ABC-CT Data Acquisition and Analytic Core
EEG Main Study
Manual of Operations
Version 2.3

Running Head: DAAC EEG MOP V2.3

Goal:
The EEG Manual of Operations will serve as the documentation of technical details for the DAAC to develop and assess the EEG acquisition and analytics for the ABC-CT. This document will primarily be of use for the DAAC for technical oversight of the sites (CIS).

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ABC-CT Network EEG Contributors include:
Adham Atyabi,
Elizabeth Baker,
Jessica Benton,
Carter Carlos,
Shou-An A. Chang
Morgan Crossman,
Casey Guilland,
Simone Hasselmo,
Gerhard Hellemann,
Caitlin Hudac,
Beibin Li,
Takumi McAllister,
Adriana Mendez,
Charlotte Muchetti,
Laura Nelson,
Max Rolison,
Damla Senturk,
Dylan Stahl

Corresponding Authors:
Sara J Webb; sjwebb@uw.edu
James McPartland; james.mcpartland@yale.edu

Acknowledgement:
The EEG Manual of Operations was directed by Sara Webb. The development and implementation of the EEG acquisition protocol (see: Data Acquisition and Analytic Core ABC-CT Main Study: EEG Acquisition Protocol) was directed by Heather Borland, the quality control procedure was directed by Megha Santhosh (ABC-CT Data Acquisition and Analytic Core EEG Main Study Quality Control Manual) and implemented by M.S., H.B., J.B., and a team of dedicated undergraduate interns. Project direction was provided by James McPartland (PI) and Sara Jane Webb (DAAC PI). Funding was provided by the National Institutes of Health (NIMH U19 MH108206). Oversight was provided by an external advisory board, NIH program science officers, and the FNIH Biomarkers Consortium.

Across the development of the project, the quality control metrics and procedures received input and review by the ABC-CT EEG contributors named and listed as additional contributors above, through bi-monthly meetings and additional workgroups. Further review and input was provided by EEG site leads who were tasked with implementing acquisition, and the site PIs.

Other support documents:
ABC-CT Data Acquisition and Analytic Core EEG Acquisition Manual
ABC-CT Data Acquisition and Analytic Core EEG Quality Control Manual
ABC-CT Data Acquisition and Analytic Core EEG Peak Picker Manual v3.1 (ABC-CT Build)
ABC-CT Data Acquisition and Analytic Core EEG ERP Pipeline and Derived Results Manual for Faces
ABC-CT Data Acquisition and Analytic Core EEG ERP Pipeline and Derived Results Manual for VEP
ABC-CT Data Acquisition and Analytic Core EEG ERP Pipeline and Derived Results Manual for Biomotion
ABC-CT Data Acquisition and Analytic Core EEG Resting Pipeline and Derived Results Manual

Version

What	File
M2.3	M2.3 ABC-CT DAAC EEG Main Study Manual 20200117 Updates and clarification
M2.2	M2.2 ABC-CT DAAC EEG Main Study Manual 20180704 Updates and clarification
M2.1	[DAAC EEG MOP v2.1] M2.1 EEG DAAC ABC-CT Main Study Manual 20171205
2	[EEG DAAC ABC-CT Manual V2] M2.0_EEG_DAAC_ABCCT_Main Study_Manual_20170202 Updates to experiments and counterbalance protocol
1	EEG DAAC ABC-CT Manual V1 [2016-07-01]
Feasibility	EEG DAAC ABC-CT Manual(s) Paradigms & Experiments Updated throughout feasibility; All feasibility manuals available upon request

Table of Contents

Administration

The ABC-CT is directed by James McPartland (james.mcpartland@yale.edu) / Yale University). The DAAC is directed by Sara Jane Webb (sjwebb@uw.edu / Seattle Children's Research Institute) with co-leadership from Fred Shic (fshic@uw.edu/ Seattle Children's Research Institute) and Catherine Sugar (csugar@ucla.edu / UCLA). The DAAC aims are listed below, but the DAAC, in general, oversees the acquisition and analytics for the ABC-CT. The DAAC is a virtual core, with members at multiple sites and includes the Lab Based (LB) measures, electroencephalography (EEG), eye tracking (ET) methodologies, and analytics. The DAAC - EEG group worked closely with the CIS - EEG team to implement the measures proposed in the grant (see grant page 171-173).

The EEG DAAC team has a project specific email for CIS-DAAC EEG interactions including file upload correspondence: daac.eeg@seattlechildrens.org

Help questions for DAAC are to be directed to: askdaac@seattlechildrens.org

Weekly coordinator calls will be conducted to support site acquisition across methodologies and members of the DAAC representing each biomarker (EEG, ET, and VT) will be in attendance.

Information and Contacts

Duke University

Geraldine Dawson, Ph.D. (Site PI)	Email: geraldine.dawson@duke.edu
Michael Murias, Ph.D. (DAAC)	Email: michael.murias@duke.edu (departed June 2019)

Harvard University/Boston Children's Hospital

Charles A Nelson Ph.D. (Site PI)	Email: charles.nelson@childrens.harvard.edu
Susan Faja, Ph.D. (Site Co-PI)	Email: susan.faja@childrens.harvard.edu
April Levin, MD (DAAC)	Email: april.levin@childrens.harvard.edu

UCLA

Shafali Jeste, MD (Site PI)	Email: sjeste@mednet.ucla.edu
Catherine Sugar, PhD (DAAC)	Email: csugar@ucla.edu

University of Washington
Raphael Bernier, Ph.D. (Site PI) Email: rab2@u.washington.edu (departed Oct 2019)
Rachel Earl (Site PI) Email: rearl@u.washington.edu (2019-2020)

Seattle Children's Research Institute
Sara Webb, Ph.D. (DAAC) Email: sara.webb@seattlechildrens.org
Frederick Shic, Ph.D. (DAAC) Email: frederick.shic@seattlechildrens.org
Heather Borland (DAAC) Email: heather.borland@seattlechildrens.org
Megha Santhosh (DAAC) Email:megha.santhosh@seattlechildrens.org
Jessica Benton (DAAC) Email: jessica.benton@seattlechildrens.org

Yale University
James McPartland PhD (Site PI) Email: james.mcpartland@yale.edu
Adam Naples, Ph.D. (DAAC) Email: adam.naples@yale.edu

Affiliated files

What	Files
U19 DAAC Grant+	U19_Budgets_Removed.PDF U19_EEG.pdf
Team Contact+	EEG ABC-CT Team Contact (Google Spreadsheet)
DAAC-DCC Meeting Notes (monthly)+	ABC-CT DAAC-DCC Main Study meeting and agenda notes (Google Documents)
DAAC EEG Meeting Notes (Biweekly)+	DAAC EEG Main Study Meeting Notes (Google Documents)
DAAC EEG Acquisition Manual*	ABC-CT Data Acquisition and Analytic Core EEG Main Study Acquisition Protocol, Version 2.5.
DAAC EEG Quality Control Manual*	ABC-CT Data Acquisition and Analytic Core EEG Main Study Quality Control Manual, Version 1.2
ABC-CT Publication Policy and Procedures+	ABC-CT Publication Policies and Procedures.6-27-17.pdf
ABC-CT Dissemination Abstract Submission Form+	ABC-CTDisseminationAbstractSubmissionForm.pdf
The Autism Biomarkers Consortium for Clinical Trials: Data Acquisition and Analytic Protocol (Manuscript)	McPartland, J., Bernier, R., Jeste, S., Dawson, G., Nelson, C., Chawarska, K., Earl, R., Faja, S., Johnson, S., Sikich, L., Brandt, C., Dziura, J., Rozenblit, L., Hellemann, G., Levin, A., Murias, M., Naples, A., Platt, M., Sabatos-DeVito, M., Shic, F., Senturk, D., Sugar, C., Webb, S. J., and the Autism Biomarkers Consortium for Clinical Trials. (2019). The Autism Biomarkers Consortium for Clinical Trials (ABC-CT): Scientific Context, Study Design, and

	Progress towards Biomarker Qualification. *MedRxiv*. https://doi.org/10.1101/2019.12.18.1901454. Webb, S.J., Shic, F., Murias, M., Sugar, C., Naples, A., Barney, E., Borland, H., Helleman, G., Johnson, S., Kim, M., Levin, A.R., Sabatos-DeVito, M., Santhosh, M., Senturk, D., Dziura, J., Bernier, R.A., Dawson, G., Faja, S., Jeste, S., McPartland, J., & The Autism Biomarkers Consortium for Clinical Trials (2019, provisional acceptance). Biomarker Acquisition and Quality Control for Multisite Studies: The Autism Biomarkers Consortium for Clinical Trials. Frontiers in Integrative Neuroscience.

*Available upon request via ABC-CT Website or email PI.
+Internal Project Support Documents

ABC-CT Grant

Excerpts from U19_Budgets_Removed.pdf

Aim 1

"Compare whether lab-based measures versus clinician and caregiver assessments of specific domains of social impairment are more sensitive indicators of (or highly correlated with) an independent assessment of overall clinical status."

Aim 2

"Evaluate whether a well-justified set of EEG and ET measures, individually or in combination, have utility as stratification biomarkers and/or sensitive and reliable measures of change. Specifically, we will assess the technical and biological variability of the biomarkers and their relationship with measures of social impairment in order to evaluate the viability of biomarkers in terms of:

a. *Construct validity* demonstrated by task-specific brain activation and signal strength (i.e., modulation of EEG spectral power and event-related potential [ERP] latency/amplitude in accordance with experimental manipulation).
b. *Test-retest reliability, consistency, and stability* in the sample overall and separately by site and diagnostic group; this will demonstrate that measurements of the potential biomarkers are replicable within subjects, are robust to practice effects, remain stable when underlying clinical status is unchanged, and can be consistently measured across experimental locations and time points, making them suitable for use in multi-site longitudinal clinical trials.
c. *Discriminant validity* reflected in sensitivity to detect differences between ASD and TD subjects' performance on the measures at each time point and across time points.
d. *Convergent validity* shown by reliable correlation (i.e., consistent patterns of inter-subject variability) of EEG and ET measures with social-communicative impairment (as assessed by LB and clinician/caregiver assessments) and clinical status (as assessed by independent rater) at each time point.
e. *Sensitivity to change,* assessed in terms of how change in EEG and ET measures corresponds to intra-subject natural course variability in social-communicative impairment and clinical status across time points. "

Aim 3

"Collect blood (DNA) samples from all subjects, including parents of ASD subjects, for future genomic analyses and share raw, processed, and analyzed data via the NDAR and NIH/NIMH Data Repositories to create a community resource accessible for use by all qualified investigators."

Scientific integration, management, and administrative responsibilities

All sites have established records of robust recruitment and high throughput characterization and well-established scientific expertise to carry out all aspects of the project, including comparable laboratories and equipment, strong expertise in the implementation of behavioral, EEG and ET measures, and demonstrated ability to work in a collaborative research project. Site Directors' administrative responsibilities will consist of hiring, training, and supervising research staff to ensure that the proper professional and technical personnel are available, including ensuring that diagnosticians are research-reliable on the ADOS-2 and ADI-R and that (1) screening and inclusion/exclusion criteria are met; (2) recruitment targets are met according to the planned timeline; (3) similar procedures are used to maximize task-specific and longitudinal compliance; (4) all LB, EEG, and ET, parent report/clinical measures, and blood are collected in a standardized, reliable manner according to reliability and training protocols, specifications, and quality control procedures overseen by the DAAC/DCC; (5) data uploaded to the DCC in a standardized, accurate and timely manner; and (6) high quality record-keeping, source documentation, and accurate data entry occur according to Good Clinical Practice (CGP) standards, as per site monitoring by the DCC. The Site Director will be responsible for direct communication with the Administrative Core and will oversee the site's participation in the Feasibility Study (described below) to demonstrate the site's ability to recruit and complete administration of all measures. In addition, the Site Directors will ensure that all requirements of the centralized IRB are met and that study procedures are carried out with clinical and ethical sensitivity to the needs of each of subject and his/her family. The Site Directors and their staff will work collaboratively with each other, the DAAC, the DCC, the overall PI, Administrative Core, and with federal and private partners to meet the goals set in this collaborative agreement. This includes participating in weekly Site Director conference calls, webinars, and in-person meetings during which aspects of the implementation, coordination, and realization of the goals outlined in the proposal are discussed and any barriers to successful completion of the project are addressed. Dr. Dawson, Duke Site Director, will serve as Site representative on the Steering Committee and will collaborate with the overall PI to communicate meeting content to CIS Directors. All Site Directors will attend the annual U19 scientific meeting, including the External Advisory Board.

U19 DAAC

"The DAAC will work together with the Sites and the Data Coordinating Core (DCC), under the oversight of the Administrative Core, and in coordination with federal and private partners in this collaborative agreement to achieve the following aims:

Aim 1

To design and implement experimental protocols for acquisition of EEG, ET and LB measures of social behavior.

During the Set-Up phase, the DAAC, in coordination with the Administrative Core and Site staff, will

develop SOPs for EEG, ET and LB experiments, including equipment technical standardization, manuals and data logs for data acquisition, and protocols for data transfer. Proper file format will be standardized during the Set-Up phase so that all data is collected uniformly and is appropriate for post-processing procedures. Equipment support and training will be provided to Sites by DAAC staff to quickly move from Set-Up to feasibility.

Aim 2

To design and implement rigorous, scientifically valid, and replicable data processing and artifact removal from biomarker data sets.

The DAAC will standardize data processing and artifact removal through the development of specialized data pipelines based on methodological and experimental best practices. All data will be processed "blind" to participant characteristics. We have conceptualized the data processing pipeline broadly such that the methodologies designed for this project will benefit the scientific goals as described in the Overall Research Strategy and can be applied to future clinical trials building from this Consortium.

Aim 3

To design and implement statistical analyses for biomarker evaluation.

The DAAC will develop the appropriate analytic strategy to address the scientific objectives. This includes: (1) Selecting, implementing, and deriving EEG and ET biomarkers and LB measurement variables with good performance metrics (construct validity, test-retest reliability, stability, and discriminant validity); (2) examining the relationship and sensitivity among EEG and ET biomarkers, LB measures, clinician/caregiver assessments, and independent measures of clinical status; (3) evaluating longitudinal change in ET, EEG, and LB measures to identify if these measures will be sensitive measures for intervention trials. "

Rationale for selection of measures - Feasibility

All consortium members (Site Directors, PI, DAAC, DCC) worked together to select measures appropriate for the current study as well as the broader goal of serving future ASD clinical trials. After a comprehensive literature review, a battery of EEG, ET, and LB measures was carefully chosen to meet the following criteria: (1) tap relevant domains of social impairment in ASD (e.g., face processing, emotion recognition, biological motion detection, preferential attention to social stimuli, proximity seeking, conversational reciprocity); (2) conform to RFA requirements of inclusion of one EEG (upright-inverted faces) and one ET (emotion-matching) task from the EU-AIMS study, a dynamic video ET task, and both visual and auditory EEG measures; (3) assess resting state EEG and functional integrity of lower-level visual processing; (4) match the abilities of ASD and TD children ranging from 4-11 years of age with IQs in the range of 50-115 to understand and comply with task demands; (5) are likely to change in response to a treatment; (6) differentiate ASD and TD children; (7) can feasibly be implemented in a large, multisite clinical trial; (8) correlate with level of social impairment as assessed by parental/clinician ratings or direct observations; (9) assess behavior in a standardized and well-normed fashion; and (10) rely on automated coding rather than resource-intensive or subjective human coding to enhance reliability across multi-site studies and for ease of implementation in future clinical trials. Clinician and caregiver assessments were chosen based on the following criteria: (1) are commonly used in ASD research to assess core symptoms and social impairment; (2) are well-standardized and normed; (3) are applicable across the entire participant age range; and (4) include a range of reporting methods (parent interview, questionnaires, clinician ratings and clinical judgment). To assess overall clinical status independent of other measures, the Clinical Global Impression (CGI)

Severity Scale and Improvement Scale will be anchored to overall level of social impairment and based on an independent rating by a licensed clinical psychologist with a high level of ASD expertise based on clinical interview with the parent and observation of the child. Dr. Lin Sikich (Psychiatrist, Duke CIS), who is highly experienced with the use of the CGI in clinical trials provided initial training and ensure cross-site reliability of the CGI ratings. She is Director of a NIH-funded multi-site clinical trials (oxytocin) that assesses social impairment as a primary endpoint and includes use of the CGI.

Main Study Changes

The changes from Feasibility to Main Study in relation to EEG includes the following:

1. Age Range: 6- 11 years
2. IQ Range:
    a. TD: 80-150
    b. ASD: 60-150
3. EEG Experiment change:
    a. Modified EU Aims Faces to replace the fixation icons with crosshair and experiment renamed to ABC-CT Faces.
    b. Eliminated Social/Non-Social Dynamic Videos and Emotional Faces experiment

Participants Goal (Target at T3 with valid biomarker data)

- 200 6-11 year olds with ASD
- 75 6-11 year olds with TD

Protocol

Time points

- T1 Baseline (day 0) & T1D2 (day 1-14)
- T2 = T1D1+6 weeks (+/- 2 weeks): T2D1 (day 28-56) & T2D2 (day 29-70)
- T3 = T1D1+24 weeks (+/- 2 weeks): T3D1 (day 154-182) & T3D2 (day 155-196
- Day 1 to Day 2, 1 to 14 days

Time point x (EEG) Session Protocol

- Day 2, one session ~ 35 min
- Counterbalance protocol within EEG order

Justification of paradigm order and counterbalancing

Feasibility

1. The feasibility study was a critical phase in determining the quality of data gathered from paradigms. Specifically, we addressed whether or not there were paradigms that consistently gave us incomplete/partial or no data.

2. In the feasibility study, EEG was conducted on both day 1 and day 2 of the protocol, with the protocol divided between the two days.

    a. We did not have a fixed order for the paradigms, as this could undermine the quality of the last paradigms. We also did not have a completely randomized design because the sample size was too small to allow such a design to answer our questions about the quality of data from each paradigm. Therefore, we established a counterbalancing scheme, in which different children receive different, yet fixed sets of stimuli (a block design). We did, however, stratify the participants by characteristics (age, sex, verbal functioning) that we thought would influence data quality. Assessment of variables related to stratification was done at screening. Any deviations from the assigned counterbalance order was recorded and treated as a protocol deviation.

Main Study

1. In the full study, we will conduct all EEG on one day (from two) and reduce the number of experiments from 6 to 4.
2. We will have a larger sample size than in Feasibility but will keep the same stratification constructs (age, gender, verbal functioning, and diagnosis), which will be assessed at screening. The stratification information will lead to a stratification group assignment, which will then specify the within method experiment order.
3. Deviations in experiment order will be reported as a protocol deviation and experiment order deviation. This will be recorded in the EEG Log Post Session Checklist (Item 6 Protocol Deviation {Yes / No}, Item 7 Please Explain; Item 10 Experiment Order Deviation).
4. The order will remain fixed across time points for each child. That is, experiment order will be the same at each time point.

Protocol Counterbalance

See "**Protocol Counterbalance**" ABC-CT DAAC EEG Main Study Acquisition Protocol

EEG Experiment Counterbalance

EEG session will be done on Day 2 of the study visit and before ET session. The estimated time of administration for the sets of tasks are listed in Table 1.

See "**EEG Experiment Counterbalance**" ABC-CT DAAC EEG Main Study Acquisition Protocol

Experiment Details

General Structure

See "**General Structure**" ABC-CT DAAC EEG Main Study Acquisition Protocol

Experiment Verbal Description & Supports

Supports should be consistent across sessions, experiments, and time points.

See "**Support During Acquisition**" ABC-CT DAAC EEG Main Study Acquisition Protocol

Background for each experiment

Resting Eyes Open

Resting EEG: The resting state condition provides a baseline for the event related EEG measures proposed below and also serves as a promising baseline biomarker of neural function in ASD. Studies of resting state EEG have not yet established a consistent biomarker in ASD as a whole (for review, see Wang 2013), but investigations have been limited by the heterogeneity of the samples, lack of detailed clinical phenotyping, and inconsistent or clear artifact mitigation and processing protocols that would allow for stratification of individuals.

Due to significant concerns about the ability to get eyes closed data from this age range, we will not do eyes closed. Prior work suggests that yield will be low due to noncompliance. For example, only ~65% of children in the NIMH ACE multisite GENDAAR (HFA 8-18) provide artifact free data (> 40 second) from 3 minutes of Eyes Closed.

Experimental Design

- Video stimuli will consist of non-social, abstract videos purchased from Shutterstock. Web 426*240 @ 25-30 fps converted to MPEG1 embedded into a black background frame with white StimTracker DIN patch present for one frame every second starting with the first frame and total video dimensions of 1920x1080 @ 30 fps. clip id: 3038821, 3041077, 3191017, 4003732, 4779302, 8398420.
    - Note: for use, independent of the ABC-CT, organizations should purchase their own licenses for use of these stimuli.
- 3 blocks, each 1 minute in length. A break can be taken between blocks.
- Each unique video (n=6) is played for 30 seconds. Videos are in random order. The videos have been clipped to 15 seconds and are played forward and then in reverse. Total video time is 1 minute per block, for a total of 3 minutes or 180 seconds.
- Flags (continuous in block) are inserted (via EPrime) every 1000 msec so that attention can be coded in 1000 ms segments (badt) via button press during EEG acquisition.
- Trial Numbers = 60 per block, 180 trials total. ("Trials" defined here as 1000 msec segments)
- Experiment will progress through the blocks based on experimenter input (keypress to start block).
- Final Display size: 9.3cm (350px) wide by 7.0cm (262px) with visual angle of 8 degrees wide by 6 degrees high.

Figure 1. Resting experiment video stimuli examples.

Pipeline Manual

See **ABC-CT Data Acquisition and Analytic Core EEG Main Study Resting Pipeline and Derived Results**

- Note: The pipeline (abstraction of derived results from the raw data) for the Resting Experiment was changed from the Main Study Interim results to the Main Study Final Results.

Primary Dependent Variable

- Slope
  - Slope represents the power x frequency distribution such that lower frequencies have higher power per Hz (delta>theta>alpha>beta>gamma) resulting in a negative slope. It has been proposed that Slope may represent "neural" signal:noise ratio. Slope was fit to a linear model (i.e., "Using Matlab's "polyfit" function; pipeline specifications are available via the pipeline and derived results manuals.
- Units:
  - Log10 (uV^2)/Log10 (Hz).
- Region:
  - The EGI HydroCel Geodesic Sensor Net includes 129 electrodes. For Slope, electrodes from all "all regions" are averaged to represent the full head, although the specific electrodes were changed between Interim and Main Study.
    - Main Study: Using ICA, the array was reduced to 18 electrodes from the 10-20 system and averaged over the electrodes.
      - Electrodes: E9, E11, E22, E24, E33, E36, E45, E52, E58, E62, E70, E83, E92, E96, E104, E108, E122, E124; see Figure 2. below
    - Interim: Initially, the DAAC used 109 electrodes (See Figure 3. Below) including those from the Regions of Interest that were specified for regional EEG analysis and for use in the ERP paradigms. The regions are:
      - Frontal Midline (4, 11/FZ, 18, 10, 16, 19)
      - Frontal Left (23, 24/F3, 20, 27, 28)
      - Frontal Right (3, 117, 123, 124/F4, 118)
      - Central Midline (7, 106, Cz, 31, 80, 55)
      - Central Left (35, 41, 36/C3, 42, 47)
      - Central Right (104/C4, 110, 103. 98, 93)
      - Posterior Midline (61, 67, 62/ Pz, 78, 77, 72)
      - Posterior-Temporal Left (58/T5, 59, 64, 65, 69)
      - Posterior-Temporal Right (91, 90, 96/T6, 95, 89)
      - Occipital Midline (70/O1, 75/Oz, 8/O2, 74, 82)

Figure 2. 18 Electrodes averaged for the resting slope at Main Study

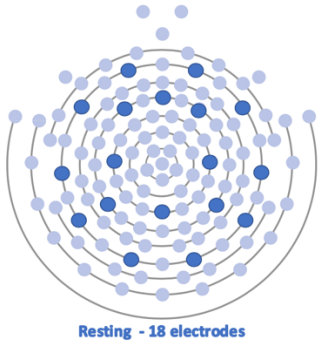
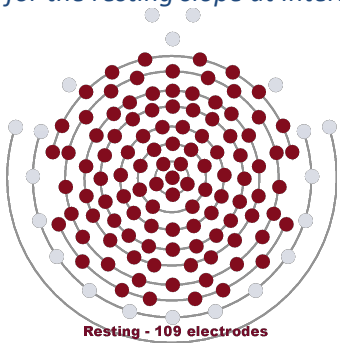


Figure 3. 109 Electrodes averaged for the resting slope at Interim



Analysis Goals: Construct Validity

- Construct Validity: Slope is negative.
- Primary Dependent Variable (DV): Slope of power spectrum for "all regions" as indicated for Interim and Main Study.

Analysis Goals: Group Discrimination, Test-retest

- Discriminate Groups: We hypothesize that individuals with ASD will, globally (i.e., across all regions of interest), have a (significantly) less negative (i.e., flatter) slope than Controls, representing reduced efficiency in signaling in ASD.
- Primary Dependent Variable (DV):
    - Main Study: Slope of power spectrum, across the whole head from 18 electrodes representing the 10-20 system.
    - Interim: Slope of power spectrum, across "all regions" by averaging across all 109 available electrodes.
- Secondary DV
    - Alpha Total is 6 to 12.99 Hz
        - Low Alpha is 6-8.99 Hz
        - High Alpha is 9-12.99 Hz
    - Gamma, due to the electrical main frequency (for US = 60Hz; for UK = 50Hz), we examine several gamma frequencies bands
        - Gamma-Low UK is 35 to 44.99 Hz
        - Gamma-Low US is 35 to 54.99 Hz
        - Gamma-High US is 65 to 79.99Hz

Faces

The proposal requested the use of the EU Aims Face experiment to allow for post-project alignment. As well, "there exists a rich literature on atypical processing of faces in ASD, as this construct represents a foundation for social perception and attention (Dawson, 2005; Webb, 2011; Webb, 2012; Neuhaus, 2015). This paradigm examines face processing and the differential neural response to face inversion (Webb et al., 2011; 2012; Neuhaus et al., 2015; Taylor et al., 2001).

Experimental Design

- The EU Aims Phase 1 experiment was modified to add in 3 houses as an object control condition. Of note, EU Aims Phase 2 added the same house upright condition to their experiment.
- Both ABC-CT and EU Aims Phase 2 used the same # trials per stimuli (3 x 24 = 72).
    - Of note, EU Aims has the goal of looking at repetition effects (memory for faces). Thus, they requested we maintain 72 trials per condition.
    - Experiment: 6 block x 36 trials for 72 per condition (face upright, face inverted, house upright) with 3 x 24 per identities within condition (3 faces, 3 houses).
- EU Aims utilized small stimuli (novel icons such as flags and objects) as fixation cues.
    - ABC-CT modified the fixation: a black crosshair on the grey background. 4.2 cm x 4.2 cm (3.8 degrees) to reduce conflict from object processing pre-stimulus.
- Trial Timing: (a) baseline fixation, random presentation timing between 500-650 msec; (b) stimulus 500 msec; (c) post trial blank screen, random presentation timing between 500-650 msec
    - Total trial time = 1500-1800 msec .
    - Note: To trigger the photocell (resulting in the insert of a digital input "DIN" in the recording file), in E-Prime the each of interest event (fixation, stimulus, blank) is divided into two event slides. E.g., fixation 1: 400-550 msec and fixation 2 100 msec. A small white/black marker is included in the slide to trigger the photocell.
- Stimulus
    - Frames were 11.3 cm width; 14.3 cm height, visual angle 12.3 degrees x 9.3 degrees
    - Faces: MacArthur (NimStim) 3 identity (black 13F, white 07F, Asian 17F) with a white background. Width and height standardized for inner face dimension. Eyebrow to chin. Note: hair is different sizes and fills a different amount of the canvas. 506*650 pixels.
    - House: Shutterstock ID 252868810, 150435080, 58015144. Scaled to have the same dimensions as the face images.
        - Note: for use, independent of the ABC-CT, organizations should purchase their own licenses for use of these stimuli.

- Units
    - Amplitude is in microVolts (µV)
    - Latency is in milliseconds (msec)
- Conditions Available:
    - Upright Faces
    - Inverted Faces
    - Upright Houses.
- Primary Region of Interest:

- Posterior Temporal Right ROI (91, 90, 96/T6, 95, 89).

Pipeline Manual

See **ABC-CT Data Acquisition and Analytic Core EEG Main Study ERP Pipeline and Derived Results Manual for the Faces Experiment**

Analysis Goals: Construct Validity

In NT, the N170 would be more negative in amplitude and faster in latency to faces than houses. Thus, in order to include a participant's data in analyses of construct validity, the participant must have both Upright Faces and Upright Houses data available.

- N170 face specificity
  - N170 latency to Upright Faces is faster than N170 latency to Upright Houses
  - N170 amplitude to Upright Faces is more negative than N170 latency to Upright Houses

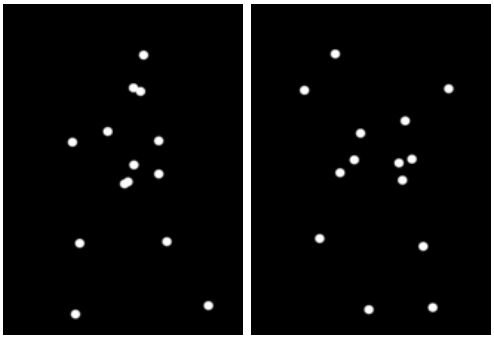
Analysis Goals: Group Discrimination, Test-retest

- Primary DV:
  - N170 latency to Upright Faces at the posterior temporal Right ROI. Latency is in msec.
    - Note: Only data from 1 UF condition (≥21 trials attended, artifact free) is necessary for this analysis.
  - Discriminate Groups:
    - We hypothesize that individuals with ASD (in comparison to TD Controls) will, at posterior temporal right region, have a slower latency (in msec) of the N170 ERP component to Upright Faces, representing slowed early stage processing of faces in ASD.
    -
  - Relation to Clinical Phenotype:
    - We hypothesize that the N170 Latency at the Posterior Temporal Left ROI will be correlated with social ability (VAB-II) and the SRS total score in ASD.
- Secondary DV:
  - N170 face specificity (N170 latency to Upright Faces – N170 latency to Upright Houses)
  - N170 face inversion effect (N170 latency to Upright Faces – N170 latency to Inverted Faces)
    - Note: The Secondary DVs require data (≥21 trials attended, artifact free) in both conditions, thus fewer participants will be available for this analysis than for the primary, which only requires data from one condition.

Biomotion

"The contrast between coherent and scrambled point-light animation activate a network of brain regions involved in social perception. Studies in ASD using both fMRI and EEG methods have demonstrated reduced neural activity to biological motion in individuals with ASD compared to typically developing controls, with focus on older high functioning children (Kroger, 2014; Kaiser, 2012). The stimuli are created from live motion capture data, and coherent biological motion displays feature an adult male walker. Scrambled motion animations are created by randomly selecting points from the biological motion displays and plotting trajectories on a black background.

Experimental Design



- Stimuli / Conditions are (A) biological motion point light display or (B) scrambled motion point light displays (e.g., white dots on a black background). Fixation is a white crosshair on a background black.
    - Static frame stimuli (60 frames) presented at 60Hz to create dynamic stimuli. Eprime Event code for first (1st) and last (60th) image.
    - Images are 150 x 240 (actual dimensions are 162x234) pixels and should display at 4 cm (w) x 7 cm (h).
    - Display goal: Images was 4.9 cm (width) by 7.2 (height) to maintain a visual angle of 4.26 x 6.18 degrees at the site-dependent subject-monitor distance. Of note: a previous study using this exact experiment (ACE GENDAAR) used stimuli presented at ~4 cm (width) x ~7 cm (height)(variability was due to different monitors per site.)
- Trials: 4 blocks of 26 trials for 104 trials; 52 per condition trials and 104 fixation trials.
    - Trial : (A) 1050-1200 msec (random length) of fixation crosshair (white on black background) (B)~1000 msec of stimulus.
    - Total trial is 2050 to 2200 msec.
- Conditions
    - Biological Motion (“biomotion”)
    - Scrambled Motion (non-biological motion)
    - Fixation
- Units
    - Amplitude is in microVolts (µV)
    - Latency is in milliseconds (msec)
- Region of Interest:
    - Posterior Temporal Right ROI (91, 90, 96/T6, 95, 89)

Pipeline Manual

See the ABC-CT Data Acquisition and Analytic Core EEG Main Study ERP Pipeline and Derived Results Manual for Biological Motion Experiment, Version 3.0

Analysis Goals: Construct Validity

- In NT, N200 would be more negative to biological motion than to scrambled motion.

Analysis Goals: Group Discrimination, Test-retest

- Primary DV:
    - N2 amplitude to Biological Motion
- Discriminate Groups:
    - We hypothesize that individuals with ASD will, at posterior temporal regions have a greater amplitude response to the biological motion stimuli than the scrambled stimuli.
- Secondary DV:
    - N2 amplitude to difference score (Biological Motion –Scrambled)
    - P3 amplitude to difference score (Biological Motion –Scrambled)

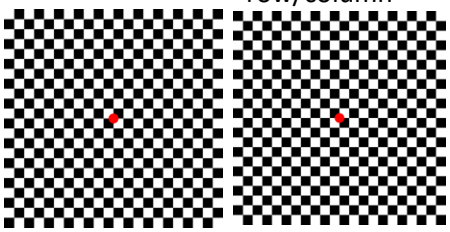
- Secondary DV: "P300" average amplitude difference (Biological Motion – Scrambled Motion).

VEP

"As a foundation for the higher-level visual paradigms that target social communication skills (see below) it is critical to quantify low level visual processing, or the functional integrity of the visual pathway (occipital cortex to lateral geniculate nucleus to optic nerves). Low level processing can be quantified by extracting visual evoked potentials (VEPs). Stimuli consist of black and white checkerboards that reverse their phase (i.e., black to white and white to black) every 500 ms. The checkerboards have a mean luminance of 80cd/m2 and a contrast of 99%.

Experimental Design

- Stimuli: modified checkerboards from Jeste & Naples, with mean luminance of 80 cd/m2 and a contrast of 99%. Fixed checkerboards size as well as row/column width/height to make each check a square as well as overall dimension square. Resulting in 20x20 black and white chessboard bounded by a grey border equal in width and height to one chessboard check (or square). Of note: Vertical height of physical monitor was limitation in viewing dimensions.



- Display Goals: Overall checkerboard dimensions, including the grey border range by site from 26.0cm2 to 29.8cm2 in width. Checkerboard itself was 26cm x 26cm. grey border was "Eprime" GREY (same as other paradigms).
- Fixation on each checkerboard was a red circle with same diameter as the length of the square check and was centered on the chessboard.
    - Checkerboard reversal was created by rotating the checkerboard by one square.
- Trial: 500 msec; 4 blocks x 52 trials for 204 trials
- Condition:
    - Checkerboard
- Units
    - Amplitude is in microVolts (µV)
    - Latency is in milliseconds (msec).

Pipeline Manual

See **ABC-CT Data Acquisition and Analytic Core EEG Main Study ERP Pipeline and Derived Results Manual for the VEP Experiment**

Analysis Goals: Construct Validity

- Construct Validity: Presence of positive P1 amplitude by visual inspection.

Analysis Goals: Group Discrimination, Test-retest

- Discriminate Groups:
    - We hypothesize that individuals with ASD will have smaller amplitude P1 than controls.
- Primary DV:
    - P1 amplitude at Midline Occipital ROI

- Secondary DV:
    - N1 amplitude at Midline Occipital RO01.

EEG EQUIPMENT: STANDARDIZATION PROTOCOL

HARDWARE

Cedrus Stimtracker Installation

See "**StimTracker**" ABC-CT DAAC EEG Main Study Acquisition Protocol

Nets

File Topic	Internal Support File Name
Site Specific Net List	Net List - Main Study (Google spreadsheet)

Net use length varies based on number and type of subjects and experimenter experience. Regular testing of nets and tracking of bad electrodes and repairs are tracked to ensure correct working equipment. Sites are required to update their net use list regularly. Bucket tests of active nets should be done monthly to ensure that the nets are in good working order. Follow EGI specifications. Please keep log at local location.

Net performance is tracked by examining which nets were used (via the EEG logs) and the impedances and signal quality.

Hardware / Software

File Topic	Internal Support File Name
Site Specific EEG Hardware	EEG ABCT Equipment - Main Study

EGI Net Station and Net Amps

Site	NetAmps Version	Computer	Net Station Version
Boston Children's	300	2.66 GHz Dual-Core Intel Xeon OS 10.6.8	4.5.4
Duke	400	3.2 GHz Quad-Core Intel Xeon	4.5.7
UCLA	300	2.66 Ghz Quad Core Intel Xeon OS 10.6.8v1.1	4.5.4
UW	400	3.2 GHz Quad-Core Intel Xeon OS 10.6.8	4.5.6

Yale	400	3.2 GHz Quad-Core Intel Xeon OS 10.6.8	4.5.7

Monitor

Dell P2314H 23 in (50.9 x 28.6)
Resolution: 1920 x 1080

EEG room light level assessment

The protocol was developed by the ABC-CT Eye Tracking Group and adapted for EEG. In short:

1. Have the room lights set as you would for a subject session.
2. On the subject monitor, have the VEP screen measurement paradigm running and set to the VEP chess board.
3. Measure from the approximate position (height and distance from the monitor) with the meter facing the subject monitor.
4. Subject monitor settings from the monitor menu (buttons on the monitor).
5. Record the monitor's brightness value.
6. Record the monitor's contrast value.
7. E-Prime PC brightness level.
8. Record the value of the computer-controlled brightness level.

Light meter readings	*Monitor off*	*Monitor on*
Boston Children's	75.0	75.8
UCLA	199	*215*
UW	*86.3*	*90.3*
Duke	*59*	*70*
Yale	104.5 lux	112.5 lux

E-Prime

This project will not standardize versions of EGI equipment at each site. Details about the E-Prime Computer, Net Station Computer, Monitor configuration, and peripherals will be tracked in the worksheet.

	E-Prime Version	Operating System	Computer
Boston Children's	EP Pro 2.0.8.90	XP Pro Service Pack 3	Dell Precision 5500
Duke	2.0.8.90	XP professional	Dell Optiplex 7010
UCLA	EP Pro 2.0.8.90	XP Service pack 3	Dell Optiplex 780
UW	EP Pro 2.0.8.90	XP Professional Service pack 3	Dell Optiplex 7010
Yale	2.0.10.356	Windows 7 Professional, Service Pack 1	Dell Optiplex 7020

Timing

EGI AV Timing Test

Although we will be using the Cedrus Stimtracker for timing, which will provide timing marks for each subject, general upkeep of the system requires regular timing tests. Please implement the EGI AV timing tests as specified in the EGI support documents.

What	File
Timing Test Tracking & reporting	EEG AV Timing Test - Main Study
AV Device	AV_050707.pdf
AV Timing Tests	MovingTimingTest.es2

See "**CIS Montly Reporting / Timing Tests**" 2.4 ABC-CT DAAC EEG Main Study Acquisition Protocol 20181004.docx

E-Prime/Net Station Software

Technical Manuals:	GES_300_tman_...	Google Drive

GES 300 Manual (GES Hardware Technical Manual" GES 400 Manual ("GES 400 Series User Manual")	GES_400_uman_....	EGI Documentation http://www.egi.com/membership-research-customers-documentation
Geodesic Net Manual	GSN_013107.pdf	EGI Documentation http://www.egi.com/membership-research-customers-documentation

See "**Net Station Software**" ABC-CT DAAC EEG Main Study Acquisition Protocol

E-PRIME Experiment Filenames

	Name Exp
Resting	Resting_M2_BCH.es2 Resting_M2_Duke.es2 Resting_M2_UCLA.es2 Resting_M2_UW.es2 Resting_M2_Yale.es2
Faces	Faces_M2_BCH.es2 Faces_M2_Duke.es2 Faces_M2_UCLA.es2 Faces_M2_UW.es2 Faces_M2_Yale.es2
VEP	VEP_M2_BCH.es2 VEP_M2_Duke.es2 VEP_M2_UW.es2 VEP_M2_Yale.es2 VEP_M2_UCLA.es2
Biomotion	Biomotion_M2_BCH.es2 Biomotion_M2_Duke.es2 Biomotion_M2_UCLA.es2 Biomotion_M2_UW.es2 Biomotion_M2_Yale.es2
VEP Screen Measurement	VEP_ScreenMeasurement_M2_BCH.es2 VEP_ScreenMeasurement_M2_Duke.es2 VEP_ScreenMeasurement_M2_UCLA.es2 VEP_ScreenMeasurement_M2_UW.es2 VEP_ScreenMeasurement_M2_Yale.es2

Data Collection

What	File
Logs for data collection	EEG_Log_Mv5.0_01Novermber2016_OrderA EEG_Log_Mv5.0_01Novermber2016_OrderB EEG_Log_Mv5.0_01Novermber2016_OrderC EEG_Log_Mv5.0_01Novermber2016_OrderD
EEG Procedures	EEG_Acquisition_Protocol_M2.4

Room Set-up

See "**Room Set Up**" ABC-CT DAAC EEG Main Study Acquisition Protocol

EEG Session Logs

The goal of these documents is to collect information during the EEG session that will allow the data to be properly identified (descriptive information) and quantified for quality control metrics. The information to be scored during the session provides valuable data about the behavior of the subject during the paradigms. Consistent reporting of this information will allow for participant as well as site specific variables to be analyzed and accounted.

Several of the items within the session log map to the NDAR eeg_sub_files01 that is required for NDAR submission. A data dictionary is available upon request.

Participant specific EEG variables (head size, net size and number, net fit, protocol, BA and parent location) will be used during analysis to look at:

1. the effect of head size on EEG,
2. to make sure the relation between head size and net size is similar across sites,
3. to account for any variability or similarity in nets (e.g., consistent bad channel),
4. that the reasons for protocol deviations are similar across sites,
5. the influence of the location of the BA/ Parent on lateralization of EEG activity.

See "**Data Logs**" ABC-CT DAAC EEG Main Study Acquisition Protocol
See "**Appendix 4a/b**" ABC-CT DAAC EEG Main Study Acquisition Protocol

Site (CIS) Roles

For details See "**Roles**" "**Experimenter**" "**Behavioral Assistant**" "**Protocol with Child In Room**"
ABC-CT DAAC EEG Main Study Acquisition Protocol

Experimenter (EXP)

The role of the data collection EXP is to guide the participant through successful data collection. The EXP is the lead for the session and will be involved in the management of the equipment, the data, and the overall protocol.

Behavioral Assistant (BA)

The role of the BA is to manage the subject's behavior during the protocol to increase compliance and reduce artifact. A BA will be in proximity to the participant and will engage with the minimum amount of actions needed to keep the participant on task. A second BA is allowed if this benefits child compliance.

Parent

The role of the parent is to manage the subjects' behavior *if* the child has additional behavioral needs that cannot be managed by the BA and/or *if* the child is of age wherein presence of the parent would be calming/supportive.

Data entry (EEG log entry)

EEG Session logs will be entered online via RexDB.

Data upload

Raw EEG data (with videos),E-Prime edat files and PDF of EEG log file will be transferred to the DAAC using RexDB large file system.

NDAR

Data is shared under NIMH Data Archive Study #2288, experiments are:

- 472 ABC-CT Resting
- 479 ABC-CT Social/Nonsocial (feasibility data)
- 480 ABC-CT Biomotion (feasibility data)
- 481 ABC-CT Emotion (feasibility data)
- 482 ABC-CT EU AIMS Faces (feasibility data)
- 483 ABC-CT VEP (feasibility data)
- 509 ABC-CT Resting v2 (main study data)
- 544 ABC-CT Faces v2 (main study data)
- 545 ABC-CT VEP v2 (main study data)
- 546 ABC-CT Biomotion v2 (main study data)
- 1229 ABC-CT Faces v3 (revised main study data)
- 1230 ABC-CT VEP v3 (revised main study data)
- 1231 ABC-CT Biomotion v3 (revised main study data)

Data uploaded to NDAR will be processed for the Resting and ERP Experiments separately. The general process is as follows:

Resting
See the ABC-CT Data Acquisition and Analytic Core EEG Main Study Resting Pipeline and Derived Results for more information.

1. Using Net Station Tools, resting segmentation tools were made for both 300 amp and 400 amp sites. Resting files were segmented at obs# is 1, obs# is 31, and obs# is 61.
2. Files were segmented 100ms before and 64,000ms after.
3. Offsets: For 300 amps, the segment was offset +8ms and for 400 amps, the segment was offset +36ms.
4. File Export - Files were exported using Net Station simple binary tool.
5. All segments, regardless of attention / behavior, are included.
    a. Attention was coded and attention codes are available for use in analysis.

ERP Experiments (Biomotion, Faces, VEP)
See the following manuals for more information about NDAR for ERP Experiment procedures:

- ABC-CT Data Acquisition and Analytic Core EEG Main Study ERP Pipeline and Derived Results Manual for the Faces Experiment
- ABC-CT Data Acquisition and Analytic Core EEG Main Study ERP Pipeline and Derived Results Manual for the VEP Experiment
- ABC-CT Data Acquisition and Analytic Core EEG Main Study ERP Pipeline and Derived Results Manual for the Biological Motion Experiment

Specifications are also available in the NDAR study experimental definition.

1. File Processing:
    a. Files were processed in batches using the YETI Yale ERP NDAR pipeline and include PREP and artifact detection.
2. File Renaming:
    a. The output MATLAB files from step iv is renamed with the subject GUID instead of study subject ID
3. File Upload:
    a. All renamed files that meet eligibility (behavioral compliance/required attended trials) are uploaded to RexDB by EEG DAAC group and then transferred to NDAR by DCC. Files that do not meet requirement will have a no data.txt file will be uploaded with “no data” description.
        i. Behavioral compliance is noted at acquisition and review criteria are available in the acquisition manual.
        ii. Attended trial #s per experiment are listed in the EEG logs for assessment during acquisition. Artifact free trials for valid data qualification are listed in the derived results and pipeline manuals.
4. Problem Files were pre-processed on a case-by-case basis by the DAAC team. Examples of problem files include: missing E-Prime Flags, missing Dins, missing video of child etc. Files where problems are unresolved, a no data.txt file will be uploaded with “no data” description.

NDAR support files	File
Protocol Definition	NDAR_eeg_protocol01.xlsx
Experiment Definition	NDAR_eeg_experiment01.xls

Data Quality Control

There are several forms of quality control that will be examined within the ABC-CT EEG data.

DAAC EEG Quality Control Manual	ABC-CT DAAC EEG Main Study Quality Control Manual

Preliminary QC (see ABC-CT DAAC EEG Main Study Quality Control Manual for detailed information)

1. QC report for counterbalance
    a. From the Screening Form and the EEG Log
    b. Includes the variables from the screening (what was assigned by the screeners) versus those recorded in the log (what was assigned and identified by the EEG staff) versus what was run.
2. QC report on the EEG
    a. From the EEG Log
        i. Includes values related to netting, distance to monitor, completion & quality (by experiment)
    b. From Net Station File
        i. Net Quality - Net quality is assessed using the Net Station video from the files uploaded to DAAC. A screenshot is taken of “netfit_start” and “netfit_end” for each day of each subject. Net fit is graded as poor, average, or excellent as defined by the ABC-CT Net Placement Guide. A power point slideshow is made for each site that shows the starting and ending picture, netfit grade, and how the net is offset (skew, off-center, etc.). These power points are shared with the sites and discussed if necessary.
        ii. Audio/video quality - Audio/ video quality is also graded as poor, average, or excellent.
        iii. Electrodes with impedances above 200 kOhm and that look bad in Net Station review are noted.
    c. Reviewed by the DAAC
        i. Includes values referencing file usability, video usability, net position, presence of DIN and Flags in NS file, electrode quality.
3. QC for EEG was reported to NIH quarterly.
    a. To ‘pass’ QC,
        i. The raw file must have all relevant flags/markers, be “readable”, and collected according to the protocol
        ii. The participant had to have average to excellent net placement, and complete 50% of the resting experiment with good or questionable data.

   b. Problem files were fixed (if possible) and added to the final list of “good files". If unresolved, problems files were marked as “invalid/failed QC”.
4. Data that passed QC was then submitted to the NDAR pipeline and then to the derived results pipeline.
   a. Data that failed QC was not subject to additional processing.