Video Article

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.

Video Link

The video component of this article can be found at https://www.jove.com/video/55596/

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 [5,6,7], 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

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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 4 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, 2 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 (IRB).

1. Recruitment

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.
2. Call potential participants and invite them to participate in the study.
    1. Explain that the study is looking at social experiences and how they relate to brain development.
    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.
    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.

1. Laboratory set up
    1. Record the EEG in an electrically shielded booth if possible to prevent interference with the EEG signal.

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) 24 inches from the table and facing the computer monitors. Leave at least 24 inches 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.
3. Position two speakers on the ground on either side of the table, directed towards the chair where the parent and infant will sit.
4. Position a tripod video camera under the table and facing up so that the infant's face is in clear view.
5. Hang an opaque 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.
6. Place a rattle, a small stuffed animal, and cereal in the booth. Use the same rattle and animal for all participants.

2. Welcoming the family
    1. On arrival, offer the parent an opportunity to feed and change the infant before beginning.
    2. Obtain informed parental consent.
    3. Explain the EEG and paradigm.
        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. Tell the parent that the infant will look at pictures on a screen and interact with the experimenter.
        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.
        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.

    4. Explain the net application procedure to the parent.
        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. 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.
        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.
        4. Ask the parent if they would like a picture taken of their infant wearing the EEG net to take home.
        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.

3. Net application
    1. Measure the infant's head in cm at the widest point using a soft tape measure. Choose the appropriate sized EEG net.
    2. Microwave an electrolyte solution (6 cc potassium chloride (KCl)/L distilled water) for 3 min. Add a teaspoon of baby shampoo.
    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.
    4. Fill pipettes with the electrolyte solution and put in the booth.
    5. Use a towel to pat off excess water from the net to prevent bridging. Carry the net on the towel and show the parent the net. Have the parent touch the net (if interested).
    6. Have the parent sit on the chair in the booth with the infant on their lap.
        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.

    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.
    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.
    9. Measure electrode impedance using the EEG recording software (each electrode should be below 50 kΩ, 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.
    10. Once impedance is at an acceptable level, save the impedance information.

4. EEG Recording
    1. Recording Parameters
        1. Record data according to manufacturer specifications. Represented data were sampled from all channels at 500 Hz.

    2. Stimuli presentation
        1. Present a series of 10 color photographs of objects on the computer monitors for 13.0 - 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).

3. Conditions
    1. Go into the booth behind the computer screens and table and face the infant.
    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.

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.
    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.
        1. For social engagement, lean on 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. For the nonsocial condition, go behind the curtain (to be hidden from infant view). Stay silent throughout the condition.
        3. For the joint attention condition, lean on 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.
            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. Look back at the infant and continue to alternate gaze between the picture and the infant's face until the trial is over.

        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.

Figure 1: Example of a Nonsocial Object. The type of object (flower) is the same across blocks, but varies in color. Please click here to view a larger version of this figure.

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?

Table 1: Order of Blocks and Script.

5. Clean up
    1. Following EEG recording, remove the net from the infant's head.
    2. Disinfect the net following the manufacturer's protocol.
    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.

6. EEG Data Processing[15,16]
    1. Notch filter the raw EEG data at 60 Hz and then apply a highpass filter of 0.1 Hz.
    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.

3. Perform artifact rejection on each epoch. Exclude electrodes from each epoch if the root mean square of the EEG voltage data exceeded 175 µV 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.
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.

7. Coding of Infant Looking Behavior[16]
   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. 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 0.80 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.
   3. Quantify infant looking behavior in the social engagement condition.
      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 x 100). This variable indexes the extent to which the infant attended to the experimenter, thus engaging in the social engagement condition as intended.

   4. Quantify infant looking behavior in the joint attention condition.
      1. Assess the percentage of time the infant followed the experimenter's point and gaze.
         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. 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).
         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).
         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. Compute the percentage of time out of the joint attention blocks that the infant looked at the experimenter.
      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 x 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 [6,18,19,20]. 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 [6,23,24,25,26,27]. The amount of useable data varied by condition. On average, infants had 78.08 s of useable data in the nonsocial condition; 82.60 s of useable data in the language-only condition; 125.75 s of useable data in the joint attention condition; and 118.36 s 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** & **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** & **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.

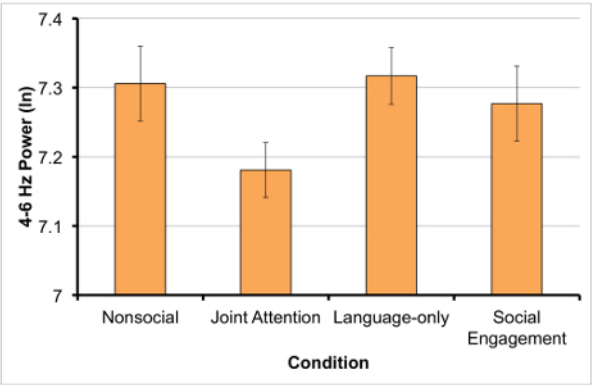

Figure 2. Mean 4 - 6 Hz Power in Each Condition. In this sample of 12-month-old infants, 4 - 6 Hz power was lower in the joint attention condition, indexing greater neural activation, compared to all other conditions. This demonstrates that the presence of language-input and face-to-face interaction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

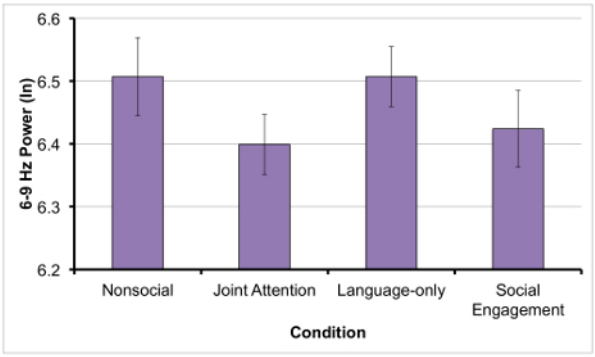

Figure 3. Mean 6 - 9 Hz Power in Each Condition. In this sample of 12-month-old infants, 6 - 9 Hz power was lower in the joint attention condition, indexing greater neural activation, compared to the nonsocial and language-only conditions. There was no difference in 6 - 9 Hz power between the joint attention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

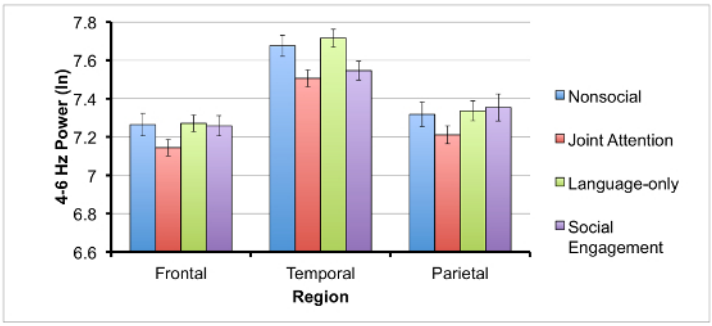

Figure 4. Mean 4 - 6 Hz Power in Each Condition and Region. In this sample of 12-month-old infants, 4 - 6 Hz power in the frontal and parietal regions was lower in the joint attention condition, indexing greater neural activation, compared with the other conditions. Temporal 4 - 6 Hz power was lower 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]. Please click here to view a larger version of this figure.

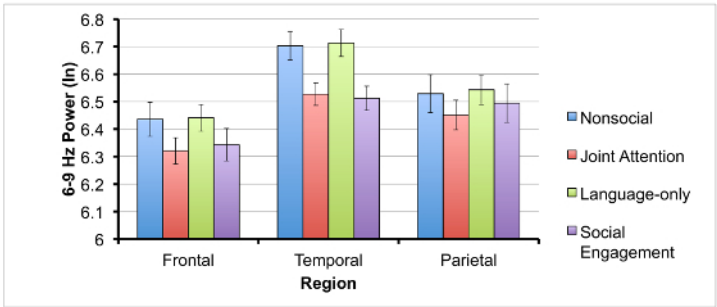

Figure 5. Mean 6 - 9 Hz Power in Each Condition and Region. In this sample of 12-month-old infants, 6 - 9 Hz power in the frontal region was lower in the joint attention condition, indexing greater neural activation, compared with the language-only and nonsocial conditions. In the temporal region, 6 - 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]. Please click here to view a larger version of this figure.

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. Please click here to download these figures.

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 8 x 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.

Disclosures

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