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The infant mirror neuron system studied with high density EEG

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Abstract

The mirror neuron system has been suggested to play a role in many social capabilities such as action understanding, imitation, language and empathy. These are all capabilities that develop during infancy and childhood, but the human mirror neuron system has been poorly studied using neurophysiological measures. This study measured the brain activity of 6 months old infants and adults using a high density EEG net with the aim of identifying mirror neuron activity. The subjects viewed both goal directed movements and non goal directed movements. An independent component analysis was used to extract the sources of cognitive processes. The desynchronization of the mu rhythm in adults has been shown to be a marker for activation of the mirror neuron system and was used as a criterion to categorize independent components between subjects. The results show significant mu desynchronization in the adult group and significantly higher ERP activation in both adults and 6 months for the goal directed action observation condition. This study demonstrate that infants as young as 6 months display mirror neuron activity and is the first to present a direct ERP measure of the mirror neuron system in infants.

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Introduction

The discovery of the mirror neuron system in monkeys (Di Pellegrino, Fadiga, Fogassi, Gallese & Rizzolatti., 1992; Gallese, Fadiga, Fogassi & Rizzolatti, 1996; Rizzolatti, Fadiga, Fogassi & Gallese, 1996a) started a search of its human homologue. As the methods differ between human and comparative studies, a range of non invasive neurophysiological methodologies have been used in the exploration of the human mirror system, such as TMS, fMRI, MEG, EEG and others (Fadiga, Fogassi, Pavesi & Rizzolatti, 1995; Grafton, Arbib, Fadiga & Rizzolatti, 1996; Rizzolatti, Fadiga, Matelli, Bettinardi, Pauulesu, Perani & Fazio, 1996b; Decety, Grèzes, Costes, Perani, Jeannerod, Procyk, Grassi & Fazio, 1997; Grèzes, Costes & Decety, 1998; Grèzes, Armony, Rowe & Passingham, 2003; Hari, Forss, Avikainen, Kirveskari, Salenius & Rizzolatti, 1998; Cochin, Barthelemy, Roux & Martineau, 1999; Nishitani & Hari, 2000; Strafella & Paus, 2000; Johnson-Frey, Maloof, Newman-Norlund, Farrer, Inati & Grafton, 2003; Buccino, Ritzl, Fink, Zilles Freund & Rizzolatti, 2004; Fadiga, Craighero & Olivier, 2005). Behavioral measures, such as gaze tracking, have also been used (Flanagan & Johansson, 2003; Falck-Ytter, Gredebäck & von Hofsten, 2006). These studies together form a solid support for the existence of a mirror neuron system in humans.

The characteristics of the mirror neuron system suggest that it plays a role for social functions such as language, gestural communication, imitation learning, action understanding and the understanding of others' emotions (Leslie, Johnson-Frey & Grafton, 2004; Kohler, Keysers, Umiltà, Fogassi, Gallese & Rizzolatti, 2002; Rizzolatti & Craighero, 2004; Buccino et al., 2004; Fadiga & Craighero, 2004; Rizzolatti & Craighero, 2004; Gallese, Keysers & Rizzolatti, 2004). These are all functions that are critically important for adults and emerge in infancy and early childhood. This makes it important to investigate the development of the mirror neuron system in infants. For example: when does the mirror system develop in infants, and how is the development of the mirror system related to the development of the infant's own action repertoire? To ask these questions have a two-fold purpose: to improve our knowledge of social and motor development and to learn more about the functioning of the mirror neuron system.

The most direct way of answering these questions would be to investigate normal infants using neurophysiological methods. However, no previous study has directly investigated the maturation of the neural

networks involved in the mirror system in infants. One obvious reason is that most neurophysiological methods are unsuitable for infant studies because of ethical problems and/or physical properties of the equipment (loud noise, restraining of subjects, specific motor responses etc.). Fortunately, EEG does not suffer from these shortcomings. By using a high density electrode net it is possible to record brain activity from infants using the same procedure as with adults. Another advantage is that EEG is directly comparable to previous studies done on adults that show responses to mirror neuron system activity.

The first connection between action observation and changes in the EEG was found by Gastaut and Bert (1954). They discovered that a 10-13 Hz rhythm was desynchronized in adults when they observed moving people (a film of boxing). The finding was confirmed and related to the "mu wave" that desynchronizes during motor planning and performance. It has also been shown that the mu rhythm desynchronize when subjects view object-directed-reaching compared to reaches into thin air (Muthukumaraswamy, Johnson, McNair, 2004), and that this change is related specifically to goal-directed actions (Muthukumaraswamy, Johnson, 2004a). This is important because it associates the mu rhythm to the mirror neuron system, which has been suggested to be tuned to goal directed actions rather than just movements. A mirror neuron that responds to a grasping movement typically does not respond to the same movement intended for scratching or grooming (Fadiga et al., 2005; Fogassi, Ferrari, Gesierich, Rozzi, Chersi & Rizzolatti, 2005). Another study shows that mu (~10 Hz) and beta wave (~20Hz) is suppressed in the motor cortex during action observation, but has an increased rebound after approximately 500-800 ms (Muthukumaraswamy & Johnson, 2004b). The same response during performance and observation has been measured and localized to the sensorimotor cortex (mu rhythm) and premotor areas (beta rhythm) by MEG (Hari & Salmelin, 1997; Nishitani & Hari, 2000). As the mirror neuron system is the only network that has been identified to be active in this area of the cortex during both performance and observation of actions, it is suggested that mu and beta wave suppression to observed actions could be used as a selective measure of mirror neuron system functioning (Oberman, Hubbard, McCleery, Altschuler, Ramachandran & Pineda, 2005).

Although debateable, there is evidence that the mu wave is also present in infants, but at lower amplitude and lower frequency (Stroganova, Orekhova & Posikera, 1999). Stroganova et al. found that the mu rhythm was 7.03±0.47 Hz at 8 months and 7.42±0.46 Hz at 11 months. Another longitudinal study estimated

the mu frequency to 6-7 Hz at 5 months, 7-8 Hz at 10 months, 8 Hz at 14-24 months, and 9 Hz at 4 years of age (Marshall, Bar-Haim & Fox, 2002). However, in none of theses studies, the functional connection between the mu rhythm and action observation was addressed.

The present study investigates infants' functional EEG response of resting mu and beta wave to observed object motion, non goal directed actions and goal directed actions. By unmixing the combined cognitive sources recorded by the EEG with an independent component analysis, this study attempts to extract components that reflect mirror neuron activity. As recommended by Babiloni et al. (1999) both the frequency response and the event related potential (ERP) is investigated, as these methods may provide complementary information. 6-months-old infants were considered to be a suitable study group of the following reasons. At this age infants have a relatively mature cortical response to visual motion (Rosander, Nyström, Gredebäck & von Hofsten, 2007; Braddick, Birtles, Wattam-Bell & Atkinson, 2005). They are also attentive for longer periods, and interested in many kinds of stimuli. But most important, at 6 months of age (and not much younger) infants can reach and grasp themselves in a goal directed manner. As the mirror neurons respond in the same way to observed action as to self performed actions, it is crucial that the actions shown can be performed by the infants. Following this, the hypothesis is that there will be functional differences in the mu or beta wave response or in the ERP as a result of mirror neuron functioning at 6 months of age.

Procedures and methods

Subjects

34 infants and 23 adults came to the lab but 13 subjects were excluded due to fussing, imitation of stimuli movements (9 infants) or high impedance (4 adults). After artefact rejection 19 infants and 15 adults passed all exclusion criteria and were fully analyzed. The infants were all born at term and were 24-26 weeks old, and all of them successfully grasped objects that were placed in front of them. The adults were between 20 and 30 years old. All parents except 5 and all adults except 2 were right-handed. Adult subjects and parents of the infants were informed about the experiment upon arrival at the lab and a written consensus was signed in accordance with the Helsinki Declaration. The experiment was approved by the Ethics committee at Uppsala University.

Procedure

An 128-electrode geodesic EEG net (EGI Corp., Eugene, Oregon) was used and adjusted so that the reference electrode (at vertex) and the ear references were correctly placed. After having attached the net to the infant's head, he or she was immediately positioned in front of a stimulus monitor at a distance of approximately 60 cm. At this distance the display covered 40° visual angle horizontally and 28° vertically. The infants sat in a special baby seat (Bumbo, SouthAfrica) that supported them in an upright sitting position that avoided leaning on the net. The light was switched off during the experiment in order to make the surrounding less distracting. While data was collected the behaviour of the infant was recorded by a web camera placed on the top of the display monitor for subsequent removal of inattentive periods. The parent and two experimenters were always in the room. When the adult subjects were measured they sat in front of the monitor, at 60 cm distance, watching the same stimuli as the infant group after an impedance control and adjustment of high impedance channels (>80kΩ).

Stimuli

The stimuli consisted of 4 short video clips, one for each condition. The video-clip also synchronized the stimulus monitor with the EEG measurements by a local white flash sequence that triggered an optic sensor. The optic sensor covered the flashes from the subjects' view, and had a response time of approximately 4μs which allowed for accurate timing of visual impression and EEG time-locking. The first condition showed a static coloured dot (dot diameter = 2 cm) against a black and white background of artificial clouds. The second condition showed the coloured dot moving against the static black and white background. The third condition showed the torso of a person that reached for a coloured object in a goal directed manner. The fourth condition showed the same model with the hand withdrawing from a position near the object. This condition was included as a non-goal directed action control. The face of the model was hidden to avoid distraction and activation from face recognition. All fingers of the model's hand were visible (bounding box = 2x2 cm), and stood out against the darker background. A grasping action was chosen in the goal directed condition for two reasons. First, mirror neurons devoted to grasping are the most common ones in area F5 of the macaque and presumably also quite common in humans Area 6-44 (Fadiga & Craighero, 2004). Second, this is the kind of action that gives the most reliable desynchronization in adult humans (Hari & Salmelin, 1997; Oberman et al., 2005). All video-

clips had durations between 3 and 3.3s, and all of them started with the stimulus being stationary. In the static condition the image remained stationary, and in the other conditions the motion started after a random 0.5s to 0.8s period to avoid an expectation response. In the reaching condition the hand touched the object exactly 1 s after motion onset. Video-clips with dots and hands were interleaved. This was done to make the display more attractive, and to elicit a strong visually evoked potential when the clips changed scenes as a control of signal quality. A whole session took 6.5 minutes, and included 32 trials of each condition.

EEG measurement and analysis

The brain electric potentials were recorded relative to the vertex, at 250 Hz. The analog filter (hardware filter, elliptical) used was 0.1 to 100 Hz (EGI Netstation, Eugene, Or). The recommendations of Picton et al. (2000) and measurement routines suggested by Johnson et al. (2001) were followed as closely as possible. After the experiment the data was transferred to the EEGLAB toolbox (version 5.03, Delorme & Makeig, 2004) in the Matlab environment for off line analysis. The video of the subject was inspected and longer intervals of inattention (>10 s) were excluded from further analysis, and shorter periods were rejected in an artefact rejection routine (described below). Even though the net was properly placed 26 channels at the lowest rows of the net often suffered from high impedance or did not keep contact to the skin. These channels were removed from all subjects, leaving 101 channels for analysis. The remaining data was segmented into trials (from -0.4s to 2.0s after timelock). The data was then band-pass filtered at 0.5 to 30 Hz and the artefact rejection routine for dense EEG arrays described by Junghöfer, Elbert, Tucker and Rockstroh (2000) was used. In short this procedure performs rejection of bad sensors and bad trials based on outliers of max amplitude, max deviation, and max standard deviation before re-referencing to average reference. This minimizes spreading of bad data when re-referencing. The artefact rejection procedure then rejects bad sensors and bad trials of the average referenced data. In the original procedure remaining bad data from single trials in single channels is spline interpolated, but as the following independent component analysis relies on independent variance between sensors this step was omitted and bad channels and bad trials were simply removed. Subjects with less than 10 trials in any condition or more than 10 rejected channels were removed (6 infants, 4 adults).

A natural-gradient logistic infomax independent component analysis was performed on the data (the runica algorithm, Makeig et al. 1997), which resulted in as many independent components as remaining

channels minus one for each subject. Artefacts coming from eye movements and eye blinks were minimized by subtracting eye related independent components from the raw EEG. Components were automatically identified as eye related if their scalp maps showed a strong far-frontal projection (this was done by normalizing the component weights so that the max absolute weight was 1 and checking if only frontal electrodes had values above 0.5). The pruned raw EEG was then high pass filtered at 2 Hz in order to prevent the independent component analysis to separate the low frequencies from mu rhythm components. The pruned and filtered data was considered clean from artefacts and trained in a second independent component analysis as recommended by the EEGLAB documentation. The resulting weights were applied to the original dataset pruned from eye component activation. This double training procedure was done to retain low frequencies in independent components with mu rhythm properties.

At the end of the pre processing the standard deviation of each component's trials max absolute amplitudes was calculated. Trials with max absolute amplitudes greater than 3 standard deviations were excluded from further analysis, as they were considered outliers. The data was also detrended by removing the best straight-line fit linear trend from each trial. Finally a z-transform was used to normalize each component's amplitudes using all data points of the component, thereby including all conditions in the same transform.

As mirror neuron activity was expected to be separated into one or a few components in each subject, these components had to be identified and selected from each subject. The fundamental "functional topography" approach (Kuhlman, 1980) infers that identification of rhythmic EEG components should be based on 3 main criteria: frequency characteristics, spatial distribution over the cortex and functional reactivity to specific conditions. The identification of mu rhythm components was implemented by ordering the components in decreasing order of variance accounted for by their projections onto the scalp, and then selecting the first component with a frequency power peak between 3-8 Hz in infants and 7-15 Hz in adults (determined by a power spectral density estimate via Welch's method, with hamming windows of 256 samples length and 128 samples overlap). A second criterion was that the mu power of the static dot condition should be higher than the mu power of all conditions. Examples of the unmixing of signals using ICA and the resulting information that was used for component selection is found in figure 1.

(Insert figure 1 about here)

By using this strategy it is still valid to compare the moving dot and action observation conditions with each other in the selected components. Visual inspection was used to control the quality of the selected components. Components with irregular scalp projections or ERP images were to be identified and discarded, but no such components were identified. The identified mu peaks in the infant group had a mean frequency of 5.4 Hz (standard deviation 0.8 Hz), and the corresponding values for the adult group was 10.4 Hz (standard deviation 1.1 Hz).

To statistically test differences between conditions three measures was used. First, the power of the individual mu frequency was calculated for each subject and condition. The time interval of the frequency analysis was between 0.8 and 1.8 s after motion onset, which covers the time period when the hand reached the object in the goal directed condition. Since previous time/frequency analyses have shown that mu responses occur mainly after the hand touch the object (Muthukumaraswamy & Johnson, 2004), the interval included more time after than before completion of the reach. The wide length of 1 s was chosen to capture the responses from both adults and infants with varying response latency. The power values was transformed to decibel relative the mean of all conditions within components, and the goal directed and non goal directed condition was compared within both groups using paired t-tests. Second, time frequency spectrograms were calculated for the goal directed and non-goal directed conditions using a Short Time Fourier Transform, with hamming windows of 128 samples length and 124 samples overlap. The frequencies of interest ranged from 0 to 30 Hz, and the time points ranged from 0 to 1.9 s relative timelock which resulted in power maps with 119 x 14 time/frequency points for each condition. The power maps were transformed to decibel change from a baseline computed as the mean power from both conditions in each frequency band. The amplitude maps were compared using pixel wise paired t-tests. As multiple significance test inflates the risk of type I errors only groups of 20 or more consecutive tests with $p < 0.05$ were considered significant. Third, the ERPs from the selected component was mean averaged for each condition, baseline corrected (from -0.4s to timelock) and low pass filtered at 10Hz to remove high frequencies that added noise variability to the signal. Comparisons between all conditions were performed for each of the 600 time points using paired t-tests. Again, only groups of 20 or more consecutive tests with $p < 0.05$ were considered significant as multiple significance tests inflates the risk of type I errors.

As a final step, the selected components were fitted in a spherical model using the dipole source localization algorithm included in EEGLAB (technical details on dipole source localization are found in Scherg, 1990). The head circumference for adults ranged from 55-60 cm in adults and 40-46 in infants. The parameters for scalp thickness and conductance were adopted from Grieve, Emerson, Fifer, Isler and Stark (2003).

Results

Mu power

In the mu power analysis, illustrated in figure 2, the adult group shows a significant difference between the goal directed and the non goal directed action observation conditions, ($p < 0.05$). There is also a significant difference between the goal directed condition and the moving dot condition ($p < 0.05$). The infants show the same pattern of desynchronization as adults between conditions, but the difference is not significant between non goal directed and goal directed condition ($p = 0.33$). The mu power differences between the moving dot and the non goal directed action is very small in both groups, with values between the static dot condition and goal directed condition values.

(Insert figure 2 about here)

Time frequency response

In the more detailed time frequency analysis, again only the adult group show significant desynchronization between the goal directed and the non goal directed condition (paired t-tests, $p < 0.05$). The desynchronization is restricted to the mu frequency band as illustrated in figure 3, between 4.0 and 13.7 Hz. The onset of desynchronization starts approximately when the hand reaches the object, but becomes significant about 0.1 s afterwards (and stays significant in the time interval 1.1 - 1.4 s after timelock). No significant desynchronization was found in the beta band, and no significant desynchronization was found in the infant group.

(Insert figure 3 about here)

ERPs

The results from the infant group ERPs is illustrated in figure 4 and the adult group in figure 5. Both groups show significant differences between the goal directed and the other conditions within 0.5s before the hand reaches the object (paired t-tests, $p < 0.05$). Both groups show significant differences after the hand touches the object when compared to the static dot condition, and the infant group when compared to a zero mean. The significant intervals in the adult group start marginally earlier (latency differences ranging from 0.1s to 0.2s). The adult intervals are also longer (length differences ranging from -0.1s to 0.2s) compared to the infant group.

The infant group also shows significant activation in the moving dot condition compared to a zero mean approximately in the interval 0.6s to 0.8s after motion onset.

(Insert figure 4 about here)

(Insert figure 5 about here)

Source localization

All 16 of the adults' components could be fitted with a residual variance below 20%. The infant skull is thinner than the adults' and gives less distortion of the EEG signal, which allows for easier dipole fitting. However, infants have much more variability in the signal, and only 15 of the 19 infant subjects' components could be fitted with a residual variance below 20%. The mean residual variance of the fitted components was 8.1% in the infant group (standard deviation of 4.1%) and 4.5% in the adult group (standard deviation 2.0%). The localization of dipoles, plotted on the mean component power projections, is illustrated in figure 6. The mean Talairach coordinates for the infant group was -9, -3, 16 XYZ (standard deviation 23, 12, 11 XYZ) and -6, 1, 7 XYZ (standard deviation 15, 15, 13 XYZ) in the adult group, which locates the individual dipoles roughly along a coronal plane through the rolandic regions. Dipoles were located in both hemispheres, as indicated by the standard deviation, with an inclination to the left hemisphere (8 of 15 dipoles in infants, 10 of 16 dipoles in adults).

(Insert figure 6 about here)

Discussion

Mu and beta responses

The conditional mu power responses in adults are in line with previous studies (Hari & Salmelin, 1997; Nishitani & Hari, 2000; Muthukumaraswamy & Johnson, 2004a). The mean mu power differences are lower in the infant group, a difference that is expected from previous studies on the resting state mu rhythm (Stroganova, Orekhova & Posikera, 1999). The desynchronization in the moving dot condition is also reported by others (Hari et al, 1998), and it is especially interesting to note that the frequency response between the moving dot condition and non goal directed condition is very similar. This could indicate that the desynchronization in these two conditions is due to a response to the visual motion rather than the parsing of the model's action. The more detailed time/frequency result show that there is an adult mu rhythm desynchronization in the goal directed condition at the time when the hand reaches the object. All this relates well to the notion that the mirror neuron system is tuned to goal directed action, and lends support that the stimuli elicits mirror neuron activity. No beta reactivity was found in either group, which can be explained by the component selection algorithm that was based on mu rhythm detection.

The lack of significant differences between the moving dot and the reaching hand conditions in the infant group may lead to the impression that the mirror neuron system is immature at 6 month of age. However, there are many factors that can suppress the mu rhythm beside the stimuli properties, thereby masking the mirror neuron activity. For example, the cortical control of eye movements is known to suppress the mu rhythm, and since the stimuli consist of translational movements, some suppression of the mu rhythm from the smooth pursuit of the targets is expected (which could explain the similarities between the moving dot and the non goal directed conditions mentioned above). This holds for adults and it is reasonable to expect the same from the infant group since 6-month-old infants track object smoothly in an adult manner (von Hofsten & Rosander, 1997; Rosander & von Hofsten, 2002). Infants also have more motor activity while watching the stimuli due to postural stabilization, which would further mask mirror neuron activity. Finally, infants get quickly tired from being in the experimental environment, and changes in attention, vigilance and fatigue also alter the mu rhythm (Cochin, Lejeune, Roux & Martineau, 1998). These factors were considered to be equal across conditions, and expected to reduce the tested differences between conditions. It therefore seems likely to

observe no difference in suppression between the conditions unless there is a strong conditional response that overcome these factors. In adults this is evidently the case, but the results from the infant group do not quite reach significant mu desynchronization in the goal directed action condition compared to the non goal directed action condition.

ERP

In contrast to the frequency analysis there are functional differences for both adults and infants in the ERP analysis. The latency is somewhat shorter in the adult group, but the significant activation from goal directed action observation starts before the hand reaches the object in both groups (approximately 0.5s before in the adult group and 0.3s in the infant group). This indicates that the subjects anticipated the goal of the action, and that the perception of others' action is predictive even in the infant group. One study by Kilner, Vargas, Duval, Blakemore, and Sirigu (2004) show predictive ERP activation in an adult group and link it to the mirror neuron system. Although that study identified the readiness potential of observed actions, the same reasoning applies that activation ahead of time may be used to interpret the intention of others. It is well known that the mirror system is considered to interpret actions on-line (Fadiga et al., 2005) with very short latency of activation, and from the original comparative studies on monkeys (Di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996a, Umiltà et al., 2001) it is possible to imagine a weak and slow ERP synchronized to when a hand reaches an object even in humans. The timing of the significant intervals in our study do show a similar time course between the ERP activation and mirror neurons tuned to reaching, but any explicit suggestions on the appearance of an expected mirror neuron ERP were not found in the literature. In this study, the measured ERP traces also vary in appearance. This is especially true in the infant group where the amplitudes of the moving dot and the ERPs in the non goal directed conditions have about the same amplitudes as in the goal directed condition. However, only the goal directed condition is significantly different from the other conditions and the close resemblance to the adult's significance intervals validates the results.

The infant groups show a significant negative peak in the moving dot condition about 0.6s after motion onset (compared to a zero mean or the goal directed condition, but not to the other conditions). Effects from moving object observation are also reported by Hari et al. (1998), who found a minor effect in the frequency domain. One possible explanation is that the independent component analysis, which finds spatio-

temporally independent sources in the data, decomposed multiple cognitive processes with overlapping brain areas to the same component. This leads to the suggestion that the measured ERP is a result of a combination of different cognitive processes, one of which being the mirror neuron system.

General discussion

Many studies have mapped the neural substrates of the adult mirror neuron system. For example, Decety et al. (1997) found increased activation in BA6 (precentral gyrus) during observation of meaningful compared to meaningless actions. They also report activation in the middle frontal gyrus (BA8 and BA9) bilaterally. Grèzes et al. (2003) found similar results, with activation in the precentral gyrus bilaterally together with activation of the intraparietal sulcus and superior temporal sulcus. This network of mirror areas in the human brain suggests that there might be large variation between subjects in both dipole localization and direction, since the signal will be influenced by the sum of different synchronized areas (the more areas the greater the possibilities for differences). Indeed, the result from the dipole analysis shows individual variation. If the dipole moments have different directions then the source signals will be mixed and distributed over different channels in different subjects during raw EEG recording. The resulting mean signal in each channel will then be suppressed or cancelled out in a group analysis, and becomes a problem for simple frequency analyses on channels or groups of channels. As pointed out by Pineda (2005), the sensorimotor cortex also seems to display a variety of mu rhythms with specific topographic and functional properties with the same type of signal suppression within subjects. By applying the independent component analysis it is possible to find a decomposition of sources that minimize these problems.

The dipole locations of the components in the present study resolve the problem of strong posterior alpha generators: EEG oscillations in the 8-13Hz frequency over occipital cortex are influenced by states of awareness and overlaps with the mu rhythm (Oberman et al., 2005; Pineda, 2005). One might argue that the independent components might reflect this posterior activity, but the localization of the dipoles suggests that the activity rather originates in the rolandic regions. Although the location of dipoles appears to be somewhat deeper than cortex, this result can be explained by the orientation of the dipoles. Radially oriented dipoles are typically deeper than the cortical source itself, and the localization is similar to the mu rhythm motor response in Makeig et al. (2004). It is worth mentioning that dipole fitting is approximate, and that groups of

dipoles should be considered a statistical sample of the location of activation (Johnson-Frey et al., 2003). Of course individual differences are included in this consideration, and only a single independent component from each subject was fitted.

One problem with the analysis of independent components is to identify components between subjects that reflect the same cognitive process. One method is to cluster components with regard to parameters such as dipole localization, scalp topography, ERP traces or frequency response. This approach is especially valuable in exploratory studies and differentiation of several cognitive processes. This study aimed at identifying components with mirror properties and investigate the functional response to the stimuli. A component selection algorithm could thus be used with criteria based on a priori knowledge of the frequency response of the mu rhythm. The sensorimotor cortex generates many mu rhythms with specific topographic and functional properties (Pineda, 2005) and if multiple components had been selected from each subject the problem with different numbers of mu rhythms from each subject would have to be considered. Also, if components that do not contain mirror neuron activity are included, it will add noise to the signal. Instead of choosing multiple components from every subject a simplistic approach was used, where the independent mu component that account for most variance in the EEG channel data was selected. As the stimuli were designed to maximize grasping mirror activity, which gives the most reliable desynchronization in adult humans (Hari & Salmelin, 1997; Oberman et al., 2005), it is reasonable to believe that this strategy of selecting components extracted the analogous mu rhythm between subjects. Also, the characteristics of the mu desynchronization, ERP appearance and dipole localization is similar to the mu rhythm results of Makeig et al. (2004), who used cluster analysis to classify independent components. In that study, the mu rhythm cluster was derived from manual response, and was convincingly linked to motor performance. There is of course other ways of identifying and selecting components, for example by ordering the components on the basis of mu power. However, artefactual components typically have strong power peaks in many frequency bands, including the defined mu rhythm bands, whereas the current ordering tends to premiere cognitive components. The method used in this study is only one of many, and further studies are clearly needed to optimize the detection of mirror neuron system activity with EEG.

The stimuli might be optimized as well, as the different actions may differ in more respects than their goal directedness. A remedy of this question would be to test more conditions, but the short attention span of the infants made this impossible in the current study. Complementary studies are therefore needed to resolve this issue. Another crucial point is that the stimuli are presented as short movie clips on a monitor. A recent article on infant's brain responses to live and televised action (Shimada, Hiraki, 2006) using near-infrared spectroscopy show increased activation in 6 months infants' motor cortex when the stimuli was presented live compared to a TV presentation (The study is interesting since it shows that infants with the same age as in the present study have increased activation in motor areas during action observation. However, goal directedness was not addressed, and the temporal resolution makes it speculative to link the activation directly to mirror neurons). Many adult studies successfully use video stimuli, but the activation may have been dampened. According to Järveläinen, Schürmann, Avikainen and Hari (2001) beta wave rebound gets suppressed 15 – 19% with video presentation, which indicates less activation of the primary motor cortex. In infants, Falck-Ytter (2006) found that 11 months olds predict the action goal of a video presented model, but the effect of video presentation and mirror neuron activity in younger infants is still controversial. To be on the safe side: the influence of monitor presentation would be eliminated by using live actors and actions would follow each other in a more ecological fashion. It is an important empirical question whether this change would further increase the conditional differences in the infant group.

The aim of the present study was to detect mirror neuron activity from action observation in infants by the frequency response and ERP. It is important to emphasis that this is only the first step in the neurophysiological mapping of the developing mirror neuron system, and there is a wealth of important findings in adults that can be investigated in infant groups. Whereas the adult brain resembles a seamlessly integrated patchwork of cortical areas, the developing infant brain may reveal how different areas are interconnected to functional systems. For example, mirror neurons are not only activated by the sight of grasping movements. By investigating audiovisual mirror neurons it might be possible to assess action recognition in infants and study how actions are transformed into abstract, modality independent representations (see Keysers, Kohler, Umiltà, Nenetti, Fogassi & Gallese, 2003 for a discussion of modalities and audiovisual mirror neurons). This is in turn related to speech perception (Skipper, Nusbaum & Small, 2004) and the

development of language and mirror neurons. Finally, developmental studies inspired by Keysers and Perret (2004) or Baldissera, Cavallari, Craighero and Fadiga (2001) can shed some light on how the infant separates (or learn to separate) its own actions from those of others.

In the introduction the question whether mirror neuron activity emerges together with specific motor acts was raised. This question is still difficult to address, even though the ERP response of the 6 months infants suggest a tight coupling between the development of performance and corresponding mirror neurons. A compatible view is provided by Falck-Ytter et al. (2006) that showed that infants predicted the goal of an observed action at 11 months but not at 6 months. The observed action in that study was to place objects in a container, and most 6 months infants would not be able to perform the same action. Certainly, to test more age groups and to conduct more elaborate studies on different actions (such as with hidden goals as Umiltà et al, 2001; impossible movements as Constantini et al., 2005; or unpredictable movements as Gangitano, Mottaghy & Pascual-Leone, 2004) could help us understand the separate development or co-development of the mirror system and the motor system in humans.

Over age it might also be possible to measure when the human mirror neuron system diverges from other species. A comparison of the human and monkey mirror systems, for example, reveal a few differences. Whereas monkeys' mirror neuron only fire if an action is geared toward an object, the human mirror neuron system can be activated by an action geared toward an imaginary object (Fadiga & Craighero, 2004). So another important consideration for future mirror neuron studies in infants is the introduction of both transitive and intransitive actions, as well as mimicking actions and meaningless actions. Without the knowledge of when and how the human mirror neuron system differ from other species it will be speculative to discuss on how the human mirror neuron system facilitates the development of theory of mind, imitation learning, language learning and other uniquely human capabilities.

Conclusions

Taken together, this study investigated mirror neuron system activity in both adults and 6 months infants using high density EEG. The results from the frequency response show that the stimuli causes mirror related mu rhythm desynchronization in adults and similar patterns in infants. By applying an alternative method of analyzing the data, using the ERP paradigm, functional differences were found in both groups. The ERP results

show significantly higher amplitudes in the goal directed action observation condition compared to non goal directed action observation and moving / static dot observation. The time course of the ERP implies that the measured effects reflect mirror neuron activity, and that the mirror neuron system can be detected directly by EEG in both adults and infants as young as 6 months. The possibility to measure mirror neuron activity in infants using this method opens up a wide range of developmental studies that can help in delineating the maturation of the human mirror neuron system.

References

Babiloni C., Carducci F., Cincotti F., Rossini P. M., Neuper C., Pfurtscheller G., Babiloni F. (1999) Human Movement-Related Potentials vs Desynchronization of EEG Alpha Rytm: A High-Resolution Study. NeuroImage 10, pp 658-665

Baldissera F., Cavallari P., Craighero L., Fadiga L. (2001) Modulation of spinal excitability during observation of hand actions in humans. European Journal of Neuroscience, Vol. 13, pp 190 - 194

Bekkering H., Wohlschläger A., Gattis M. (2000) Imitation of Gestures in Children is Goal-directed. The quarterly Journal of Experimental Psychology, 53A (1), pp 153 – 164

Braddick O.,Birtles D.,Wattam-Bell J., Atkinson J. (2005). Motion-and orientation specific cortical responses in infancy. Vision Research, 45(25-20), pp 3169-3179

Buccino G., Vigt S., Ritzl A., Fink G. R., Zilles K., Freund H-J., Rizzolatti G. (2004) Neural Circuits Underlying Imitation Learning of Hand Actions: An Event-Related fMRI Study. Journal of Cognitive Neuroscience, 16, pp 114-126

Cochin S., Barthelemy C., Roux S., Martineau J. (1999) Observation and execution of movement: similarities demonstrated by quantified electroencephalografy. European Journal of Neuroscience, 11, pp 1839-1842.

Constantini M., Galati G., Ferretti A., Caulo M., Tartaro A., Romani G. L., Aglioti S. M. (2005) Neural Systems Underlying Observation of Humanly Impossible Movements: An fMRI Study. Cerebral Cortex, November, 15, pp 1761 - 1767

Decety J., Grèzes J., Costes N., Perani D., Jeannerod M., Procyk E., Grassi F., Fazio F. (1997) Brain activity during observation of actions, influence of action content and subjct's strategy. Brain, 120, pp 1763 – 1777

Delorme A., Makeig S. (2004) EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics, Journal of Neuroscience Methods, 134, pp 9-21

Di Pellegrino G., Fadiga L., Fogassi L., Gallese V., Rizzolatti G. (1992) Understanding motor events: a neurophysiological study. Experimental Brain Research, 91, pp 176 - 180

Fadiga L., Fogassi L., Pavesi G., Rizzolatti G. (1995) Motor facilitation during action observation: a magnetic stimulation study. Journal of Neurophysiology, 73, pp 2608-2611

Fadiga L., Craighero L. (2004) Electrophysiology of Action Representation. Journal of Clinical Neurophysiology, Vol 21, Number 3, pp 157 – 169

Fadiga L., Craighero L., Olivier E. (2005) Human motor cortex excitability during the perception of others' action. Current Opinion in Neurobiology 15, pp 213 – 218

Falck-Ytter T., Gredebäck G, von Hofsten C. (2006) Infants predict other people's action goals. Nature Neuroscience, Vol 9, Number 7, pp 878 - 879

Flanagan, J.R. and Johansson, R.S. (2003) Action plans used in action observation. Nature, 424, pp 769–77

Fogassi L., Ferrari P. F., Gesierich B., Rozzi S., Chersi F., Rizzolatti G. (2005) Parietal Lobe: From Action Understanding to Intention Understanding. Science, Volume 308, pp 662 - 667

Gallese V., Fadiga L., Fogassi L., Rizzolatti G. (1996) Action recognition in the premotor cortex. Brain, 119, pp 593-609

Gallese V., Keysers C., Rizzolatti G. (2004) A unifying view of the basis of social cognition. Trends I Cognitive Sciences, Vol 8, No 9, pp 396 – 403

Gangitano M., Mottaghy F. M., Pascual-Leone A. (2004) Modulation of premotor mirror neuron activity during observation of unpredictable grasping movements. European Journal of Neuroscience, Vol 20, pp 2193 – 2202

Gastaut H. J., Bert J. (1954) EEG changes during cinematographic presentation (moving picture activation of the EEG). Electroencephalography and Clinical Neurophysiology, 6, pp 433 – 444

Grafton S.T., Arbib M.A., Fadiga L., Rizzolatti G. (1996) Localization of grasp representations in humans by PET: 2. Observation compared with imagination. Experimental Brain Research, 112, pp 103-111

Grèzes J., Costes N., Decety J. (1998) Top-down effect of strategy on the perception of human biological motion: a PET investigation. Cognitive Neuropsychology, 15, pp 552-582

Grèzes J., Armony J. L., Rowe J., Passingham R. E. (2003) Activations related to “mirror” and “canonical” neurones in the human brain: an fMRI study. NeuroImage 18, pp 928 – 937

Grieve P. G., Emerson R. G., Fifer W. P. Isler J. R., Stark R. I. (2003) Spatial correlation of the infant and adult electroencephalogram. Clinical Neurophysiology 114, pp 1594 – 1608

Hari R., Forss N., Avikainen S., Kirveskari E., Salenius S., Rizzolatti G. (1998) Activation of human primary motor cortex during action observation: A neuromagnetic study. Proc. Natl. Acad. Sci. USA. Neurobiology, Vol. 95, pp 15061 – 15065

Hari R., Salmelin R. (1997) Human cortical oscillations: a neuromagnetic view through the skull. Trends in Neuroscience, Vol 20, No 1, pp 44 – 49

Järveläinen J., Schürmann M., Avikainen S., Hari R. (2001) Stronger reactivity of the human primary motor cortex during observation of live rather than video motor acts. Neuroreport, Vol 12, No 16, pp 3493 – 3495

Johnson M.H., de Haan M.,Oliver A., Smith W., Hatzakis H., Tucker L.A., Csibra G. (2001). Recording and analyzing high-density event-related potentials with infants. Using the Geodesic sensor net. Developmental Neuropsychology, 19, 295-323.

Johnson-Frey S. H., Maloof F. R., Newman-Norlund R., Farrer C., Inati S., Grafton S. T. (2003) Actions or Hand-Object Interaction? Human Inferior Frontal Cortex and Action Observation. Neuron, Vol 39, pp 1053 – 1058

Junghöfer M., Elbert T., Tucker D. M., Rockstroh B. (2000) Statistical control of artifacts in dense array EEG / MEG studies. Psychophysiology, 37, pp 523 - 532

Keysers C., Kohler E., Umiltà M. A., Nanetti L., Fogassi L., Gallese V. (2003) Audiovisual mirror neurons and action recognition. Experimental Brain Research, 153, pp 628 - 636

Keysers C, Perred D. I. (2004) Demystifying social cognition: a Hebbian perspective. Trends in Cognitive Sciences, Vol 8, No. 11, pp 501 – 507

Kilner J. M. Vargas C., Duval S., Blakemore S-J., Sirigu A. (2004) Motor activation prior to observation of a predicted movement. Nature Neuroscience, Vol 7, No 12, pp 1299 – 1301

Kohler E., Keysers C., Umiltà M. A., Fogassi L., Gallese V., Rizzolatti G. (2002) Hearing Sounds, Understanding Actions: Action Representation in Mirror Neurons. Science, Vol 297, pp 846 – 848

Kuhlman W. N. (1980) The mu rythm: functional topography and neural origin. In: Pfurtsheller G, editor. Rythmic EEG activities and cortical functioning, Amsterdam: Elsevier. Pp 105-120

Leslie K. R., Johnson-Frey S. H., Grafton S. (2004) Functional imaging of face and hand imitation: towards a motor theory of empathy. NeuroImage 21, pp 601 – 607

Makeig S., Delorme A., Westerfield M., Jung T, Townsend J., Courchesne E., Sejnowski T. J. (2004) Electroencephalographic Brain Dynamics Following Manually Responded Visual Targets. PLoS Biology, June, Vol 2, Issue 6, pp 0747 – 0762

Marshall P. J., Bar-Haim Y., Fox N. A. (2002) Development of the EEG from 5 month to 4 years of age. Clinical Neurophysiology 113, pp 1199 – 1208

Muthukumaraswamy S. D., Johnson B. W. (2004a) Changes in rolandic mu rhythm during observation of a precision grip. Psychophysiology 41, pp 152 – 156

Muthukumaraswamy S. D., Johnson B. W. (2004b) Primary motor cortex activation during action observation revealed by wavelet analysis of the EEG. Clinical Neurophysiology 115, pp 1760 – 1766

Muthukumaraswamy S. D., Johnson B. W., McNair N. A. (2004) Mu rhythm modulation during observation of an object-directed grasp. Cognitive Brain Research 19, pp 195 – 201

Nishitani N., Hari R. (2000) Temporal dynamics of cortical representation for action. Proc. Natl. Acad. Sci., USA, January, Vol 97, No 2, pp 913 – 918

Oberman L. M., Hubbard E. M., McCleery J. P., Altschuler E. L., Ramachandran V. S., Pineda J. A. (2005) EEG evidence for mirror neuron dysfunction in autism spectrum disorders. Cognitive Brain Research, 24, pp 190 - 198

Picton, TW., Bentin, S., Berg, P., Donchin, E., Hillyard, SA., Johnson, R., Miller, GA., Ritter, W.,Ruchkin, DS., Rugg, MD., Taylor, MJ. (2000). Committee report Guidelines for using human event-related potentials to study cognition: Recording standards and publication criteria. Psychophysiology 37, 127-152.

Pineda J. A. (2005) The functional significance of mu rythms: Translating “seeing” and “hearing” into “doing”. Brain Research Reviews, in press.

Rizzolatti G., Fadiga L., Fogassi L., Gallese V. (1996a) Premotor cortex and the recognition of motor actions. Cognitive Brain Research, 3, pp 131-141

Rizzolatti G., Fadiga L., Matelli M., Bettinardi V., Pauulesu E., Perani D., Fazio F. (1996b) Localization of grasp representations in humans by PET: 1. Observation versus execution. Experimental Brain Research, 111, pp 246-252

Rizzolatti G., Craighero L. (2004) The Mirror-Neuron System. Annu. Rev. Neurosci., 27, pp 169 – 192

Rosander K., Nyström P., Gredebäck G., von Hofsten C. (2007) Cortical processing of visual motion in young infants. Submitted.

Rosander, K., von Hofsten, C., (2002). Development of gaze tracking of small and large objects. Experimental Brain Research, 146, 257-264.

Scherg M. Fundamentals of dipole source potential analysis. In: Auditory evoked magnetic fields and electric potentials. (eds. F. Grandori, M. Hoke and G.L. Romani). Advances in Audiology, vol. 6. Karger, Basel, pp 40-69, 1990.

Shimada S., Hiraki K. (2006) Infant's brain responses to live and televised action. NeuroImage, 32, pp 930 – 939

Skipper J. I., Nusbaum H. C., Small S. L. (2005) Listening to talking faces: motor cortical activation during speech perception. NeuroImage, 25, pp 76 – 89

Strafella A. P., Paus T. (2000) Modultaion of cortical excitability during action observation: a transcranial magnetic stimulation study. Neuroreport, 11, pp 2289-2292

Stroganova T. A., Orekhova E. V., Posikera I. N. (1999) EEG alpha rythm in infants. Clinical Neurophysiology 110, pp 997 – 1012

Umiltà M. A., Kohler E., Gallese V., Fogassi L., Fadiga L., Keysers C., Rizzolatti G. (2001) I Know What You Are Doing: A Neurophysiological Study. Neuron, Vol 31, pp 155 – 165

von Hofsten, C., and Rosander, K., (1997). Development of smooth pursuit tracking in young infants. Vision Research, 37, 1799-1810

Figure captions

Figure 1. Examples of signal before and after independent component analysis, with logarithmic mean values of the spectral power densities (0 dB means 1$\mu V^2/Hz$). Blue colours are mean mu rhythm power of all conditions; red colours are mean of the static dot condition. Thin lines in top plots show individual channels of the ROIs used by Muthukamaraswathy (2004a) that covers areas around standard C3 and C4 electrode positions. Information like in the bottom plots was used to select the first desynchronizing component from each subject (the critical frequency range is highlighted).

Figure 2. Mu rhythm power in the four conditions in decibel relative all conditions' mean power. Error bars indicate confidence intervals of 95%. In the adult group the goal directed action condition differs significantly from the non goal directed action and moving dot condition ($p < 0.05$). The static dot condition is significantly higher than the other conditions in both groups.

Figure 3, Time frequency spectrogram differences (goal directed – non goal directed conditions) showing desynchronization in blue colours. Pixel wise statistical probability maps are overlay and highlight significant areas ($p < 0.05$). Non significant values are shown in faint colours. The color scale show decibel change from baseline (computed as the mean power from both conditions in each frequency band).

Figure 4. Infant ERPs with z-transformed amplitudes (μV) within subjects. Single ERP traces are tested sample wise against zero using t-tests, double ERP traces are tested against each other using paired t-tests. Significant intervals ($p < 0.05$) are highlighted in yellow. Shaded areas are standard error of ERP trace. Dotted lines mark timelock (motion onset, time = 0s) and time when hand reaches object in the goal directed condition (time = 1s).

Figure 5. Adult ERPs with z-transformed amplitudes (μV) within subjects. Single ERP traces are tested sample wise against zero using t-tests, double ERP traces are tested against each other using paired t-tests. Significant intervals ($p < 0.05$) are highlighted in yellow. Shaded areas are standard error of ERP trace. Dotted lines mark

timelock (motion onset, time = 0s) and time when hand reaches object in the goal directed condition (time = 1s).

Figure 6. Mean topographic plots of the independent components' power projections (component weights, arbitrary unit). Black circles mark the axial projections of independent components' dipole localization (one per subject). Black lines show the projection of dipoles' directions with normalized length. The color scale show the z-transformed component weights.

Figure 1

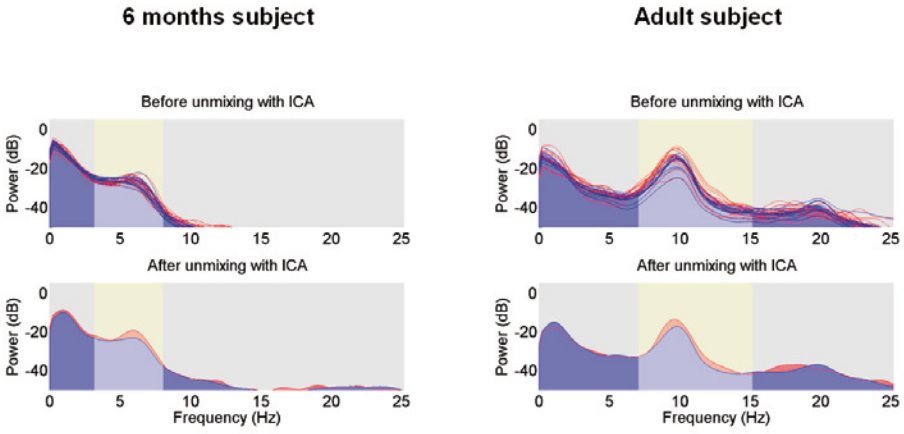

6 months subject
Adult subject
Before unmixing with ICA
After unmixing with ICA
Power (dB)
0
-20
-40
0
5
10
15
20
25
Frequency (Hz)

Figure 2

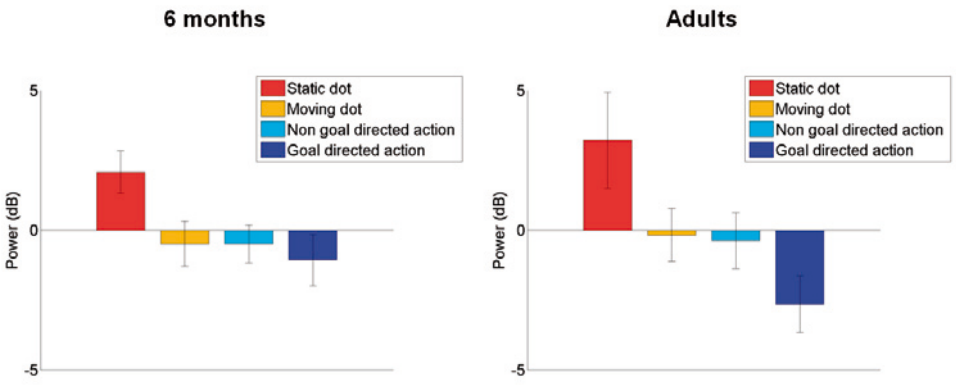

6 months
Adults
Static dot
Moving dot
Non goal directed action
Goal directed action
Power (dB)
5
0
-5

Figure 3

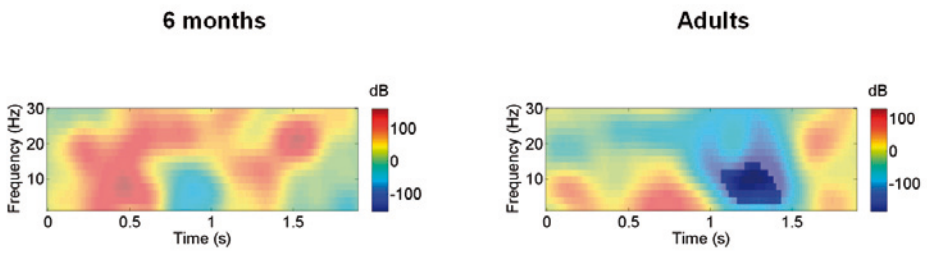
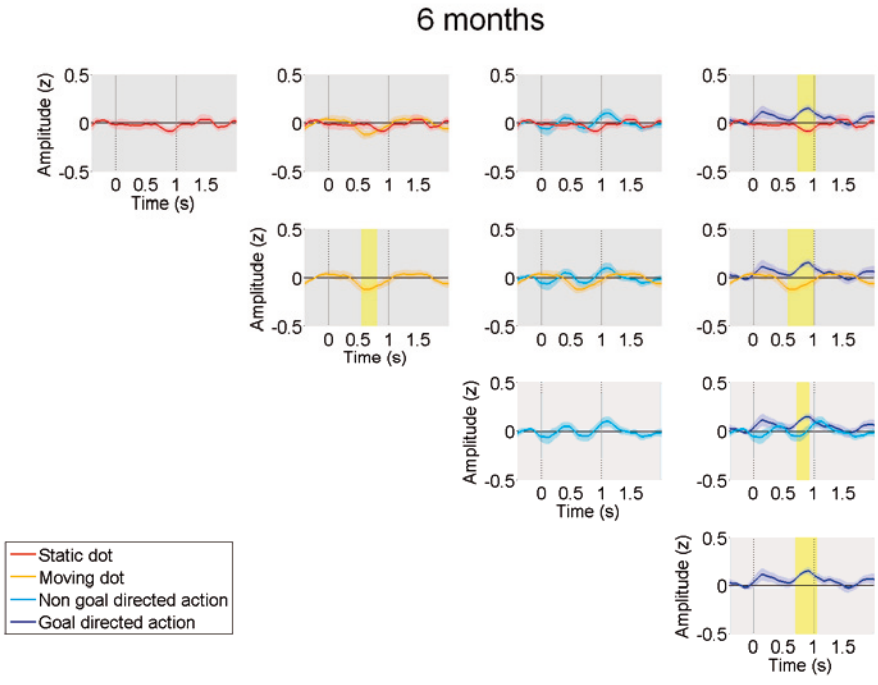

6 months
Adults
dB
100
0
-100
Frequency (Hz)
30
20
10
0
0.5
1
1.5
Time (s)

Figure 4


6 months
Amplitude (z)
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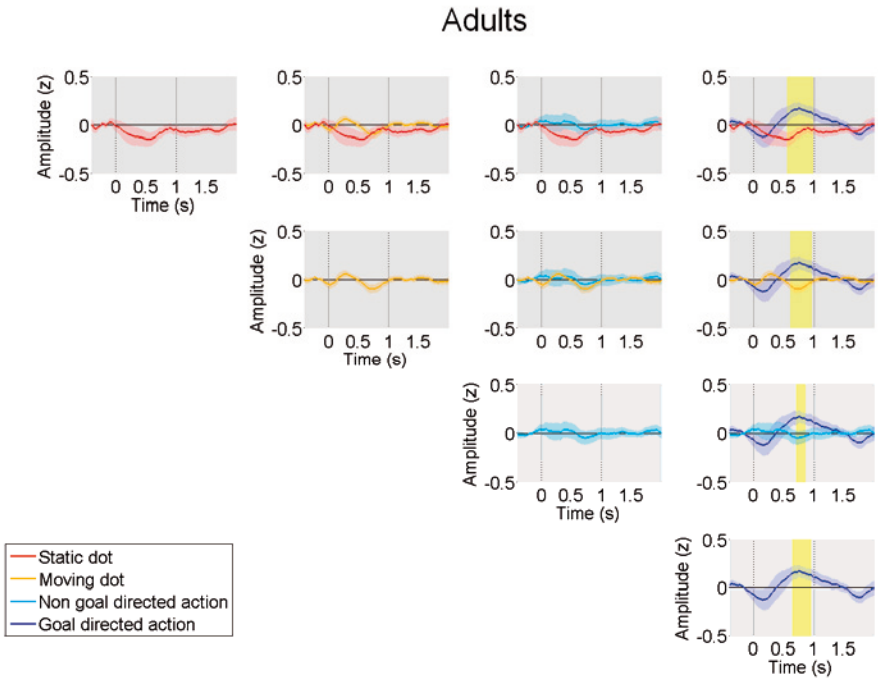
0
-0.5
0 0.5 1 1.5
Time (s)
Static dot
Moving dot
Non goal directed action
Goal directed action

Figure 5


Adults
Amplitude (z)
0.5
0
-0.5
0 0.5 1 1.5
Time (s)
Static dot
Moving dot
Non goal directed action
Goal directed action

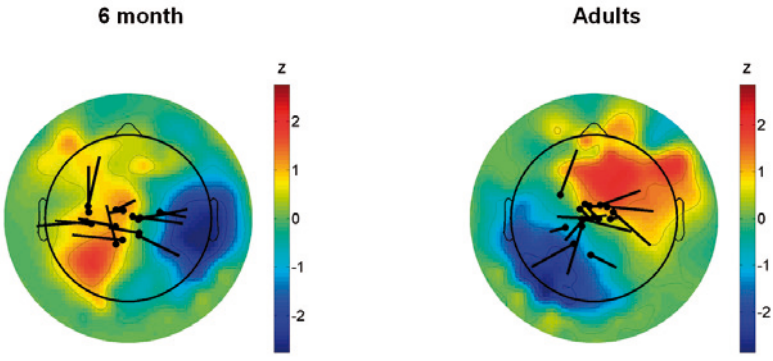



Figure 6