

Selective Cortical Mapping of Biological Motion Processing in Young Infants

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Abstract

■ How specialized is the infant brain for perceiving the facial and manual movements displayed by others? Although there is evidence for a network of regions that process biological motion in adults—including individuated responses to the perception of differing facial and manual movements—how this cortical specialization develops remains unknown. We used functional near-infrared spectroscopy [Lloyd-Fox, S., Blasi, A., & Elwell, C. Illuminating the developing brain: The past, present and future of functional near-infrared spectroscopy. *Neuroscience and Biobehavioral Reviews*, 34, 269–284, 2010] to investigate the ability of 5-month-old infants to process differing biological movements. Infants watched videos of adult actors moving their hands, their mouth, or their

eyes, all in contrast to nonbiological mechanical movements, whereas hemodynamic responses were recorded over the their frontal and temporal cortices. We observed different regions of the frontal and temporal cortex that responded to these biological movements and different patterns of cortical activation according to the type of movement watched. From an early age, our brains selectively respond to biologically relevant movements, and further, selective patterns of regional specification to different cues occur within what may correspond to a developing “social brain” network. These findings illuminate hitherto undocumented maps of selective cortical activation to biological motion processing in the early postnatal development of the human brain. ■

INTRODUCTION

The ability to decode social cues from motion, such as eye gaze shifts, emotional expression, articulation of the mouth, and manual gestures, provides the foundation of social perception and allows us to comprehend and interpret the intentions, language, emotions, and desires of others. Indeed, the capacity to attribute these simple cues to complex social states is a principle characteristic of what it is to be human and is thought to be a contributing factor to the development of our culture and civilization (Adolphs, 2009). Biological motion perception is an important process that contributes to the perception and interpretation of social cues. Many neuroimaging studies have investigated biological motion perception in children and adults highlighting distinct areas of the cortex sensitive to such cues (for a review, see Pelphrey, Morris, Michelich, Allison, & McCarthy, 2005; Allison, Puce, & McCarthy, 2000). These regions of the cortex are located within a network referred to by Brothers (1990) as the “social brain,” including the medial pFC, the frontal cortical regions including the inferior frontal gyrus (IFG), and the STS. These three regions have been the focus of much work on biological motion and have been identified to respond to a host of cues such as eye gaze (i.e., Hoffman & Haxby, 2000), mouth movements

(i.e., Calvert et al., 2003), gestures (i.e., Lotze et al., 2006), and whole-body motion (i.e., Grèzes et al., 2001) as well as to more complex social abilities such as inferring others’ mental states (i.e., Saxe & Wexler, 2005) and interpreting intention in actions (i.e., Pelphrey, Morris, & McCarthy, 2004; Saxe, Xiao, Kovacs, Perett, & Kanwisher, 2004). Further, a recent study by Klin, Lin, Gorrindo, Ramsay, and Jones (2009) showed that at 2 years of age, unlike typically developing children, those with autism orient to non-socially contingent movements rather than biological motion movements, implying that atypical biological motion detection could be contributing to the impairment that individuals with autism show in their interpretation of their social environment. Identifying the developmental origin of our capacity to perceive biological motion and social cues will enable us to investigate the atypical trajectories of development in infants who later develop disorders such as autism.

For many years, the earliest age that biological motion perception had been identified was at 4 months (Fox & McDaniel, 1982). In a preferential looking study, Fox and McDaniel (1982) found evidence that infants of 4 months, but not 2 months, of age showed a preference for displays of upright point light human walkers compared with scrambled motion. Recently, however, it has been reported in newborns. Simion, Regolin, and Bulf (2008) used a preferential looking paradigm to show that infants between 1 and 5 days of age preferred to look at a point light display

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containing a walking chicken compared with displays of both random motion and inverted biological motion. They suggest that these findings provide further support that rudimentary perception of biological motion may be an intrinsic capability of the visual system. In contrast, Slaughter, Heron, and Christie (in preparation) suggest that expertise in the perception of human form develops gradually, being initially stimulus dependent and then becoming more generalizable over time. However, they do accept that there may be some innate mechanism that directs our attention to biological motion. Specifically, their findings suggest that younger infants (4–9 months of age) require more complex stimuli containing dynamic, lifelike/live content to discriminate typical and scrambled human bodies (Christie & Slaughter, 2010; Heron & Slaughter, 2010), whereas at a later age infants of 18 months are able to discriminate bodies on the basis of line drawings (Slaughter, 2002). The behavioral studies of biological motion perception outlined earlier highlight the uncertainty over the age at which infants are first able to perceive biological motion. However, very little is currently known about the brain bases of this phenomenon during early development, and this could help elucidate stimulus- and age-related differences in activation.

To date, there have been very few neuroimaging studies directly testing the cortical origin of this perceptual ability largely because it can be very difficult to use fMRI with young awake infants. A small number of EEG studies in infants have shown cortical responses to biological motion cues, for example, to point light displays of upright figures in the right parietal/occipito-temporal region (Hirai & Hiraki, 2006; Reid, Hoehl, & Striano, 2006), to eye contact and gaze perception (Stahl, Parise, Hoehl, & Striano, in press; Rigato, Farroni, & Johnson, 2009), and to referential gaze perception in the frontal-temporal (Hoehl, Reid, Mooney, & Striano, 2008; Reid, Striano, Kaufman, & Johnson, 2004) and occipito-temporal area (Senju, Johnson, & Csibra, 2006). Although these findings evidence cortical activation to biological motion cues from a young age, conclusions regarding spatial localization from EEG findings are limited by the inverse mapping problem. Functional near-infrared spectroscopy (fNIRS) provides an elegant solution to bridge this methodological gap. fNIRS is inherently a very similar technique to fMRI in the regard that it measures hemodynamic response to neuronal activation. Although the fNIRS technique provides data on a less detailed spatial scale than fMRI, research from adults has shown a high degree of correlation between simultaneous recordings of hemodynamic responses with fNIRS and fMRI (Steinbrink et al., 2006).

A recent fNIRS study of social perception in 5-month-old infants (Lloyd-Fox et al., 2009) revealed selective cortical activation to social dynamic videos with nursery rhymes accompanied by manual gestures (such as “Peek-a-boo” and “Incy-Wincy Spider”) in two bilateral areas thought to correspond to the inferior frontal and superior temporal regions of the cortex. These findings provide evidence of

social perception processing of complex communicative visual information. However, it is unknown which of the elements within the complex and dynamic stimuli caused this activation and whether infant cortex would respond to pared down biological motion cues such as isolated facial or manual movements. In an fMRI study with adults, Pelphrey et al. (2005) found activation to passive viewing of mouth, hand, and eye movements in distinct areas of the cortex corresponding to the posterior-temporal-occipital region (STS; right lateralized), which corresponds with the area of activation found in the infant fNIRS study (Lloyd-Fox et al., 2009). Further activation was also found in response to these biological movements in the IFG, a region that may correspond to the frontal-temporal activation found in the infant social dynamic fNIRS study. Within these areas, there was also evidence of selective regional activation to the different biological movements. By adapting the paradigm used by Pelphrey et al. to an fNIRS design, we were able to explore whether young infants also respond to these different biological movements or whether at this age they require more complex dynamic stimuli (such as in Lloyd-Fox et al., 2009) or combinations of cues presented together (see Grossmann et al., 2008). Further, if evidence of cortical activation was found in these infants, we would be able to explore whether distinct areas of the inferior frontal and superior temporal regions show specialization for different types of biological movement or whether this specialization may develop later in life.

We tested 5-month-old infants, comparing responses to full-color life-size video clips of female actors (the actor’s face is approximately the size it would be in real life as viewed from an equivalent distance) moving either their eyes, mouth, or hand, all in contrast to nonbiological naturalistic, mechanical movements of pistons, cogs, and toys. This baseline condition was chosen because nonbiological movement has also been found to activate areas associated with biological motion perception (i.e., STS, as found in Schultz, Imamizu, Kawato, & Frith, 2004; Beauchamp, Lee, Haxby, & Martin, 2002, and ventral frontal cortex as found in Imaruoka, Saiki, & Miyauchi, 2005).

Predictions for the active areas of the arrays were informed by the spatial resolution of fNIRS (light transport models; Fukui, Ajichi, & Okada, 2003; structural infant MRI; Salamon, Raynaud, Regis, & Rumeau, 1990) and from the findings of previous work on biological motion processing in infants (Lloyd-Fox et al., 2009; Grossmann et al., 2008). The channel separations used in the current study were predicted to penetrate up to a depth of approximately 2 cm from the skin surface in infants of 5 months of age, potentially allowing measurement of both the gyri and the parts of the sulci near to the surface of the cortex. According to these previous findings in infants, we predicted that activation in the current study would be localized to the frontal-temporal, posterior-temporal, and frontal regions of the arrays, if the infants were able to distinguish biological from nonbiological motion content of the cues. The fNIRS headgear was aligned to scalp and anatomical

landmarks, consistent with the 10–20 electrode placement system (Jasper, 1958), so that general comparisons could be made with adult findings from previous fMRI research. Because of the spatial limitations of fNIRS relative to fMRI, comparisons of activation are limited to regions of the cortex at superficial locations within the brain. Given the results in the study by Pelphrey et al. (2005), if the infants' brain responses were similar to those in adults, we predict (1) stronger or more widespread response in the right hemisphere, (2) responses in areas corresponding to the inferior frontal and superior temporal regions of the cortex, and (3) some degree of specialized cortical activation for the different conditions. If these predictions are not upheld, we may conclude that the cortical representation of facial and manual gestures is less specialized in young infants than adults—perhaps indicating that this specialization develops over time as the cortex develops and matures through internal processes and external interactions and experiences with their social environment (Johnson, 2001).

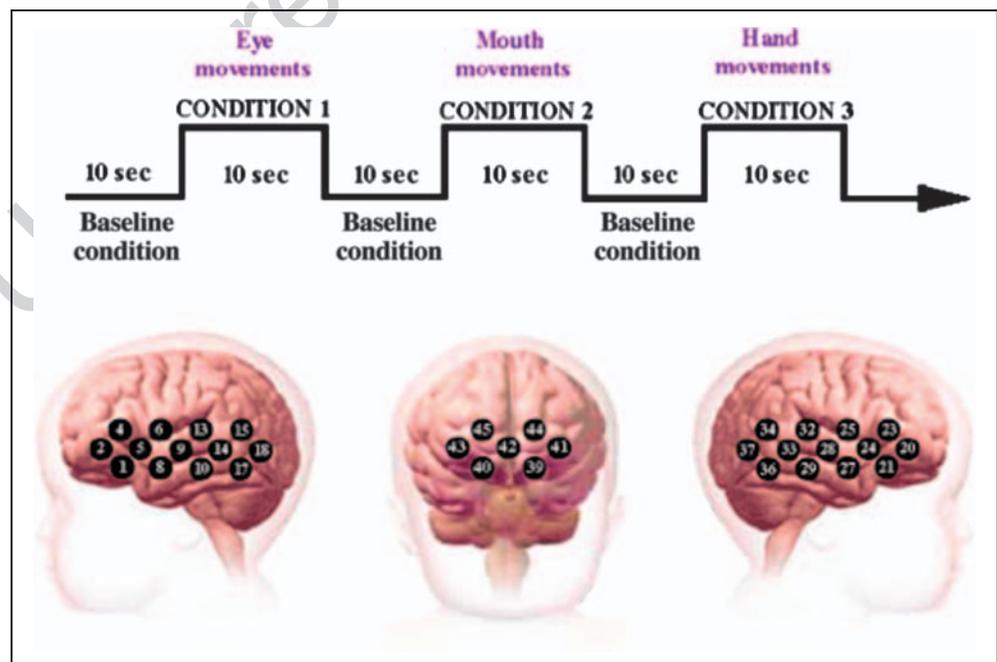
METHODS

Stimuli and Design

The experimental design comprised three experimental conditions and one baseline condition (see Figure 1A). The experimental stimuli consisted of full-color approximately life-size video clips of female actors (the actor's face

being approximately the size it would be in real life as viewed from an equivalent distance and subtended a visual angle of approximately 28°) in three conditions: Condition 1, the actors moved their *eyes* left, right, opened, or closed; Condition 2, the actors moved their *mouth* open, closed, or in mimed vowel production; and Condition 3, the actors moved their *hand* by closing their palm to form a fist and opening again or by retracting and flexing their fingers. These stimuli were adapted from an fMRI study of biological motion perception with adults (Pelphrey et al., 2005). The baseline stimuli consisted of full-color video clips of mechanical movements. These stimuli were different video clips of moving pistons, cogs, and toys. These images of multiple-nested, coordinated, and meaningful motion were chosen to provide a complex and dynamic but nonbiological contrast to the human movements. We purposefully chose not to use mechanical pincer human-like movements because these have been shown to activate some areas of the social brain network (Pelphrey et al., 2003). The baseline condition provided a reference response from which to compare the activated period during the experimental conditions. The overall surface area of the displayed experimental stimuli and baseline stimuli were equivalent. In addition, during every third trial (baseline and experimental combined), music was played to help maintain the infant's overall engagement with the task. If necessary, occasional alerting sounds were used to draw the infant's attention back to the screen. To ensure

Figure 1. Illustrations of the procedure used in this experiment. (A) The experimental design showing the order and timing of stimulus presentation. (B) A schematic infant head showing the approximate location of the three arrays and channels there within. Although the exact underlying anatomical brain structures may vary across individuals, this positioning allowed recording from prefrontal, frontal-temporal, and posterior-temporal brain regions. Note that in addition to the 2-cm channels shown on this schematic, there are 4.5-cm channels located at a lower depth underneath this set of channels at the following locations; under Channel 5 are Channels 3 and 11; under Channel 9 are Channels 7 and 16; under Channel 14 are Channels 12 and 16; under Channel 24 are Channels 22 and 30; under Channel 28 are Channels 26 and 35; and under Channel 33 are Channels 31 and 38.



that these sounds were balanced over trial types, each time an alerting sound was used during the baseline trial, the following experimental trial would also include a sound.

Participants

Thirteen healthy 5-month-old infants (8 women; mean age = 150.38 days, range = 140–161 days) participated in this study. A further 11 infants participated but were excluded from the study because either they failed to look at the minimum three trials per experimental condition ($n = 6$) or they had thick hair that prevented data collection ($n = 3$). This attrition rate is within the standard range for infant NIRS studies (see review by Lloyd-Fox, Blasi, & Elwell, 2010). All parents gave informed consent before the study, and the ethics committee at Birkbeck, University of London, approved the study design.

Procedure

Infants wore custom-built NIRS headgear consisting of three source-detector arrays (see Figure 1B) containing a total of 45 channels (source-detector separations; 29 at 2 cm, 4 at 2.5 cm, and 12 at 4.5 cm) and were tested with the University College London topography system (NTS2; Everdell et al., 2005). This system used two continuous wavelengths of source light at 770 and 850 nm. The different channel separations allowed the measurement of activation at different depths into the cortex. On the basis of an understanding of light transport and given that the cortex is approximately 0.5 cm from the skin surface in this age group (measure taken from structural MRIs; Salamon et al., 1990), the 2- to 2.5-cm separations will reach up to a depth of 0.5 cm into the cortex, and the 4.5-cm separations will reach a little deeper. Before the infants began the study, measurements of their head circumference and distance between glabella, ears, and inion were taken, and the location of the channels and arrays relative to these anatomical landmarks was recorded. The distance from the midpoint of the headband over the forehead (the glabella) to the midpoint of the temporal arrays (Channel 9 left hemisphere and Channel 28 right hemisphere) is fixed at 11 cm and is aligned approximately with T3 and T4 of the 10–20 system on an average 5-month-old infant head (43 cm; unpublished observation from the 200+ infants of this age range tested at Birkbeck). Measurements from this group of infants showed that the distance from the glabella to the ear (T3/T4) ranged from 10.5 to 12 cm (mean = 11.1 cm, $SD = 0.47$ cm). Therefore, across infants, the position of the channels varied no more than 1 cm, supporting the investigation of spatially contiguous groups of significant channels to account for individual differences in anatomy.

The infants sat on their parent's lap and were encouraged to watch the stimuli displayed on a 117-cm plasma screen with a viewing distance of approximately 100 cm. The experiment ended when the infants became bored or fussy

as judged by the experimenter who was monitoring their behavior. The parent was also instructed to refrain from interacting with the infant during the stimuli presentation, unless the infant became fussy or sought their attention. The sequence of stimulus presentation is illustrated in Figure 1A. The session began with a rest period (30 sec), during which the infants were shown shapes on the screen to familiarize them with the general experimental setup. After this, the trials alternated one after the other, beginning with a 10-sec baseline trial followed by a 10-sec experimental trial. The three types of experimental trials (eyes, hand, or mouth) were presented pseudorandomly to prevent anticipatory effects and to ensure the infant was presented with an equal number of trials per condition after every 12 trials. To assess whether the infants were looking during each trial, we made digital video recordings of their eye movements and coded looking time off-line. For a trial to be considered valid, the infant had to be looking at the screen for a minimum of 80% of the experimental trial (following a protocol developed in previous work, see Lloyd-Fox et al., 2009; Blasi et al., 2007). A minimum of three valid trials per experimental condition was required to include an infant in the study.

Data Processing and Analysis

Within each optical array, light reaching the detectors will have travelled from the sources through the skin, the skull, and the underlying brain tissue. The NIRS system measured the absorption of this light, from which the changes in oxyhemoglobin (HbO_2) and deoxyhemoglobin (HHb) concentration (μmol) were calculated and used as hemodynamic indicators of neural activity (Obrig & Villringer, 2003). The reference for the hemodynamic change observed in response to the biological motion videos was obtained from the baseline condition. Therefore, the resulting activation was specific to the nature of the biological stimuli rather than motion per se.

Initially, the recorded near-infrared attenuation measurements for each infant were analyzed, and trials or channels were rejected from further analysis on the basis of the quality of the signals using artifact detection algorithms (Lloyd-Fox et al., 2009). For each infant, the channels that survived these rejection criteria were entered into further group analyses. Inclusion criteria required each channel to contain valid data in all three conditions. A minimum number of three valid trials per condition was set as a threshold for inclusion within infants, and the maximum number of rejected channels could not exceed 10 (of the thirty-two 2- and 2.5-cm channels). Given the lower level of light at the 4.5-cm channels, the signal-to-noise ratio was expected to be lower, and therefore the threshold was set higher with no minimum limit imposed. This resulted in three of the twelve 4.5-cm channels (12, 19, and 31) being excluded from further group analyses.

For each infant, the attenuation signal (from the reflected near-infrared light) was low-pass filtered, using a

cutoff frequency of 1.8 Hz. The data were then divided into blocks consisting of 4 sec of the baseline trial before the onset of the 10-sec experimental trial plus the following 10-sec baseline trial. The attenuation data were detrended with a linear fit between the first and the last 4 sec of each 24-sec block. The data were then converted into changes in concentration (μMol) in HbO_2 and HHb using the modified Beer Lambert law (Delpy et al., 1988) and assuming a differential path-length factor for infants (5.13; based on Duncan et al., 1995). The maximum hemodynamic changes in both HbO_2 and HHb concentration were analyzed, but consistent with other studies with infants, HbO_2 was found to be a more sensitive measure than HHb (for a review, see Lloyd-Fox et al., 2010). Either a significant increase in HbO_2 concentration or a significant decrease in HHb are commonly accepted as indicators of cortical activation in infant work (Lloyd-Fox et al., 2010). If HbO_2 and HHb were to either increase or decrease significantly in unison, the signal was considered unreliable and not included in the data set.

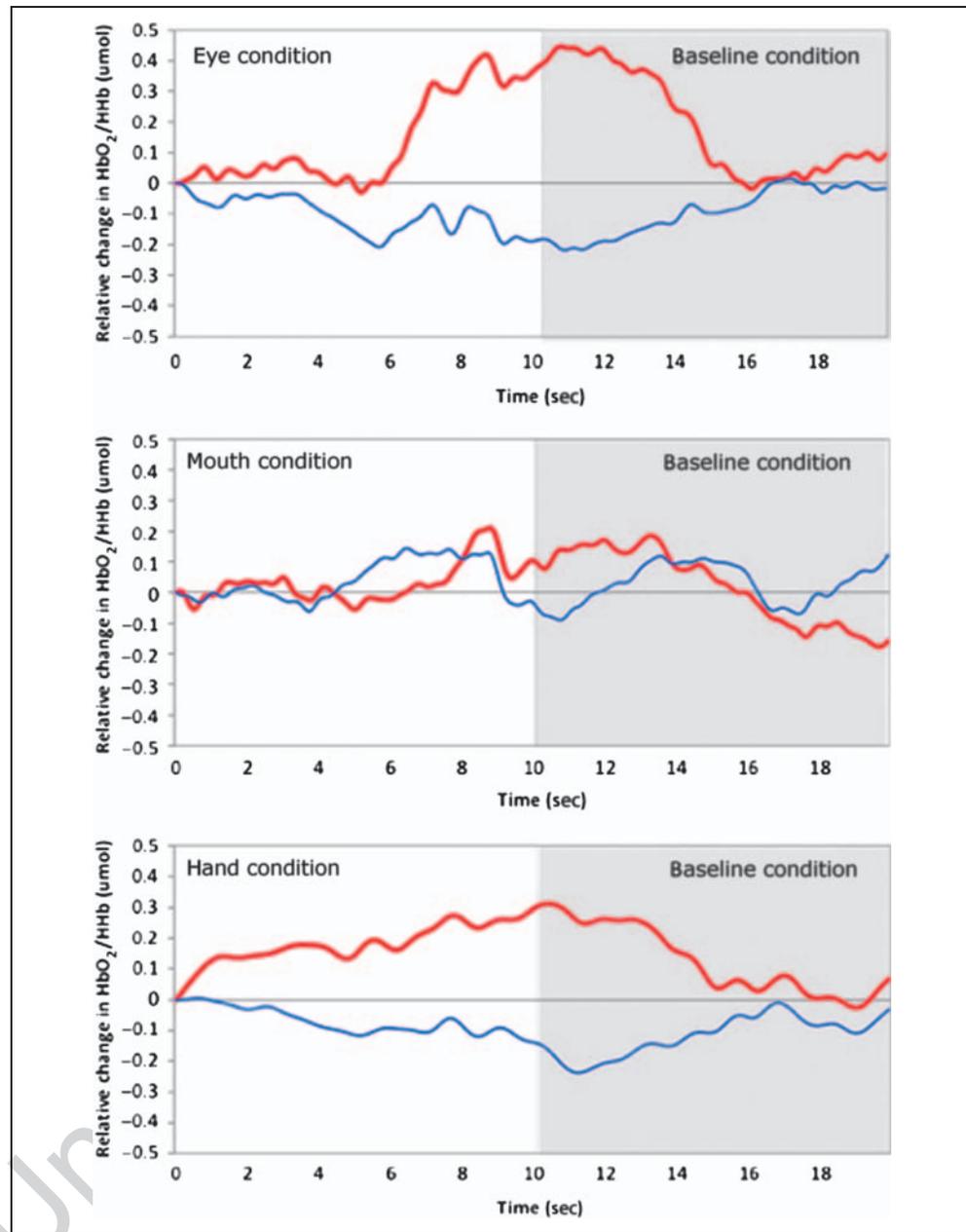
After this, valid experimental stimulus trials for each condition were averaged together within channels for each infant, and a time course of the mean concentration change in HbO_2 and HHb was compiled for each channel. These average time courses for each infant were then compiled into grand-averaged time response curves of the hemodynamic responses (across all infants) for each channel. A time window was selected between 8 and 16 sec postexperimental stimulus onset. This period was selected to include the range of maximum concentration changes observed across infants for HbO_2 and HHb. Statistical comparisons of the response to experimental versus baseline trials across all infants were made using the valid data for each channel. One-sample t tests were performed on the group data during the specified experimental trial time window to compare the maximum change (or amplitude) in HbO_2 and HHb with the reference concentration level obtained from the baseline trial. To ensure reliability, we only reported activation in regions of the arrays where significant results occurred in clusters of two or more neighboring channels; the statistical likelihood of two or more spatially contiguous (neighboring) channels producing false-positive results is very low ($p = .013$). This method was applied as an alternative to multiple comparison corrections. Please note that the degrees of freedom may vary across channels as individual infants may have invalid data in some channels (this is especially the case for the larger channel separations—4.5 cm—where the signal is weaker in some individuals). Finally, post hoc comparisons were made between stimulus conditions within the group of infants. This secondary analysis was designed to explore further the findings of the primary analysis and was not intended as a stand-alone measure of activation. Following a method on the basis of that used in fMRI studies (i.e., Joseph, Gathers, & Bhatt, in press; Grill-Spector, Saynes, & Ress, 2006), biological motion preference indices were calculated for each channel within each infant on the basis of the maximum hemody-

amic concentration changes in HbO_2 (μM) observed during the experimental conditions relative to the baseline. This method used the following preferential index formula: eye-selective index = $(\text{eye} - ((\text{hand} + \text{mouth}) / 2)) / ((\text{hand} + \text{mouth}) / 2)$; mouth-selective index = $(\text{mouth} - ((\text{hand} + \text{eye}) / 2)) / ((\text{hand} + \text{eye}) / 2)$; hand-selective index = $(\text{hand} - ((\text{eye} + \text{mouth}) / 2)) / ((\text{eye} + \text{mouth}) / 2)$. We adjusted the concentration values to take into account negative responses in the data (as advised by Simmons, Bellgowan, & Martin, 2007). Please note that this procedure does not change the relationship between the conditions but rather prevents inflation of the preferential values. These indices were then averaged across infants, and the stimulus condition that showed the highest preferential index was selected for each channel. For these preference indices, a threshold was set at 0.02 (representing a 2% higher response in the preferred channel compared with the average signal change across conditions).

RESULTS

In an initial analysis, the grand-averaged hemodynamic responses of all 13 infants were assessed. Figure 2 shows the grand-averaged hemodynamic time course of Channel 23, showing the relative change in HbO_2 and HHb for each of the three experimental conditions. The t tests (with a threshold of $p < .05$) compared the stimulus-related changes in HbO_2 and HHb (during the time window of activation described in the Methods section) evoked by the eye, mouth, and hand movements (Experimental Conditions 1, 2, and 3) relative to the mechanical movements (baseline condition) in each channel. A channel was deemed reliably active if at least one spatially contiguous channel was also significant. Single isolated channels were not considered further in the analysis. Because the placement of the NIRS array for each infant was not informed by known underlying brain anatomy (as would be the case in TMS using an MRI scan) but rather by scalp anatomical landmarks, identifying pairs or clusters of active channels provided a more robust method for accounting for individual variability in NIRS measurements. The significant hemodynamic responses (in spatially contiguous channels) to the biological motion conditions relative to the mechanical movement condition are illustrated in Figure 3 and Table 1. For the eye condition, a significant increase in HbO_2 concentration (μMol) was localized to the frontal-temporal region of each lateral array, which is centered approximately over the inferior frontal region of the cortex. For the mouth condition, a significant increase in HbO_2 concentration (μMol) was observed in a middle-temporal region of the right lateral array, which is centered approximately over the anterior superior temporal region of the cortex. Finally, for the hand condition, a significant increase in HbO_2 concentration (μMol) was found in the bilateral frontal-temporal region of each lateral array in a similar area to the results from the eye condition and in a further region in the frontal array. The results also show a stronger involve-

Figure 2. An example of the grand-averaged hemodynamic response curves for the group of infants (Channel 23—right frontal temporal). The experimental conditions are represented on three separate graphs. The white area indicates the time in which the experimental conditions were displayed, and the gray shaded area is the baseline condition. The red lines are changes in oxyhemoglobin (HbO_2), and the blue lines are changes in deoxyhemoglobin (HHb).



ment of the right hemisphere in distinguishing biological motion from mechanical motion, with seven channels showing significant hemodynamic changes in HbO_2 in the right hemisphere compared with four channels in the left hemisphere.

In a secondary analysis, post hoc comparisons were made between experimental conditions within the group of infants. Biological motion preference indices were calculated for each channel within each infant on the basis of the maximum hemodynamic changes in HbO_2 concentration (μMol) observed during the experimental conditions (for further details, see the Methods section). These indices were then averaged across infants, and the stimulus condition that showed the highest preferential index was selected for each channel (results plotted in Figure 4).

The average preferential index values selected ranged from 0.026 to 0.178 (average of 0.064). There were seven channels that did not reach this threshold and were therefore not included in the summary plot (see Figure 4). This secondary analysis was designed to reveal broad trends in activation across the set of channels, which were not evident in the more stringent t tests.

The overall preference map (see Figure 4) revealed that the bilateral frontal-temporal areas preferentially responded to eye movements (and in the lower depth to hand movements), which supported the effects revealed by the primary data analysis. The right middle temporal region showed a preferential response to the mouth movements, and the frontal region showed a preferential response to hand movements in line with the results of the prior t test

analysis. Therefore, the preference map largely supports the results of the channel by channel t tests. One pattern of preference in the bilateral posterior-temporal regions for the hand condition was not evident in the channel-by-channel analysis. However, note that there was one significant channel (37) in the right posterior-temporal region, which showed a significant increase in HbO₂ relative to baseline during the hand condition, but this has been excluded from consideration in the primary analysis because there were no neighboring channels which reached significance.

DISCUSSION

In this study, we identified regions of the infant cortex that responded to biological motion stimuli in areas thought to be involved in biological motion perception in adults. The perception of hand and eye movements produced bilateral cortical activation in a frontal-temporal area of the lateral arrays located approximately over the inferior frontal region of the cortex. The perception of mouth movements produced right lateralized cortical activation in a middle temporal area located approximately over the anterior

superior temporal cortex or primary motor cortex. Further, the hand movements elicited greater cortical activation relative to the other biological motion in the frontal array located over the right prefrontal region of the cortex. Overall, there were a higher number of active channels in the right hemisphere compared with the left. Further, the results indicate that 5-month-old infants show partially separable patterns of responses according to the type of biological motion perceived, as has been found in adults (Pelphrey et al., 2005). Preferential responses were found to the eye condition in the bilateral frontal-temporal regions, to the hand condition in the bilateral posterior-temporal regions and prefrontal region, and to the mouth condition in the right middle-temporal region of the arrays. These findings provide strong evidence for the early specialization of regions of the human cortex involved in the perception of facial and manual biological motion. Further, these regions correspond to areas associated with the social brain network in adults.

With the use of age-appropriate infant structural MRIs, anatomical scalp landmarks, and the 10–20 system, we can approximate the location of the broad underlying cortical regions for the infants and draw tentative comparisons

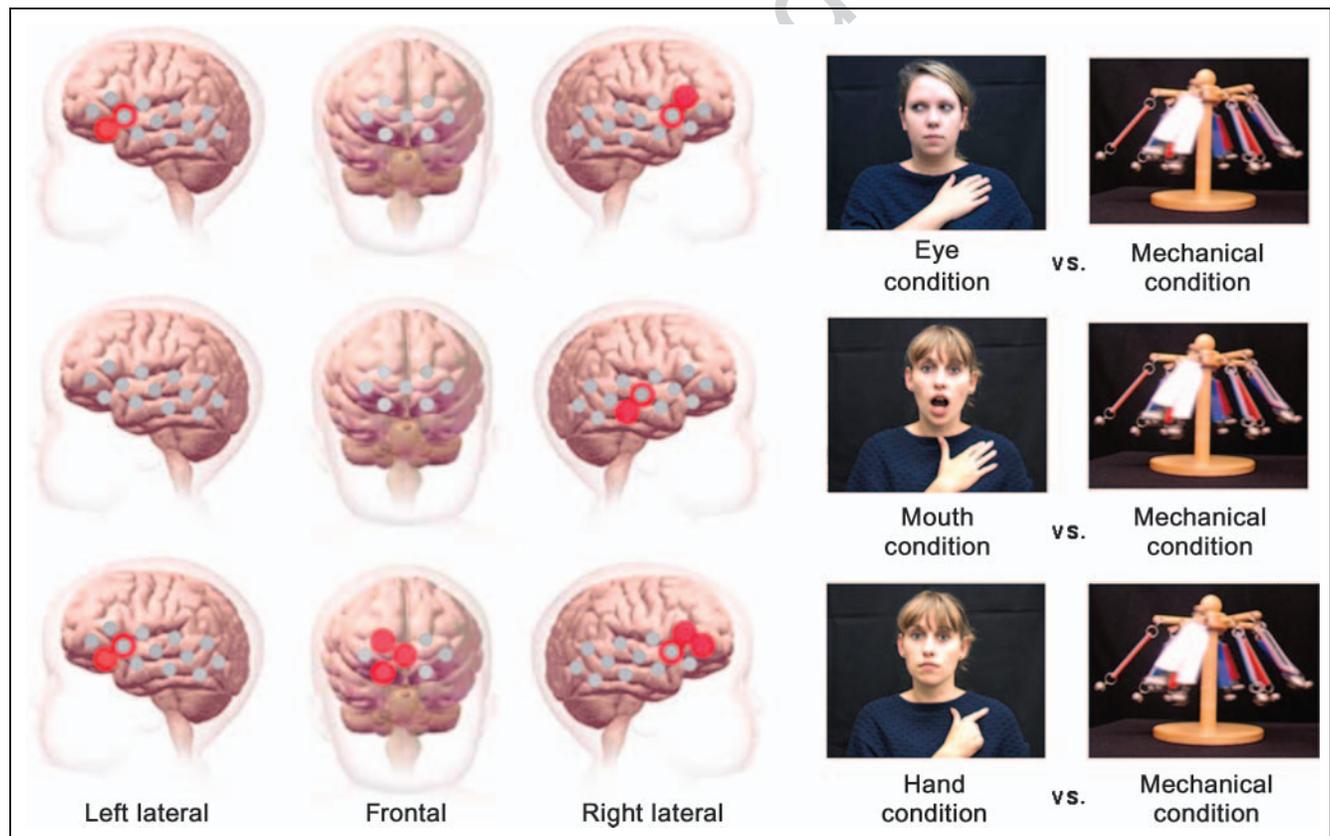


Figure 3. A schematic infant head showing the statistically significant effects ($p < .05$) in the channel-by-channel t test analysis for each experimental condition. Channels are plotted following the same layout as in Figure 1B. The red circles indicate those channels that revealed a significant increase in HbO₂ concentration during the specified time window of activation relative to the baseline condition in the shallow channels (2 and 2.5 cm). The red rings around the channels indicate those channels that revealed a significantly greater increase in HbO₂ concentration during the specified time window of activation relative to the baseline condition in the deeper channels (4.5 cm). Note that the schematic is used for illustrative purposes only and does not imply mapping of channels onto brain anatomy.

Table 1. Results from the *t* Test Channel-by-Channel Analysis across the Three Experimental Conditions

Left Lateral Probe				Right Lateral Probe				Frontal Probe			
Ch	t	p	e	Ch	t	p	e	Ch	t	p	e
<i>Eye Movement Condition</i>											
1	6.04	.00008	0.87	23	2.99	.011	0.65				
3	4.06	.022	0.76	30	6.48	.0029	0.88				
<i>Mouth Movement Condition</i>											
				26	6.83	.0064	0.89				
				29	2.23	.048	0.54				
<i>Hand Movement Condition</i>											
1	2.48	.031	0.58	20	3.87	.0038	0.75	40	2.63	.027	0.60
3	7.81	.0044	0.91	22	6.18	.0085	0.87	42	2.47	.035	0.58
11	9.77	.00062	0.94	23	2.20	.047	0.54	45	2.18	.05	0.53

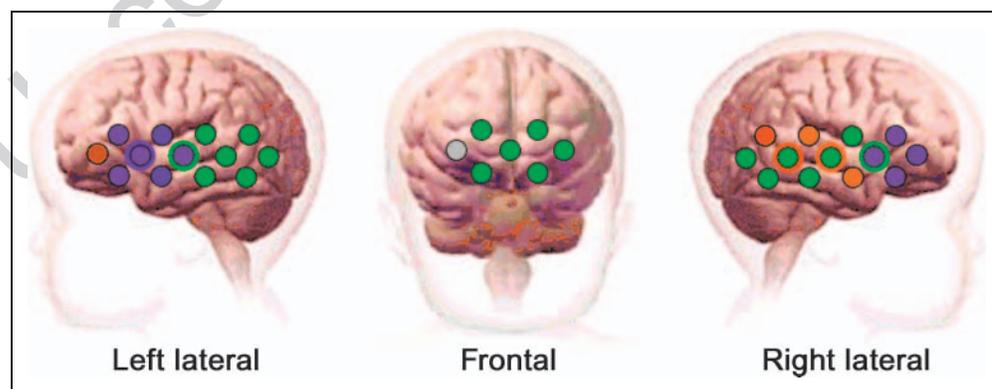
The spatially contiguous channels that displayed a significant increase in HbO₂ concentration are included in this table.

of general regional activation with findings from adult populations. Even accepting certain limitations of this approach, there are striking similarities between our findings and those of a previous fMRI study in adults of responses to biological motion (Pelphrey et al., 2005). The adult fMRI study (Pelphrey et al., 2005) that found clusters of maximum activation for each condition was found in an inferior frontal region corresponding to the frontal-temporal area

identified in our current findings with infants. Moreover, a posterior area of the superior temporal-occipital region that responded to the hand movements in adults may correspond to the bilateral posterior-temporal region identified as having a preferential response to the hand movements in infants. Finally, as with the adult study, there was evidence in our study that the response was more widespread and prevalent in the right compared with the left hemisphere. This finding of right lateralized activation to social and biological motion stimuli has also been evidenced in other EEG and fNIRS studies with infants (Grossmann et al., 2008; Hirai & Hiraki, 2006; Reid et al., 2004).

A notable difference between our findings and those of the study with adults is evident in the posterior-temporal half of the arrays that broadly corresponds to the posterior superior temporal region of the cortex. Previous research has clearly shown evidence of cortical activation to eye, mouth, and hand movements in the posterior superior temporal region, both in infants (Lloyd-Fox et al., 2009; Grossmann et al., 2008) and in adults (i.e., Lotze et al., 2006; Allison et al., 2000; Puce, Allison, Bentin, Gore, & McCarthy, 1998). However, our findings only show a trend toward a preferential response in one condition (hand) in this region. In contrast to the majority of neuroimaging studies with adults (including Pelphrey et al., 2005), the cortical activation reported in this study in response to the biological motion stimuli was relative to the activation resulting from the perception of naturalistic dynamic mechanical stimuli. The mechanical dynamic stimuli were contrasted with static nonbiological stimuli in a previous study (Lloyd-Fox et al., 2009), and no activation was evident in the posterior-temporal regions of the probes. However, the superior temporal region is known to respond to move-

Figure 4. A schematic infant head showing the locations of the conditions that revealed the highest preferential response within each channel (hand condition = green, eye condition = purple, mouth condition = orange) according to the post hoc analyses. The colored rings represent data in the deeper channels (4.5 cm), which lie underneath the shallower channels (2 cm) in the temporal arrays. The preferential index values plotted here ranged from 0.026 to 0.178, with an average of 0.064. The channels in gray did not reach the threshold for inclusion in this plot ($\geq 2\%$ preferential index). Note that the schematic is used for illustrative purposes only and does not imply mapping of channels onto brain anatomy.



ments of other stimuli such as tools (Beauchamp et al., 2002), intentional actions of objects (Schultz et al., 2004), and computer-animated mechanical motion (tempo and orientation matched to biological movement; see Carter & Pelphrey, 2006; Pelphrey et al., 2003), so perhaps this weakened the effect of the experimental conditions. To our knowledge, there has not been any fMRI work that has used such a stringent contrast of baseline-corrected signals from a dynamic naturalistic control stimuli period (although for work with a dynamic artificial shapes control stimuli, see Puce et al., 1998) for the investigation of differing facial and hand movements. It would be pertinent in light of the current findings and those of Carter and Pelphrey (2006) and Pelphrey et al. (2003) to repeat this infant paradigm with adults and to investigate whether the responses change in relation to the previous adult findings (Pelphrey et al., 2005).

Cortical activity observed in response to the hand and eye movements in the inferior frontal region of the cortex compares well with other infant (Lloyd-Fox et al., 2009; Reid et al., 2004) and adult research (Lotze et al., 2006; Pelphrey et al., 2005; MacSweeney et al., 2002; Neville et al., 1998) on facial and manual movements. For example, Reid et al. (2004) found EEG activity in the right frontal-temporal region in response to gaze directed toward an object in 4-month-old infants. Furthermore, evidence has been accumulating in recent years that the IFG contains a motor representation of hand movements (i.e., Iacoboni et al., 1999) and may be associated with the putative mirror-neuron system (as suggested by Rizzolatti & Craighero, 2004). Recently, Southgate, Johnson, Osborne, and Csibra (2009) have shown neural responses to anticipatory observation of another's goal-directed hand movement in an EEG study with 9-month-old infants. It is unknown whether this ability is present at birth, whether it arises during development, and whether factors exist to promote this specialization (such as our own motor activity). Further developmental work with fNIRS will help answer these questions.

The pattern of activity in response to the hand movements included the pFC, an area known to be involved in many social cognitive tasks (Mundy & Newell, 2007). Although the purpose of this study was to investigate cortical activation to biological motion perception in young infants, the different biological motion stimuli may have also elicited differing levels of attention or perceived social relevance. We suggest that the prefrontal activity observed in the current study may be due to enhanced attention or perceived social relevance for the hand movements. Five-month-old infants are at a stage in development when they are learning to use their hands to explore their environment and to involve this environment in their social interactions (Striano & Stahl, 2005). We speculate that the manual movements and accompanying direct gaze that the infants viewed may have triggered areas of the brain that process communicative intent (Amodio & Frith, 2006) and socially relevant input (i.e., Capek et al., 2008; Lotze et al., 2006; Schilbach et al., 2006; Kampe, Frith, & Frith, 2003). In

contrast, we propose that the eye movements as presented in our experiment provided weaker ostensive signals for communication or joint attention and therefore did not trigger strong activation in the pFC. The majority of joint attention studies have shown that young infants only follow gaze when accompanied by other social cues such as infant-direct speech, head turns, and gestures (Senju & Csibra, 2008; Striano & Reid, 2006). When the eye gaze is in isolation, however, joint attention is not elicited until 18 months of age (Hood, Willen, & Driver, 1998). In further support of this account, a study that used EEG and NIRS with 4-month-old infants (Grossmann et al., 2008) found that the pFC is involved in the processing of gaze and expressive facial gestures (eyebrow raise and smile), but only in the context of direct and not averted gaze. Therefore, a combination of more than one social cue or enhanced attention may be necessary to elicit cortical responses in the pFC of infants of this age. Possibly the isolated eye gaze shifts in the current study did not elicit sustained cortical activation over repeated trials because infants quickly perceived that the actor's repeated gaze movements were not goal oriented or communicatively relevant. In contrast, during the hand condition, the actor maintained eye contact and performed novel isolated hand movements, which may have engaged and sustained the infants' attention more effectively. Because our study did not vary the degree of social communicative cues or attention getting directly, our speculation about the different responses in the pFC remains tentative and should be explored in future work.

To our knowledge, there have been no neuroimaging studies of silent articulation of the mouth with infants. From the first weeks of life, infants are able to distinguish different tempos, intervals, and rhythms in the speech and sounds in their environment (Lewkowicz & Turkewitz, 1980). Further, Kushnerenko, Teinonen, Volein, and Csibra (2008) have shown that 5-month-old infants are able to perceive the McGurk effect, in which the auditory speech does not match the lip movements, showing that they can interpret these mouth movements as socially relevant and communicative. Speech is multimodal, and the infants in our study will have rarely if ever seen a face with direct gaze articulating their mouth in speech but with no auditory output. It is possible that the very novel nature of the stimuli in the current study (open and closing of mouth, or articulation of vowel movements) resulted in the different pattern of activity observed for the mouth compared with the hand and eye conditions. However, the activity observed in the anterior superior temporal cortex does compare well with research with adults on speech production (for a review, see Hein & Knight, 2008) and perception of silent lip movements (Calvert et al., 1997).

Finally, although we observed significant group effects, there was also evidence that the pattern of brain responses evoked by the perception of these biological motion cues differed between individual infants. Although some infants showed a robust and widespread pattern of cortical activation to the hand condition, this response was absent

in other infants who, in contrast, responded maximally to the mouth and eye condition (face-specific cues). The degree of artifact caused by movement and the number of valid trials per infant did not differ across infants, suggesting that these individual differences were not due to variability in the quality of data acquisition. It is possible that the looking behavior of individual infants was directed at different cues between these two groups, causing the shift in activation in response to the different conditions. Alternatively, the different patterns of brain responses observed across infants could be correlated with the infants' own motor capabilities. Possibly as infants learn to explore their environment with their own hands, the perception of another's hand movements becomes more relevant to the infant and promotes cortical specialization. Whether it is the perception of their hands during movement or the development of their own fine motor development that is correlated with this specialization has yet to be investigated in infants of this age (although interestingly a link between fine motor skills and visual discrimination of biologically possible and impossible human movements has been found in 8-month-olds, see Reid, Belsky, & Johnson, 2005). Further, the fNIRS work is currently underway in combination with eye-tracking technology to more accurately monitor their eye movements and behavioral measures to assess each infant's own motor capabilities and to investigate these effects further.

Conclusions

The current findings suggest that young infants can extract biological motion from other dynamic information. Further, the resulting brain responses reveal partially separable patterns of regional specification to different cues within a network of biological motion-sensitive cortical regions identified in adults. Whether we are predisposed to respond to these cues in predefined areas of the cortex from birth or whether rapid specialization occurs in response to the vast quantity of cues that we are exposed to postnatally remains unknown. Although the present results cannot resolve this issue, they do reveal the surprising capacity that human infants possess for the discrimination and processing of biologically relevant information. Although the degree of specialization of these areas remains to be investigated in more detail in relation to age of onset, individual differences, social relevance of the stimuli, and their relation to other developmental milestones, these findings illuminate hitherto undocumented maps of cortical activation to biological motion processing in what may be an early developing social brain network.

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S. L.-F. contributed to all aspects of this experiment: design, data collection, development of data analysis methods, and data analyses. A. B. developed the data analysis methods and contributed to data collection. N. E. developed the NIRS system and gave advice on data quality analysis. C. E. and M. J. contributed to the

design and data analysis. All the authors contributed to interpreting the results and to writing the manuscript.

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