Dear Editor & Reviewers,

Below you will find your suggestions and guidance to improving this manuscript, followed by a reply to each comment and suggestion. I thank you all very much for taking the time and effort to review this manuscript and for providing your guidance.

Very Best,

Jacqueline Turcios

**Changes recommended by the JoVE Scientific Review Editor:**

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

* *Thank you for your comments. Please see the changes made to the manuscript described in response to your comments as well as reference to the line items that are relevant to the suggested changes.*

• **Abstracts:** Please re-word the Long Abstract to more clearly state the goal of the protocol.

* *Changes in wording have been made to the long abstract, so that it states that the familiarization protocol was created in order to reduce anxiety and increase the likelihood of successful participation in research.*

• **Protocol Language:** Please ensure that all text in the protocol section is written in the imperative tense as if you are telling someone how to do the technique (i.e. “Do this”, “Measure that” etc.) Any text that cannot be written in the imperative tense may be added as a “Note”, however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

* *We have reviewed the protocol to ensure that it is in imperative language.*

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please add more details to the following protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure that all additional details in the protocol section are written in the imperative tense, as if you are telling someone how to do the technique (i.e. “Do this”, “Measure that” etc.).

1. 4.2: Please check the figure reference, this should likely be figure 3.

*Number changed in line 253.*

1. 4.3: Please check the figure reference.

*Now step 4.4, image reference has been repaired, thank you.*

1. 4.4: How is this assessed? Do you ask them participants to rate themselves on the emotion rating scale as in 4.2? You state “both emotion-rating scales”, but only one scale is present.

*Now step 4.5. The second scale will be attached as a figure; figure 4. Further explanation on how to use the scales is now described on step 4.5, line 276.*

1. 4.5: Is the consent form presented to the child or the guardian?

*Now step 4.6. Clarification has been made under this item, see line 281.*

1. 4.9: You state “both emotion-rating scales”, but only one scale is present. Again, is this performed as in 4.2?

*Again, the second scale is now available as a newly attached figure.*

1. 4.10.1: Do you read this out to the participant? If not, it should likely be un-highlighted.

*Yes, the social narrative is read to the participant. Now stated in the text 4.12.1, line 321.*

• **Protocol Numbering:** Please adjust the numbering of your protocol section to follow JoVE’s instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.

* *Numbers have been adjusted to follow journal instructions. We have justified all text to the left. However, sub-steps cannot be entirely moved to the left, because this disrupts the numbering convention.*

• **Protocol Highlight:** After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. Please see JoVE’s instructions for authors for more clarification.

For continuity, 4.10.2 should be highlighted.

*This is now 4.12.2 and it has been highlighted as, suggested.*

• **Results:** Do you have data to show what percentage of children wore the net without the familiarization approach for comparison? Also, can the average wearing time be provided for all samples?

* *We have included an additional pie chart to show improvements in running participants through the protocol post-implementation of the familiarization protocol. See Figure 9. A note is included in line 360-361, mentioning the approximate wearing time of the EEG net during the actual experiment.*

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

* *The protocol now contains these headings, in order to insure that none of this information is missed.*

• **Figures:**: (change table to Figure)

1. Please ensure that figures are called out in the correct order. Currently 5 and 7 are referenced before 3.

*All images are now in the correct order.*

1. Fig 3: How does the researchers scale look? How is it different from this?

We attached a figure, Figure 4, so that you can see the scale that was used.

* *See Figure 4*

• **References:**Please make sure that your references comply with JoVE instructions for authors. Citation formatting should appear as follows: (For 6 authors or less list all authors. For more than 6 authors, list only the first author then *et al.*): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. *Source*. **Volume** (Issue), FirstPage – LastPage, doi:DOI (YEAR).]

1. Please abbreviate all journal titles.

*All journal titles have been abbreviated*

1. Please include volume, issue numbers, and DOIs for all references.

*DOIs that were available have been included.*

• **Commercial Language:**JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are Electrical Geodesic Inc., NetAmps 300, Hydrocel.

1. Please use MS Word’s find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names.

*All references to EGI have been removed from the protocol.*

• Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space *between the numerical value and unit.*

* *We ensured that all reference to time units were changed as requested.*

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

**Comments from Peer-Reviewers:**

**Reviewer #1:**

*Manuscript Summary:*

This manuscript by Turcios and colleagues discusses a pervasive but often under discussed aspect of ASD research-- how to consistently get good data from a vulnerable population while also ensuring that participants are comfortable and continuously consenting to the sometimes demanding research procedures. This protocol would be useful to inform not only EEG-specific experiments with children with ASD, but nearly any protocol with children who have any communication deficits or developmental delays.

*Major Concerns:*

I have no major concerns about this manuscript.

*Minor Concerns:*

- What is iconicity?

* *Thank you for making us aware of the need to clarify this. Iconicity refers to level with which an image matches the object that it represents. We have added a brief definition on lines 180-181.*

- It's stated that the visual schedule and social narrative should be consistent, but not in what way. Should the icons used be the same, etc.? For anyone who has heard of social stories/visual schedules but never made them, I think a little extra direction here would be helpful.

* *The visual schedule and social narrative should be consistent, in a way that the images and language, used in the two tools, are the same. See lines 219-221.*

- The protocol seems well suited for younger/less verbal children, but certain aspects (especially re-reading, multiple administrations of the emotion scale) may be less needed for older/more-verbal children (at the discretion of the researcher). Are there any adaptations the researchers would suggest for participants who are older children/quick to pick up the procedure? This might be useful as well in terms of control groups that are often included in these studies, TD children will likely benefit from some of these strategies but may not need all of them.

* *This certainly needed further clarification. Images and language can be adapted to appeal to children of different ages and cognitive abilities is now stated under “Modifications and trouble shooting,” see lines 494-508.*

*Additional Comments to Authors:*

N/A

**Reviewer #2:**

*Manuscript Summary:*

The authors present a protocol designed specifically to assist researchers in adapting children with ASD to the demands of neuroimaging studies and participating in research protocols. The basic concepts presented, although not new, are presented in a logical manner and described in a stepwise manner with appropriate places for decision making by the subject to withdraw from further testing. I have two major concerns and a few minor comments listed below:

*Major Concerns:*

1)The emotions chart listed in the study is far too narrow and mostly focused on anxiety and fear. There are many other emotions that children may feel during testing which are more useful for the evaluator. For example: grumpy, impatient, angry, sleepy, frustrated, shy, excited, annoyed, unsure or embarrassed. Did the reviewers pick these five, very similar, emotions for a particular reason or could the chart be redesigned with more informative emotional states?

* *Thank you for making a note of this. The chart was designed in this manner because these are the emotions that were observed early in the research project. Children were coming in exhibiting anxiety about the steps of the procedure. Therefore, the focus of this protocol is to reduce anxiety. However, we recognize that there are more emotions that could arise in this context. Therefore, we have mentioned that the Researcher Rating Scale (see lines 501-505) can include other emotions in the self-rating scale in other research situations. For our study, these are the emotions that were consistently present in our participants.*

2)At step 5.2.4 the authors tell the participant to "sit still like a statue" during the EEG reading. Although there has been extensive training during the early phases of study for putting on the sensor net, they have not suggested any training at all for sitting still. In fact, sitting still for 5-10min could be the most difficult task you can ask a child with ASD to perform. They should re-think this issue and add places in the protocol for playing a "sit still" game with visual cues.

* *This is a great idea and we have included it as a modification: see line 507-509.*

*Minor Concerns:*

1. The materials table could be just listed in a Methods section of the written portion of this article.*[Editor's Note: Please ignore this comment.]*

2) Table #1 is not useful - it should be a pie chart figure instead.

*Thank you for the suggestion, we have reviewed this section to include pie charts from a cohort tested prior to the familiarization protocol and after adopting the protocol, see Figure 9.*

3) What is the "stress brain" referred to in Figure 3? Also, check that the text matches the stated "what I can do" section of this figure - it does not.

* *A stress brain is a stress ball in the shape of a brain, is now changed to stress ball in a changed chart of the self-rating emotion scale, and now the language starts with “I can do.” See modified image 3. Thank you.*

1. The section written after Table #1 and before the Discussion is confusing and should be re-written to some extent. For example, the sentence on line 448 implies that some of the boys "were unable to do so,…" but really they were unable to go to the appointment.
   1. *This paragraph has been changed. We are only including the information about the results and removed the phrase that you mention in this comment. Lines 466-473. It wasn’t that they did not attend the appointment, but did not receive the familiarization protocol. Thank you.*

*Additional Comments to Authors:*

None

**Reviewer #3:**

*Manuscript Summary:*

This paper presents a familiarization protocol for involving children with ASD in EEG/ERP research. I am enthusiastic about this work being presented and that it will be a useful tool for many in the field. My main questions are those that relate to the generalizability of the procedures, and some instances where the rationale for certain decisions could be made more clear.

*Major Concerns:*

The authors use the term "EEG" throughout the paper, when it seems that their paradigm is actually an ERP study. It might be helpful early in the paper to describe how these terms will be used, as the suggestions are generally appropriate for both techniques.

* *Our current experimental approach includes analysis of both EEG and ERP, this is now explicitly stated. Further, we now clarify that because ERPs are extracted from the ongoing EEG, we will be referring to procedures for EEG data collection throughout the manuscript.*

The paradigm described is for kids of a generally moderate ASD severity level and of a certain age range. It would be very helpful to briefly describe which aspects of the protocol could be used in children who are older or younger, and more or less severe on the ASD spectrum.

* *Excellent point! This is now explicitly laid out at the end in the “Modifications and trouble shooting” portion, lines 495-510.*

*Minor Concerns:*

In the short abstract, the authors say that EEG is useful for identifying biomarkers. I think that this effort has not actually been very successful, and that EEG/ERP can do more than this. Consider rephrasing.

* *We agree with the reviewer, and we have changed our wording to refer to our broader goal of better understanding the neural bases of AV speech processing in typically developing children and those with developmental disabilities. Space limitations, and the focus on procedures for familiarization and preparation in the current manuscript, preclude a longer discussion of our approach and motivation for the use of EEG and ERP specifically.*

As I mentioned, several points could use a bit more explanation as to why the given approach is chosen. These include 1.1.3 (using powerpoint to show social narrative), the experimenter's rating of child's state (lines 351-354).

Adjust awkward wording in section 4.10.4.

* *Step 1.1.3, we added more explanation to say that it is in powerpoint mode so that it can be used as a story on presentation mode. Of course, other programs can be used as well.*
* *This is now 4.12.6 and the wording has been changed to read more clearly, see lines 352-354*

What is to be done if the child's comfort/anxiety is increased after wearing the cap? (4.11)

* *A note has been made to provide further instruction, see lines 367-368*

I have a few additional suggestions that many colleagues find helpful: soliciting information from the parent in advance about their child's likes and preferences. For example, a child who has a given interest can be rewarded with that interest. Parents can also bring a child's favorite movie, a stuffed animal or blanket, and extra layers to keep the child comfortable.

I also find it works well to never mention the effect of eye blinks on the data, as kids tend to think about it and blink more. I do find it helpful to show the child their EEG in real time and let them see how body motion or touching the electrodes make their brain waves go wiggly and hard for the researchers to understand.

It's likely that the research team may already employ these strategies given their expertise, and I defer to them to add these to the protocol as they see appropriate.

* *Thank you for your excellent suggestions, we have added some of these as consideration for modifications to the protocol in lines 499-514. Further, we do indeed show older children their EEG and the effects of movement, however we do not do this consistently as some, particularly younger and/or highly anxious children, are easily distracted by this, and we have tried to only include procedures that are consistently employed with every child.*

*Additional Comments to Authors:*

N/A

**Reviewer #4:**

*Manuscript Summary:*

Paper by Turcios et al provides recommendations on familiarization of the young participants with ASD with behavioral and EEG experimental protocols to improve compliance and increase participation. The procedure can be applied to children with other developmental disorders and to typically developing young children.

This is a useful paper for those who works with developmentally disordered children. It may appear particularly instructive for the researchers who just start working with the ASD participants. Turcios formalizes the steps to increase compliance of the subjects, their satisfaction, and participation outcome, and suggest useful means, such as e.g. visual schedule of upcoming activities (Fig. 2) and 'How I feel' scale (Fig. 3).

I think that the content of the paper can be used to produce a valuable educational video. I have just a few comments.

*Major Concerns:*

N/A

*Minor Concerns:*

1. The authors report the '% participation' outcome of their study that used this protocol. They though do not report IQs and autism severity of their participants. Please, do that since it would give the reader/viewer a better understanding of which group could benefit most of this approach.

*Thank you for pointing this out, we have noted that our participants had an average IQ.*

1. Children with ASD may have difficulties understanding emotions. Do they have difficulties interpreting the 'How I feel' emotional scale? In what % of you cases there were discrepancies between subject's and researcher ratings?

*We did not do a discrepancy analysis. The purpose of using the pictures was to increase the child’s understanding of the different emotions. We have now added the researcher scale as Figure 4, this scale was used to ensure that the outward state of the child showed that he/she wanted to continue participation. We did not come across a situation in which a child was unable to rate how they felt.*

1. Figure 1 implies a monetary reward upon finishing the experiment. I wonder if is would be appropriate for all participants? Young children and those with developmental delay may prefer to choose a toy from a collection of small toys as a reward. This can be done during both the 1st and the 2nd visit to increase the motivation. The 'toy reword' does not exclude the monetary reward for the participating family.

*Great point. We have included a toy reward as a modification for younger children*

1. It is a bit difficult to judge without the video how the familiarization takes place and how the participants use the means depictured in figures 2 and 3 in the experimental settings. I hope it will be clear from the video.

*We hope so too, thank you!*

1. How do you use the 'How I feel' information (Fig. 3) with those children who cannot read? Would it be better to use the cartoons without the text printed?

*This is now more explicitly explained in steps 4.1 to 4.3. See the text below. In the manuscript these are lines 248-256.*

* *Ask the participant to point to the picture that best represents how they feel on the 5-pt emotion-rating scale (see Figure 3).*
* *The researcher asks the participant “how do you feel” and supports the participant in saying “I feel \_\_\_\_\_.” While pointing to the corresponding emotion on the scale (see Figure 3).*