**Editorial comments:**  
Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

2. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”

3. Figure 1: Please include a space between the number and its corresponding unit (i.e., 500 ms, etc.)

4. Please define all abbreviations before use.

5. Please include a space between all numbers and their corresponding units: 14 cm, 37 °C, 60 s; etc.

6. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

7. Please include single-line spaces between all paragraphs, headings, steps, etc.

8. There is a 2.75 page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.  
9. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

*We have highlighted in yellow the steps that we believe are best for filming.*

10. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique

d) The significance with respect to existing methods

e) Any future applications of the technique

11. References: Please do not abbreviate journal titles.

**Reviewers' comments:**  
  
  
**Reviewer #1:**  
Manuscript Summary:  
This is an interesting study with a visual motivator paradigm to study anticipatory reward ERPs in children with and without autism. The researchers found that children with high functioning ASD showed poorer anticipatory ERP to the social condition compared to TD, in support of the social motivation hypothesis.  
  
Concerns to be addressed

Methods:

1. More details on face photographs needed (how many male/female, adult/children).

*We have added this information to the procedure.*

2. Please make more explicit the rationale behind scrambling faces, or how it was 'matched'.

*We have added the following to the discussion section:*

*“This protocol is useful for measuring anticipatory brain activity in children with and without ASD, and these data provide evidence that this type of brain activity can be reliably and successfully elicited in children over 6 years old. Furthermore, this method allows social and non-social conditions to be directly compared without confounds related to reward properties (as the reward for “correct” responses was goldfish in both conditions). In the current protocol, faces were scrambled and an arrow shape was created. This procedure was chosen to preserve the physical stimulus properties of faces in the non-social (non-face) condition.”*

3. How long is each stimuli presented for and is there a time-out option for when children don't respond.

*We have included this information, and thank the reviewer for this suggestion. There is a ‘time-out’ option that triggers if children do not respond within 3 seconds.*

4. How do you deal with movement artefact, is that included in the instructions given to children (apart from that controlled for in your preprocessing)?

*We have added this information into the protocol. We did not give children explicit instructions to remain still, but did emphasize that breaks could be used to “get the wiggles out!” or “move around if you need to”.*

5. You mention that 'Channels that were problematic', how were these identified and were there more or the same in ASD?

*We have added this information to the manuscript. We did not notice an increase in bad channels in children with ASD versus TD children.*   
  
Introduction :

1. There should be more explanation about the reward anticipation ERP (the Stimulus Preceding Negativity; SPN), how it is usually used, how it is identified (eg subtraction). Also, would the SPN be expected to vary by severity of the condition or age?

*The SPN is conceptualized as anticipation of reward (and is sensitive to reward magnitude). The SPN does not appear to vary by age (though the current protocol was the first to measure the SPN in children). However, since we hypothesize that children with more severe ASD will be less rewarded by faces, it follows that the SPN would differ with severity.*

*We have added the following to the introduction to provide more information about the SPN:*

*“To explore differences between children with and without ASD in reward anticipation, the stimulus related negativity (SPN) was measured. The stimulus preceding negativity (SPN) is a slow wave component that reflects the expectation of reward stimuli (Damen and Brunia, 1987). The significance of the SPN is typically conceptualized as emotional anticipation (Chwilla & Brunia, 1992; Kotani, Hiraku, Soda, & Aihara, 2001; Kotani et al., 2003), and is thought to reflect activity in the insula (Kotani et al., 2009; Kotani et al., 2015). The SPN is often measured after participants make a motor response and before feedback onset in a decision-making task (Brunia, van Boxtel, & Bocker, 2012; van Boxtel & Bocker, 2004). The SPN is sensitive to reward magnitude, and is consistently larger in reward versus no-reward conditions (Kotani et al., 2001; Kotani et al., 2003; Ohgami et al., 2004). Though the SPN is typically measured in decision-making tasks, previous research has reported that the SPN can be observed when anticipating affective upcoming stimuli without a task (Takeuchi, Mochizuki, Masaki, Takasawa, & Yamasaki, 2005; Parker & Gilbert, 2007; Poli, Sarlo, Bortoletto, Buodo, & Palomba, 2007).”*

2. Some important references missing that can add depth to the Introduction/Discussion. E.g. An apt reference is a similar study (EEG-MEG, Yau et al 2015 Frontiers in Neuroscience) where the social and non-social sounds were matched and nonverbal children were found to have no ERP response in the brain to non social-sounds. Collectively, your results and theirs would suggest a core social motivation deficit which becomes more severe in the most profoundly children. It could be argued that matching the stimuli in the way you have is potentially more sensitive task that distinguishes those with and without language (cite in Intro or Discussion).

*We thank the reviewer for bringing this paper to our attention. It is certainly interesting to consider using speech versus non-speech in reward anticipation. We have included information about this in the Discussion.*   
  
Results :

Please insert a legend in Figure 2 to more clearly indicate results. Perhaps another figure separating the within groups and between groups results would make the visuals clearer. *We have edited the figure by making Figure 2 into parts a and b.*

Discussion :

The conclusion is reasonable but could be further strengthened by having a couple of sentences speculating the use of this paradigm in lower functioning ASD. Despite the paradigm being tested only in higher functioning ASD, it seems a shame to not mention its potential in the individuals who make up a large portion of the spectrum. Especially as this new method is fairly 'passive' (i.e. visual, not demanding) and could tease out variation between the low (no language) and high functioning groups.

*This is an excellent point. We have added the following to the discussion:*

*“Some modifications to make the current protocol appropriate for younger children (e.g. toddlers) are currently being investigated. These modifications include making the task passive (e.g. having children watch stimuli that appear at predicable intervals in a block design), and/or using an S1, S2 paradigm (e.g. Poli et al., 2007). In such a design, the content of S1 reliably provides information about the content of S2 (e.g. if S1 is a square then S2 will be a face; if S1 is a circle, then S2 will be an arrow).”*  
  
  
**Reviewer #2:**  
Manuscript Summary:  
This manuscript describes a protocol for measuring the neural correlates of social reward processing, particularly as it pertains to aspects of social communication impairments in autism spectrum disorder (ASD). This protocol was used in prior work that identified reduced anticipatory responses in ASD via the stimulus preceding negativity (SPN) and feedback related negativity (FRN; Stavropoulos & Carver, 2014), as well as atypical alpha suppression asymmetry (Stavropoulos & Carver, 2018).  
  
Please read and incorporate specific comments below to improve future versions.  
  
Major Concerns:

(1) Although mentioned in the discussion, please clarify that this task is only intended for individuals with average to high cognitive performance within the introduction as well as the representative results (e.g., "age and cognitively-appropriate …"). It is presumed that this restriction (in Behavioral Procedures #2) is due to the requirement of a button press and understanding directions. It would be of interest to describe possible modifications within the discussion section that may be used to improve the generalizability of the task. For instance, considering that correct/incorrect responses are not link to behavioral choice, could it possible to use this as a passive task? If so, are there any modifications that you would suggest? For instance, other groups have had success working with lower functioning individuals (and infants) by incorporating auditory components to maintain attention for visual ERPs. Would it be possible to use auditory cues?

*We have added information about why we chose to make an IQ of 70 the “cut-off” for the current study. In addition, we added information in the introduction about how the current protocol was designed for “high functioning” children with ASD. Finally, we added the following to the discussion:*

*“However, we note that modifications to make the current protocol appropriate for younger children (e.g. toddlers) or individuals with intellectual disability is currently being investigated.” Because our research group is investigating the feasibility of utilizing this task in toddlers and younger children, we cannot be certain about whether making the task passive will be successful.”*

(2) Please justify the use of bilateral mastoid re-referencing (Processing EEG Data, #3), which could impact topographic results as mean mastoid reference can overly influence lateral ERP components. Also, please describe procedures if the mastoid electrodes were noisy or problematic during acquisition.

We have added the following information to the Processing EEG Data section:

*“Mastoid electrodes were chosen as the reference due to not having a dense electrode array (in which case, experts sometimes suggest using the average reference17), and because using the average of both mastoid electrodes is less problematic for laterality effects versus a single mastoid18*

*If using the average of mastoid electrodes for re-referencing, those two electrodes must provide a clear signal. If either mastoid electrode had a poor quality signal (e.g. high impedence, lost contact with skin during recording), the participant’s data was not used for analysis.”*

(3) Acquisition of the EEG data with a bandpass of .1-10 Hz seems odd. Please justify.

*We apologize for this typo, it was an error. EEG acquisition was done with a 70Hz low-pass filter, a DC filter, and a 60Hz notch filter. All other filtering was done offline and described in the analysis section.*

(4) Considering both ERP and EEG analyses can be conducted using this protocol, please clarify any processing differences for EEG analyses (i.e., do not post-hoc filter?)

We have added the following information about ERSP analysis (it is section 5):

1. **Processing differences for ERSP analysis**
   1. Begin with the file created in Step 4.11 (e.g. the epoched file with all artifact rejection procedures complete).
   2. Utilize the EEGlab ‘newtimef’ plug-in, which yields a time x frequency transform with values for each time point, frequency, and trial.
   3. In order to measure anticipatory alpha band activity, the average values between 8-12 Hz were calculated.
   4. Average activation prior to feedback onset (e.g. -2200 to -100 ms, baseline of -2200 to -2000 ms) was calculated in the same electrodes as utilized for the SPN.
   5. In order to calculate alpha asymmetry prior to feedback, log power in the left hemisphere was subtracted from the right hemisphere.
   6. Note that in order to calculate ERSP activation after feedback onset, data would need to be re-analyzed and re-epoched using a different time window (e.g. -200 to 800 ms, with -200 to 0 ms as the baseline).

Minor Concerns:

(1) It would be helpful to cite the following paper that provides a nice overview of the social motivation hypothesis in ASD.  
a. Chevallier, C., Kohls, G., Troiani, V., Brodkin, E. S., & Schultz, R. T. (2012). The social motivation theory of autism. Trends in cognitive sciences, 16(4), 231-239.

*We have added this reference to the introduction, and thank the reviewer for this suggestion.*

(2) Do the authors have any suggestions regarding content of stimuli (i.e., emotional faces)? It seems that the authors used the Tottenham et al., (2009) stimuli set in which the mouth is closed and full shoulders/face are present. Are there any suggestions regarding different stimuli options? For instance, many face processing ERP studies opt to eliminate the hair and shoulders by using an oval mask so that only the eyes, nose, and mouth are present.

*This is an excellent question, and one that should be directly compared in future research (e.g. are there systematic neural differences observed when stimuli are presented with the oval mask versus a full face with hair?) In the current protocol, we wanted to create a task that would most accurately be able to answer questions related to social motivation (rather than lower-level perception). We therefore decided it seemed more important to preserve stimulus characteristics than to make sure each face was shown with an identical oval mask.*

(3) Relatedly, Tottenham et al (2009) had the stimuli normed by college students. Considering that this protocol is developed for children age 6-11 years, it may be more appropriate to suggest a child-normed stimuli set.

*This is an excellent point that should be considered. We have added the following to the protocol (where we originally mention the stimulus set):*

*“It is important to note that the NimStim set of facial expressions were rated and normed by adults. It is possible that children or adolescents may perceive the facial expressions differently than the adults who normed the images.”*

*We have also added this information to the discussion section as a potential future direction.*

(4) Were only Caucasian faces used? Despite converting the images to black and white and having the scrambled arrow comparison condition, the race of the faces should be noted, considering that other races may have differential perceptual effects on the ERP depending on contrast levels.

*We have added this information to the methods section. We used Caucasian, Asian, and African American faces. Analyses were conducted collapsed across race, as the difference between social versus non-social images was our primary research question.*

(5) Why a resolution of 72 pixels/inch? Is it more difficult to scramble the face for the arrow stimuli with a higher quality image?

*When the images (from NimStim) were opened using photoshop on the laboratory computer, the resolution was shown to be 72 pixels/inch. We decided not to edit this value because we did not want any distortion to occur. Thus, we did not make the images higher or lower quality than they were on our computer (though I cannot be 100% sure that this level of resolution would have been identical on a higher quality or newer computer monitor).*

(6) Is it possible to provide a macro or batch script for Photoshop that can be used once the "scramble" plugin is installed? In Photoshop, you should be able to record a series of actions, including opening every file in a folder and proceeding with Steps 8-20. It would be helpful to host these instructions and the action file on a website.

*Unfortunately, we do not have anyone in the lab who has that type of knowledge (to provide a script). All of the images were scrambled/edited by hand. This would be very helpful in the future, but at this time we are not able to provide it.*

(7) What should be the border distance between the goldfish cracker and the images?

*When viewed at 100% on a 13 inch laptop computer, the approximate distance between the goldfish and the image is 1-2mm.*

(8) Please provide the timing information for the EEG presentation of the stimuli (Step 4, #28). Considering that this is an anticipatory reward task, it is vital to standardize the duration of the fixation cross, response period, and feedback. Also, please clarify whether the button press terminates the stimuli or if the image remains on the screen for the full duration.

*We apologize for this oversight in the initial submission of the manuscript, and have included this information.*

(9) What is the anticipated final visual angle of the stimuli?

*The anticipated visual angle is the following: horizontal visual angle of 14.5 degrees, and vertical visual angle of 10.67 degrees. We have added this to the EEG presentation section.*

(10) Clarify that the counterbalance order refers to which block of stimuli is used (social versus nonsocial) in EEG recording section, #11.

*We have included this information in the EEG recording section.*

(11) Why is the SPN analyzed from -210 to -10 ms rather than -200 to 0 ms?

*We have addressed this in the EEG recording section. This decision was made to avoid any “edge effects” of including 0ms (e.g. the exact onset of feedback stimulus) in our analyses.*

(12) Is there baseline correction? This step appears to be missing. \*\* see EEG processing, step 5).

*We have included information about baseline correction in the EEG processing section.*

(13) In the discussion, it would be helpful to expound briefly upon why "these results are exciting" and how they future our understanding of the underlying neural mechanisms in ASD.

*We have added more information to the discussion section, including reasons why these findings are of interest in ASD.*