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A Within-Subjects Experimental Protocol to Assess the Effects of Social Input on Infant EEG

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Abstract:	Despite the importance of social interactions for infant brain development, little research has assessed functional neural activation while infants socially interact. Electroencephalography (EEG) power is an advantageous technique to assess infant functional neural activation. However, many studies record infant EEG only during one baseline condition. This protocol describes a paradigm that is designed to comprehensively assess infant EEG activity in both social and nonsocial contexts as well as tease apart how different types of social inputs differentially relate to infant EEG. The within-subjects paradigm includes four controlled conditions. In the nonsocial condition, infants view objects on computer screens. The joint attention condition involves an experimenter directing the infant's attention to pictures. The joint attention condition includes three types of social input: language, face-to-face interaction, and the presence of joint attention. Differences in infant EEG between the nonsocial and joint attention conditions could be due to any of these three types of input. Therefore, two additional conditions (one with language input while the experimenter is hidden behind a screen and one with face-to-face interaction) were included to assess the driving contextual factors in patterns of infant neural activation. Representative results demonstrate that infant EEG power varied by condition, both overall and differentially by brain region, supporting the functional nature of infant EEG power. This technique is advantageous in that it includes conditions that are clearly social or nonsocial and

	allows for examination of how specific types of social input relate to EEG power. This paradigm can be used to assess how individual differences in age, affect, socioeconomic status, and parent-infant interaction quality relate to the development of the social brain. Based on the demonstrated functional nature of infant EEG power, future studies should consider the role of EEG recording context and design conditions that are clearly social or nonsocial.
Author Comments:	We attached multiple supplemental files. The first is a list of the nonsocial objects (file names) that are in each block. We also attached all of the nonsocial objects, which are the stimuli presented on the computer monitors during the paradigm.
Additional Information:	
Question	**Response**
If this article needs to be "in-press" by a certain date, please indicate the date below and explain in your cover letter.	

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December 12, 2016

Dear Dr. Mani,

Enclosed please find the revision of our manuscript JoVE55596, “A Within-Subjects Experimental Protocol to Assess the Effects of Social Input on Infant EEG.” We appreciate the time that you and the reviewers have spent with this manuscript and the thoughtfulness of your comments.

We have made revisions based on the editorial and reviewer comments, which has helped us greatly improve the manuscript. Based on reviewer feedback, we have clarified points in the protocol including how to talk to parents about EEG and net application as well as providing additional detail on artifact rejection parameters. In the discussion, we have added additional modifications such as appropriate ages for the protocol; alternative ways of analyzing the EEG data; and adapting the protocol to use with only one computer screen. We have also expanded our discussion of future directions including assessing the neural bases of social interactions in conjunction with other developmental domains; using additional neural measures with this paradigm; and expanding the relevance of using this paradigm with clinical populations at high risk for social difficulties.

Thank you and we look forward to hearing from you.

Best,

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TITLE:
A Within-Subjects Experimental Protocol to Assess the Effects of Social Input on Infant EEG

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KEYWORDS:
Infant; Electroencephalography (EEG) power; EEG; social interaction; social engagement; joint attention; social brain; child development

SHORT ABSTRACT:
This novel protocol is designed to assess the neural bases of social interaction in infants. The paradigm is designed to tease apart how various social inputs such as language, joint attention, and face-to-face interaction relate to infant neural activation. Infant EEG power is recorded during both social and nonsocial conditions.

LONG ABSTRACT:
Despite the importance of social interactions for infant brain development, little research has assessed functional neural activation while infants socially interact. Electroencephalography (EEG) power is an advantageous technique to assess infant functional neural activation. However, many studies record infant EEG only during one baseline condition. This protocol describes a paradigm that is designed to comprehensively assess infant EEG activity in both social and nonsocial contexts as well as tease apart how different types of social inputs differentially relate to infant EEG. The within-subjects paradigm includes four controlled conditions. In the nonsocial condition, infants view objects on computer screens. The joint attention condition involves an experimenter directing the infant's attention to pictures. The joint attention condition includes three types of social input: language, face-to-face interaction, and the presence of joint attention. Differences in infant EEG between the nonsocial and joint attention conditions could be due to any of these three types of input. Therefore, two additional conditions (one with language input while the experimenter is hidden behind a screen and one

with face-to-face interaction) were included to assess the driving contextual factors in patterns of infant neural activation. Representative results demonstrate that infant EEG power varied by condition, both overall and differentially by brain region, supporting the functional nature of infant EEG power. This technique is advantageous in that it includes conditions that are clearly social or nonsocial and allows for examination of how specific types of social input relate to EEG power. This paradigm can be used to assess how individual differences in age, affect, socioeconomic status, and parent-infant interaction quality relate to the development of the social brain. Based on the demonstrated functional nature of infant EEG power, future studies should consider the role of EEG recording context and design conditions that are clearly social or nonsocial.

INTRODUCTION:

Social interactions are crucial for infant neural development [1,2]. Although recent research has begun to focus on the development of the social brain [3,4], the neural processes involved in social engagement are not well understood. The goal of the reported method was to assess how infant electroencephalography (EEG) power, a measure of voltage released from neuronal communication, varies across controlled social and nonsocial contexts. This method allows for assessment of how specific aspects of social input differentially relate to neural activation and has implications for future studies to consider the role of recording context when assessing functional neural activation.

EEG is a well-suited method to measure infant brain activity, as it is noninvasive and robust to infant movement. A cap composed of electrodes is placed on the infant's head to record electrical activity from the cerebral cortex released during neuronal communication. EEG power is a measure of voltage at each electrode site over a period of time. EEG is a functional measure of neural activity and thus reflects in part the immediate context under which EEG is recorded. Due to its functional nature, EEG power has the potential to be compared across contexts using a within-subjects design and thus to index context-specific activation. Therefore, EEG can be used to assess both the neural underpinnings of social interactions specifically and of context-specific activation more generally. However, this potential has not been fully realized as infant EEG is often recorded during only one condition.

Many studies have recorded infant EEG power during a "resting state" or baseline, which does not always clearly differentiate between social and nonsocial input. In some cases, EEG is recorded as infants watch an experimenter spin a bingo wheel [5–7], watch an experimenter blow bubbles[8] or watch an experimenter shake a rattle [9,10]. However, infants can attend to either the experimenter or the object, and infant characteristics could influence how they direct their attention. Thus, for some infants the baseline could be social if they are attending to the experimenter and for other infants the baseline could be nonsocial if they attend primarily to the object. As EEG reflects the recording context, observed individual differences in baseline EEG that researchers might interpret as stable or developmentally meaningful could simply be due to differences in what the infants were attending to at the time of recording. Indeed, one study recorded EEG while infants watched a woman singing while holding an object [11]. Infant EEG power varied depending on whether the infant paid attention to the woman or the object. This demonstrates both the functional nature of infant EEG power to assess the neural bases of social interaction and also the methodological importance of using controlled conditions during EEG recording.

Social interaction is complex and multi-faceted. Therefore, if EEG was recorded during a naturalistic interaction, it could be difficult to tease apart the neural processing of different aspects of the interaction (*e.g.*, hearing language, interacting face-to-face, or engaging in joint attention). A strategy to address this issue involves including different conditions that each involve a certain aspect of social interaction. Thus, this paradigm is designed to systematically compare how EEG power varies according to the specific type of social input.

The reported within-subjects paradigm involves recording infant EEG during four conditions. The conditions were designed both to examine the functional nature of infant EEG power – how it varies depending on recording context – and to assess the roles of specific types of social inputs. First, a nonsocial condition was included where the infant saw objects on two computer screens. By presenting objects on computer screens instead of having an experimenter manipulate an object, this condition is clearly nonsocial and involves no form of social input. Next, a joint attention condition was included where the experimenter directed the infant's attention to pictures and talked about the pictures. The joint attention condition thus involves three types of social input: face-to-face interaction, language input, and the added component of joint attention. Therefore, the nonsocial and joint attention conditions differ on three dimensions (face-to-face interaction, language input, and the presence of joint attention). Thus any differences in EEG power between the nonsocial and joint attention conditions could be attributable to any of these three social inputs. Therefore, two additional conditions were included to tease apart which aspect of social input explained any observed differences in neural activity between the nonsocial and joint attention conditions. To assess the effect of language, a language-only condition was included where the infant could hear the experimenter comment on the pictures on the computers, but could not see the experimenter. Thus, if EEG power was similar during the joint attention and language-only conditions compared to the nonsocial condition, this effect could be attributed to language. Lastly, to assess the effect of face-to-face interaction, a social engagement condition was included where the experimenter was face-to-face with the infant and contingently engaged with the infant. If EEG power was similar during the joint attention and social engagement conditions compared to the nonsocial condition, the difference between the joint attention and nonsocial conditions could be attributed to face-to-face interaction. If the difference between the joint attention and nonsocial conditions was not explained by the language-only and social engagement conditions, this would suggest that the presence of joint attention specifically was explaining differences in EEG power. This paradigm was piloted with 12-month old infants, as this is an age when the capacity for joint attention is well established[12]. In addition, joint attention during this time is particularly important for language development in the 2nd year of life[13,14], so neural activation in this context was of particular interest at this age.

The paradigm is designed to maintain infants' interest while also ensuring that the conditions are standardized and only differ in the type of social input. Each of the four conditions is repeated once for a total of eight blocks, which alternate between experimenter being present (joint attention and social engagement conditions) or absent (nonsocial and language-only conditions).

To maintain consistency, photographs of objects are presented in all conditions and the same utterances are used across blocks. During each block, 10 photographs of nonsocial objects appear sequentially on computer screens. There are 10 categories of objects (e.g. flower, glove) and four colors of each object. Thus, the same 10 categories of objects are presented in each block with the color of the objects varying across blocks. The stimuli were selected to be interesting for the infants. During the joint-attention and language-only conditions, the experimenter makes a scripted utterance as each object appears on the screen. There are 10 specific utterances (with specified directions to point to the left or right computer screen in the joint attention condition). Utterances are the same for the joint attention and language-only conditions but are said in a variable order to maintain infant interest and to prevent associating particular categories of object with particular utterances. The order of utterances is the same for the first joint attention block and first language-only block. The order then changes for the second joint attention and language-only blocks. Lastly, the direction of pointing varies for each joint attention block and is pseudo-randomized so that infants cannot anticipate the direction.

PROTOCOL:

All procedures were approved by the Boston University Institutional Review Board.

1. Recruitment

1.1) Identify potential participants through participant databases (if available), through publicly available state birth records, through online advertising, and through face-to-face recruitment events.

1.2) Call potential participants and invite them to participate in the study.

1.2.1) Explain that the study is looking at social experiences and how they relate to brain development.

1.2.2) Explain that a visit would involve using EEG to get a measure of the infant's brain activity using a stretchy cap made of soft sponges. Explain that the infant would sit on the parent's lap while looking at pictures on a computer monitor and playing peekaboo with an experimenter, and that this allows to see brain activity.

1.2.3) Explain how long the visit lasts and that a visit can be scheduled whenever is most convenient for the parent, based on the infant's schedule. Provide information about transportation options (*e.g.*, whether there is free parking or reimbursement for public transportation) and about compensation (if any is provided).

2. Running a visit

Note: Schedule the visit for a time when the infant will be alert and well rested. Have two experimenters available. The lead experimenter will do the net application and administer the conditions during EEG recording. The second experimenter will help with net application, control the EEG recording and stimulus presentation computers, and monitor the raw EEG as it is recorded.

2.1) Laboratory set up

2.1.1) Record the EEG in an electrically shielded booth if possible to prevent interference with the EEG signal.

2.1.2) In the booth, place two adjacent computer monitors (measuring 14.5 x 12 inches) on a table. Place the monitors 18 inches apart. Place a chair (where the parent and infant will sit) 48 inches from and facing the computer monitors. Leave three feet between the table and the back wall of the booth (so the experimenter can stand behind the table facing the infant). Ensure that the chair and table are at a height such that the computer monitors are at the infant's eye level.

2.1.3) Position two speakers on the ground on either side of the table, directed towards the chair where the parent and infant will sit.

2.1.4) Position a tripod video camera under the table and facing up so that the infant's face is in clear view.

2.1.5) Hang a black curtain immediately behind the table for the experimenter to stand behind to be hidden from the infant's view during the nonsocial and language-only conditions.

2.1.6) Place a rattle, a small stuffed animal, and cereal in the booth. Use the same rattle and animal for all participants.

2.2) Welcoming the family

2.2.1) On arrival, offer the parent an opportunity to feed and change the infant before beginning.

2.2.2) Obtain informed parental consent.

2.2.3) Explain the EEG and paradigm.

2.2.3.1) Explain that EEG involves a net made of soft sponges that are soaked in warm water, which measures brain activity. Explain that the EEG net does not emit anything, but just measures electrical activity released from the neurons in the brain communicating with each other.

2.2.3.2) Tell the parent that the infant will look at pictures on a screen and interact with the experimenter.

2.2.3.3) Explain that sometimes the experimenter will be behind the curtain, hidden from view. Emphasize that it is important for the parent not to be social with the infant, such as talking to the infant or making face-to-face contact.

2.2.3.4) Explain that 20 min can be a long time and that if the infant gets bored or fussy, the parent can give him a toy (*e.g.*, the rattle or stuffed animal) or cereal.

2.2.4) Explain the net application procedure to the parent.

2.2.4.1) Inform the parent that the EEG net is like a swim cap and that infants typically do not like hats.

Note: When the experimenter puts the net on, most infants will fuss and try to pull the net off. Tell the parent not to worry and emphasize that this is normal and infants typically get used to the net, stop crying and calm down quickly.

2.2.4.2) Explain to the parent that their help is important to make sure the infant does not pull on the net and if the infant moves their arms up towards their head, to gently push their arms down and away from the net.

2.2.4.3) Explain that the experimenter is very practiced at putting the net on quickly. Explain that the second experimenter will distract the infant during net application using the rattle and stuffed animal.

2.2.4.4) Ask the parent if they would like a picture taken of their infant wearing the EEG net to take home.

2.2.4.5) Inform the parent that there may be faint pressure marks that look like little circles on the infant's head when the net is removed, but that they will fade quickly. Also, mention that the infant's hair will be slightly damp.

Note: For this study, parents were compensated $40.

2.3) Net application

2.3.1) Measure the infant's head in cm at the widest point using a soft tape measure. Choose the appropriate sized EEG net.

2.3.2) Microwave an electrolyte solution (6 cc potassium chloride/liter distilled water) for 3 min. Add a teaspoon of baby shampoo.

2.3.3) Soak the correct-sized high-density net (this study used a 128-lead net; a 64-lead net is also suitable) for 10 min in the heated electrolyte solution. This will facilitate electrical contact between the scalp and electrodes.

2.3.4) Fill pipettes with the electrolyte solution and put in the booth.

2.3.5) Carry the net on a towel and show the parent the net. Have the parent touch the net (if interested).

2.3.6) Have the parent sit on the chair in the booth with the infant on their lap.

2.3.6.1) Remind the parent that the infant may become upset and that this is normal. Remind the parent that the second experimenter will distract the infant with the rattle and stuffed animal. Remind the parent to keep the infant from touching or pulling on the net. Remind the parent not to engage in social interaction with the infant during EEG recording.

2.3.7) Place both hands inside the net, gently stretch the net and lower it so that it fits over the infant's head. Place the Cz electrode on the vertex of the head. Keep both hands inside the net during positioning.

2.3.8) When the net is positioned on the infant's head, remove hands from inside the net and tighten the chinstrap so that the net is secure. Inspect the net for correct positioning and make adjustments as necessary.

2.3.9) Measure electrode impedance using the EEG recording software (each electrode should be below 50 k Ω, if using a high impedance system). Administer the electrolyte solution using the pipettes by squeezing a few drops on to the electrodes with poor contact. If necessary, gently move the infant's hair so that the electrodes are in better contact with the scalp.

2.3.10) Once impedance is at an acceptable level, save the impedance information.

2.4) EEG Recording

2.4.1) Recording Parameters

2.4.1.1) Record data according to manufacturer specifications. Represented data were sampled from all channels at 500 Hz.

2.4.2) Stimuli presentation

2.4.2.1) Present a series of 10 color photographs of objects on the computer monitors for 13.0 to 14.5 s, at variable interstimulus intervals (0.5–2.0 s). Display the same photograph on both screens. Present the photographs as similar sizes in middle of the screens (see supplementary materials for list of stimuli in each block and stimuli files).

Note: A subtle and brief clicking sound alerts the experimenter to stimuli onset. The photographs are of common nonsocial objects (*e.g.*, flower, glove) and the same 10 categories of objects are repeated across blocks. The individual objects within a category vary in terms of color. Their distribution across the blocks is counterbalanced and the same colors are represented in each block to maintain consistency in the infant's viewing experience (see Figure 1 for example).

[Place figure 1 here]

2.4.3) Conditions

2.4.3.1) Go into the booth behind the computer screens and table and face the infant.

2.4.3.2) Open the curtain during the joint attention and social engagement conditions so that the infant can see the experimenter. Close the curtain so the experimenter is hidden from the infant's view during the nonsocial and language-only conditions.

2.4.4) Administer four conditions: nonsocial, joint attention, social engagement, and language-only. Present each condition twice for a total of eight blocks. Administer each block for 2.5 min to maintain infant attention.

2.4.4.1) Administer the blocks in the following sequence: social engagement, nonsocial, joint attention, language-only, joint attention, nonsocial, social engagement, and language-only. Present a white screen on the computers and use a bell sound to alert the experimenter to start the next block.

2.4.4.1.1) For social engagement, kneel behind the table between the computer monitors. Be face-to-face with the infant. Maintain the infant's attention so that the infant does not focus on the screens (as identical objects continue to appear). Look only at the infant throughout the condition. Do not look or point at the screens.

Note: If the infant points at the pictures, try to pull the infant's attention back, but do not follow the infant's gaze. Ensure that joint attention is not present. Have a positive affect and respond contingently. In each block, sing children's songs with hand motions, (*e.g.*, Itsy Bitsy Spider, The Wheels on the Bus) and play peek-a-boo. Adapt as necessary to maintain the infant's interest. Maintain positive affect throughout the block.

2.4.4.1.2) For the nonsocial condition, go behind the curtain (to be hidden from infant view). Stay silent throughout the condition.

2.4.4.1.3) For the joint attention condition, kneel behind the table between the computer monitors and be face-to-face with the infant. Direct the infant's attention to the pictures on the monitors and comment on the pictures. Follow the specified script of utterances and pointing directions for each trial as described in Table 1. See Table 1 for list of blocks and specific utterances for each trial.

2.4.4.1.3.1) At the start of each trial, make eye contact with the infant and continue bids for the infant's attention until the infant looks. Turn in a pre-specified direction (left or right), look at the appropriate screen, and point to the picture while simultaneously saying the specific utterance for each trial.

2.4.4.1.3.2) Look back at the infant and continue to alternate gaze between the picture and the infant's face until the trial is over.

2.4.4.1.4) For the language-only condition, go behind the curtain and comment on the pictures on the computer screens. Follow the specified utterances listed in Table 1 for each trial (Table 1; language-only). Use the same tone of voice as in the joint attention condition.

[Place Table 1 here]

2.5) Clean up

2.5.1) Following EEG recording, remove the net from the infant's head.

2.5.2) Disinfect the net following the manufacturer's protocol.

2.5.3) Save the EEG recording file. In this paradigm, the EEG recording file is automatically saved when the program automatically closes at the end of the paradigm.

2.6) EEG Data Processing[15,16]

2.6.1) Notch filter the raw EEG data at 60 Hz and then apply a highpass filter of 0.1 Hz.

2.6.2) Segment the raw data into shorter length epochs.

Note: This paradigm segmented the data into 30 s epochs, as the goal was to assess infant EEG power during different states of engagement. If too much data loss is a concern, shorter epochs such as 1-3 s could be used.

2.6.3) Perform artifact rejection on each epoch. Exclude electrodes from each epoch if the root mean square of the EEG voltage data exceeded 175 μ V or if the amplifier is saturated at any time within the epoch. Reject epochs with > 20 excluded electrodes from further analyses. Re-reference the EEG to the average reference of the remaining electrodes.

2.6.4) Use a Fourier transformation to compute EEG power for each electrode in each epoch for the chosen frequency bands. Compute EEG power averages for regions of interest. Average good epochs to yield average power values for each condition, region, and frequency band. Log transform power values using the natural log.

Note: For infants to have useable EEG data in a given condition, they must have at least one useable 30 s epoch of data in that condition. This protocol was developed using a high-density EEG system with liquid-saline based electrodes (see the materials Table). Other EEG systems are appropriate, but specific steps may vary.

2.7) Coding of Infant Looking Behavior[16]

2.7.1) Use behavioral coding software with the ability to code videos frame-by-frame (every 30th of a second). Add codes to the video file of each infant to mark when each block starts (e.g. use the bell sound that indicates block transitions in the paradigm).
Note: For the joint attention blocks, mark the beginning of each trial (use the trial onset sound that is in the paradigm) and the direction that the experimenter points (left or right; use the script which outlines which direction the experimenter points on each trial).

2.7.2) Code where infants look during the social engagement and joint attention conditions.

Note: For example, in this study some of the codes (or events) included: “infant looking at the left screen”, “infant looking at the right screen”, “infant looking at the experimenter”, “infant looking at parent’s face”, “infant looking elsewhere” (e.g. when infant is looking anywhere other than the screens, experimenter, or parent), and “missing” (when the infant was out of camera view). Train coders to a reliability threshold of .80 kappa and double code 20 % of videos to assess inter-rater reliability. Use coding software to assess the extent to which the two coders code the same looking behaviors with the same durations. Use a tolerance window of one second so that onset or offset of looking behaviors within one second of each other will be scored as an agreement.

2.7.3) Quantify infant looking behavior in the social engagement condition.

2.7.3.1) Out of total time of the social engagement blocks, compute the percentage of time the infant looked at the experimenter (time looking at the experimenter/total time of the social engagement blocks * 100). This variable indexes the extent to which the infant attended to the experimenter, thus engaging in the social engagement condition as intended.

2.7.4) Quantify infant looking behavior in the joint attention condition.

2.7.4.1) Assess the percentage of time the infant followed the experimenter’s point and gaze.

2.7.4.1.1) Select the time in seconds of the video when the experimenter pointed left. Within that time window, compute the time the infant looked at the left screen and the right screen. Next, select the time when the experimenter pointed right and compute the time the infant looked at the left screen and the right screen.

2.7.4.1.2) Sum the time the infant looked at the correct screen (i.e. the time when the experimenter pointed left and the infant looked at the left screen and the time when the experimenter pointed right and the infant looked at the right screen).

2.7.4.1.3) Sum the time the infant looked at the incorrect screen (i.e. the time when the experimenter pointed left and the infant looked at the right screen and the time when the experimenter pointed right and the infant looked at the left screen).

2.7.4.1.4) Compute the percentage of time that the infant looked at the correct screen and the incorrect screen, out of the total looking time for the joint attention blocks. This variable indexes the extent to which the infant accurately followed the experimenter’s bids for joint attention.

2.7.4.2) Compute the percentage of time out of the joint attention blocks that the infant looked at the experimenter.

2.7.4.3) Sum the percentage of time the infant spent looking at the correct screen and the experimenter. This variable indexes the extent to which the infant engaged in joint attention.

REPRESENTATIVE RESULTS:

Infant Looking Behavior

Representative results are from 73 12-month old infants[12]. Conditions were effective in changing infants' looking behavior[16]. In the social engagement condition, infants spent the majority of the time looking at the experimenter, as intended (on average, 60.06% of the time during the social engagement condition). Further, every infant looked at the experimenter more than 50% of the time. In the joint attention condition, infants accurately followed the experimenter's gaze and pointing: infants spent 2.88 times more looking at the correct screen where the experimenter had pointed compared to the incorrect screen. Infants also spent the majority of time engaging in joint attention, defined as the amount of time both looking at the experimenter and the correct screen (on average 67.93 %; for more details, see original article[16]). The paradigm was also effective in maintaining infant interest among infants of varying temperaments. Specifically, there were no relations between infant temperament as assessed with a parent report measure[17] and the amount of useable EEG data for each condition. This demonstrates that the paradigm is not biased to yield different amounts of useable EEG data depending on individual differences in temperament.

EEG Data Analysis Strategy

Repeated measures analyses of variance (ANOVAs) with condition and region as repeated measures and post-hoc comparisons with Bonferroni corrections were used in the present study. However, mixed modeling is also appropriate. In the initial model, the joint attention and nonsocial conditions were included as they differ on several dimensions: joint attention includes language, face-to-face interaction, and the presence of joint attention while the nonsocial condition has none of these inputs. Separate models were used for each frequency band. Whenever condition main effects or interactions with condition were observed in this initial model, the model was repeated two times: once adding the language-only condition and a second time adding the social engagement condition. This is to determine which dimensions of social input explain the difference in EEG power between the joint attention and nonsocial conditions. Including the language-only condition in the model is to assess whether language input explains the difference in EEG power between the joint attention and nonsocial conditions. If EEG power in both the language-only and joint attention conditions differs from the nonsocial condition, this suggests that the difference between the joint attention and nonsocial conditions is partially explained by the neural processing of language input. Including the social engagement condition in the model is to examine whether face-to-face interaction explains the difference in EEG power between the joint attention and nonsocial conditions. If EEG power in both the social engagement and joint attention conditions differs from the nonsocial condition, this suggests that face-to-face interaction explain the difference between the joint attention and nonsocial conditions.

EEG Power

Infant EEG power (both overall and within regions) varied by condition in the expected pattern[16], validating the presented paradigm. Infant EEG power was assessed in the 4-6 Hz and 6-9 Hz frequency bands, which are widely used in infant research [6,18–20]. In infants, these frequency bands are thought to reflect slow wave brain activity, so lower power in these frequency bands is thought to index greater neural activation [6,18,19,21,22]. Infant 4-6 Hz and 6-9 Hz power was assessed in frontal, temporal, and parietal regions, based on the proposed involvement of these regions for social interaction[6,23–27]. The amount of useable data varied by condition. On average, infants had 78.08 seconds of useable data in the nonsocial condition; 82.60 seconds of useable data in the language-only condition; 125.75 seconds of useable data in the joint attention condition; and 118.36 seconds of useable data in the social engagement condition. The amount of useable data in each condition was unrelated to infant EEG power.

Results were similar for each frequency band[16]. Joint attention and the nonsocial conditions were included in the initial model. Power was lower in the joint attention condition compared to the nonsocial condition both overall and within each region. Therefore, the language-only and social engagement conditions were added to the model to tease apart whether language-input and face-to-face interaction were contributing to the power difference between the joint attention and nonsocial conditions (see Figures 2 and 3). The frontal regions are involved in orienting and shifting attention[24,27] and power recorded from frontal scalp regions was lowest, indexing greater activation, in the joint attention condition compared to the other conditions. This is consistent with the demands in the joint attention condition (for results in each region and condition see Figures 4 and 5) and demonstrates that language-input and face-to-face interaction do not explain the difference in frontal scalp power between the joint attention and nonsocial conditions. The temporal regions play a role in facial processing[26] and power recorded from temporal scalp regions was lowest, indexing greater activation, in both of the conditions with face-to-face social interaction (joint attention and social engagement) compared with the nonsocial condition. This demonstrates that the difference in neural processing between the joint attention and nonsocial conditions in the temporal scalp region can be attributed to face-to-face interaction. The parietal regions are involved in spatial orientation and gaze following[6,23,25,26], demands which were unique to the joint attention condition as the infant had to respond to the experimenter's bids for attention. Accordingly, power recorded from parietal scalp regions was lower, indexing greater neural activation, in the joint attention condition compared to all other conditions. Thus language-input and face-to-face interaction do not explain the difference in parietal scalp power between the joint attention and nonsocial conditions. The power values (transformed using the natural log) ranged from 7.21-7.71 in 4-6 Hz and 6.32-6.71 in 6-9 Hz. These are consistent with past research that used the same EEG recording system and comparable processing parameters[28]. However, EEG power values may vary based on factors such as the EEG system used and choices of reference and artifact parameters.

Figure Legends:
Figure 1: Example of a nonsocial object. The type of object (flower) is the same across blocks, but varies in color.

Figure 2. Mean 4-6 Hz power in each condition. In this sample of 12 month-old infants, 4-6 Hz power was lower in the joint attention condition, indexing greater neural activation, compared to all other conditions. This demonstrates that the presence of language-input and face-to-face interaction do not fully explain the difference in power between the joint attention and nonsocial conditions. The error bars represent standard errors.

Figure 3. Mean 6-9 Hz power in each condition. In this sample of 12 month-old infants, 6-9 Hz power was lower in the joint attention condition, indexing greater neural activation, compared to the nonsocial and language-only conditions. There was no difference in 6-9 Hz power between the joint attention and social engagement conditions. The error bars represent standard errors.

Figure 4. Mean 4–6 Hz power in each condition and region. In this sample of 12 month-old infants, 4–6 Hz power in the frontal and parietal regions was lower in the joint attention condition, indexing greater neural activation, compared with the other conditions. Temporal 4–6 Hz power was lower in both the joint attention and social engagement conditions compared with the nonsocial condition. The error bars represent standard errors. This figure has been modified from[16].

Figure 5. Mean 6–9 Hz power in each condition and region. In this sample of 12 month-old infants, 6–9 Hz power in the frontal region was lower in the joint attention condition, indexing greater neural activation, compared with the language-only and nonsocial conditions. In the temporal region, 6–9 Hz power was lower in both the joint attention and social engagement conditions compared with the nonsocial condition. The error bars represent standard errors. This figure has been modified from[16].

Table 1: Order of Blocks and Script.

Supplemental Figures. Stimuli used in the paradigm. The supplemental file 'List of photos in each block' includes the names of stimuli files that accompany each block in the paradigm. The stimuli files are also included.

DISCUSSION:

First, it is critical that the net application is correct and that impedances are lowered. Second, it is important to explain to the parent what the EEG net application and paradigm will entail and how the parent can help calm the infant if they become fussy without speaking to or making eye contact with the infant, which would blur the lines between the social and non-social conditions. Further, instruct parents to keep infants from pulling on the net, which can affect the EEG data and cause damage to the net. Third, consistent paradigm administration is crucial. This includes using the same tone of voice and affect during all conditions and with all participants; making sure to get the infant's attention before beginning each joint attention trial; and keeping the infant focused on the experimenter during the social engagement condition. If there are multiple experimenters, their tone of voice and affect should be similar so as not to introduce experimenter effects. Fourth, follow the alternating sequence of social and nonsocial conditions to sustain infant cooperation throughout the protocol. Lastly, confirm that infants engaged in the conditions as intended by coding infant looking behavior during the social engagement and joint attention conditions.

There are multiple modifications to this technique. If the infant becomes fussy, it is possible to modify the paradigm by stopping halfway through. The entire paradigm includes eight 2.5-min blocks (with each condition repeated twice) and each condition happens once in the first four blocks. Therefore, if necessary for the infant or parent, it is possible to end the paradigm half way and still have completed each condition once. The order and administration of blocks in this protocol was the same for all infants. If researchers are concerned with making certain that the order of the blocks does not influence neural activity, the order of the blocks can be counterbalanced across participants. However, based on piloting, counterbalancing requires two constraints: (1) each condition needs to be represented in the first four blocks in case the paradigm needs to end early and (2) the blocks need to alternate between social (joint attention and social engagement conditions) and nonsocial (language-only and nonsocial conditions) to maintain infant interest.

This paradigm has only been piloted with 11-14-month-old infants. However, if researchers are interested, this paradigm could be used at other ages. The time window that this paradigm is most likely to be appropriate is from 6 months, when joint attention and capacity for gaze following are well established [29], through 24 months. This age range is when joint attention is the most important developmentally for nonverbal social communication and language learning[13,14]. If using the paradigm for other ages, modifications of the conditions are likely not needed. However, the most effective block length may vary depending on infant age. Specifically, the blocks may need to be shortened for younger ages to maintain infant cooperation and attention.

If a laboratory's technical capabilities do not allow for the presentation of two computer screens, it is possible to modify the paradigm to be completed with only one screen. The main consideration is whether interests lie in assessing whether infants are complying with the experimenter's directions to look at the left or right screen. This requires two screens to allow for an assessment of the amount of time each infant looks at the correct or incorrect screen, which would not be possible if only using one screen. If individual differences in responding to adult joint attention bids are not part of the research question, a single screen could suffice.

To further disentangle how EEG power varies by social context, infant EEG could be parsed within the joint attention and social engagement conditions based on whether the infant was attending to the experimenter or the computer screens. In addition, infant EEG could be analyzed when the infant was engaging in the condition as intended, such as only including EEG data from the social engagement condition when the infant was looking at the experimenter.

While parents were mostly compliant with the instructions to not socially interact with their infant, researchers may be concerned with parent interference during EEG recording. A modification would be for the parent to wear headphones and/or a visor so that they would not know what was happening during EEG recording. Further, as the infant was sitting on their parent's lap, the infant could be affected by the parent's body language. The infant could sit in a high chair, instead of the parent's lap, however this may lower the amount of time the infant can tolerate the EEG recording. Another option would be to use an event marker to mark in the EEG data when the parent engaged with the infant and not include this data. However, it is notable that despite the potential for parent interaction, there are significant condition differences in the representative data. Lastly, in the presented protocol, trials and blocks for coding were marked in the video post-hoc, based on the auditory tones signifying block and trial onsets. An alternative would be to synchronize the video to the EEG and mark transitions during EEG recording.

Recording EEG with infants is challenging and a limitation of this protocol is that not all infants will have useable EEG data in each condition. In this sample, 73 out of 85 infants (85.88%) of whom EEG was successfully recorded had useable data for at least one condition. To maximize the potential of useable EEG data, blocks are frequently alternated to maintain infant interest. However, it can be a challenge for infants to maintain interest for the entire protocol (20 min) and infants were fussier during the conditions where the experimenter was behind the curtain (nonsocial and language-only conditions). Of the 73 infants with useable EEG data, 78.1 % (57 infants) had useable EEG in the nonsocial condition and 71.20 % (52 infants) had data for the language-only condition. In contrast, 91.80 % (67 infants) of infants had useable data in the joint attention condition and 87.85 % (63 infants) had useable data in the social engagement condition. Finally, it is possible that infant affect could vary across blocks. To address this, infant affect could be coded and compared across conditions. An overall limitation of EEG research is that it is difficult to know exactly where the EEG activity recorded from the scalp is generated from in the cortex. However, it is noteworthy that the reported pattern of regional differences in EEG activity across conditions is consistent with adult fMRI research[24,27,30,12].

The primary significance of the reported EEG paradigm is the inclusion of controlled social and nonsocial conditions to systematically examine functional neural activation during social interactions. Conditions were designed to tease apart the effects of different elements of social interaction - such as the presence versus absence of language and face-to-face interaction - to understand the contributions of different social inputs in patterns of infant EEG power. The conditions were validated by coding infant looking behavior to ensure infants socially engaged as intended. Many infant EEG studies use “resting state” or baseline recording conditions that include both social and nonsocial elements[5,8,9]. This paradigm demonstrates significant differences in infant EEG power between the social and nonsocial conditions, suggesting the relevance of this paradigm for assessing the development of social engagement in infancy. Further, it demonstrates the importance of using clearly social or nonsocial conditions during EEG recording to maximize consistency across infants, as there could be variability in what infants are attending to if both social and nonsocial stimuli are present during recording.

This paradigm and results demonstrate how context affects functional neural activation, assessed with EEG power. Future studies can leverage this technique to examine functional neural development taking into account the role of recording context. This includes using clearly social or nonsocial conditions as well as using multiple contexts to have a more comprehensive and thorough understanding of functional neural activation. In addition, future research should build on the results from this paradigm by using other measures such as EEG coherence or cross frequency coupling to further examine the differential patterns of brain activity associated with social input. Further, different domains of infant development are likely linked. Thus, this paradigm assessing the neural bases of social interactions could be used with other EEG paradigms tapping cognitive and motor development[31]. Assessing infant EEG across these multiple domains as well as using multiple neural measures, would provide a broader picture of infant development and further an understanding of how these domains are related in the brain. In addition, pairing EEG with other neural techniques such as fMRI would help to better understand how patterns of cortical activity relate to underlying brain regions.

This paradigm took a first step in teasing apart factors that might underlie differences between the joint attention and nonsocial conditions including language input and face-to-face interaction. However, joint attention is complex and multi-faceted. It includes components such as gaze following, alternating gaze, and pointing. Future research could break down these components into different conditions, such as having a pointing-only condition and a condition of the experimenter only alternating gaze, to further parse how the different components of joint attention relate to infant EEG. Further, assessing how individual differences such as infant age and socioeconomic status relate to patterns of infant EEG power and coherence during each condition of this paradigm is another important future direction. It could be, for example, that EEG recorded during social versus nonsocial contexts has differing sensitivity to environmental factors such as poverty or culture. Thus, examining EEG across several controlled recording contexts may allow for a more nuanced picture of environmental influences on infant functional neural activation.

In the reported paradigm, the experimenter socially engaged with the infant. Having the infant socially interact with their caregiver during EEG recording would allow for examination of how individual differences in the interaction quality of parent and infant relate to patterns of EEG power. In addition, clinical diagnoses such as autism spectrum disorder (ASD) are typically associated with impairments in social interactions. Using this paradigm with clinical populations at high risk for social difficulties would further an understanding of the neurobiological underpinnings of certain diagnoses, such as ASD. Moreover, many studies use baselines that are not clearly social or nonsocial, which would also be especially relevant when comparing the EEG of typically developing individuals to those with ASD. It is possible that differences in EEG between these groups could in part be a function of differences in where the groups are looking during the baseline (e.g., at the experimenter or at an object). Lastly, the concept and design behind the reported paradigm could be applied to other states beyond social inputs. In this paradigm, the nonsocial and joint attention conditions differed in three ways: the joint attention condition had language-input, face-to-face interaction, and the presence of joint attention. Additional conditions were included to tease apart which social inputs drove the difference between the joint attention and nonsocial conditions. This within-subjects design of using conditions to separate out different environmental contributions could be applied in other domains.

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DISCLOSURES:

The authors have no disclosures to report.

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1 - Example of a Nonsocial Object

Click here to download Figure Figure 1 - Nonsocial objects.pdf

Figure 2

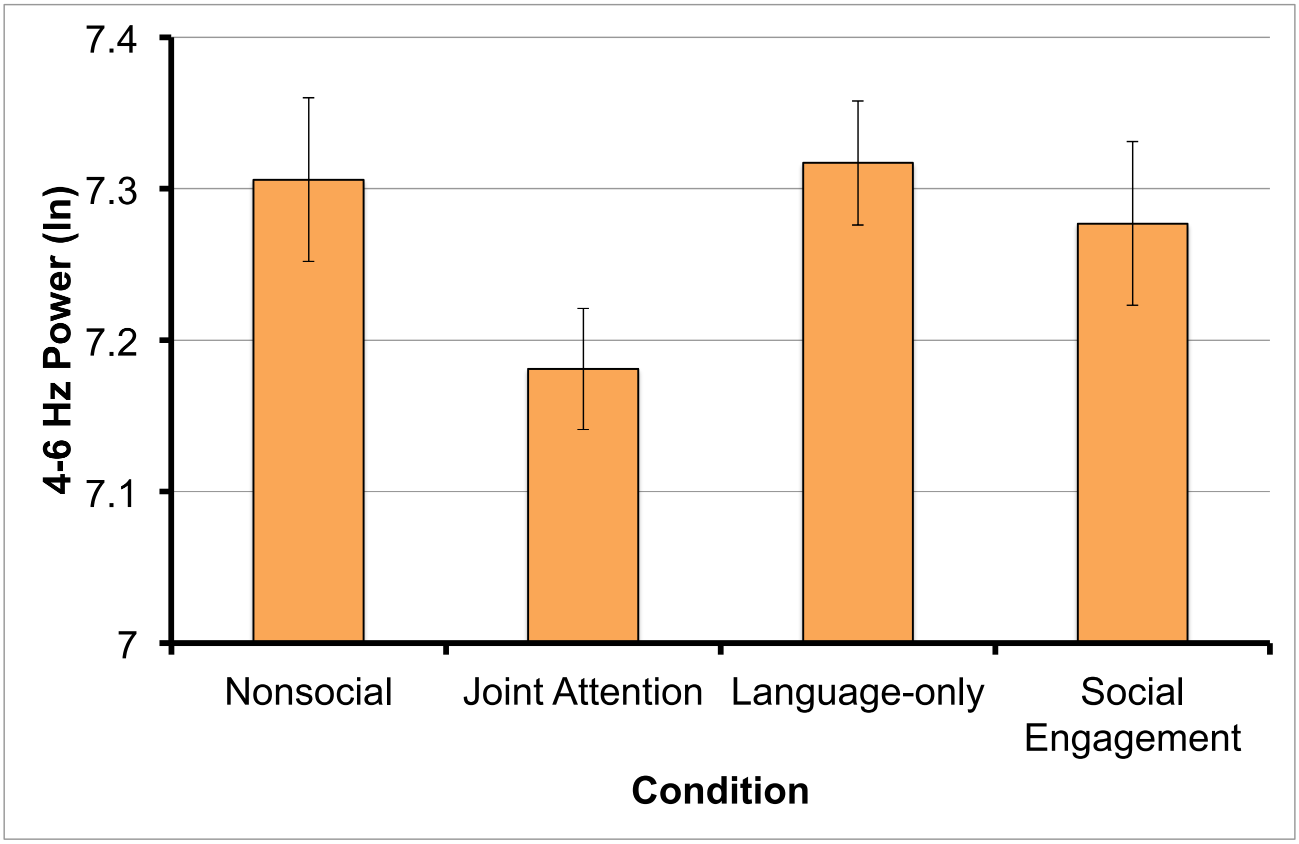

7.4
7.3
7.2
7.1
7
4-6 Hz Power (ln)
Nonsocial
Joint Attention
Language-only
Social Engagement
Condition

Figure 3

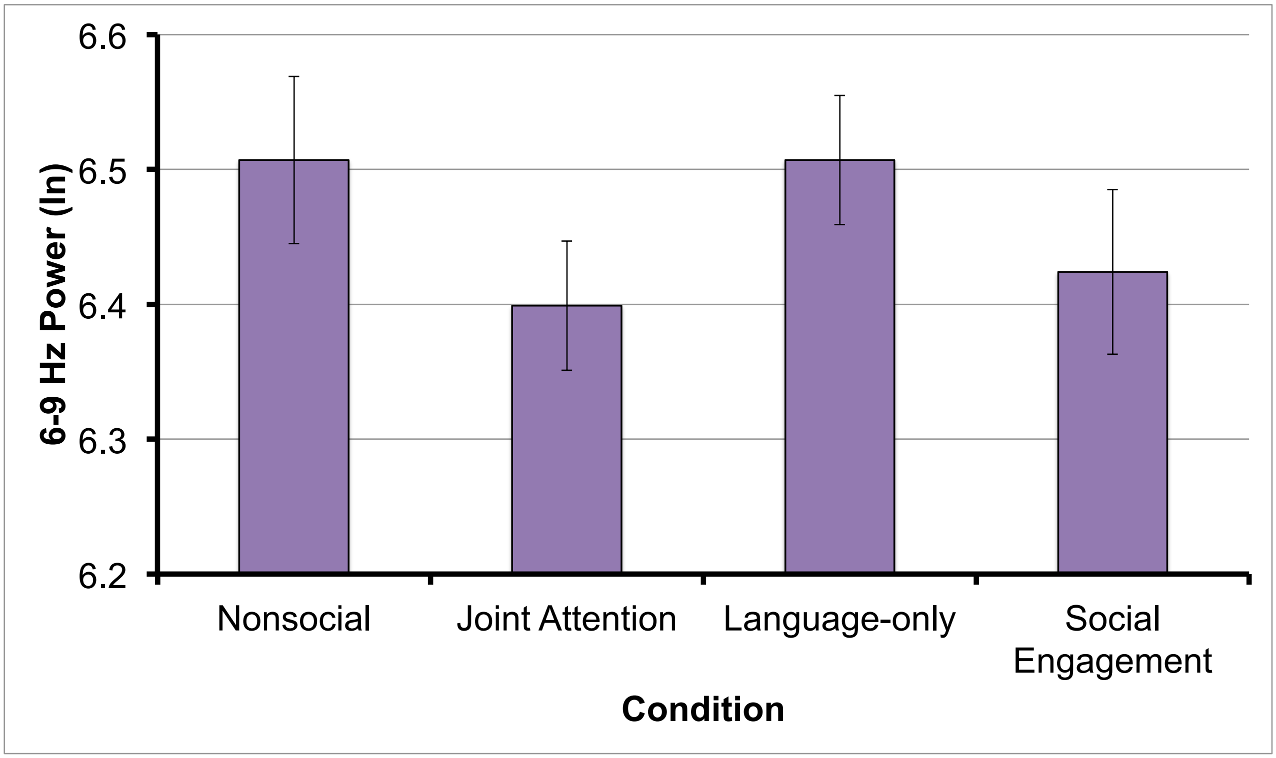
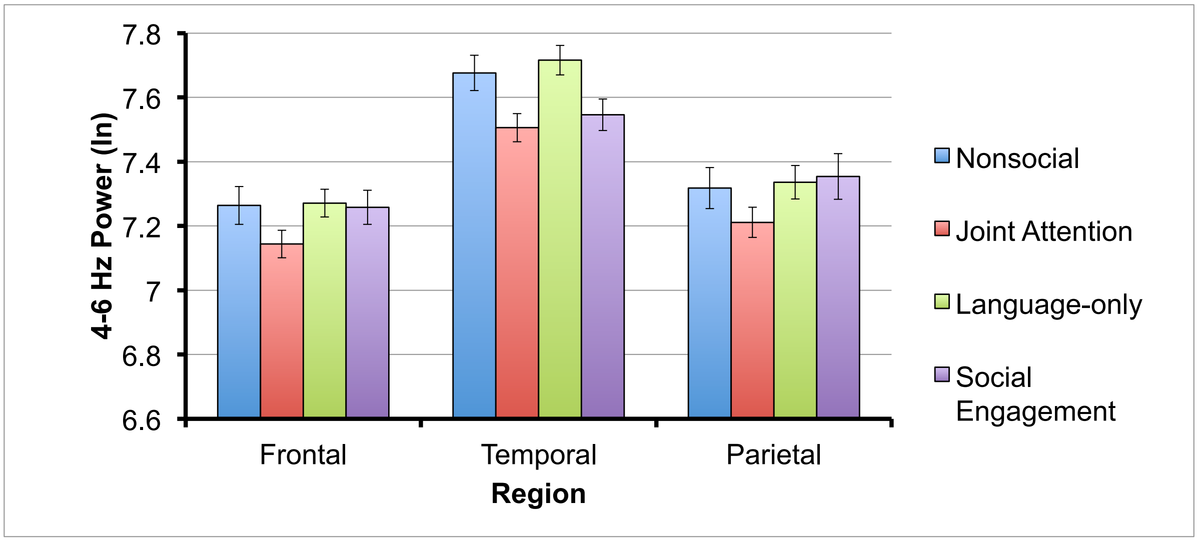

6.6
6.5
6.4
6.3
6.2
6-9 Hz Power (ln)
Nonsocial
Joint Attention
Language-only
Social Engagement
Condition

Figure 4



7.8
7.6
7.4
7.2
7
6.8
6.6
4-6 Hz Power (ln)
Frontal
Temporal
Parietal
Region
Nonsocial
Joint Attention
Language-only
Social Engagement

s

Figure 5

Click here to download Figure Figure 5 bar graph regions_6-9 Hz.pdf

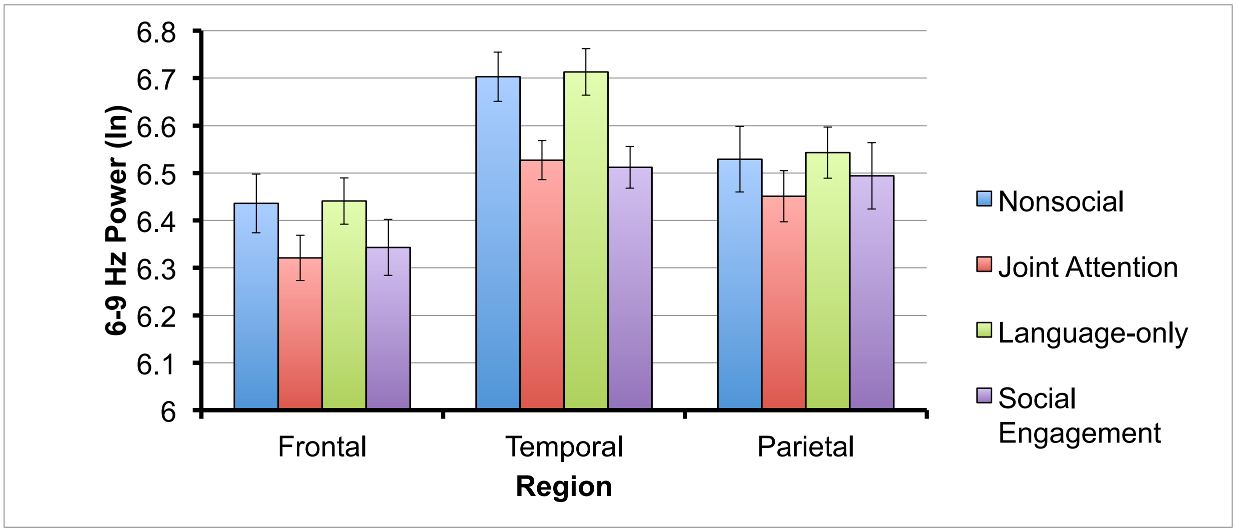


Table 1

Click here to download Table Table 1_Table of blocks and script_revision.xlsx

Table 1: Order of Blocks and Script

1. Social engagement (curtain open): Maintain the infant's attention. Talk warmly to the infant and respond contingently. Strategies include interactive children's songs with gestures such as the itsy-bitsy spider, and the wheels on the bus.
2. Nonsocial (curtain closed): Stay quiet.
3. Joint attention (curtain open) R=look at the right screen; L=look at the left screen. Specify that its right of the experimenter. 1. Look at the cool picture over here! Do you think it's a cool picture too? (R) 2. I really like this picture over here. What do you think? (L) 3. Hi, let's both look at this screen together! Can you see the picture I'm pointing to? (L) 4. Wow, this picture is really great! Do you like the picture too? (R) 5. Hey, look over here! Isn't that a silly picture? (L) 6. Look at the cool picture over here! Do you think it's a cool picture too? (R) 7. I really like this picture over here. What do you think? (L) 8. Hi, let's both look at this screen together! Can you see the picture I'm pointing to? (R) 9. Wow, this picture is really great! Do you like the picture too? (L) 10. Hey, look over here! Isn't that a silly picture? (R)
4. Language-only (curtain closed) 1. Look at the cool picture over here! Do you think it's a cool picture too? 2. I really like this picture over here. What do you think? 3. Hi, let's both look at this screen together! Can you see the picture I'm pointing to? 4. Wow, this picture is really great! Do you like the picture too? 5. Hey, look over here! Isn't that a silly picture? 6. Look at the cool picture over here! Do you think it's a cool picture too? 7. I really like this picture over here. What do you think? 8. Hi, let's both look at this screen together! Can you see the picture I'm pointing to? 9. Wow, this picture is really great! Do you like the picture too? 10. Hey, look over here! Isn't that a silly picture?
5. Joint attention (curtain open) 1. Wow, this picture is really great! Do you like the picture too? (L) 2. Hey, look over here! Isn't that a silly picture? (R) 3. Hi, let's both look at this screen together! Can you see the picture I'm pointing to? (L) 4. I really like this picture over here. What do you think? (R) 5. Look at the cool picture over here! Do you think it's a cool picture too? (R) 6. Wow, this picture is really great! Do you like the picture too? (L) 7. Hey, look over here! Isn't that a silly picture? (L) 8. Hi, let's both look at this screen together! Can you see the picture I'm pointing to? (R) 9. I really like this picture over here. What do you think? (L) 10. Look at the cool picture over here! Do you think it's a cool picture too? (R)
6. Nonsocial (curtain closed): Stay quiet.

7. Social engagement (curtain open): Maintain the infant's attention. Talk warmly to the infant and respond contingently. Strategies include interactive children's songs with gestures such as the itsy-bitsy spider, and the wheels on the bus.
8. Language-only (curtain closed) 1. Wow, this picture is really great! Do you like the picture too? 2. Hey, look over here! Isn't that a silly picture? 3. Hi, let's both look at this screen together! Can you see the picture I'm pointing to? 4. I really like this picture over here. What do you think? 5. Look at the cool picture over here! Do you think it's a cool picture too? 6. Wow, this picture is really great! Do you like the picture too? 7. Hey, look over here! Isn't that a silly picture? 8. Hi, let's both look at this screen together! Can you see the picture I'm pointing to? 9. I really like this picture over here. What do you think? 10. Look at the cool picture over here! Do you think it's a cool picture too?

Name of Reagent/ Equipment	Company	Catalog Number
EEG Amplifier	EGI	N/A
EEG Sensor Nets	EGI	N/A
EEG Recording Software	Netstation	N/A
EEG Recording Computer	Apple	N/A
Stimulus Presentation Computer	Dell	N/A
Stimulus Presentation Software - E-Prime 2.0 Professional Edition	Psychology Software Tools, Inc.	http://www.psychology-software-tools.mybigcommerce.com/e-prime-2-0-professional/
Stimulus Presentation Monitors	Dell	N/A
Potassium Chloride	Sigma-Aldrich	http://www.sigmaaldrich.com/catalog/product/sigma/p9541?lang=en&region=US

Pipettes	Karter Scientific Labware Manufacturing Co.	http://www.kartersci.com/7ml_Volume_3ml_Graduated_Transfer_Pipette_Karter_p/206h2.htm
Disinfectant-Control 3 Disinfectent Germicide	Maril Products Inc	https://www.amazon.com/Control-Disinfectant-Germicide-Cntrl3-Concntr/dp/B007AZ37VC
EEG Processing Software	MATLAB	https://www.mathworks.com/products/matlab/
Data Analysis Software	SPSS	https://www.ibm.com/marketplace/cloud/statistical-analysis-and-reporting/purchase/us/en-us#product-header-top
Coding Software - The Observer XT	Noldus	http://www.noldus.com/

General Note: This equipment list includes what was used in the presented study, however other systems and products with the same capabilities are also appropriate.

Comments/Description

We used a net amps 300 system. Contact EGI for more information or to purchase. https://www.egi.com/

We used HDGSN 130 nets with 128 channels in pediatric sizes. Contact EGI for more information or to purchase. https://www.egi.com/clinical-division/geodesic-sensor-nets

Contact EGI for more information or to purchase. https://www.egi.com/clinical-division/net-station

An apple computer is required to run the Netstation software. The operating system just has to match the version of Netstation used.

E-Prime 2.0 is compatible with PCs running Microsoft Windows
XP SP3, Vista SP1, 7 SP1, 8/8.1 and 10

LCD monitors are appropriate.

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Title of Article: A within-Subjects Experimental Protocol to Assess the Effects of Social Input on Infant EEG

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Article Title: A Within-Subjects Experimental Protocol to Assess the Effects of Social Input on Infant EEG

Signature: Ashley Date: 10/13/16

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We have removed all commercial sounding language. For the Table of Materials, we contacted EGI and they do not provide catalogue numbers for their products and their website is not designed to easily browse their different products. Therefore, we included the specific names of all EGI materials so researchers can contact EGI to purchase the products we used.

• Please remove the trademark symbols (TM/R) from the Table of Materials.

Trademark symbols have been removed.

• Please use h for hour(s), min for minute(s) and s for seconds throughout the manuscript (including figures and tables).

We have made this adjustment.

• In the Long Abstract (150-300 words), please include a statement that clearly states the goal of the protocol. For example, "This protocol/manuscript describes…"

We have added a statement that clearly states the goal. We write, "This protocol describes a paradigm that is designed to comprehensively assess infant EEG activity in both social and nonsocial contexts as well as tease apart how different types of social inputs differentially relate to infant EEG."

• 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.). Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. 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.

• Please re-write the following in the imperative tense: 1.3.4, 2.2.1, 2.2.2, 2.2.3, 2.3.2, 2.3.3, 2.3.4, 2.3.5, 2.3.5.1, 2.3.8.2, 5.1

We have adjusted all text in the protocol section so that it is written in the imperative tense and have adjusted all points noted above.

• Line 153- Ethics statement: Please mention the name of the University (review board).

This information is now in the manuscript.

• Step 1.2.4: Please note that this step cannot be filmed. Please remove the highlighting.

We have removed the highlighting.

• 1.3.1: How is the infant's head measured?

The infant's head is measured with a soft measuring type. We have added this information to the protocol.

• **1.3.9: How is the electrode impedance assessed? How is the electrolyte solution administered?**

Electrode impedance is assessed with the EEG recording software. The electrolyte solution is administered using the pipettes (which were filled with electrolyte solution) and squeezing a few drops of solution onto the electrodes with poor contact. We have added this information to the revised manuscript.

• 1.3.10: How is the information saved?

The way that this information is saved likely varies by EEG system. In Netstation, there is a button to "save" impedance information. We have added this information to the protocol.

• 2.1.1: Please provide a reference for recording data.

For this study, we use equipment from Electrical Geodesics and followed their specifications for EEG recording. We can cite their manual, but did not do so previously because of the limitation of including manufacturer names in the manuscript. Please let us know how to proceed.

• 2.2.2: Please re-write in the imperative tense or make this a Note.

We have made this information a note.

• 2.2.4: Please make this a Note.

This is now a note.

• 3.3: How is this done?

Our paradigm is designed such that our EEG recording software (Netstation) automatically closes after the paradigm is completed. The recording file is automatically saved during this process. We have adjusted this in the protocol.

• Section 4: Please provide a reference for EEG data processing.

We have added references.

• 4.2: If this step is to be filmed, please add stepwise detail on how to segment the raw data and exclude artifact. Please note that data analysis using only graphical user interface can be filmed. Alternatively, provide a reference and remove the highlight.

We have removed the highlight for this step. We included a reference for the "EEG Data Processing" section, which is applicable to all sub-steps.

• 4.6: This step (calculation) cannot be filmed.

We have removed the highlight for this step.

• Section 5: This section cannot be filmed as there are no action items. This section should be moved to the Results section.

We have moved this section to the results section.

• Section 6: This section does not use a graphical user interface. We are unable to film coding and calculations. Please un-highlight this section and provide appropriate references. If possible, please provide the codes used in your studies as a Supplemental code file (via Editorial Manager).

We apologize for not making this section clear. We used a behavioral coding software, which does have a graphical user interface. The researcher watches the video of the infant during EEG recording and manually assigns behavioral “codes” to where the infant was looking. Codes refer to not computer code, but to different classifications of looking behavior. In other words, the software is a way to log events during the video. For example, in this study some of the codes/events included: “infant looking at left screen”, “infant looking at right screen”, “infant looking at experimenter.” Therefore, the researcher would watch the video and when the infant looked at the right screen, they logged appropriate code/event and when the infant looked at the experimenter, they logged the appropriate code/event. The end result is a list of behavioral looking codes/events that are matched with the video. The software then allows for analysis of this coded data/events so it is possible to assess how long the infant spent looking at different things.

• 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. If your protocol is longer than 3 pages, please highlight (in yellow) 2.75 pages or less of text (which includes headings and spaces) to identify which steps should be visualized to tell the most cohesive story of your protocol steps. See JoVE’s instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.

We have highlighted 2.75 pages of text.

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We have uploaded a word document including an email that says we do not need permission to use the adapted figures.

• Figures 2-5: Please provide these figures as tiff or pdf files.

We have attached the figures as pdf files.

• Please define the error bars (SD, SEM, etc.) in the legend.

We have defined the error bars as standard errors in the legend.

• Please include a supplemental figure legend (below figure 5).

Are you referring to a figure legend for the supplemental files that include a “List of the stimuli used in each block” to accompany the paradigm as well as the stimuli files? If so, we have added this below figure 5. We are happy to adjust if this is not what you are looking for.

• Please ensure that your 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.

We have made sure the discussion covers all topics listed.

• References: Please abbreviate all journal titles.

We have abbreviated all journal titles.

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We have added DOIs to the references, however the following did not have DOIs: Nichols et al., 2005; Jones et al., 1997.

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

We have proofread the manuscript.

• Grammar:
-Please revise the protocol text to avoid the use of any pronouns (i.e. "we", "you", "your", “our” etc.).
-Step 2.3.5: responds contingently, continued appearing.
-2.3.5: Please correct the run-on sentence.
-2.3.7: directs the infant’s

We have corrected all grammar comments.

Reviewers' comments:
Reviewer #1:
Manuscript Summary:
This manuscript presents a new method for measuring infant EEG in social and nonsocial contexts. The study has several strengths. First, live presentation is used, which has been demonstrated previously to be critical in maximising social brain activity. The authors present several conditions varying in key parameters (e.g. social input, language etc), to allow greater finesse in interpretation than has been possible previously. This area has also traditionally suffered from a lack of standardization (as the authors point out); whilst standardised social/nonsocial videos are becoming more available, live paradigms are still very variable.

Major Concerns:
1. The set-up (with two screens) is perhaps challenging for some labs to accomplish - it might be worth discussing any possibility of simplification with just one screen?

Thank you for this suggestion. Simplifying the paradigm to be used with just one screen is possible and we have added this modification to the discussion (see p.14, lines 699-705). The main consideration in using one or two screens is whether the researcher would like to look at concordance of the infant following the experimenter's directions to look at the left or right screen. If concordance is a main question, then two screens are necessary to assess whether infants are following the experimenter's instructions. We were interested in assessing whether infants complied with the experimenter's directions, so we included two screens. Thus, we were able to compare how often the infants looked at the correct screen (the one the experimenter directed their attention to) and the incorrect screen. If the researcher is interested in how much infants are looking at the one screen, it would not be possible to attribute this to joint attention. However, if interests do not involve concordance with gaze, then a single screen would be fine to use.

2. The authors could expand the relevance of this paradigm to studies of populations at high risk for social difficulties, eg. ASD, where resting state is often looked at with sub-optimal paradigms.

We appreciate this suggestion. We have expanded the relevance of this paradigm to populations at high risk for social difficulties (see p.16, lines 790-797).

3. Consider suggesting that EEG is also analysed during epochs of visual attention to the social or nonsocial content of the probe (not just overall during the condition)- this may allow investigators to more specifically pull out social brain activity.

Thank you for this recommendation. We have added as a modification that it would be possible to analyze the data depending on whether the infant is looking at the screens or the experimenter (see p.14, lines 707-709). However, the joint attention and social engagement conditions still have social content, even when the infant is looking at the screen. For example, during the joint attention condition, if the infant is looking at the same screen as the experimenter, this is part of the social experience of joint attention. Also, in both of these social conditions, the infant hears the experimenter's voice. Therefore, even if they are not looking at the experimenter, there is still social content to the interaction.

4. Muscle/eye movement noise should be considered - this will be important in conditions where lots of shifting attention is expected (e.g. joint attention), This will contaminate higher frequencies and needs to be explicitly considered.

We have added a step to the protocol, which outlines our artifact rejection parameter (see p. 9, lines 466-468).

5. The interpretation of higher 4-6Hz power as 'less activation' is puzzling - typically theta power is interpreted as greater being greater activation. This should be discussed in the manuscript, with references to support the interpretation and selection of different frequency bands.

This information can be found in the "Representative Results" section before we discuss the EEG power results (see p.12, lines 589-592).

6. The power values look really high - can the authors contextualise these with respect to previous work, in terms of what investigators should expect? Usually I would expect more like 2ish for ln(power) in the alpha range, and 3ish in the theta range. Sometimes very high values can reflect substantial artifact so this needs to be considered.

Our values are consistent with past research (Tierney, Gabard-Durnam, Vogel-Farley, Tager-Flusberg, & Nelson, 2012) that used comparable EEG recording and processing methods to compute EEG power (high-density net referenced to Cz and then re-referenced to the average reference). There is much variability in infant EEG research in terms of the EEG system used for recording as well as processing choices such as artifact parameters and choice of reference, which can affect EEG power values. Further, there have not been enough studies with identical methods to establish concrete norms of expected values. In the representative results section, we explain that our results are consistent with data processed in the same way, but that values may vary depending on the processing methods used (see p.13, lines 623-627).

Minor Concerns:
7. Segment lengths should be specified.

We have now specified the segment lengths we used in the protocol (see p.9, lines 462-464).

Additional Comments to Authors:
I would like to commend the authors for working on this paradigm - this is a challenging area and I think this study makes an important contribution.

Reviewer #2:
Manuscript Summary:
N/A

Major Concerns:
N/A

Minor Concerns:
What ages is this paradigm most appropriate for? Do the different social and non-social conditions need to be modified due to infant age?

Thank you for this question. Although we have only tested this paradigm on infants ranging from 11-14 months, we anticipate that this paradigm would be appropriate for 6 months through 24 months of age and that the conditions would not need to be modified. When considering the youngest appropriate age, the main consideration is that infants are old enough that they are able to follow gaze and thus are able to engage in joint attention. Six-month olds have established joint attention (Morales et al., 2000; Morales, Mundy, & Rojas, 1998), so this would most likely be the youngest age that this paradigm is appropriate for. On the older side, 12-24 months is the age range when joint attention is particularly important for language learning (Markus, Mundy, Morales, Delgado, & Yale, 2000; Mundy et al., 2007). If using this paradigm with younger ages, it is possible that the blocks may need to be shortened to maintain infant interest. We have added this information to the discussion (see p.14, lines 690-697).

In the representative results, a sample size of 70+ infants was analyzed. What sample size is appropriate for this task?

The sample size appropriate for this task will vary depending on the research questions being asked and the analyses planned. The main consideration is expected attrition, thus accounting for how many infants will likely have useable EEG data. Of the 85 infants with whom we successfully recorded EEG during the paradigm, 73 had useable data in at least one condition (85.88%). Within these 73 infants, 78.1 % (57 infants) had useable EEG in the nonsocial condition; 71.20 % (52 infants) had data for the language-only condition; 91.80 % (67 infants) of infants had useable data in the joint attention condition; and 87.85 % (63 infants) had useable data in the social engagement condition. Thus, researchers would need to take this level of attrition into account when deciding on their sample size for EEG analyses.

Title on cover page is different from title on 1st page.

We apologize for the inconsistency; we have corrected the title so that it is the same on all pages.

In the analyses sections, were multiple comparisons controlled for? Or were only the 4-6Hz and 6-9Hz frequency bands analyzed?

Only 4-6 Hz and 6-9 Hz frequency bands were analyzed, as these bands have been widely used in infant research (Calkins, Fox, & Marshall, 1996; Henderson, Yoder, Yale, & McDuffie, 2002; Marshall, Bar-Haim, & Fox, 2002; Mundy, Card, & Fox, 2000). Within a given model, we used Bonferroni corrections.

EGI is specifically mentioned and used in this paradigm, would this paradigm be appropriate for other EEG systems?

Yes, this paradigm would be appropriate for other EEG systems. We have added a “note” in the protocol that addresses this point (see p.10, lines 487-489).

Additional Comments to Authors:
N/A

Reviewer #3:
Manuscript Summary:
The major strength of this manuscript and proposed methods video is the nicely articulated argument for disentangling the neural correlates of social behavior in infants by using four carefully designed conditions rather than what may be a biased baseline: (1) nonsocial, (2) joint attention, (3) language-only, and (4) social engagement. The authors show that their paradigm examining EEG power using a within-subjects design in a sample of 73 12-month-olds is able to discern differences in neural activity across these four conditions. My suggestions are directed at clarifying steps in the procedure, and adding additional potential applications for the protocol.

Procedural:
- The authors could consider adding some detail about techniques for recruiting infants for EEG studies that they have found to be successful, such as how EEG is discussed with parents and any incentives used.

Thank you for this suggestion. We have added a section to the protocol outlining techniques for recruiting infants for EEG research (see p.4, lines 171-190). We have also added additional details to the protocol on how to discuss EEG with parents when they are in the lab for a visit and incentives that are helpful (see p.5-6, lines 228-268).

-Was a pointing-only condition considered? I understand it may be difficult to squeeze in a fifth condition type, but perhaps it could be a suggestion for future work, as pointing cannot be parsed out from other elements of joint attention.

This is an excellent idea. We did not include a pointing-only condition in this initial protocol, given that there was a limit to how many conditions we could include and still get the majority of infants through the paradigm. However, we certainly agree that this would be a helpful future direction to parse how infant EEG power may vary by the specific elements of joint attention. We have added this to the discussion of future directions (see p.16, lines 776-779).

- Section 2.3.8 starting on line 305 suggests that the experimenter only needs to talk behind the screen. However, Table 1 has L/R directions of where the experimenter should look in the language-only condition. Please reconcile.

You are correct that the experimenter is talking from behind the screen, we apologize for the confusion. We have removed the L/R directions from the language-only condition.

Application:
- It may not be necessary for this type of paper, but the authors could state why they targeted 12-month-olds for their pilot. I would be interested in their thoughts as to whether this procedure

would work in younger infants in particular. More broadly, what is the age range that the technique could be or should be used?

We targeted 12-month olds for our pilot, as this age is a time when joint attention is particularly important for language development (Markus, Mundy, Morales, Delgado, & Yale, 2000; Mundy et al., 2007). Therefore, we thought that assessing EEG during joint attention at this age would be especially relevant.

We have added this information to the introduction (see p.3, lines 136-140). Reviewer two also had a question about what ages would be appropriate to use for this protocol, see our detailed response above on p. 6. In sum, this protocol is likely appropriate for 6 month olds-24 month olds.

- The authors could consider some brief discussion on how to expand their method from dyadic to triadic social interactions.

We are not sure what type research questions the reviewer has in mind, but we are happy to include any suggestions you have. One consideration may be the logistical difficulty of a triadic interaction, as it would likely involve an additional experimenter in the booth. We worked to make the experience of each infant as similar as possible, which may be more difficult in a triadic interaction.

- The authors may want to take a look at Gonzalez et al. (2016) Front. Psychol. 7:216, which is focused on motor, rather than social, development but discusses using EEG measures like power as well as coherence and mu desynchronization to make broader connections across domains, and could be used in support of their concluding remarks on p.14.

Thank you for this helpful reference. We have added a discussion of the importance of assessing EEG using multiple measures in multiple domains to broaden our understanding of infant development (see p.16, lines 765-771).

Minor Comments:
- p.1, line 45: should be "In the nonsocial condition…"
- p.4, line 183: I would also add 'and change' the infant before starting.
- p.6, line 264: For better readability, I would suggest one sentence with the open curtain instruction and a new sentence with the closed curtain instruction.
- p.11, line 499: "on the net" is repeated in the sentence
- p.19: Step 8 is missing a closing bracket in block 5 (Joint attention).

We have corrected all of the minor comments cited.

Major Concerns:
N/A

Minor Concerns:
N/A

Additional Comments to Authors:
N/A

Reviewer #4:
Manuscript Summary:
The paper describes an electroencephalography (EEG) experiment where infant participants' brain activity was recorded in conditions with or without social interaction. Brain activity was indexed with EEG power and was shown to depend on the social interaction in the experiment. The study is valuable in providing novel methodology and evidence for infant brain research using realistic face-to-face communications to study the development of social neurocognition. A

video-based demonstration will greatly add to the transparency and replicability of the study as well as to the application of the method to further research using social settings in EEG acquisition. I suggest that the paper will be accepted after minor revision according to the points detailed below. The Introduction gives adequate background and motivation for the present study. The hypotheses are stated and related to the differences between experimental conditions.

The protocol is methodologically sound, adheres to ethical standards and is described in great detail. However I was somewhat concerned about the temporal order of experimental conditions. Starting from line 272: "Blocks follow the sequence: social engagement, nonsocial, joint attention, language-only, joint attention, nonsocial, social engagement, and language-only." Does this mean that the order of conditions was the same for all participants? If so, I think this may have influenced the results and could constitute a confounding effect. I would strongly recommend permuting and balancing the order of conditions across participants.

We appreciate this point. However a random order of the blocks would not allow for maximum useable EEG data for two key pragmatic reasons: (1) Our pilot testing showed that it was necessary to alternate between social (when the experimenter was present-joint attention and social engagement) and nonsocial (when the experimenter was absent-nonsocial and language-only) blocks to ensure that infants stayed engaged throughout the paradigm and (2) to maximize getting useable data in each condition, the first four blocks need to include all four conditions in case infants are not able to complete the protocol. Based on the challenges of conducting EEG research with infants, especially with a long paradigm, those constraints are important. However, we agree that within those constraints, the order of blocks could be permuted across participants. We have added this information to the manuscript (see p.14, lines 682-688).

Major Concerns:
I have no major concerns.

Minor Concerns:
1. Account of movement artefacts. The experiment included conditions involving face-to-face communication and social engagement. I would believe that such conditions can arouse more bodily movement and head movement in the infant participants than for example watching stimuli on a computer screen. The authors further state on Line 531 that "infants were fussier during the conditions where the experimenter was behind the curtain". Do the authors have protocol for assessing the presence of movement during EEG acquisition and the contribution of such movement to the EEG power? Should video-based quality control be included in the "Protocol" section of the paper?

You are absolutely correct that it is important to cut out data when there is movement artifact. To detect and reject artifacts, we used an artifact threshold (*e.g.,* data is rejected when it exceeds a certain threshold in micro-volts), which is common practice (Bell, 2002; Brito et al., 2016; Tierney et al., 2012; Welch et al., 2014). We have now included our artifact rejection criteria in the protocol (see p. 9, lines 466-468). As you noted, infants were fussier during the conditions when the experimenter was behind the curtain and this data was cut out by our automatic artifact rejection as infants had less useable EEG data in these conditions compared to the conditions when experimenter was present (see our response to your next point for more details).

2. Number of averaged EEG data frames. How many data frames were averaged per condition in the study? How did this differ across conditions?

Each of the eight blocks lasted 2.5 minutes and each condition was repeated twice. Therefore, EEG was recorded during 5 minutes for each condition. This data was then segmented into 30-second epochs, which were subject to artifact rejection. For an infant to have useable data in a given condition, the infant had to have at least one useable/good 30-second epoch. The average number of useable epochs for each condition was:

-Nonsocial condition = 2.60 epochs (78.08 seconds) on average
-Language-only condition = 2.75 epochs (82.60 seconds) on average
-Joint attention condition = 4.19 epochs (125.75 seconds) on average
-Social engagement condition = 3.95 epochs (118.36 seconds) on average

Thus, there was more usable data in the social face-to-face conditions (joint attention and social engagement) compared to the nonsocial conditions (nonsocial and language-only). The amount of usable data was tested as a possible covariate and was unrelated to EEG power values. We have added this information to the manuscript (see p.12, lines 594-598).

3. Abstract. "In then nonsocial condition, infants view objects on computer screens." Should be "In the nonsocial condition…"

We apologize for this typo, we have corrected it in the revised manuscript.

Additional Comments to Authors:
N/A

Reviewer #5:
Manuscript Summary:
The authors outline an infant EEG paradigm designed to pinpoint changes in EEG activity related to specific aspects of social interactions: language, face-to-face interactions, and 'joint-attention'. They discuss the importance of having multiple conditions that are tightly controlled in order to 'tease apart' these various elements of social functioning, and the associated underlying changes in EEG.

Major Concerns:
***The apparent variability in the social-engagement condition is concerning. It is designed to assess the property of 'face-to-face' interaction. However, the description discusses 'suggestions' for how to maintain infants' engagement that include songs, games with hand actions (itsy-bitsy spider), and 'talking warmly', peekaboo. There is tremendous variability in the auditory and visual experience of the infant across the suggestions mentioned for this condition, (song vs. speech, hand actions/gestures vs. not, potential differences in facial expressions of emotion such as neutral vs. happy) making interpretation of the eeg activity during this condition difficult in and of itself, and thus making its use as a 'control' condition for a joint attention condition much less effective. The authors' main argument is about the importance of tightly controlled conditions in order to 'tease apart' the activity specific to different aspects of social interaction. With such variability within a given condition, the confidence in the 'tightly controlled' aspects of each condition, and thus the aim of the experiment, is undermined.**

We see your concern. Our goal in this condition was to keep the infant engaged and focused on the experimenter's face. Our description may have not made it clear that each social engagement block was standardized to include songs with hand motions, peek-a-boo, and positive affect. In piloting, we found that a sequence including multiple songs with hand motions and peek-a-boo was necessary to maintain infants' attention (as opposed to just one song or just peek-a-boo). In other words, to sustain infant attention on the experimenter's face for the duration of the 2.5-minute block, it was necessary to maintain novelty. Further, the experimenter was trained to always maintain the same level of positive affect throughout the block. The experimenter was contingent and did respond to the infant because we wanted contingent interaction. While it would be possible to have only one context (*e.g.,* only songs) during the block, our goal was for the infant's experience of social engagement (*e.g.,* focused on the experimenter's face and always hearing the experimenter) to be controlled across subjects. We have adjusted the protocol to make it clear that in each block of social engagement, the experimenter should use songs with hand motions and play peek-a-boo (see p.8, lines 374-385). Our paradigm and results show that context matters. If researchers were interested, they could zoom in more and further parse the social engagement condition into songs versus peek-a-boo, to see if there were differences in neural activation.

***I see an issue with 'tight control' across conditions on a global scale in this paradigm. Joint attention is indeed a complex characteristic that includes, as the authors point out, language, the presence of at least one other social agent, and some entity that engages the agents' attention. But there are many more subtle yet potentially critical aspects of this complex social interaction that may be driving eeg activity and a difference from a non-social condition, that would need to be 'controlled' in order to make specific claims about what aspects are associated with underlying changes in EEG. Subtle differences such as direct or averted eye-gaze, gaze that alternates between infant and object, etc., could be critical in eliciting changes in the EEG. The additional conditions did not systematically account for these alternative yet vitally important aspects of joint attention, and thus it is difficult to determine what any differences in EEG that occur b/w joint attention and non-social conditions really indicate.**

Your point is well taken. We agree that joint attention is a complicated social interaction. Joint attention is defined as coordinated attention with another person with respect to an object or event (Mundy et al., 2000) and we wanted the condition to be a realistic experience for the infants. We recognize that there are multiple aspects of joint attention that could be driving the difference between the joint attention and nonsocial conditions. This paradigm is a first step in understanding what broad social factors (*i.e.,* language and face-to-face interaction) could underlie differences between the joint attention and nonsocial conditions. Given the time constraints of maintaining infant attention for long periods of time while also getting useable EEG data, we did not have the capability to add in additional conditions to tease apart the different components of joint attention. We agree that there are other more subtle differences, such as alternating gaze and the role of pointing, that we did not elucidate in the current paradigm and that would be important to explore in future studies. For example, other studies could include a condition of the experimenter only alternating gaze between the infant and screen and a condition of the experimenter only pointing at the screen. These conditions would allow a further parsing of how different components of joint attention may relate to differences in neural activation. We have added this as a direction for future research (see p.16, lines 776-779).

***Relatedly, the authors write in lines 350-351: "joint attention includes language, face-to-face interaction, and the presence of joint attention". How is the 'presence of joint attention' actually operationalized? Is it possible that there was 'joint attention' in the social engagement condition? If both baby and experimenter were looking at the experimenter's hands as they made a 'spider crawl up a waterspout', how can it be determined that no 'joint attention' occurred in the social engagement condition?**

Joint attention was operationalized as the experimenter directing the infant's attention to objects on the computer screens and commenting on the objects. Specifically for each trial, the experimenter made eye contact with the infant, then turned in a pre-specified direction, looked at the appropriate screen, and pointed at the object while commenting on the object. The experimenter then alternated gaze between the infant and object until the end of the trial. Joint attention was only present in the joint attention condition and was not present in the social engagement condition. We agree that in a normal social interaction, joint attention is likely to occur. However, in our social engagement condition, the experimenter kept her eyes on the infant and was trained to only look at the infant throughout the block. Thus, the experimenter did not look at their hands and kept their attention directed only at the baby so as not to create this confound. We have clarified this point in the protocol (see p.8, lines 374-385).

***How is it ensured that mothers' reactions to the conditions are not influencing the infants' reactions? Infants are incredibly attuned to their mothers' body language, tension etc. and sitting on the mother's lap puts the infant in close contact with the mother. Unless mothers' vision and hearing have been blocked, it is highly possible that mothers' own reactions to the conditions will influence the infant, and thus the infants' eeg.**

This is a challenging issue. We agree that it is possible mothers' reactions relate to infants' reactions and thus their EEG. Based on the length of our paradigm and the goal of getting useable EEG data for every infant in each condition, we needed mothers to be visually aware of their infant so that they could quickly prevent the infant from pulling on the net. Thus, we did not have mothers wear visors for the pragmatic reason of maintaining data quality. We did instruct mothers not to say anything to their infants or socially interact. It would be a reasonable modification to have the mothers wear headphones and this is in the discussion of the manuscript (see p.15, line 715). We agree that having infants on their mothers laps introduces a confound. However for our set-up, this was necessary to ensure useable data throughout the paradigm as we find that when infants sit in a high chair at this age, they are less tolerant to the length of the paradigm. We have addressed this point in the discussion (see p.15, lines 713-719). It is an interesting empirical question how mothers' reactions may influence infant EEG and future studies could code the mothers' body language to assess this question.

***I am confused about the analytic approach outlined, as well as the conclusions drawn from analyses. Seeing lower versus higher eeg power in certain scalp regions does not necessarily lead to the conclusion that brain regions underlying that portion of the scalp were 'more active' during a given condition. Volume conduction of the EEG signal makes it very difficult to determine where in the cortex signals are generated, and thus conclusions about functions of the eeg scalp activity based on functions of certain brain regions (e.g., frontal cortex as important for attention and orienting; lines 455-456) are tenuous at best.**

Additionally, higher versus lower power is not necessarily inherently meaningful, especially when different frequency bands are considered. An interpretation of what higher and lower power means across the conditions in this experiment is also made more difficult by the less convention analysis of raw power. In more conventional event-related analyses, power is calculated as a change from non-task related EEG to task-related eeg in each condition.

We agree that caution is needed when drawing conclusions about EEG and that it is difficult to know exactly where EEG scalp activity is generated from in the cortex. We have adjusted the representative results section to clarify that we are referring to "regional scalp power" (see…). However, the EEG power recorded from scalp regions in our study is consistent with fMRI studies (Mundy & Jarrold, 2010; Redcay, Kleiner, & Saxe, 2012; Schilbach et al., 2009; Williams et al., 2005). In addition, our interpretation of attributing functional significance based on regional EEG patterns is in line with common practice (Bell & Diaz, 2012; Fox et al., 2001; Gonzalez, Reeb-Sutherland, & Nelson, 2016; Grossman, 2015; Henderson et al., 2002; Mundy et al., 2000; Mundy & Jarrold, 2010; Paulus, Kühn-Popp, Licata, Sodian, & Meinhardt, 2013). We recognize that a caveat is needed and we have added this issue as a limitation in the discussion (see p.15, lines 737-740). Pairing EEG with fMRI or fNIRS would be an important future step to better understand links between scalp activity and underlying regions and we have added this to our future directions (see p.16, lines 770-771).

In response to your point about higher versus lower power, our interpretation of lower 4-6 Hz and 6-9 Hz EEG power indexing greater activation is consistent with infant EEG research, including studies that recorded EEG in only one condition (Calkins, Fox, & Marshall, 1996; Davidson, 1988; Henderson, Yoder, Yale, & McDuffie, 2002; Mundy, Card, & Fox, 2000). Moreover, as we were comparing four conditions, using ANOVAs allowed us to directly compare the mean level of ln(4-6 Hz and 6-9 Hz power) across the conditions, information which would be hidden by using change scores, as a change score could reflect higher power in one condition or lower power in a different condition. We agree that change scores are the convention in some event-related research, such as the mu rhythm (Cuevas, Cannon, Yoo, & Fox, 2014; Fox et al., 2015). However, our analytic approach of using ANOVAs with condition as a within-subjects factor is common practice in infant EEG research that records EEG in multiple global conditions, as opposed to second by second changes in event-related EEG (Bell, 2002; Davidson & Fox, 1982; Jones, Venema, Lowy, Earl, & Webb, 2015; Orekhova, Stroganova, Posikera, & Elam, 2006; Santesso, Schmidt, & Trainor, 2007).

***The difficulties in infant eeg paradigms are often addressed with many subtle details in setup and administration. For example: the type of toys used to distract infants, the timing of those distractions as an experimenter moves to execute different aspects of EEG application, the specific instructions given to parents to help them be calm during application and thus be able to keep infants calm, the ways in which parents restrict infants from touching the EEG cap. These elements of the methods are presented, but on a broad and vague scale. This is unfortunate given that these specific details often make the difference between successful and unsuccessful eeg application and recording in infant studies. For a methods journal, I would think these details would be particularly relevant.**

This is a good point, thank you for the suggestion and we are happy to include more information. We have expanded the relevant sections of the protocol by adding all relevant details including: how we explained EEG to the parent (see p.5-6, lines 228-266); the types of toys used (see p.5, lines 219-220); who used the toys to distract the infant (see p.6, lines 242-243 and 258-259); what instructions were given to parents before net application (see p.5-6, lines 228-266); and instructions for the parents for during EEG recording (see p.5-6, lines 228-266).

***Relatedly, analytic details may be beyond the scope of the journal, but the specific thresholds and other parameters selected for artefact rejection that determine what "good" epochs are included in analyses are critical details that novice infant EEG researchers would want and need to know. Recommendations on whether any infants or blocks should be rejected based on not enough 'good' data could also be included.**

Other reviewers had similar questions about the artifact rejection threshold. We have now included this information in the protocol (see p. 9, lines 466-468). To clarify, each block was segmented into 30-second epochs and artifact rejection was performed on each epoch. For an infant to have included EEG data for a given condition, they had to have at least a minimum of one useable 30-second epoch of data in that condition. However, most infants had more useable epochs of data for each condition. On average, infants had 2.60 useable epochs (78.08 seconds) in the nonsocial condition; 2.75 useable epochs (82.60) in the language-only condition; 4.19 (125.75 seconds) useable epochs in the joint attention condition; and 3.95 (118.36) useable epochs in the social engagement condition.

***It is also critical to know how portions of the eeg in which infants are not paying attention to the desired stimuli are dealt with. Analyzing EEG data only during which the infant is attending to the specified stimulus is a critical aspect of accurate and interpretable infant EEG work. Were blocks dropped in which a certain percentage of the block included infants' being distracted? These are additional critical details necessary for appropriate replication and analysis of the paradigm.**

Our research goal was to understand the larger role of recording context and to see broadly what the infant EEG power differences are based on overall context. We therefore designed our conditions to more tap differences in global recording context, as opposed to parsing EEG data based on exactly what the infant is doing. We segmented each block into 30-second epochs and ran artifact rejection on each epoch. Blocks were therefore excluded based on artifact rejection parameters only. For the nonsocial and language-only conditions, the main consideration was that the conditions be nonsocial, which was accomplished by having the experimenter hidden from view (behind the curtain). For the joint attention and social engagement conditions, we validated that infants were engaging in those conditions as intended by coding their looking behavior. For this paradigm, we were also interested in whether individual differences in looking behavior related to infant EEG power. We included the infant looking measures in the EEG models and there was no relation between the looking measures and infant EEG power in the joint attention and social engagement conditions. This may be because infants were for the most part engaged in the conditions as intended, as we set up the conditions to maximize engagement. Your suggestion could also be of interest. For example, a researcher could cut out data when the infant is looking away during the social engagement condition. We have added this as a modification in the discussion (see p.14, lines 709-711).

Minor Concerns:
***EEG "power" is not defined up front. This is not necessarily a known construct and should be explained for readers upon its first use.**

We have now defined EEG power upon its first use (see p.2, lines 71-72).

***From the methods description, I think the paradigm setup is such that categories are identical across each block, and that though the specific color of a category item may be different in each block, the overall collection of colors across the 10 items within a block is identical (e.g., blue flower, green glove in block 1; green flower, blue glove in block 2). The point about identical collections of colors in every block should be made explicit so the reader understands the extent to which the viewing experience is matched across conditions/blocks.**

We have added this information to the protocol (see p.7, lines 329-335).

***Steps 1.3.3 - 1.3.10 are things that one would find in a training manual for their specific EEG system. So I wonder how useful it is to include here. Indeed, the instructions for EEG net preparation appear specific to EGI, or other liquid saline-based passive electrode set-ups. These instructions will not apply to active-electrode gel-based systems. It would be useful to make it more clear that the instructions are for one specific type of eeg system, or else make the instructions more general so as to apply to other types of systems as well.**

Thank you for this suggestion, reviewer 2 had a similar point. We have added a note to the protocol that explains our paradigm was developed using a high-density system with liquid saline-based electrodes and that the steps could vary depending on the EEG system used (see p.10, lines 487-489).

List of photos in each block

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Supplemental code file (if applicable)
apple1.BMP

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shoe4.BMP