in, the temperature can drop anywhere between 5-10C, depending upon how long the door is opened. To account for this temperature loss, heat the incubator to about 55C.

- When the temperature reaches 55C, open the incubator door and place all saliva tubes standing upright on the incubator tray.
- **IMPORTANT NOTE:** the incubator is extremely sensitive to movement - the saliva samples are likely to fall over with any large tap and/or placement of saliva samples on the tray inside. Be very careful placing samples onto the tray, closing the incubator door, and opening the incubator door once the incubation has completed.
- Close the incubator door *very* carefully and turn the knob as needed to regulated the temperature towards 50C. If the heat has dropped significantly, turn to level 10. If the heat has dropped slightly, move towards 6-8. If the heat is still too high, drop to level 2-4. If the heat is about right, keep the knob at level 5.
- Note down the current time and set an alarm for 2 hours to continue processing. Continue to check the incubator regularly to ensure temperature level is still at 50C and heat level does not need adjusting.

Steps for processing:

- Once the tubes have incubated for 2 hours, retrieve the cryovials, test tube rack, pipettes, pipette tips, and sample log notebook with pen.
- **Very carefully**, open the incubator door and remove five samples to place in the test tube rack. Close the incubator door. (NOTE: the samples are not very hot, so no additional safety protection is needed beyond gloves. If extremely sensitive to heat touch, use a paper towel to grab the samples out of the incubator.)
- Carry the five samples in the test tube rack to the vortex machine. Place one sample on the vortex machine and press down, holding for 20 seconds while the sample is shaken.
- After each sample is vortexed, turn the sample upside down and back up to see if any is still too viscous. If so, vortex for an additional 20 seconds.
- Carry the samples back to the Pipettes in the BAB area of HPL. Select the 1000uL pipette and spin the dial until the numbers on the back of the pipette read 1000. Open the box of pipette tips.
- **PIPETTING SAMPLE:** Locate the labeled cryovials for the sample you will process. Open the sample tube and place back down into the test tube holder. Open the cryovial and hold tube with one hand. Grab the pipette, push down into a new pipette tip, place into sample, press down until first stop, release to suck in first 1000uL. Release the sample into the cryovial, pressing down until second stop. Place pipette down on its side. Close the cryovial and place back into transfer box. Open the second cryovial, pipette what is left from the saliva sample into the second cryovial before placing into the transfer box. When there is no saliva left in the sample tube, close it and dispose in the biohazard bin. Release the pipette tip by pressing on the button located on the back of the pipette into the biohazard bin. Write down the sample quality and location in the sample log notebook.

- Wipe down the table with ethynol before replacing gloves, disposing in biohazard bin.
- Return to the incubator for the next five samples, repeating the **PIPETTING SAMPLE** step above for the rest of the samples.
- Once the samples have been processed, transfer the samples to the -20 freezer in the saliva box.
- Turn the knob on the incubator back to zero, and turn the incubator off. If researcher would like to return the incubtaor to room temperature quick, the incubator door may be opened to release heat faster.
- Take a picture of the sample log notebook and transfer notes to the sample log on Box

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4.7.3 W2 Protocol - Storing Hair Sample

- Store the sample in a dry area at room temperature

4.7.4 W2 Protocol - Storing Stool Sample

4.7.4.1 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.

4.7.4.2 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:

  - 2.0mL cryogenic vials

  - Stool samples in biohazard bag

  - Test tube racks
  - Transport box with divider
  - Sharpie for labeling

Steps:

- Clean workspace prior to placing shield down and paper towel over shield
- Untwist two 2.0mL vials and place them on the side of the shield to prepare
- 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 label with industrial sharpie
- Take a photo of the sample to store on Box
- 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 an industrial sharpie, ensure it has the participant ID and Wave 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 the -20 upright freezer in HPL
- 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
- Update the Stool Quality on REDCap

4.7.4.3 Taking out the Hazards

-Take the Hazards key with you from the lock box in the Lab. Make sure there are two keys attached- a large gold key and a small gold key. -Head to HPL.

Once inside, put on PPE. -Find the large gray cart in the back room, and wheel into the main HPL lab space. This will assist you in carrying the Hazards bin to the waste area. Put the entire Hazards bin (with the hazards bag full of materials) onto the gray cart.
-Ensure you either leave the HPL keys in the lockbox on the door or take them with you. -Wheel the cart to the elevator and take it down to level A. Turn right into the hallway and take another right out to the double doors that lead to the loading dock. Exit the building and be sure to close both doors- these doors cannot be propped open. -Wheel the cart to the gate. Use the small gold key to open the lock holding together the chains that lock the gate closed. Once you have the gate open, carry the hazards bin inside. -Take out the hazards bag and tie it closed. Leave the tied hazards bag in one of the red bins in the corner. -Bring the hazards bin back to the gray cart. Use the gold key to lock the chains and gate back up.
-Use the large gold key to reenter through the double doors. Take the elevator back to floor 1. Head back to HPL. -Once back in HPL, find a new hazards bag- the Brain and Body Lab hazards bags are located on the first shelf above our space in the corner. If we run out, Dr. Robles kindly invited us to use the Hazards bags in the HPL space which are located underneath the tables by the refrigerator.
-Discard your gloves into the newly replaced hazards bag.
-Make sure to return the Hazards keys to the lock box in BABLAB.

4.7.5 W2 Protocol - Tasso Blood Sample Storage

- Lab manager should try to store sample within one day of receiving sample
- Take the blood samples to HPL
- Take out the blood sample and twist off the button (counterclockwise until it stops, then release the sample from the button), leaving only the blood sample itself to be stored
- Discard the button in the biohazard bin in HPL
- Place silver Tasso biohazard bag with blood sample and silica gel in its designated space in the freezer box (in accordance to the Sample Storage Log Diagram) in -20 freezer
- Update MBB log
- Log the sample in the Sample Storage Log

4.7.6 W2 Protocol - Data Entry & Data Quality

4.7.6.1 Data Entry

4.7.6.2 Data Quality

4.7.6.3 Relevant Protocols

How to burn a CD - https://bablab.github.io/wiki_bablab/lab-protocols.html#burning-cds

How to make a high to low resolution video - https://bablab.github.io/wiki_bablab/lab-protocols.html#high-to-low-res-video

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4.7.7 W2 Protocol - Data Review & Data Audit

4.7.7.1 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.

4.7.7.2 Data Review

https://youtu.be/z_mQGyguaEY

- 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
  - blood 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.

4.7.7.3 Data Audit

Before reaching out to participants

It is important to check that information marked as missing on the participant's Trello card is *actually* missing before reaching out to participant to request it.

- Check for the missing information in the participant's folder on Box
- If a REDCap survey is missing check if it was maybe just not input on REDCap but is there in participant folder
- Look through email threads with participant for info on why a file might be missing or if they sent it to us in the past
- If it is info the researcher collected during the session (height, weight, waist) check if researcher has that info

IF the information is actually missing then you can go ahead and reach out to the participant

- You will be sending them editable copies of the files they are missing. It is advised you create some way to track each file (ex. you can name file as date of session and then delete that name later on)
- There is an email template available in the BAB email under templates titled "MBB_O - Data Audits Mind Brain Body Study"

Timeline for Data Audits:

1) Call participant and request missing information from them (surveys, contact list, etc). After calling (and leaving a voicemail if they don'tt answer) send them an email requesting they fill out missing info and upload it to our Box folder

2) *One week later* call them again and send another email. Leave a voicemail if they don't answer.

3) *One week later* call them again and send a third email. Leave a voicemail if they don't answer. At this point if you have not heard from them *at all* you can end the audit for that person.

    - if you *have* heard from participant but haven't gotten the missing info then extend the audit a week or two at your discrection if you think they will complete the missing info.

4.7.7.4 Documenting the audits

The folder for Box uploads is in BABLAB/Studies/Mind_Brain_Body/Data/Wave_1_onl
Check it to see if participant's have uploaded the missing info

After each contact

- Make sure you write in the description the date you reached out to participant and any notes from the contact
- If the participant did not seem willing to provide the missing info make a note
- If participant emailed add date of contact into description too

Successful Audit

1) Add the missing info to the participant's folder on Box.
2) Make a note on participant's Trello card that you received the missing info.
3) Add the info to the ammend list on the Trello card.

3.1) If you know how to input the information into REDCap and have time to do so you can add the info and then mark that item on amend list as completed.

- **If you input the missing data then go to the participant log and mark it as complete on the Data Quality Check tab**

4) If there are any items that need to be amended add an amend label to the Trello card
5) Move the Trello card to the appropriate Done column of Audit dashbaord

Unsuccesful Audit

1) Make a note in the description of participant's trello card that the data was not received
2) Add the no response label to the card.
3) Move to the appropriate Done pile of Audit board

4.7.7.4.1 Getting A Code From REDCap

1. Log onto REDCap and click on "record status dashboard"
2. Click on designated participant
3. Click on the first incomplete questionnaire for the parentself, parentproxy, or child questionnaire sets
4. Click on Survey Options
5. Click Survey Access Code and QR Code
6. Copy and paste web address and code + send to email to participant

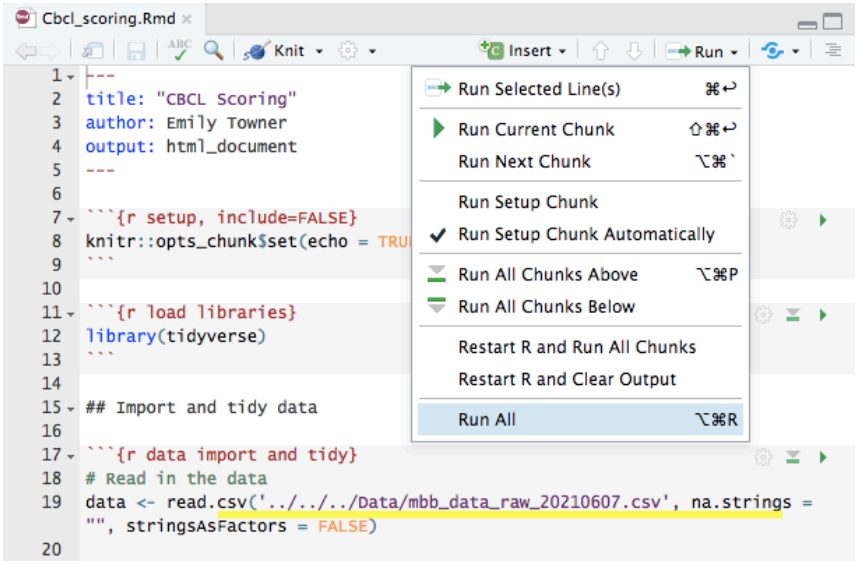
Refer to Wave 1 protocol for more details

4.7.8 W2 Protocol - Report Card

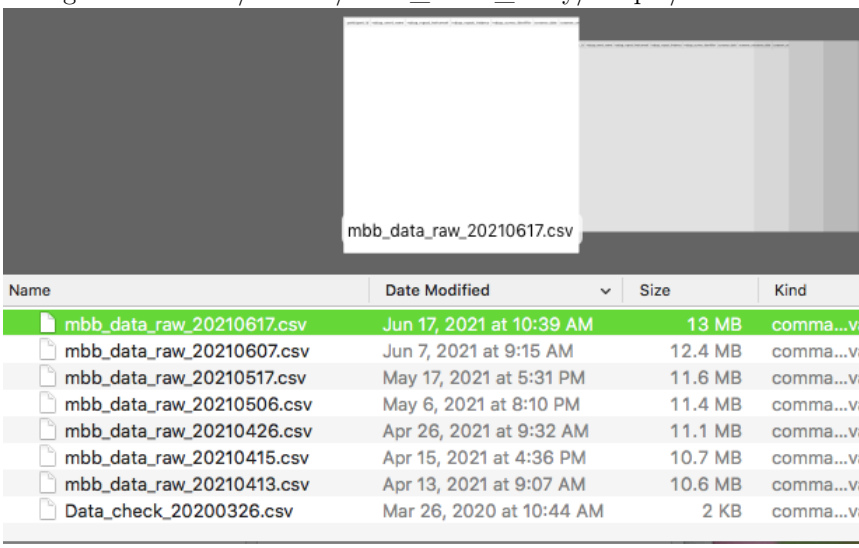
Scored Cbcl data for the report cards can be found in BABLAB/Studies/Mind_Brain_Body/Scripts/Wave_2/Data_

Running script to get cbcl data ready

1. Ask lab manager to update REDCap raw data file.
2. Open the R script for scoring cbcl using RStudio. **Make sure you open with desktop Box** BABLAB/Studies/Mind_Brain_Body/Scripts/Wave_2/Data_scoring/Cbcl/cbcl_scoring
3. Change the line with file name so it has the most updated file.

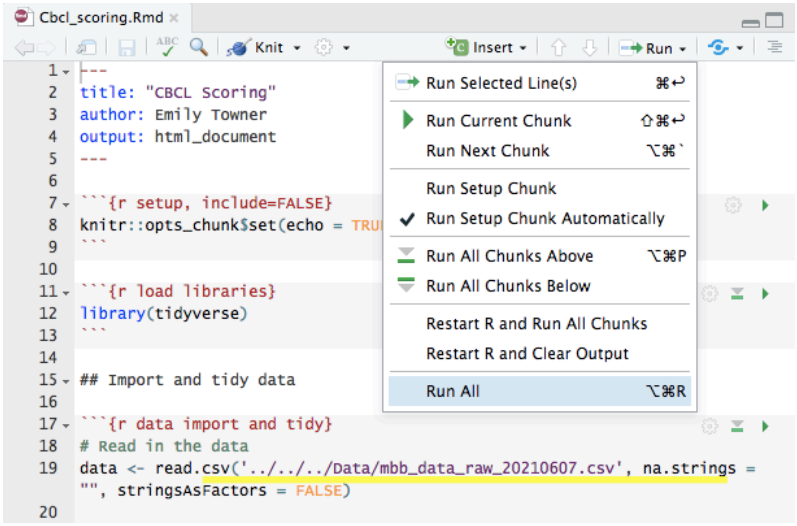


3.1 To access the raw data file name go to BABLAB/Studies/Mind_Brain_Body/Scripts/Data and look for most recent date.



4. Run the script by clicking run all.

    - *if there is an error saying data file couldn't be found make sure the file name is correct*
    - *Make sure you have all the "packages" needed for the script down-*



loaded in your RStudio

Making report card

1. Open a participant data folder - BABLAB/Studies/Mind_Brain_Body/Data/Wave_2/Wave_2_data
2. Navigate to the report card folder and rename the template file - MBB999 to the relevant participant - and open the file

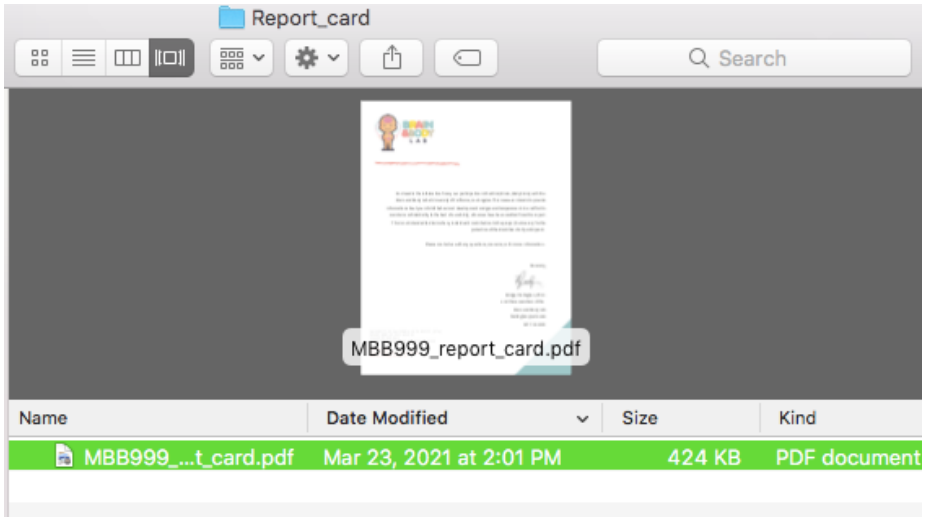


Figure 4.6:

3. 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.

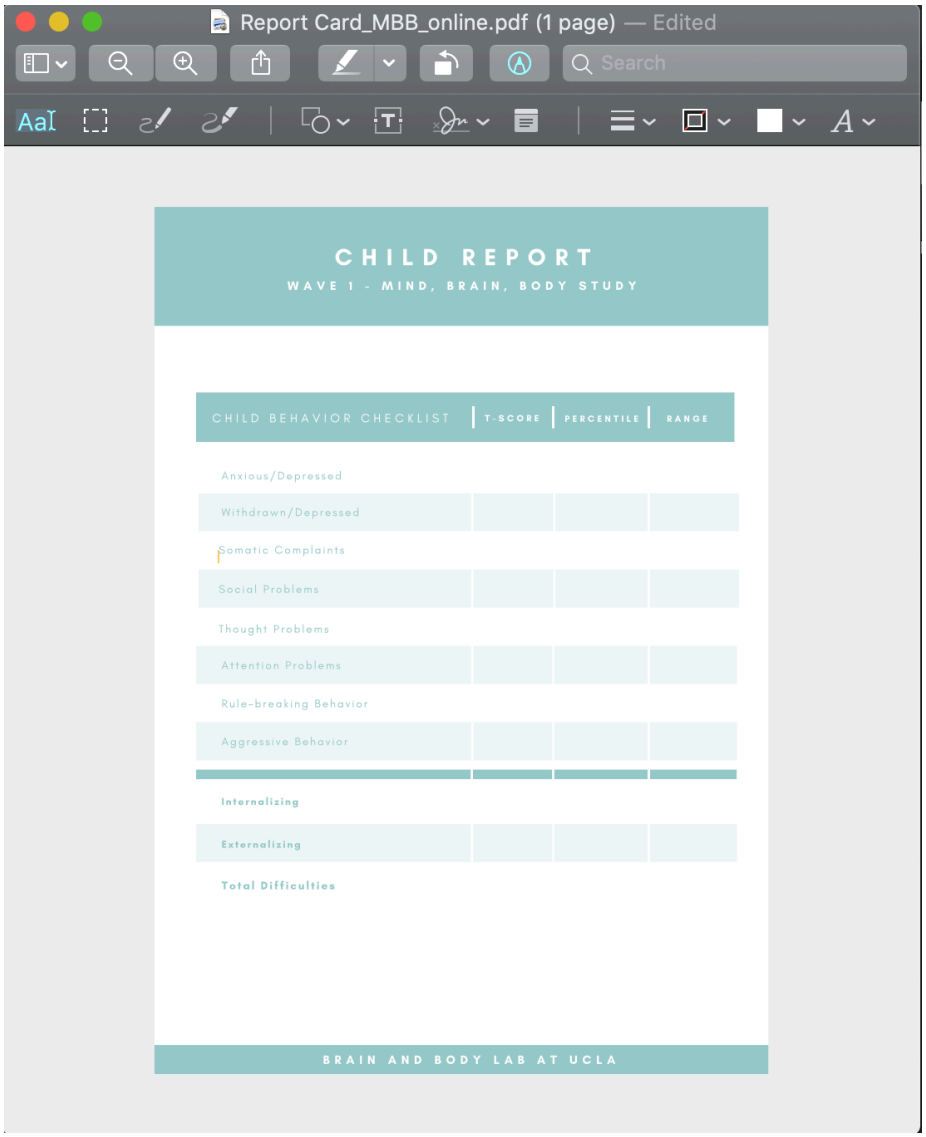

Report Card_MBB_online.pdf (1 page) — Edited
Search
CHILD REPORT
WAVE 1 - MIND, BRAIN, BODY STUDY
CHILD BEHAVIOR CHECKLIST
T-SCORE
PERCENTILE
RANGE
Anxious/Depressed
Withdrawn/Depressed
Somatic Complaints
Social Problems
Thought Problems
Attention Problems
Rule-breaking Behavior
Aggressive Behavior
Internalizing
Externalizing
Total Difficulties
BRAIN AND BODY LAB AT UCLA

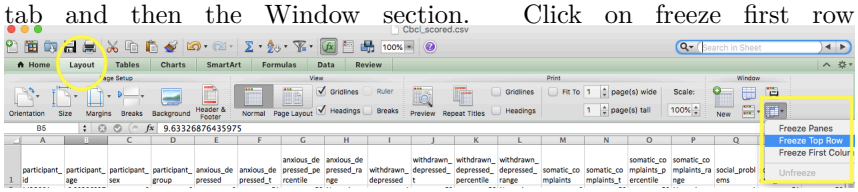
Figure 4.7:

4. The data you are inputting into the report card comes from the file titled "Cbcl_scored.csv" which can be found in BABLAB/Studies/Mind_Brain_Body/Scripts/Wave_2/Data_scorin

4.1 Some info about the scored cbcl data file

- *Make sure you do not save any changes you make to the cbcl file. Open the file on Desktop Box*

- You can freeze the first row of the data by going to Layout tab and then the Window section. Click on freeze first row



- Make the names of the columns fully visible by clicking on the 1 to highlight row 1 then clicking Wrap text.

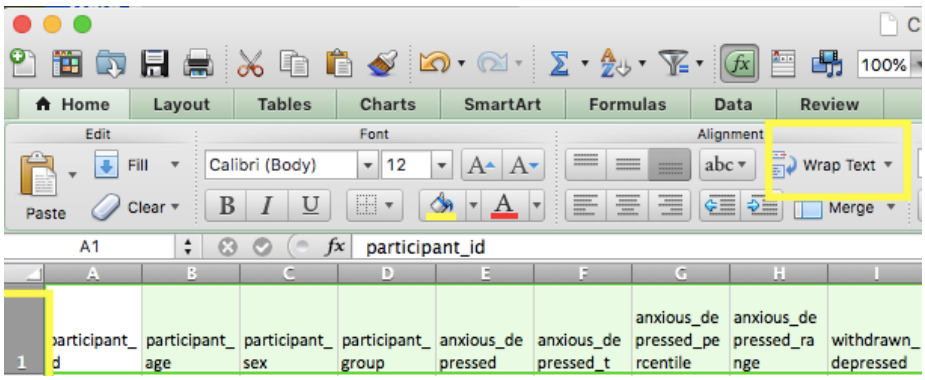
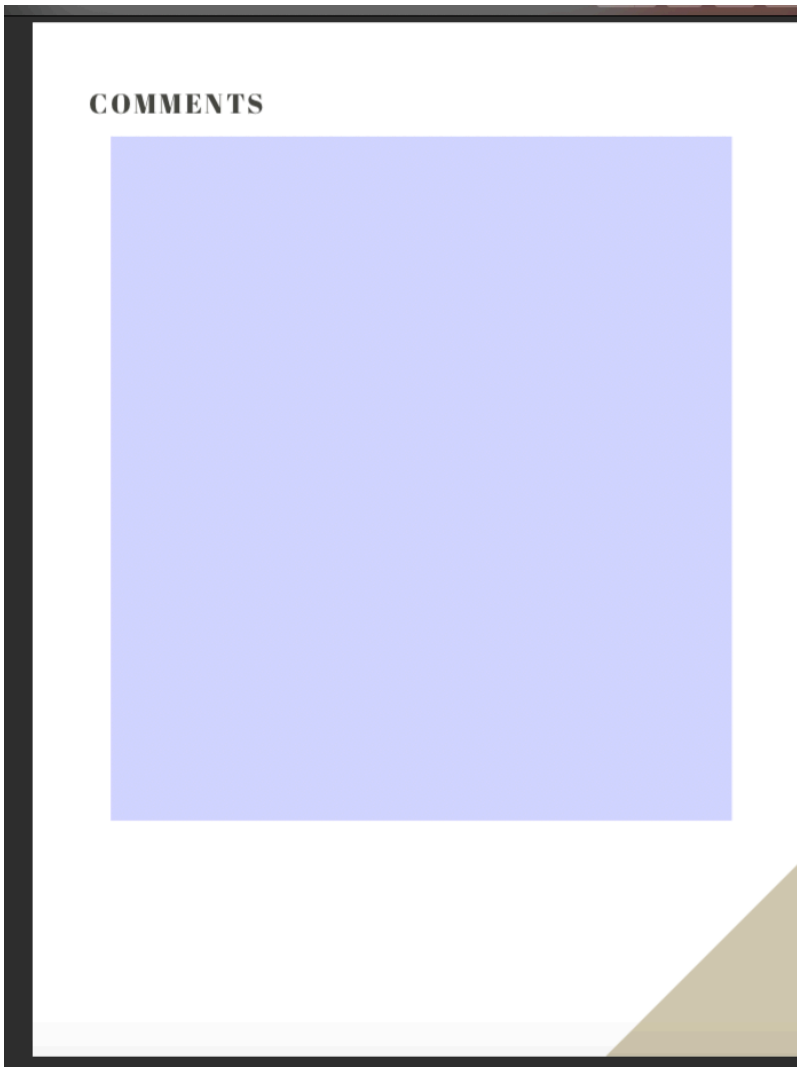


Figure 4.8:

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.



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

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

Notes:

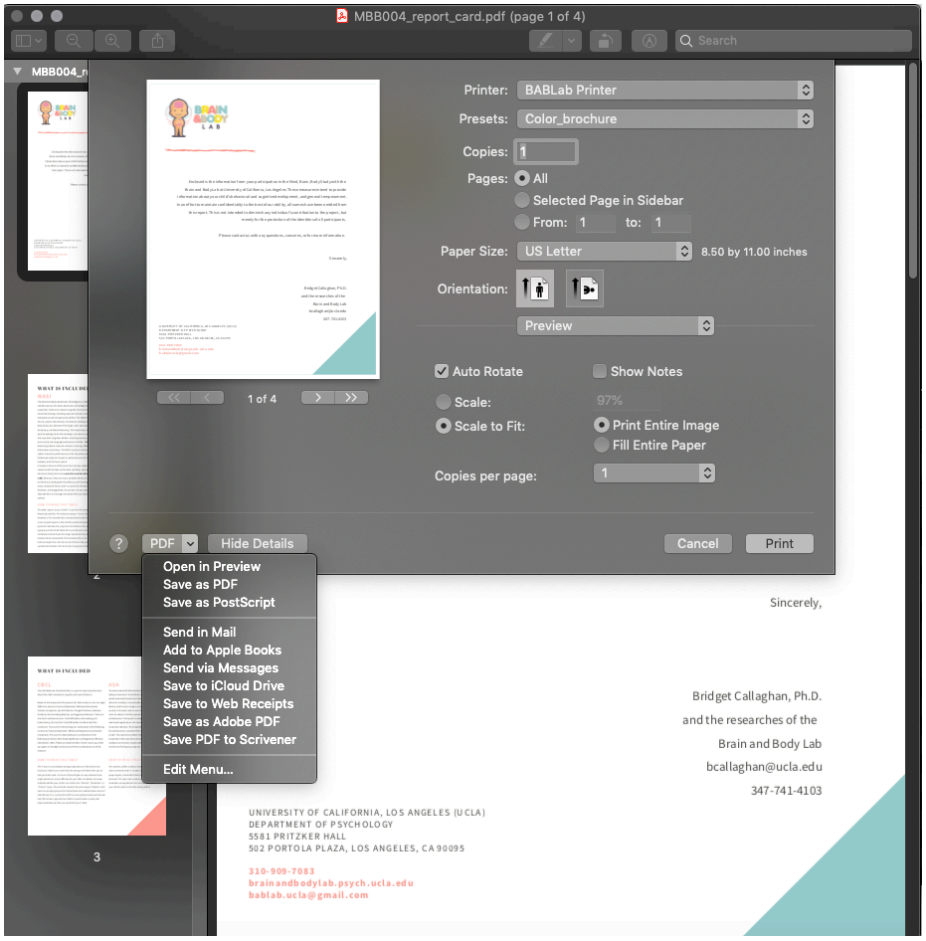

MBB004_report_card.pdf (page 1 of 4)
Search
Printer: BABLab Printer
Presets: Color_brochure
Copies: 1
Pages: All
Selected Page in Sidebar
From: 1 to: 1
Paper Size: US Letter 8.50 by 11.00 inches
Orientation:
Preview
Auto Rotate
Show Notes
Scale:
Scale to Fit:
Print Entire Image
Fill Entire Paper
Copies per page: 1
1 of 4
PDF
Hide Details
Cancel
Print
Open in Preview
Save as PDF
Save as PostScript
Send in Mail
Add to Apple Books
Send via Messages
Save to iCloud Drive
Save to Web Receipts
Save as Adobe PDF
Save PDF to Scrivener
Edit Menu...
Sincerely,
Bridget Callaghan, Ph.D.
and the researchers of the
Brain and Body Lab
bcallaghan@ucla.edu
347-741-4103
UNIVERSITY OF CALIFORNIA, LOS ANGELES (UCLA)
DEPARTMENT OF PSYCHOLOGY
5581 PRITZKER HALL
502 PORTOLA PLAZA, LOS ANGELES, CA 90095
310-909-7083
brainandbodylab.psych.ucla.edu
bablab.ucla@gmail.com

Figure 4.9:

- If the scores for a particular participant seem off check in with the lab manager so they can verify there isn't something wrong with the cbcl script
- The fill in spots for the aggressive behavior row don't match the rest of the document so there are two small text boxes where you can put in the values

Sending report card email

1. Make a copy of certificate template on your desktop, type in participant name, and save as a pdf
    - certificate is in BABLAB/Studies/Mind_Brain_Body/Payment/Wave_2/Wave_2_print
    - when you save it as a pdf open it then rotate it so the pdf is oriented correctly
2. Open participant's report card pdf and save a copy to your desktop *without the participant ID* which will be attached to email
3. Open up report card email template & fill in highlighted parts
4. Attach thank you letter, cerificate, and report card to email
    - thank you letter is in BABLAB/Studies/Mind_Brain_Body/Payment/Wave_2/Wave_2_print

---

4.7.9 W2 Protocol - Payment

Payment package contents:

- Payment box
- Type in participant's name and print copy of certificate
- Print thank you letter
- include prize chosen by participant (check REDCap session 2 checklist for choice)
- Include Amazon gift card payment
- send gift card codes in payment email
- Check stool sample quality- if poor, send another stool kit

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

Payment emails

1. In the same email thread that you've been emailing participant open up the payment email template

2. Fill in highlighted parts.

3. Add in the gift card codes
   - gift card codes are under last name of caregiver in BABLAB/Studies/Mind_Brain_Body/Payı

4. If we're still missing anything from them (a survey, contact list, etc) also request it in the payment email

5. Mark payment email as complete on the MBB participant log in column called " Send payment confirmation email to participant"

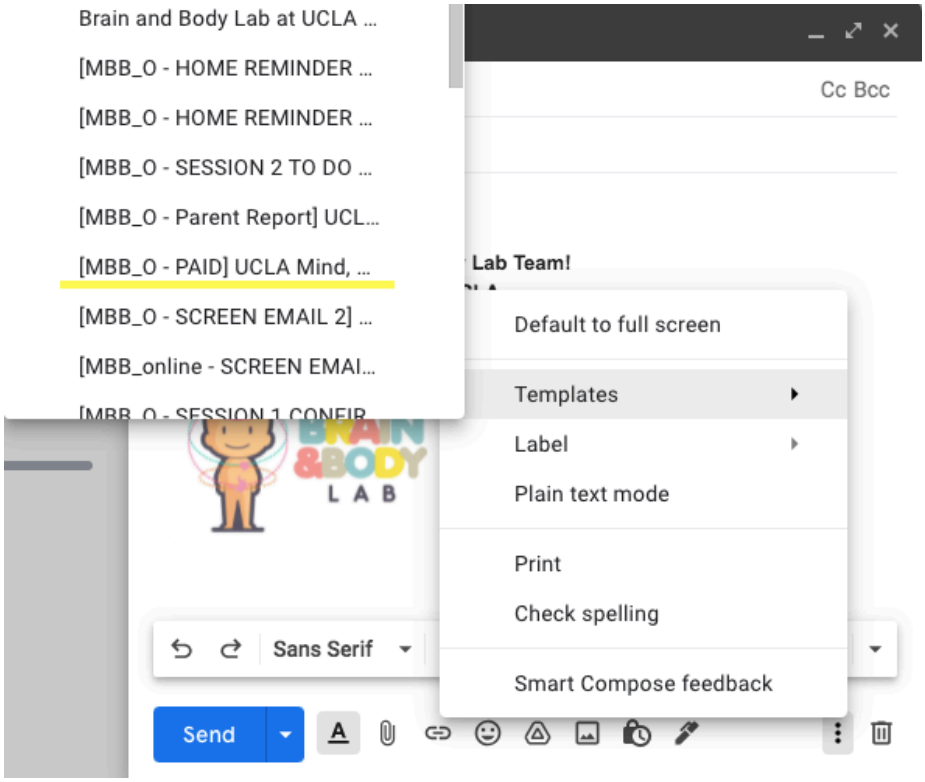


Figure 4.10:

[MBB_O - PAID] UCLA Mind, Brain, Body Study (online)

Dear [NAME],

Thank you for completing Wave [#] of the Mind, Brain, Body study!

We at the Brain and Body Lab cannot thank you and your family enough for your dedication and contribution to our research. We hope that our findings will help make a difference in children's lives here in our local community and around the world.

We have sent via mail the payment (in the form of an Amazon gift card) as well as educational activity kits for your child. If you do not receive them in the next week or so, please let us know!

We have included the gift card codes below for your convenience:

-

If you are able, we also request that you please complete **[Missing Requirment]**. Please fill it out and upload it here to our Box portal.

If you have any questions feel free to contact us. We hope that you will join us next year for Wave [#]!. If you have any comments or concerns and would like to leave anonymous feedback, please click here.

All the best,

Figure 4.11:

Chapter 5

Troubleshooting

5.1 Troubleshooting - Links

5.1.0.1 If links in chat are not working...

[Get them to have the links email open just in case]

You should have received an email from us right before this session full of links for today. Can you please pull that up to have it ready just in case? I will be sending you all of these links through the Zoom chat, but it will a good reference in case we have any technical difficulties! Once you have it up, you can co ahead and minimize the window.

5.2 Troubleshooting - Internet Issues

5.2.0.1 If internet/wifi seems to be malfunctioning...

[Try to get the best internet connection possible.]

Are you working on a computer that is connected via ethernet cable? We want to ensure you have the best internet connection possible for the session, as we will be doing many activities today that may need strong internet connection. Can you possibly transfer to a computer that is connected to an ethernet cable?

[If so, proceed]

[If not, say] *Thats alright! If possible, can you please move to the best area of your house for internet connection?*

[If this does not work, we are not IRB approved to use FaceTime or conduct session via phone yet. May need to reschedule session and/or contact Lab Manager for troubleshooting help.]

5.3 Troubleshooting - PC Interactions

5.3.0.1 Plan B to Zoom recording on PC interaction

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.

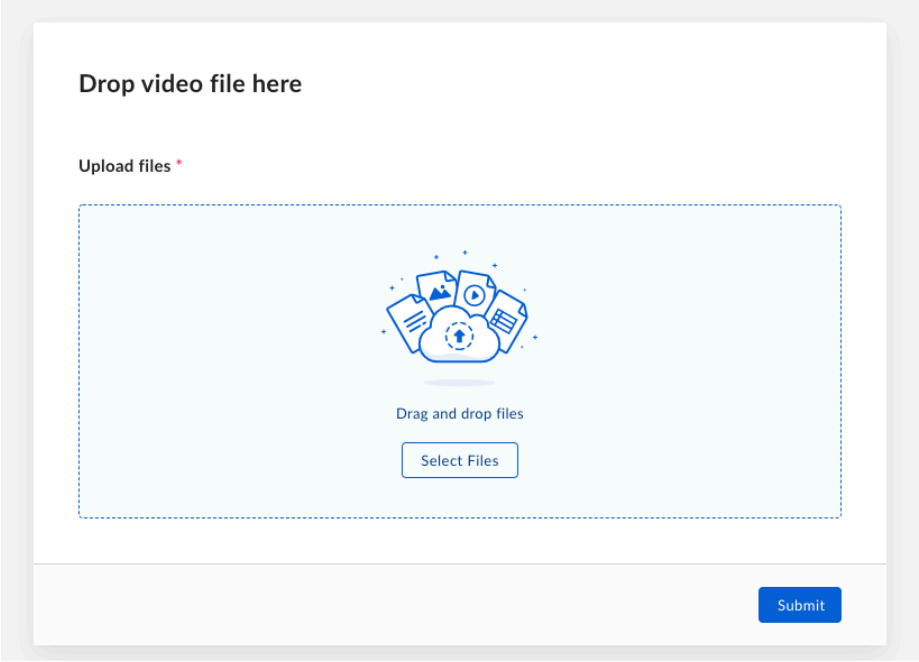


Figure 5.1:

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.

5.4 Troubleshooting - Protocol Deviations

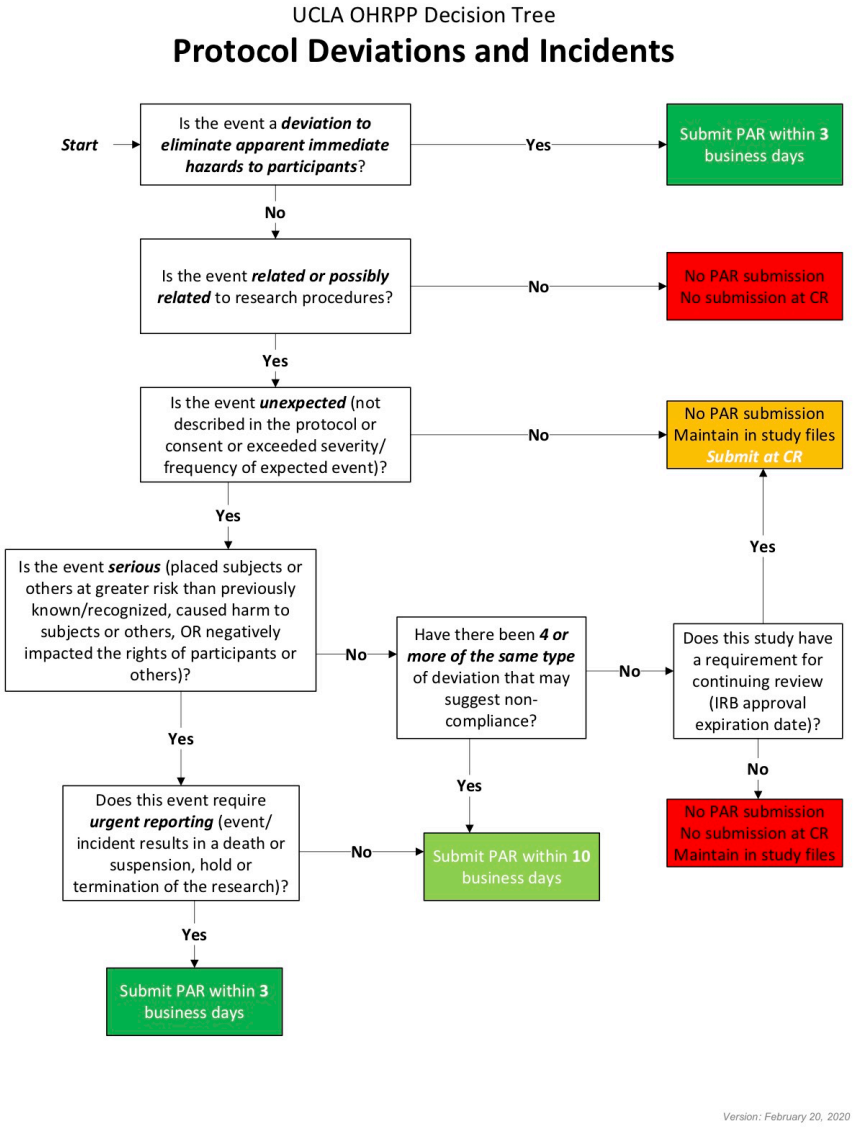

UCLA OHRPP Decision Tree
Protocol Deviations and Incidents
Start
Is the event a deviation to eliminate apparent immediate hazards to participants?
Yes
Submit PAR within 3 business days
No
Is the event related or possibly related to research procedures?
No
No PAR submission
No submission at CR
Yes
Is the event unexpected (not described in the protocol or consent or exceeded severity/ frequency of expected event)?
No
No PAR submission
Maintain in study files
Submit at CR
Yes
Yes
Is the event serious (placed subjects or others at greater risk than previously known/recognized, caused harm to subjects or others, OR negatively impacted the rights of participants or others)?
No
Have there been 4 or more of the same type of deviation that may suggest non-compliance?
No
Does this study have a requirement for continuing review (IRB approval expiration date)?
Yes
No
Yes
Yes
Does this event require urgent reporting (event/ incident results in a death or suspension, hold or termination of the research)?
No
Submit PAR within 10 business days
No PAR submission
No submission at CR
Maintain in study files
Yes
Submit PAR within 3 business days
Version: February 20, 2020

Figure 5.2:

Chapter 6

Between Waves Protocol

Protocol relevant to moving from one wave of MBB to the next wave of study.

6.0.1 Between Waves - Database Formation

6.0.2 Between Waves - Redcap

Reminders on what to change in REDCap (NOTE: this is not an exhaustive list, just some things Lab Manager should remember. Reach out to Kristen for questions):

- do NOT click the branch button that says "apply to all." If you copy a survey and make a change on branch logic, sometimes it will ask you if you want to apply the change to more than one item. This is dangerous because the logic does not work 100% accurately- it may change items you do not want to shift the branch logic on, and it applies to every survey where these items are related, so it is VERY hard to change back.

Figure 6.1:

- applied to all surveys that are age specific, you need to remember change surveys that should only be given to each ages
- need to make sure any branch logic on a specific variable that is branching from a different survey (age, what wave, etc.) is redone between waves because its on a different arm

6.0.3 Between Waves - Gorilla

6.0.4 Between Waves - Ordering

6.0.5 Between Waves - Bio Sample Shipping

6.0.5.1 Shipping with Dry Ice

supplies need to obtain:

```
- styrofoam box (bioshipper) - HPL or recycling bin
- newspaper
- rubber bands
- gallon ziplock bags
- dry ice
- box for the outer later of styrofoam - tyler or recycling bin or purchase
- class 9 label for dry ice - black and white diamond - obtain from dry ice dept or ty
- markings for UN1845/dry ice/weight - tyler
- Air waybill - tyler
```

Reorganize Samples

- extract storage boxes from the freezer
- place samples on dry ice
- take first sample of every participant for stool and saliva, place in new box (if only shipping 1 sample rube per participant to collabator)
- re-label boxes
- create new spreadsheet detailing new location of every sample

Packing

determine dry ice amounts and sizing:

- determine volume of specimens to pack per size of bioshipper
- determine amount of dry ice per volume of samples, and ensure it all fits within bioshipper(s)

packaging it up:

- secure each storage box with rubber bands
- place boxes inside biohazard zip bloc bags
- weigh and record amount of dry ice per box:
  - weigh shipping box prior to adding dry ice
  - surround freezer boxes with dry ice (about 2 blocks worth per box)
  - weigh freezer box + dry ice together
- subtract point 1 from point 3 to get total weight of dry ice and record it on shipping label
- place required amount of dry ice in bottom of insulated shipper and cover with crumpled newspaper
- place biohazard zip bloc bag with storage boxes inside on top of newspaper
- fill any remaining space on sides and between boxes with crumpled paper
- place newspaper on top of specimens
- place remaining dry ice for volume on top and place lid on box
- do not overstuff box, do not tape the lid, do not tape the box the bioshipper goes in
- fill out and place dry ice sticker on outside of bioshipper?
- print and add other labeling (ask tyler) to regular box, that styrofoam box goes in
- attach fedex courier forms to outside of box

shipping

- coordinate with Tyler about where and when to pick it up – typically needs to be by 11 am on either Monday or Tuesday of the week

6.0.6 Between Waves - Analysis

6.0.6.1 Saliva Sample Analysis

Notes from Dr. Meyer: Please note the following regarding the data: (1) sample MBB073 had too little hair to process (you will not be charged for that sample); (2) several of the samples were shorter than 3 cm, which is noted in the file; this is just informational ….you don't have to do any correction for the shorter length in your data analysis; same thing with the one sample that showed the presence of hair dye; (3) we recommend performing a statistical test for outliers and considering removing those data points from subsequent analysis; (4) lastly, hair CORT distributions are often non-normal due to right skewness, even after removal of outliers; we recommend checking for that, and if present, the most common approach is to perform a log transformation of the data before further analysis.

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.

Brunec, 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.

Norrholm, 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.

Norrholm, 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.