

Using mu rhythm perturbations to measure mirror neuron activity in infants

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Abstract

The Mirror Neuron System hypothesis stating that observed actions are projected onto the observer's own action system assigns an important role to development, because only actions mastered by the observer can be mirrored. The purpose of the present study was to investigate whether there is evidence of a functioning mirror neuron system (MNS) in early infancy. High-density EEG was used to assess the mu rhythm perturbations in an action observation task where the infants viewed a live model. To reduce noise ICA decompositions were used. The results show a higher desynchronization of the mu rhythm when 8-month-old infants observe a goal-directed action than when they observe a spatially similar non-goal-directed movement. This provides evidence that the MNS is functioning at this age level. Importantly, the results show that mu rhythm perturbations can be used as a tool for studying MNS activity in infants.

Introduction

It has been suggested that other people's actions are understood by mapping them onto one's own motor representation of that action (Flanagan & Johansson, 2003, Rizzolatti, Fadiga, Gallese, 1996). It is suggested that this mapping is accomplished by a neuronal system, the Mirror Neuron System (MNS), activated both by the execution of one's own goal-directed actions and by the perception of someone else performing the same actions (Rizzolatti et al., 1996; Fadiga & Craighero, 2004). It has even been suggested that the MNS plays a crucial role in many social activities, like imitation learning (Buccino et al., 2004), theory of mind, empathy, and the development of language (Théoret & Pascual-Leone, 2002). Thus, the mirror neurons principle could provide a unifying framework for human social cognition (Gallese et al., 2004, Oberman et al, 2007), and has consequently attracted attention from many research fields (Berthouze & Metta, 2005).

Neurophysiological evidence indicates that the MNS is a distributed system with at least three primary nodes, the ventral premotor area (Area 44 in humans), the STS, and the IPL. It was in the left ventral premotor area that the mirror neurons were first observed. Rizzolatti et al. (1996) showed that the same neuron fired when the monkey picked up a peanut and when they saw someone else do it. Fogassi et al. (2005) found that most motor IPL neurons coding a specific movement (e.g., grasping) showed markedly different activations when this act was part of different actions (e.g., for eating or for placing). Studies using fMRI have found elevated activation in STS, IPL and Area 44 in adult humans during action observation (Rizzolatti & Craighero, 2004). Using MEG, Nishitani and Hari (2002) showed that observed and imitated lip movements activated first the STS, then the IPL, and finally the Area 44.

In order to understand other people's actions in the way proposed by the MNS hypothesis, that is, in terms of one's motor representations, the corresponding movements must be mastered by the subject. In other words, actions that are not mastered cannot be understood in this way. This makes the MNS hypothesis very interesting from a developmental point of view because it states that motor development plays a crucial role for the understanding of other people's actions. If mastery of actions is essential for the understanding of other people's actions, it should develop in parallel with or ahead of such understanding. The problem is that direct evidence of brain activity in young children from fMRI and MEG is generally not available below preschool age. The importance of this question, however, is reflected in several explicit requests for empirical neurophysiological data to start to resolve speculations (Lepage & Théoret, 2007; Bertenthal & Longo, 2007; Kilner & Blakemore, 2007).

One indirect piece of evidence for the MNS is found in people's proactive eye movements during action observation. When subjects perform actions, they move the eyes to the goal proactively (Land & Hayhoe, 2001). The purpose of such eye movements is to guide the hand to the goal. If the MNS hypothesis holds, the proactive gaze shifts performed during action performance should be produced during action observation as well. Because the eyes are free to move when observing such actions, the MNS hypothesis predicts that subjects should produce eye movements similar to those produced when they perform the tasks. In accordance with this, Flanagan and Johansson (2003) found that adult subjects performed proactive eye movements to the goal of a displacement action both when they performed the action themselves and when they observed someone else perform the action. Such indirect behavioral evidence for MNS activity in infancy was obtained by Falck-Ytter, Gredebäck and

von Hofsten (2006). They found that 12-month-old but not 6-month-old infants looked proactively at the goal when a hand transported an object there. For both age groups of infants, the eyes just tracked the object when the same object motion was shown outside the context of an action. It should be noted that 12-month-olds but not 6-month-olds displace an object they have grasped to a different location in a goal-directed way. They might move the grasped object to the mouth, however. In accordance with this, Kochukhova and Gredebäck (2009) found that observed hand movements involved in eating actions were accompanied by proactive eye movements to the mouth of the model in 6-month-old infants.

Other studies of social development have found that towards the end of the first year of life, infants rapidly develop social skills requiring understanding of other people's actions. For instance, from about 11 months of age they begin to point to objects in the surrounding that they want other individuals to attend to (Butterworth, 2003; Liskowski, Carpenter & Tomasello, 2006; Tomasello, Carpenter, & Liskowski, 2007). Infants have been shown to imitate other people's actions from 6 months of age (Barr, Rovee-Collier & Campanella, 2005; von Hofsten & Siddiqui, 1993) and to perform deferred imitation from 9 months of age (Meltzoff, 1988). From around 8 months of age, infants babble in social settings (Ejiri, 1998). In summary, the present results suggest that the development of the mirror neuron system either precedes the emergence of these social functions or develop in parallel with them.

A neurophysiological method suitable for developmental research is EEG, due to its light-weight and few physical constraints, and that it therefore can be applied to awake subjects of all ages. MNS activity has been identified in adults using EEG by analysis of the mu rhythm (Pineda, 2005; Muthukumaraswamy et al., 2004a; related experiments has been performed by using MEG as well, e.g., Nishitani & Hari, 2000; Hari et al., 1998). There are at least three reasons to associate the mu rhythm with the MNS. First, these studies show that the mu rhythm oscillates at approximately 9-13 Hz in adults but gets suppressed both when a subject performs an action and when the subject observes someone else perform the same action. Second, an important feature of mirror neurons is that they are tuned to goal-directed actions. The mu rhythm is also modulated by the goal-directedness of actions. It is suppressed more when adults either perform or observe goal-directed actions compared to non-goal-directed actions (Muthukumaraswamy & Johnson, 2004; Muthukumaraswamy, Johnson & McNair, 2004). This functional response has also been found in 4-11-year-old children (Lepage & Théoret, 2006) and has recently been observed in infants (Southgate...). Third, the mu rhythm is a sensorimotor rhythm that appears to consist of several rhythms with different origins both in motor areas and in parietal sensory areas (Pineda, 2005). This is consistent with the locations of mirror neurons. Mu rhythm suppression can therefore be considered being an established measure of MNS activity.

The infant alpha rhythm between 5-9 Hz show strong resemblances to the adult mu rhythm, and it has therefore been suggested that it is the infant mu rhythm (Stroganova et al., 1999; Marshall et al., 2002). Orekhova et al. 2006 found that the action related part of the alpha rhythm emerge at around 5-8 months and increase in frequency and amplitude over age. Other studies have also studied infant alpha rhythms (Orekhova, Stroganova & Posikera, 2001) although no study has explicitly tested the functional reactivity of the infant alpha e mu rhythms in both action execution and action observation tasks (Southgate, 2009a, 2009b; . Only one study has previously shown significant mu rhythm perturbations associated with the MNS in infants (van Elk et al., 2008) but then only in an action observation paradigm. One reason why EEG studies of the mu rhythm in an action performance condition has not yet

been reported is that infants tend to introduce large artefacts into the EEG signal when they move. Is it then possible to measure the infant mu rhythms without a performance condition? The best option would be to investigate mu rhythm suppression during action observation at mu sources in motor and sensory areas, and determine whether the suppression is synchronized with the goal of the action. With this paradigm it might be possible to identify infant mu rhythms. This study will therefore assume that the infant mu rhythms have the same characteristics as the adult mu rhythms and investigate the mu perturbations in an action observation situation.

Another reason for the sparse literature on the infant mu rhythms is probably that infant EEG is plagued by short recording sessions (due to short attention spans) and eye movement artefacts since infants do not want to sit and watch passively (Thierry, 2005). In a traditional EEG paradigm where the channels receive mixed signals from different parts of the brain as well as muscle and eye movement artefacts, the result is often a very low signal to noise ratio where the mu rhythm is masked. By using a blind source separation technique, such as the independent component analysis (ICA), many of these problems can be solved. In principle ICA can be viewed as an oblique rotation method for PCA dimensions or sphered raw data. ICA is thus similar to principal component analysis (PCA), but whereas PCA aims at finding the factors that explains the most variance, ICA aims at finding components that are most statistically independent of each other (Delorme & Makeig, 2004). Since the MNS in infants (measured by the desynchronizing mu rhythm) cannot be expected to account for much variance but still has unique statistical properties, ICA appears to be a suitable tool. Unfortunately, ICA introduces a component selection problem. Because the mu rhythm can be expected to decompose into one or a few components from each subject, only these components should be considered in the analysis. We used 3 conditions to solve the independent component selection/testing problem. In one condition, the base-line mu rhythm activation was estimated when the subject observed a non-moving human model. In the other two conditions, the model was moving. In one of the movement conditions, the model reached for and grasped an object and in the other he moved his arm in a similar way but just placed his hand on a table-top at the end. Then, the difference in mu rhythm activation between the baseline and the two movement conditions was used for the selection of EEG sources. Finally, the difference between these two conditions was analyzed. If the MNS is activated by the goal-directed reaching movement, then the mu rhythm should be more desynchronized in this condition compared to the observation of a simple arm displacement.

A recent study of 6-month-old infants used a similar method to extract mu rhythm activation elicited by video presentations of a passive and an active actor (Nyström, 2008). Although no evidence of a consistent difference in the mu rhythm was obtained for these two situations, an ERP analysis of the same data showed different responses for goal-directed and non-goal-directed movements. The insignificant mu rhythm desynchronization could either be due to the young age of the infants, the suboptimal viewing conditions, or both. In the present study we optimized the experiment in two ways. First, 8-month-old instead of 6-month-old infants were studied. If MNS develops in infancy, its indicators should get more distinct with age. Secondly, live actors instead of video recorded ones were used. In earlier studies of adults (Järveläinen et al., 2001; Shimada & Hiraki, 2006) and 7-month-old infants (Shimada & Hiraki, 2006), live actors were found to elicit stronger MNS activation compared to video taped ones. In fact, although Shimada and Hiraki (2006) found that action observation gave rise to activation in the motor areas in both the live and the TV setting, only the activation in the live setting was significant. With these two improvements the present study investigated whether infant MNS activity could be assessed by mu rhythm perturbations.

Methods

Participants

In the present study 36 healthy 8-month-old infants participated. The infants were tested on a simple reaching task, and all infants reached successfully for objects presented to them. The parents were given written information upon arrival to the lab and signed a written consent form in accordance with the Helsinki Declaration. The experiment was approved by the Ethics committee at Uppsala University. Two infants were excluded before analysis due to fussing or inattention, and 2 infants were excluded due to technical problems. All parents received a gift certificate of 100 Swedish kronor (approximately € 9) at a local toy store.

Procedure

The infants were seated in front of the scene at a distance of approximately 1 meter where the events were shown and a high density 128 channels EEG net (EGI, Corp., Eugene, Oregon) of the appropriate size was applied to the infant's head. The infants were shown from a lateral viewpoint a live male model sitting in front of a table. On the table, a short railway track with a toy train was placed (see Figure 1). First, the model was shown a baseline condition where the model sat passively (static condition). Then, two hand movement conditions were shown. In one of them (goal-directed condition), the model grasped the toy train and moved it from a starting position to a new position further away from the model. When the toy was in the new position the model performed the second condition. In this condition (non-goal-directed condition), the model moved his hand toward the first toy position (now empty) and simply placed the hand flat on the table. As the railway track was slanted toward the original starting position, the train returned there when an electronic trigger was released at the end of these events. Except for the very first condition that for practical reasons was always a goal-directed movement, these conditions were presented in randomized order by sometimes interleaving the static condition and sometimes not, or presenting the goal-directed or non-goal-directed condition twice before proceeding to any of the other conditions. The conditions were repeated until the subject was no longer interested or started to fuss.

EEG recordings and processing

The 128 EEG channels were sampled at 250 Hz, with an analogue hardware band pass filter at 0.1 to 100 Hz (EGI, Corp., Eugene, Oregon). During recording, the infants observed the model either sitting still or performing the two actions described above. The EEG was time-locked by the model when the hand touched the toy or the table, or after approximately 1 second of sitting passively. Manual triggers hidden from the infants' view were used to time lock the events. The triggers were operated by the model and triggered by the hand that performed the action (the triggers were hidden under a cloth on the table and under the models foot behind the table for the static condition). The model released the trigger with the moving hand as it arrived at the table-top or grasped the object. In the case where the object was grasped, the trigger was released by the little finger of the grasping hand situated on the far side of the object and thus not visible by the subject. The grasping action was performed by the thumb and three of the other fingers. After training the triggering over a few hundred trials during pilot testing, the temporal synchrony between the grasping and the triggering was quite high. The timing precision was determined in a separate experiment where a trigger was

placed on the object reached for in addition to the trigger used during the action observation. For a sequence of 182 control measures of the trained experimenter, the mean time difference was 9.7 ms and the standard deviation 27.1 ms.

The experimenter observed the behaviour of the subject during the whole session, and conditions were never presented unless the infant was in an attentive and passive mode. The resulting EEG recordings consisted of between 10 – 49 trials in each condition from every subject (mean = 21.06; SD = 7.40). The data was transferred to the MATLAB v.7.2 environment and analyzed using the EEGLAB v5.03 toolbox. First, 29 of the outermost sensors were removed due to bad contact in most infants, although the net was properly positioned. The continuous EEG was then band pass filtered from 2 to 20 Hz using two-way least-squares FIR filtering to remove noise and to focus on the frequencies where most brain related signals appear. The data was segmented into trials from -1s to 1s after time-lock (touch of toy or table, or after approximately 1 second of resting). A modified artefact rejection routine for high density EEG (Junghöfer et al., 2000) removed bad trials and sensors based on their maximum values, standard deviations and range values. Next, the EEG was transformed to average reference as recommended by the EEGLAB guidelines. Modifications of the artefact rejection routine were removal of interpolation, which could violate the assumption for the ICA of independent measurements from each channel. Bad trials and channels were simply excluded from the dataset to prevent spreading of bad data when re-referencing. A natural-gradient logistic infomax independent component analysis was performed on the data (the Runica algorithm, (Delorme & Makeig, 2004), which resulted in as many independent components as remaining channels minus one for each subject (mean = 88, ranging from 78 – 98 components).

Selection of independent components

Although each subject's data was decomposed into many components only a few of them were assumed to reflect mirror neuron activity. The other components were assumed to mask the signal of interest by introducing noise and neural signals not related to the MNS. To reduce noise and to focus on components with mirror neuron properties we performed the two step component selection procedure described below. First we excluded components that reflected artefacts by the following criteria. Components with any abnormal ICA weight, >2.7 SD of all weights within a component, were considered artefactual and were excluded. The value of 2.7 SD was decided by visual inspection of all components to retain components with dipole like scalp projections and to exclude components originating from channel pops or movement artefacts. Two EEG experts knowledgeable of dipole source projections and four novices visually inspected the components that showed mu suppression. The area under the receiver operating characteristic (ROC) curve (AUC) was 0.95, and justified the use of a maximum of 2.7 was SD in any ICA weight as a rather conservative threshold. Also, the maximum absolute amplitudes of components were calculated to identify outlier values that could bias the subsequent frequency analysis. Trials with abnormal values (>3 SD) were thus excluded and also components with less than 10 trials in any condition. Secondly, we selected components related to mu rhythm desynchronization from the remaining components. Frequency spectra of the three conditions were extracted. To speed up the computations we used Welch's method (hamming windows of 256 samples length and 128 samples overlap) instead of the EEGLAB timef function at this point. The results were converted to dB values across a 1 Ohm reference load to simplify visual presentation of the power spectra. Components with a power peak greater than 1 dB in the static condition and a decrease in the

power peak from this value greater than 1 dB for the two movement conditions were selected for further statistical analysis. This procedure selected components that showed mu desynchronization in the live movement conditions but without making any distinction between the goal-directed and non-goal-directed condition. The peak power was calculated as the max difference between the power spectrum and the linear interpolation of the power values at the boundaries of the 8-month-olds mu band (5-9 Hz). This definition of the peak power would account for overall differences in the power spectra between conditions. The definition of the 5-9 Hz interval was based on previous studies of infant alpha rhythms (Stroganova et al., 1999). An example of the frequency spectra used to select components is shown in Figure 2 together with the mean spectrum of all selected components. It should be noted that the highest amplitude in this frequency interval was 7 Hz in agreement with Stroganova et al. (op.cit.). In total 43 components with 10 – 49 trials (mean = 21.06; SD = 7.40) from each condition originating from 23 subjects were selected. Each subject contributed with between 1 and 5 components (mean = 1.87; SD = 1.12). If the ICA did not decompose any component with mu characteristics the subject was excluded. There could be several reasons. First, the mu rhythm is subject to individual differences. Second, the mu rhythm might not have developed in some subjects. Third, the measurements could have been contaminated by some minor artefacts that the ICA could not decompose. In either case the subject would not have any signal to contribute with, and would have to be excluded.

The components that were not selected were subtracted from the raw EEG to create datasets pruned from noise, artefacts and neural activity that were not related to mu desynchronization. Thereby only the selected components were represented in the scalp channel activity of the pruned datasets. These components accounted for 1.4% of the variance in the raw data (each component ranging from 0.1% - 3.7%).

To control the validity of the selection procedure, the selected components could be interchanged with random components. The resulting datasets should not show any significant effects, as randomly selected signals tend to cancel out each other.

Statistical analysis

First, the statistical procedure was performed channel wise on the raw EEG datasets ($n = 32$). The same procedure was then repeated on the pruned datasets ($n = 23$) as a more elaborate analysis of the signal, and five times using randomly selected components. A time/frequency analysis using discrete wavelet transforms were performed on each channel's (or component's) conditions using the standard EEGLAB `timef` function. The window size was 64 samples (256 ms) wide, and Hamming windows was applied 200 times at an average step of 2.191 samples (8.76 ms). The EEGLAB `timef` function returned 20 frequency bands ranging from 2.0 Hz to 49.8 Hz and 200 time points ranging from -800ms to 800ms from time lock. However, as we were mainly interested in the 5-9 Hz frequency band, this interval was averaged and the goal-directed and non-goal-directed conditions were compared with reference to this measure at every time point. As multiple significance test inflates the risk of Type 1 errors only clusters of 10 or more adjacent significant p-values ($p < 0.05$) were considered. The most prominent channel was then analysed in further detail using pixel wise t-tests in all frequency bands between 2 and 20 Hz. To control for multiple testing of these 200x20 time/frequency points only clusters of 20 or more adjacent p-values ($p < 0.05$) were considered significant.

Results

The experimental design was very successful as indicated by the low exclusion rate. Only 2 infants out of 34 were excluded because of insufficient data. The remaining 32 infants watched the model attentively during more than 10 trials in each condition. The data from these infants was analysed using the raw data as well as the data from selected independent components. In the analysis of selected components', the t-tests between the goal-directed and non-goal-directed conditions showed a significant desynchronization of the mu rhythm band for the goal-directed condition when the hand touched the object. A global power minimum of -1.6 dB was found approximately 10 ms after time lock ($p=0.0007$) for channel number 105 in the EGI Geodesic Sensor Net 128 channel v2.0. This minimum lies in a significant interval that starts approximately 60 ms before time lock and remains until 55 ms after time lock. The channels with the largest difference between the goal-directed and the non-goal-directed movement conditions are located on the right frontal lobe and in central areas, as shown in Figure 3 (channel numbers 103, 104, 105, 107, 110, 111 and 123). Two more channels with significant desynchronization were located in the left frontal lobe (over prefrontal cortex, channel numbers 29 and 35) and finally two over the occipital lobe (channel numbers 71 and 76). A magnified view of the most significant channel is presented in Figure 4, which also shows the amplitudes of 5-9 Hz for all conditions. A detailed time/frequency analysis of the most significant sensor is presented in Figure 5. The difference between the goal-directed and non-goal-directed condition had a global minimum of 1.6 dB 10 ms after the hand touches the object for the frequency 6.8 Hz ($p=0.0002$). The significant effects start at 75 ms before time lock and remain until 170 ms after time lock.

To test the validity of the t-tests a bootstrap analysis using 5000 permutations was performed. The bootstrap test was chosen as it does not rely on normally distributed data. The significant t-tests were all included in the significant bootstrap results. Using the raw EEG data, point wise statistical tests (two tailed t-tests, $n = 32$) showed no significant desynchronization in any of the channels analyzed. The 5-9 Hz amplitude difference between the goal-directed and non-goal-directed condition of each channel is shown in Figure 6. The p-values are thresholded at $\alpha = 0.05$ (adjusted for multiple testing by removing intervals with less than 10 significant p-values). No significant effects were found in the five runs with EEG control data using randomly selected components.

Discussion

The results from the selected components show a greater desynchronization of the mu rhythm in 8-month-old infants when they observe goal-directed actions compared to when they observe non-goal-directed actions. In relation to studies performed on adults the desynchronization onset is very similar (Muthukumaraswamy & Johnson, 2004b). The significant effect remains for about 120 ms. It is a conservative estimate, as individual variation in processing time between trials will decrease the effects when the mu suppression does not overlap. Thus, 8-month-olds display adult like mu rhythm perturbations when observing goal-directed actions, which indicates that they have a relatively mature MNS.

The raw data showed no such desynchronization. This is probably a consequence of many ongoing and interfering neural processes. The fact is that the selected components based on mu rhythm desynchronization only account for 1.4 % of the variance of the raw data. While

this is not much, we might not have reasons to expect more from a small subset of neurons competing with other active brain areas. It should be noted that the strength of the signal of interest is not related to the reliability, validity or significance as long as it can be detected and extracted from the surrounding noise. One way to reduce noise is to collect and average much raw data. As it was impossible to gather more information from each infant (due to attention, fussing and fatigue) we believe that problems with mixed signals are best solved by using blind source separation techniques such as ICA.

At the piloting phase of this study, an execution condition was included in which the infants reached themselves. However, this condition always introduced strong motion artefacts and the ICA analysis did not decompose any condition into useful components. The execution control condition was therefore not included in the main experiment but as a final demonstration of MNS activity in it is of crucial importance to include such a condition. Since we conducted the present research, however, Southgate et al. (2009) were able to measure mu rhythm desynchronization both during self produced actions and during action observation in 9-month-old infants. Similar desynchronization occurred during both conditions demonstrating that infants exhibit sub-threshold motor activity during action observation that matches directly the neural signal occurring during their own actions.

Another important issue is whether any other self-movements by the subjects could cause mu suppression and thereby contaminate the measurements. The infants did move occasionally but these movements were usually small, evenly distributed over the whole recording session, and any resulting unwanted mu suppression would just reduce the grand averages (just like artefacts in conventional ERPs). The strong time locking of the mu suppression implies that the significant effects are not related to randomly occurring movements by the subject. However, if the significant effects are the result of systematic self movement, this in itself would be a strong argument that the MNS has been measured. Since the mu suppression is precisely time locked to the touch of the object, the infant must then have predicted the goal of the action and started to plan that motor movement in advance. Also, a synchronized motor response could indicate an immature inhibition of mirror neuron motor resonance.

In contrast to the raw data, the analysis of the selected components shows a high degree of consensus. Many adjacent channels show a significant desynchronization in the goal-directed condition compared to the non-goal-directed condition (Figure 3). This desynchronization is most significant over central areas where we can expect projections from motor areas and where the mu rhythm has been assessed by previous studies (see Pineda, 2005). There are also significant effects for sensors over frontal areas in the right hemisphere. The timing of the desynchronization from the time of touch until less than 100 milliseconds after suggests that the desynchronization is tuned to the goal of the action.

The results of the present method depend on the component selection procedure. The procedure assumes that the infant MNS is functioning in a similar fashion to the adult MNS, and uses the characteristics of the mu response of adults who observe stationary models and models performing goal-directed actions. As the MNS hypothesis states that goal-directed action will result in more mu rhythm desynchronization than just observing movements in general, we can focus on independent components with mu rhythm. In this process most extracted components are discarded, but without any loss of validity. For example, some of the excluded components can show an opposite effect in the statistical test, but since they do not show any mu increase in the baseline they are not related to the MNS. An issue of greater

concern has to do with the inclusion of components that are not related to MNS activity, or exclusion of components that really are related to MNS activity. In both cases we decrease the signal to noise ratio and end up with a too conservative measure of the MNS.

While this analysis of EEG source dynamics as independent components does not require an explicit head model one might argue that the signal could reflect posterior alpha waves within the same frequency band (Marshall et al., 2002). One argument against posterior alpha confounding is that visual differences (that would affect the alpha waves) between the tested conditions were minimized. All objects were present in the scene in all conditions, and the time-locking of the EEG occurred when the hand had decelerated and touched the toy train or the table. Furthermore, the significant sensors in the occipital area could be explained by the dipole field of the stronger signal from the right frontal lobe. In the enlarged plots (Figure 4 and 5) all conditions are shown. These plots show that the mu rhythm power has the greatest amplitude when the infant observes a passive model, that it is lower when the infant observes a simple arm movement, and that it is significantly lower when the infant observes a model grasping an object. All these characteristics have been related to mu perturbations in adults and the adult MNS. This robust desynchronization represents the infant equivalence of the adult mu rhythm and that it is tightly linked to MNS activity. It is in agreement with previous adult mu rhythm studies with reference to the timing of desynchronization, the significant frequencies, and the source locations (Pineda, 2005; Muthukumaraswamy et al., 2004a; Nishitani & Hari, 2000; Hari et al., 1998; Oberman et al., 2005). Furthermore, it is also in agreement with earlier studies on the frequency of infant mu rhythm (Orekhova et al., 2006; Gelisse & Crespel, 2006). Thus, the current study yields a valid measure of MNS activity in infants.

Although the present study reports how the mu rhythm is modulated by the goal-directedness of the action, it raises the question how the static baseline relates to the other conditions. Since the adult MNS has been shown to respond to non-object directed actions, also called intransitive movements (Rizzolatti & Craighero, 2004), it is possible that also the infant MNS would respond in a similar way. To test this we compared the non-goal-directed trials with the static trials using the raw data. We also pooled the goal-directed and non-goal-directed trials and compared them with the static trials to test the mu modulation between the baseline and hand movements. This test showed strong mu suppression during hand movements both in frontal and parietal areas.

While reliable mu rhythm desynchronization was obtained in the present study, this was not the case in Nyström (2008) when testing 6-month-old infants. The difference between these two studies, however, was not only a question of age. Video recorded action sequences were used in Nyström (2008) while a live model was used in the present study. Several earlier studies have shown a weaker mu rhythm desynchronization to televised actions in adults (Järveläinen et al., 2001). Using NIRS on 7-month-old infants, Shimada and Hiraki (2006) found that the activation of the motor areas was only significant in the live setting. Thus, it is possible that a live model would also induce mu rhythm desynchronization in 6-month-olds.

On the other hand, Falck-Ytter et al. (2006) did not find evidence that 6-month-old infants produce eye movements that predicted the outcome of observed manual displacement actions. Instead they tracked the object to the goal. This indicates that displacement actions are not within the repertoire of infants of this age. We have not been able to get infants that young to perform manual displacement actions. Six-month-olds rarely move objects to external goals

while 12-month-olds do it all the time. According to the MNS hypothesis, only observed actions that are established in the subject's own motor repertoire are mirrored. The grasping action demonstrated in the present study is clearly within the repertoire of the 8-month-old subjects. Most infants perform grasping successfully at 4 months of age and at 8 months they are quite skilled at various grasp configurations (von Hofsten & Rönnqvist, 1988; Newell, MacDonald, & Baillargeon, 1993). Another implication of this hypothesis is that the more an action is exercised, the more is the MNS activated when that specific action is observed. Recently, this implication received support from a study of crawling toddlers (van Elk, et al., 2008). To investigate the effect of natural motor experience on motor resonance during action observation, 14- to 16-month-old infants' EEG was recorded during observation of action videos. Stronger mu- and beta desynchronizations were found for observation of crawling compared to walking videos and the size of the effect was strongly related to the infant's own crawling experience.

Beside van Elk et al. (2008) and Southgate et al. (2009) the present study is the first to demonstrate significant mu rhythm desynchronization to observed actions in infants. The van Elk et al. (op. cit.) study was conducted on 14-month-old infants and the obtained contrast between observation of crawlers and walkers was strong enough to be identified with a simple time-frequency analysis indicating a more mature mirror system in these infants than in the 8-month-olds of the present study. Southgate et al. (2009) also used a time-frequency analysis to identify desynchronization relative to baseline. What is unique with the present study is that, using a source analysis, we showed more desynchronization when the observed action was distinctly goal-directed (grasping an object) than when the model just placed the hand on a table. The results strongly indicate that the MNS is functional in 8-month-old infants. Behavioural data support these results. Using the habituation/dishabituation paradigm, Woodward (1998) found that 6-months-old infants are sensitive to the action goal of others but only when performed by a human agent (Woodward 1998). Sommerville and Woodward (2005) found that 10-months-old infants identify the goal of action sequences but only to the extent that they can perform them.

The concept of the MNS system is not uncontroversial. Some studies using fMRI have failed to identify such activity in adults (Dinstein et al. 2009; Lingmau et al., 2009) while others have found it (Chong et al. 2009). The question is whether fMRI is an optimal method for studying these processes. Our data indicate that the neural processes during action observation that matches the neural signal occurring during their own actions are confined to a rather short time interval. Thus, the difficulty in detecting MNS activity with fMRI may be due to its low time resolution. When using MEG, robust evidence of activation from the motor cortex during action observation is obtained (Hari et al. ,1998).

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Figure captions

Figure 1.

Photo of the experimental setup from the subjects' viewpoint. Top, the non-goal-directed condition when the hand touches the tabletop. Bottom, the goal-directed condition just before touch of the object.

Figure 2.

Power spectra of component activity. The highlighted interval (5-9 Hz) was used for determining the mu rhythm power. The red line mark the "static"-condition and the two blue lines mark the two movement conditions ("goal-directed action" and "nongoal-directed action"). Top, example of mu rhythm power peak estimation in a single component. Bottom, mean of all selected components power spectra.

Figure 3.

Mean amplitude differences of the 5-9Hz frequency band between the goaldirected and non-goal-directed conditions in the pruned datasets containing only the selected independent component projections. Each curve represents a channel and the layout describes the spatial relations of the sensors on the scalp (nose pointing upwards). Significant differences between conditions are marked with yellow intervals, and these channels are shaded ($n = 23$, $p < 0.05$ and corrected for multiple testing). The significant sensors in right central areas are channel numbers 103, 104, 105, 107, 110, 111 and 123. Two more channels with significant desynchronization were located in the left frontal lobe (over prefrontal cortex, channel numbers 29 and 35) and finally two over the occipital lobe (channel numbers 71 and 76). The channel with longest significant interval is marked with a red circle. This sensor is presented in more detail in Figure 4 and 5.

Figure 4.

A presentation of the separate conditions in the most significant sensor (marked with a red circle in Figure 3). The left row shows data from the raw datasets, and the right row shows data from the pruned datasets that only contains the selected independent component projections.

Figure 5.

A presentation of the separate conditions in the most significant sensor (marked with a red circle in Figure 3) using the time/frequency decomposition returned by the EEGLAB timef function. The frequencies are truncated above 20Hz since these frequencies were filtered out before the ICA. The left row shows data from the raw datasets, and the right row show data from the pruned datasets that only contains the selected independent component projections. The bottom row shows the point wise statistical t-tests' p-values (left plot $n = 32$, right plot n

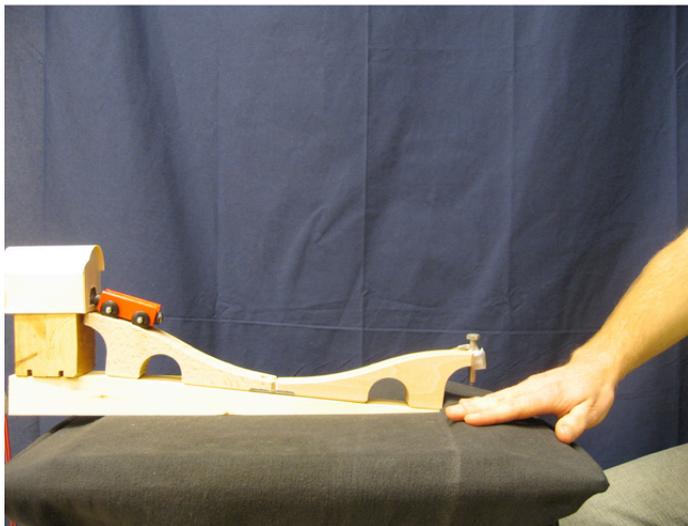
= 23), thresholded at $\alpha = 0.05$, adjusted for multiple comparisons (200x20 tests) by removing significant clusters smaller than 20 pixels.

Figure 6.

Mean amplitude differences of the 5-9Hz frequency band between the goal-directed and non-goal-directed conditions in the raw datasets ($n = 32$). Each curve represents a channel and the layout describes the spatial relations of the sensors on the scalp (nose pointing upwards). There was no significant desynchronization in any condition.

Figure 1

Non-goal-directed condition



Goal-directed condition

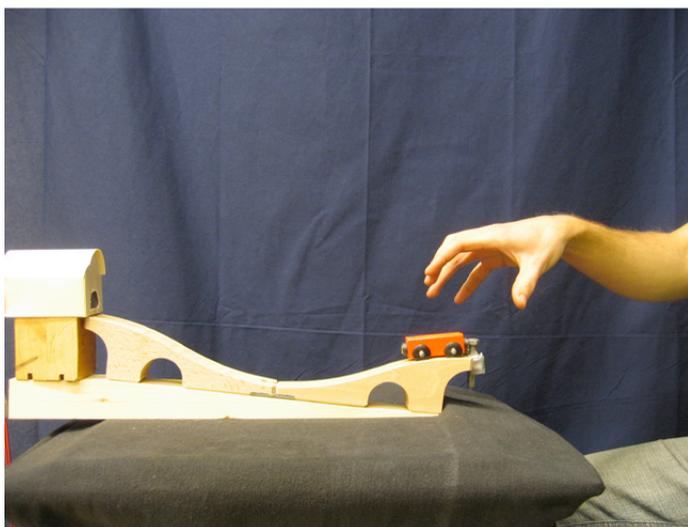


Figure 2

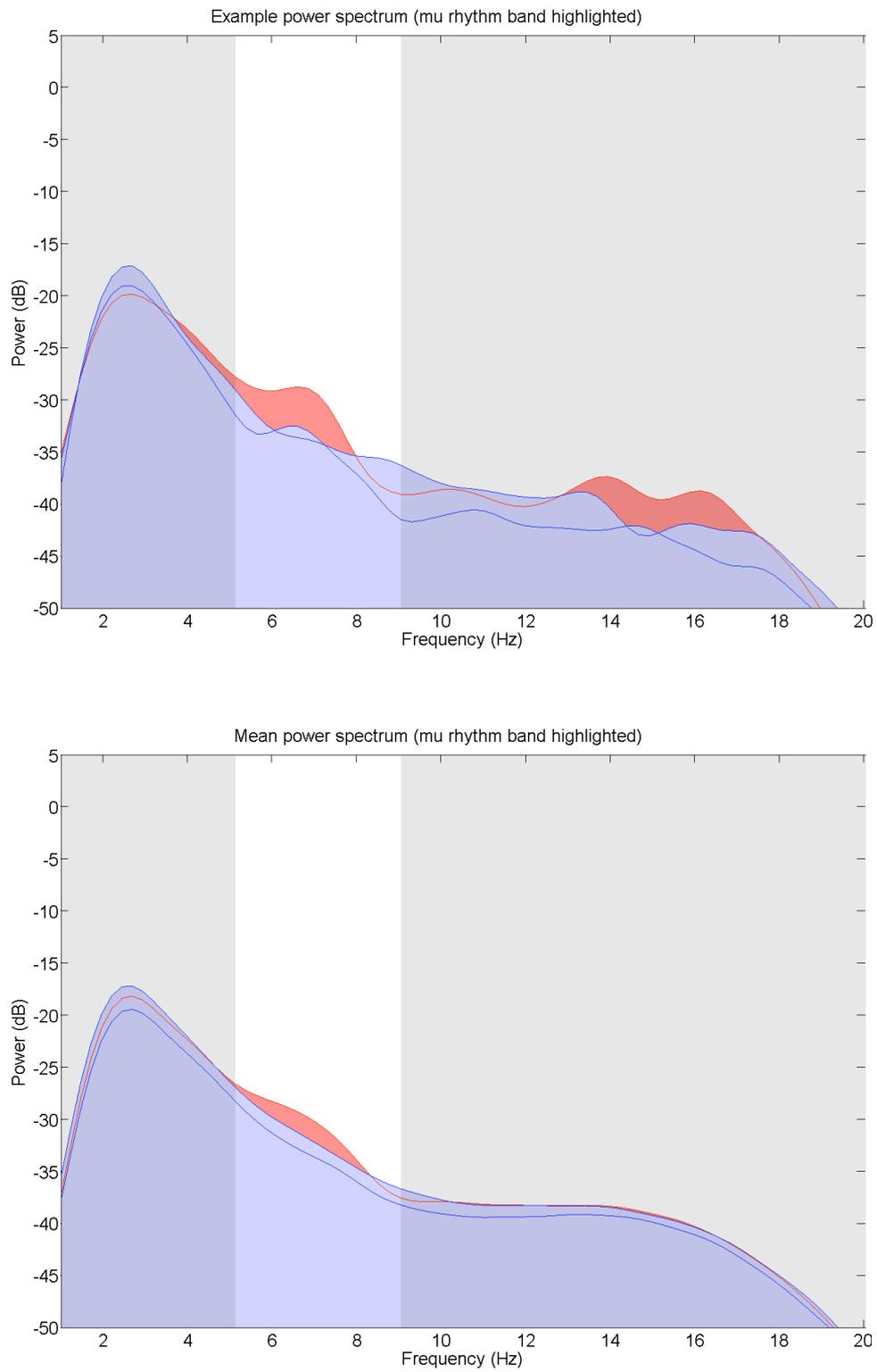


Figure 3

Goal - nogoal on selected components

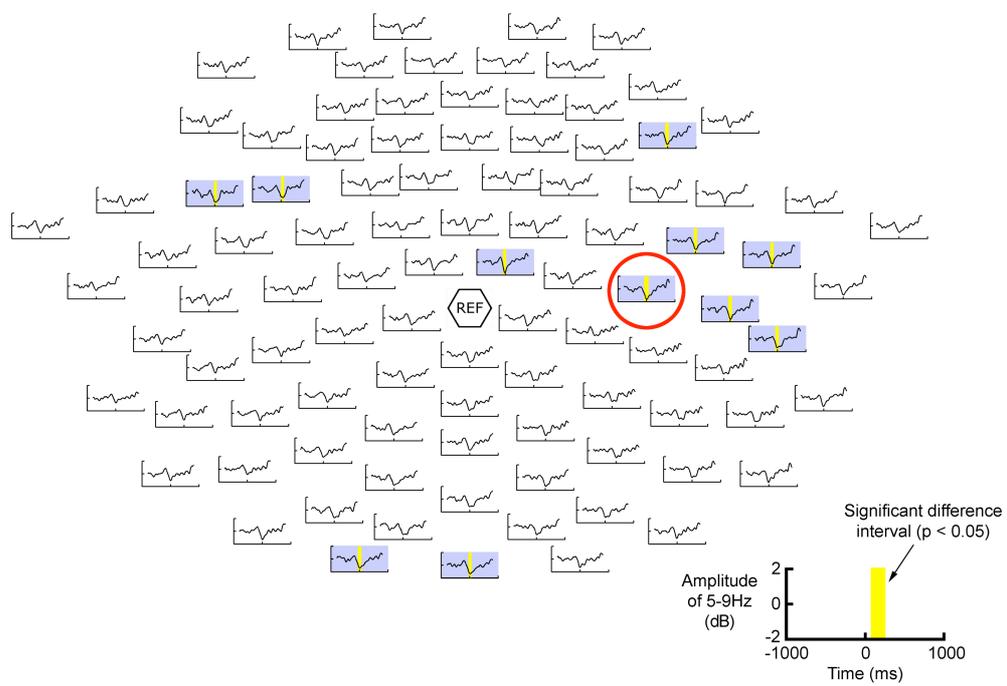


Figure 4

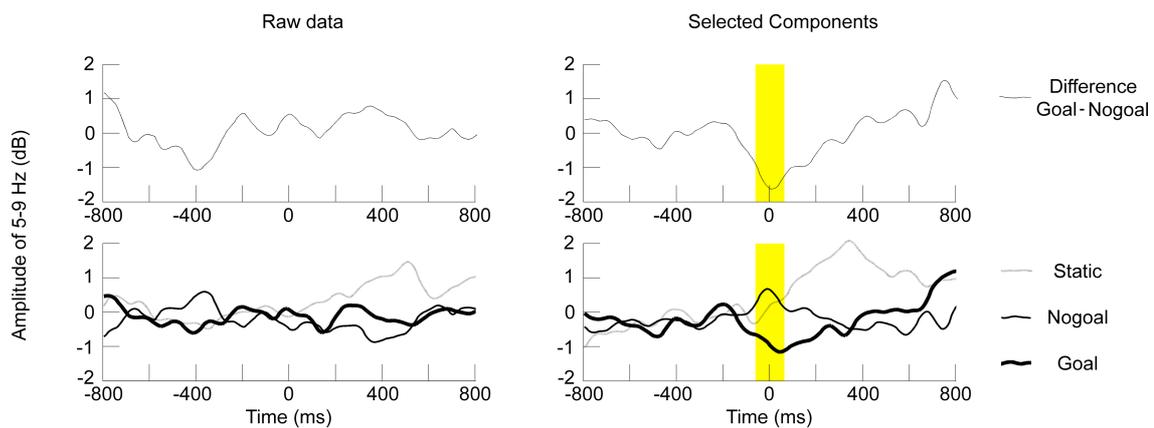


Figure 5

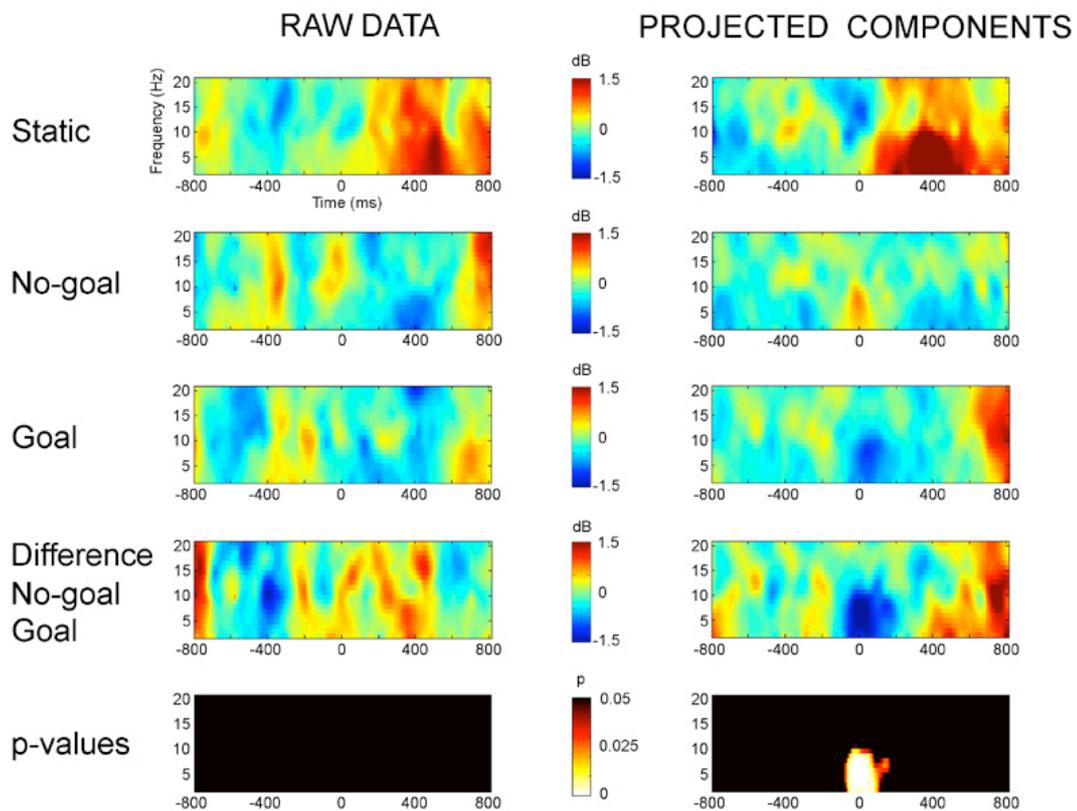


Figure 6

Goal - nogoal on raw data

