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From Motion to Movements

Revelations by the Infant EEG

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Abstract
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The introduction of high density EEG (hd-EEG) nets for easy application on subjects of all ages has improved the possibilities to investigate the development of the infant neurophysiology. This dissertation consists of three studies (I – III) that investigate the visual motion system and mirror neuron system of the infant, and methodological sections that outline the bioelectrical background and the characteristics of the methods used.

Study I covers the maturation of cortical areas involved in motion perception in adults and infants using an ERP paradigm. Over three age groups (2, 3 and 5 month olds) the cortical activation increased dramatically. All infant groups showed significant activation when moving displays was contrasted to static displays on a video screen. The study shows that 5-month-old infants and older can be expected to process motion in a similar fashion as adults.

Study II covers the infant mirror neuron system (MNS). In adults the mu rhythm perturbations is considered a reliable measure of activation of the MNS. This study presented both a mu rhythm analysis and a ERP analysis to detect MNS activity in 6-month-olds and in adults. This study concludes that the infant MNS can be measured using ERPs and that the development of mu rhythm perturbations requires further study.

Study III focused on exploring the mu rhythm suppressions. 8-month-olds observed a live actor that performed goal directed reaches and non-goal directed hand movements. The results show robust mu rhythm perturbations time-locked to the grasping moment. The study concluded that the MNS activity is possible to evaluate by analysis of mu rhythm perturbations and that the MNS show mature characteristics at the age of 8 months.

In summary, Study 2 and 3 present new methods to investigate the infant mirror neuron system and shows that the infant MNS is active at 6 months of age. At 8 months of age the infant MNS show mature EEG responses to simple actions such as reaching. How the MNS development relates to the infants' motor development, and how the MNS interacts with the development of social skills requires further studies that could benefit from the methods presented here.

Keywords: infant, brain, EEG, mirror neurons, ERP, ICA, MNS

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This thesis is dedicated all parents and children who helped us in our pursuit.

List of papers

I Rosander K., Nyström P., Gredebäck G., von Hofsten C. (2007) Cortical processing of visual motion in young infants. Vision Research
Volume 47, Issue 12, June 2007, Pages 1614-1623

II Nyström P. (in press). The infant mirror neuron system studied by high density EEG. Journal of Social Neuroscience.

III Nyström P., Ljunghammar T., Rosander K., von Hofsten C. (submitted) Using mu rhythm perturbations to measure mirror neuron activity in infants.

Contents

Abbrevations and common concepts

Dipole	An electric dipole consists of two charges of opposite signs that are close to each other. The charges give rise to an electrical field that is extended in space with a characteristic spatial orientation. See figure on page 24.
Dipole moment	The dipole moment describes the orientation and magnitude of the dipole electric field.
EEG	Electroencephalography is the measure of electrical fields produced by the brain. The electrical fields can be measured by sensors (electrodes) placed on the scalp. The raw data consists of one waveform from each sensor of potential differences over time.
ERP	Event Related Potential. See figure on page 32.
ERSP	Event Related Spectral Perturbations. See figure on page 32.
fMRI	Functional Magnetic Resonance Imaging
ICA	Independent Component Analysis is a signal processing solution that separates different signals that are maximally statistically independent of each other (Example is found on page 27). It is primarily used to remove artifacts from MEG and EEG signals.
Independent component	A dimension of data that accounts for a signal that is maximally statistically independent from other dimensions in the same data.
ITC	Inter Trial Coherence. See figure on page 32.

MEG	Magnetoencephalography is the measure of magnetic fields produced by the brain.
Mirror neurons	Mirror neurons are motor neurons that are activated when we perform our own goal-directed actions as well as when we observe somebody else perform the same action. This direct matching may provide a mean to understand other peoples' actions.
MNS	The Mirror Neuron System
MT/MST	A motion sensitive area in the junction between occipital, parietal and temporal areas.
Mu rhythm	A sensorymotor rhythm that oscillates at approximately 8-13 Hz in adults and approximately 5-9 Hz in infants. In adults the rhythm disappears during movement and during action observation. It has therefore been linked to MNS activity.
PET	Positron emission tomography
Source localization	An estimation of the 3-D dipole location of an EEG signal.
STS	Superior Temporal Sulcus, a brain region involved in perception of biological motion.
TMS	Transcranial Magnetic Stimulation is a non-invasive method to activate neurons in the brain by inducing weak currents by changes in magnetic fields.

Introduction

Anyone who is interested in the functioning of the brain will find an overwhelming literature describing the adult brain processes. There are many successful methods of functional brain mapping used on adults (PET, fMRI, and MEG among others) which have greatly increased our knowledge of the brain. Although the knowledge of the adult brain has important implications for individuals as well as society in whole, there are at least two reasons to investigate the developing brain in early childhood.

First, the adult brain is a complex system of cognitive, perceptual and emotional processes that work simultaneously. In this parallel distributed network it can be hard to determine which processes builds on which, as the information streams seamlessly between different brain processes. Also, these processes may or may not be separated into distinct brain areas which can be measured independently. In these cases, when the causal relationships between processes are impossible to disentangle, the maturation of different brain areas in development may reveal how they become interconnected into functional systems.

Second, and more important, in the case of developmental disorder it is important to be able to diagnose and intervene at an early stage. Of course, to tell what is abnormal it is necessary to know what is normal development and normal variation between individuals.

Then, how much do we know of the developing brain? Today relatively little is known about infant brain processes. The reason for this ignorance is that most of the successful methods used in adults cannot be applied to small children for practical and ethical reasons. All these methods are very sensitive to movement artefacts and, in addition, PET requires a radionuclide to be injected into the brain. However, there are a few methods suitable for functional studies of infants' brains. One of these methods is electroencephalogram (EEG), and the

advent of modern EEG equipment and analysis makes a detailed infant brain mapping possible.

Figure 1. An infant is waiting to get a high density EEG net prepared. The 128 sensors should be placed with good contact to the head to capture the electrical fields that are generated in the brain and continue outside the scalp.

EEG measures electrical fields that are generated when a brain area is activated. These fields extend through the skull and can be measured using two or more electrodes that are placed on the scalp. The sensors are very light weight (which allows the subject to move quite freely) and the temporal resolution is excellent. Early use of EEG utilized few sensors, since each sensor was applied separately. This gave information of the time-course of activation, but did not distinguish different brain areas very well. Later, sensor caps were designed to make application easier and measure specific points on the scalp (usually the 10-20 system). However, the spatial resolution of this setup is very crude. Today a typical sensor cap has between 64 and 256 sensors, arranged in a geodesic grid over the scalp (as in *Figure 1*). Such a high-density EEG (hd-EEG) is applied like an ordinary cap and takes less than a minute to apply on infants.

One important feature of the high-density-EEG (hd-EEG) is that it allow for estimation of the distribution of electrical fields, and not only the time course of activation potentials at specific scalp locations. This means that underlying electrical dipole locations in the brain can

be estimated, thus giving a 3 dimensional point of activation origin. While this point still is an approximation of an area, the spatial resolution of hd-EEG is much higher than the preceding EEG methods.

Since hd-EEG is a relatively new, there are many questions that could benefit from this technique. This thesis concerns two important issues of infant cortical activity. One issue concerns the cortical activation related to motion perception and the other issue concerns activation related to perception of others' movements and actions. These issues are related to each other in a fundamental way: motion perception is a prerequisite for movement perception, and thereby also for action perception and action understanding.

Cortical activation by visual motion

Much of our perception involves vision. At the lowest level, vision is about detecting static contrasts in the instant view of the visual field. However, as soon as the eyes are redirected, the whole visual field moves. Also, the surrounding environment change constantly as objects and people move around. It is therefore important to be able to detect and process visual motion during interaction with the surrounding.

Visual motion has been studied both in adults and in infant populations, but the brain areas that have been related to different aspects of motion perception mainly comes from adult and comparative studies. One area of particular interest is the MT/MST area that lies at the junction of the occipital, temporal and parietal lobe. This area is also sometimes referred to as MT+ or V5. The MT/MST is activated by visual motion, processes perceived motion direction and is crucial for the control of smooth pursuit eye movements. Patients with brain lesions that include the MT area have impaired motion perception (Zeki, 2004) and cannot perform smooth pursuit eye movements (Schoenfeld, Heinze, & Woldorff; 2002).

As smooth pursuit eye movements develops rapidly between 6 and 14 weeks of age (von Hofsten & Rosander, 1997; Rosander & von Hofsten, 2002) it can be argued that the MT/MST complex via the primary visual pathway is not functioning before that age. There are also EEG studies that investigate at what age the cerebral cortex starts to

process visual motion. Braddick, Birtles, Wattam-Bell, and Atkinson (2005) studied motion direction sensitivity in young infants and concluded that the cortical response becomes progressively stronger between 5 and 18 weeks of age. Also, between 6 -14 weeks of age the infants' ability to discriminate motion direction improves rapidly (Wattam-Bell, 1991; Atkinson, 2000). However, in these studies only few sensors were used, and it was not possible to determine how the visual motion information was propagated between different brain areas.

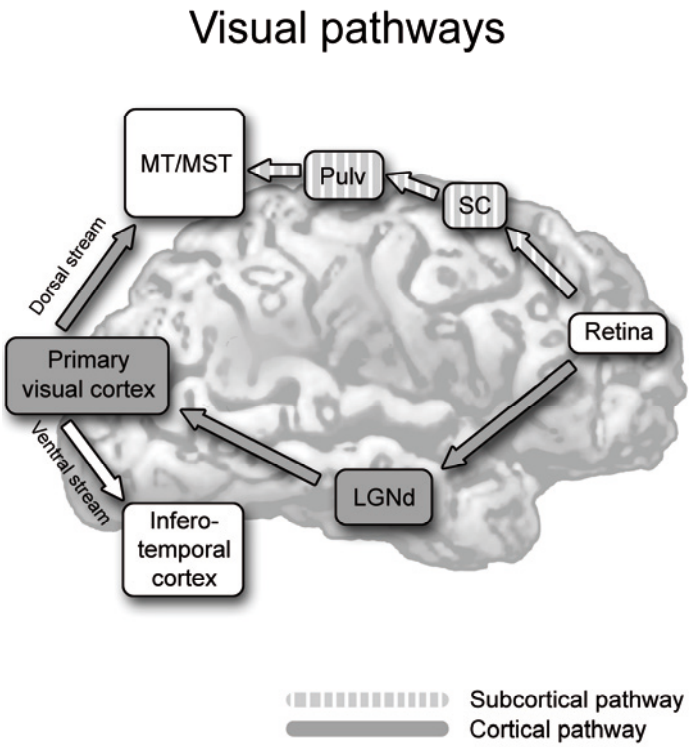


Figure 2. Schematic diagram of two parallel visual pathways.

The MT/MST area mainly receives information from two parallel visual pathways (see *Figure 2*). The retina, at the back of the eye, sends visual information both to the primary visual cortex (V1, V2 and finally to V5) in the occipital lobe via lateral geniculate nucleus (LGN) of the thalamus, and to a subcortical pathway to MT/MST via the superior colliculus (SC) and pulvinar (Sincich, Park, Wohlgemut, &

Horton, 2004, ffytche, Guy, & Zeki, 1995; Buchner et al., 1997; Schoenfield et al., 2002; Callaway, 2005; Schneider & Kastner, 2005). The pathway via SC is suggested to be a phylogenetic old pathway, functioning for non-conscious fear (Morris et al., 1999) and fast moving stimuli (Buchner et al., 1997, ffytche et al., 1995).

However, the primary visual pathway is not only activated by motion. In addition, cells that detect mere contrast changes (or flicker) are activated as the visual scene change. It is therefore necessary to distinguish motion activation from flicker activation in order to determine the development of motion perception. One way to do this is to investigate the proportion of activations in different brain areas. As flicker sensitive cells are more common in V1 than in MT/MST, a flickering stimulus should elicit more activation in V1. In the MT/MST of adult subjects, the flickering response is only about 20-50% compared to V1 (Sunaert et al., 1999).

By using high density EEG it is possible to determine when the different visual pathways mature during infancy, and how information is propagated between interconnected areas. Also, the spatially different distributions of cells sensitive to motion and to flickering makes it possible to evaluate the degree to which visual motion activates these two different kinds of cells in young infants. These questions were considered in study I of this thesis.

A functional motion processing system is necessary to perceive movements, but it is not sufficient to understand the meaning of the visual input. The first issue of this thesis regards visual motion. The other issue of this thesis extends to how we parse the movements of other people into meaningful actions.

Activation related to the mirror neuron system

Most people appreciate their relations to other people as one of the most rewarding and important aspects of life. Imagine yourself without the ability to understand the actions and intentions of the ones you love, without sharing their feelings and perhaps even without recognizing their words. These social skills should not be taken for granted; they do not come from nowhere but are rather a part of a complex cognitive system in the brain. As such they are exposed to the real

world and could be damaged by trauma, disease or developmental disruptions. As such they can also be studied by scientific methods to help us understand the functions of the systems, and how to aid treatments of dysfunction.

During the last decade a growing set of studies has investigated the adult mirror neuron system (MNS), which has been suggested to lie at the core of human social cognition. The MNS has been suggested to facilitate action understanding by a direct matching process where the observer engages the same cognitive functions as the action performer. At the cellular level of the MNS the individual mirror neurons share this characteristic: mirror neurons typically fire when a subject performs a goal directed action, but also when the subject observes someone else perform the same action. The mirror neuron system will be described in the following section.

The adult mirror neuron system

The mirror neurons were first discovered by Rizzolatti and collegues (Di Pellegrino, Fadiga, Fogassi, Gallese & Rizzolatti., 1992; Gallese, Fadiga, Fogassi & Rizzolatti, 1996; Rizzolatti, Fadiga, Fogassi & Gallese, 1996a) in prefrontal areas by single cell recording in the macaque brain. Particulary area F5, which corresponds to Broca's area in humans, and motor cortex were found to contain a high number of mirror neurons. It was estimated that approximately one third of the motor neurons used for grasping also showed mirror properties. Also, if was found that observation of grasping and lip movements elicited the most reliable mirror neuron activations (Fadiga & Craighero, 2004).

Later, further single cell studies found other brain areas that contained mirror neurons. These were located in the parietal lobe and in the superior temporal sulcus (Rizzolatti & Craighero, 2004).

One important finding is that the mirror neurons are tuned to goal directed actions rather than body movements. For example, a mirror neuron that fires when a monkey observes somebody grasp an object will not fire when the monkey observes the same grasping movement into thin air (Fadiga & Craighero, 2004). The object can also be situated out of sight, but if the monkey knows it is there, and the hand

reaches for it, the mirror neurons will still fire. Also, it has been found that a familiar sound from a goal directed action can trigger mirror neurons, without any visual cues (Keysers, Kohler, Umiltà, Nenetti, Fogassi & Gallese, 2003).

These findings propose that mirror neurons are primarily involved in action understanding. But the direct matching properties of mirror neurons also suggest a link to other social phenomena such as intention understanding and empathy (Théoret & Pascual-Leone, 2002; Gallese et al., 2004). It is therefore no wonder that the discovery of mirror neurons in monkeys started an intense search of the human homologue.

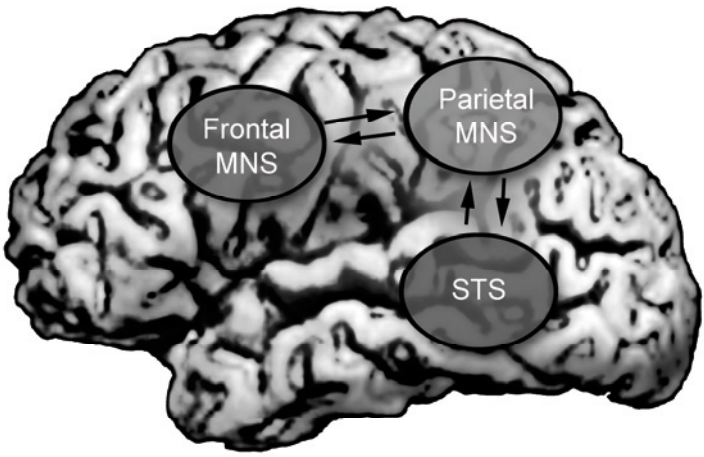


Figure 3. Schematic view of the areas included in the mirror neuron system.

While the mirror neurons have been studied with single cell recording in monkeys, almost all human findings come from neural population studies. In these studies groups of mirror neurons are assumed to increase the activation at different brain areas. This activation can then be studied. Today there is robust support than humans have mirror neurons much in the same way as monkeys do. The evidence come from numerous studies using different neurophysiological methodologies such as TMS, EEG, MEG, fMRI and PET (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). Mirror neuron activity in human adults have primarily been found in premotor cortex (motor areas and Broca's area), the inferior parietal lobule and in the superior temporal sulcus (Rizzolatti & Craighero, 2004). Together these areas form a mirror neuron system (MNS), as depicted in *Figure 3*. These studies confirm the findings in monkeys; the MNS is activated both when a subject perform an action and when the subject observes somebody else perform the same action. Also, the human MNS responds stronger to goal directed actions than to non-goal-directed actions (Muthukumaraswamy & Johnson, 2004; Lepage & Théoret, 2006). Although this is very similar to the macaque MNS there are some important differences.

It appears as if the human MNS is activated not only when the observed action is geared toward a physical object, but also when the observed action is an abstract gesture. Actions that involve an object have been termed transitive actions, and actions without a physical object are termed intransitive actions (Fadiga & Craighero, 2004). The monkey MNS only parse transitive actions and is not activated by intransitive actions. The human MNS is activated by both.

The fact that the human MNS is also activated by abstract actions, such as someone mimicing tea-drinking, has led to the speculation that the human MNS may underlie abstract and symbolic communication. It is enough to see the abstract gesture of tea-drinking to activate our own tea-drinking motor representation and thereby understand the meaning of the gesture. Also, the sensitivity of mirror neurons to lip movements suggests a link to speech perception. Mirror neurons could thus play an important role in uniquely human abilities such as language (Théoret & Pascual-Leone, 2002; Gallese et al., 2004).

There have also been studies of higher cognitive functions such as emotional understanding and theory of mind in relation to mirror neurons. For example, Gallese, Keysers & Rizzolatti (2004) showed mirror activation in the amygdala during emotion expression and emotion observation. This is partially related to theory of mind, where people

infer other people's beliefs and desires by subconsciously simulating the mental states of the other person. Problems with this kind of social understanding are typical symptoms in subjects with autism spectrum disorder. Indeed, several studies indicate that autistic patients often have reduced mirror neuron activity or mirror neuron dysfunction (Dapretto et al., 2006; Nishitani, Avikainen & Hari, 2004; for a review, see Williams et al., 2001 or Lepage & Théoret, 2007). This supports the hypothesis that mirror neurons play an important role in social cognition. However, autism is characterized by problems in multiple fields (social, communicative and motoric) and it may be hard to disentangle the role of mirror neurons from other factors.

Taken together, the MNS seems to be involved in action- and intention understanding, language, empathy and theory of mind. It has therefore been suggested to provide a unifying framework for all social cognition (Gallese, Keysers & Rizzolatti, 2004), which has both practical and theoretical implications. While the importance of mirror neurons has been mostly hypothetically or speculatively stated, we can turn to child development to get more concrete evidence of how mirror neurons relate to social skills. Considering that these social skills appear in infancy and early childhood, we can relate the appearance of MNS activity to the appearance of various social skills. Such studies can validate or falsify the importance of the MNS. Infancy is also an important period when MNS dysfunction could be detected and intervened. So, what do we know from infant studies?

The infant mirror neuron system

Unfortunately our understanding of the infant MNS and the development of the MNS is very poor. Only recently have studies started to assess the infant MNS, and very few of these have been direct measures of MNS activity. The main evidence of MNS activity in infants comes from indirect behavioural studies.

One line of MNS evidence is related to imitation research. While mirror neurons are not sufficient for imitation, they may be necessary (Rizzolatti, 2005). If this holds true then infants that imitate should have a functional MNS. Imitation in infants has been shown at different ages. The most debated case is newborn facial imitation, where infants increase their number of tongue protrusions after seeing an

adult do this. It has been suggested that this is evidence of an innate mirror neuron system. However, the tendency of increased tongue protrusions could also be elicited by other stimuli such as moving objects. The effect could thus be explained by a higher state of arousal. Also, the tendency of newborns to imitate facial movements disappears after a few weeks and returns in a different form some month's later (Lepage & Théoret, 2007). More clear-cut evidence of action imitation has been found from 6 months of age (von Hofsten & Siddiqui, 1993). At 9 months of age infants perform deferred imitation (Meltzoff, 1988), and shortly before one year of age the tendancy to imitate increases very much (Tomasello, 2000).

Other studies have focused more on the MNS itself rather than on imitation. Falck-Ytter, Gredebäck & von Hofsten (2006) used gaze tracking and predictive looking as a measure of action understanding. They found that 12-month-old but not 6-month-old infants looked proactively at the goal of a hand transporting an object there. When the same object motion was shown without the hand, the eyes just tracked the object. Proactive looking at the goal is functional when subjects generate the action themselves but not when they just observe it. If, however, the observation of an action is projected onto the action system of the observer as the MNS hypothesis states, also the proactive looking should be a part of it. This study raises a question of MNS and motor co-development: 6-month-olds can reach for objects but do not transport them to a goal. They take the object to their mouth or simply drop the object. Perhaps the 6-month-olds have a MNS but since they do not have the action representations of transporting they cannot match the observed action onto themselves? If the action was too complicated for the 6-month-olds this study indicates that 12-month-olds have a functional MNS but leaves the onset of a functional MNS open.

Direct neurophysiological measures of the mirror neuron system have rarely been performed in an infant population. There have been EEG studies of children with autism (Lepage & Théoret, 2006), but since autism is not diagnosed until approximately 3 years of age these studies shed little light on the early development of the MNS. There is only one study that touches upon the infant MNS. Shimada & Hiraki (2006) used near infrared spectroscopy (NIRS) to measure concentration of oxygenated relative deoxygenated blood (degree of oxygenation) in motor areas during the observation of live and televised ac-

tion. They found that 6-month-olds have a higher degree of oxygenation in the motor areas during action observation, and they suggest that this may be a consequence of mirror neuron activity. However, the focus of the study was to investigate the differences between stimuli presented on a TV screen and presented live. Therefore they did not fully use their empirical data to demonstrate MNS activity in infants.

No matter how convincing the theoretical discussions have been about the importance of the MNS for social skills, many arguments remain speculative. For example, if the MNS lies at the core of social functioning the MNS should develop before or at the same time as the social skills. Few studies have sought empirical support for this hypothesis or tested the predictions that could be stated. Today this kind of evidence is wanted, and there have been explicit requests of data from the MNS developmental path (Lepage & Théoret, 2007).

Aims of this thesis

The aim of this thesis is to present empirical neurophysiological data from the infant electroencephalogram (EEG). The focus of interest will range from low level motion perception (study I) to action perception and MNS activation (study II and III).

Study I aims at finding characteristics of motion sensitive areas in the infant brain. While the visual system has been intensely studied in infants before, the development of the spatiotemporal distribution of cortical activation from visual motion has never been investigated. Also, functional motion perception is a prerequisite for functional action perception as the perception of movements will build on perception of motion.

Study II investigates differences between observation of goal-directed and non-goal-directed movements. The difference in activation is assumed to be related to infant MNS activity, and is a first step toward a better understanding of the development of the MNS.

Study III also investigates differences between observation of goal-directed and non-goal-directed movements. In contrast to study II, which used monitor presentation, the stimuli were presented live. Study III specifically investigates mu rhythm perturbations as a marker of infant MNS activity.

This thesis also aims at overcoming some of the difficulties of EEG recording of the infant MNS by presenting new methods of analysis. This is done by using independent component analysis (ICA) and selecting components of interest based on mu rhythm properties (study II and III). However, before we can turn to the actual experiments the bioelectrical background of the EEG will be explained. This background is necessary to understand the studies fully.

Bioelectrical basics

When neurons in the brain gets activated, the ion channels in the cell membrane start transporting electrically charged ions in and out of the cell. This generates an activation potential within the cell that starts at approximately -50 mV and peaks at 50 mV in less than a millisecond. The electrical field of individual neurons is not what is commonly measured by scalp surface EEG since the magnitude cannot compete with other surrounding electrical fields. Rather, only when several neurons fire simultaneously they sum together to measurable EEG signals. Due to the columnal organization of the cortex, the current sources (+) and sinks (-) are placed perpendicular to the cortical surface (see *Figure 4*). Since the distance between current sources and sinks are small compared to the distance to the recording sensor, the electrical field from each column can be approximated as a dipole field (Scherg, 1990). When a patch of cortex becomes activated the electrical field becomes the field sum of many cell columns.

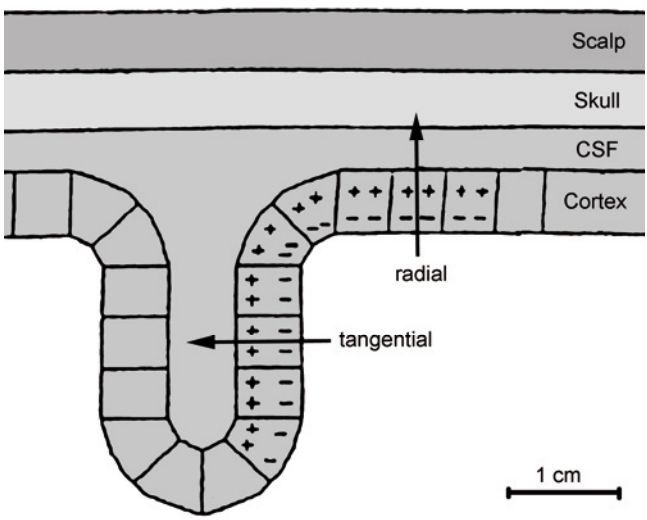


Figure 4. Section cut of a cortical fold. Each cell column can be considered as an equivalent dipole that is orientated perpendicular to the cortical surface.

In *Figure 4* we can consider two perpendicular dipole fields, one radially oriented to the skull and one tangential. Given the spatial properties of the dipole field (*Figure 5*) we can expect very different scalp measurements from the two dipoles. Although the dipoles are located close to each other the orientations of the dipoles determine the scalp topography of the electrical potentials. An example of this is shown in *Figure 6*, where the radial and tangential dipoles are measured separately and in a superposition condition where the signals are summed together.

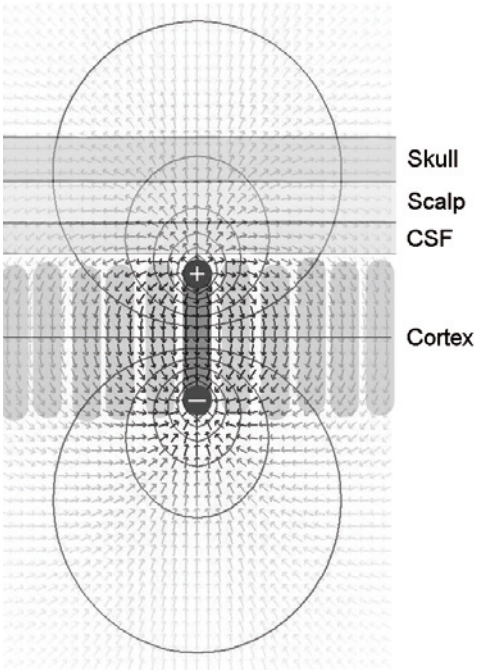


Figure 5. Spatial distribution of an electrical dipole field. The small arrows describe the orientation of the fieldlines, and rounded curves show equipotentials. If the dipole were oriented tangential to the skull (e.g. rotated 90 degrees, as in a sulcus) the potentials on the skull would be both positive and negative. The orientation of the dipole is therefore an important characteristic of the dipole.

The example in *Figure 6* illustrates the problem with the complex distribution of the electrical fields. In the case of the tangential dipole, the sensor closest to the activated brain area does not register the strongest signal. The topographic signature is nevertheless very characteristic, and with this knowledge it is possible to approximate the localization of the activated brain area. In the mixed condition it be-

comes very difficult to decompose the measured signal into its constituents. Traditional EEG measures often overlook these complex patterns and rely on the fact that the electrical fields decay rapidly with distance. It is therefore assumed that a stronger signal reflects a shorter distance to the activation source. This assumption is most valid when only a few brain areas are activated or the locations of activated brain areas are very similar between subjects.

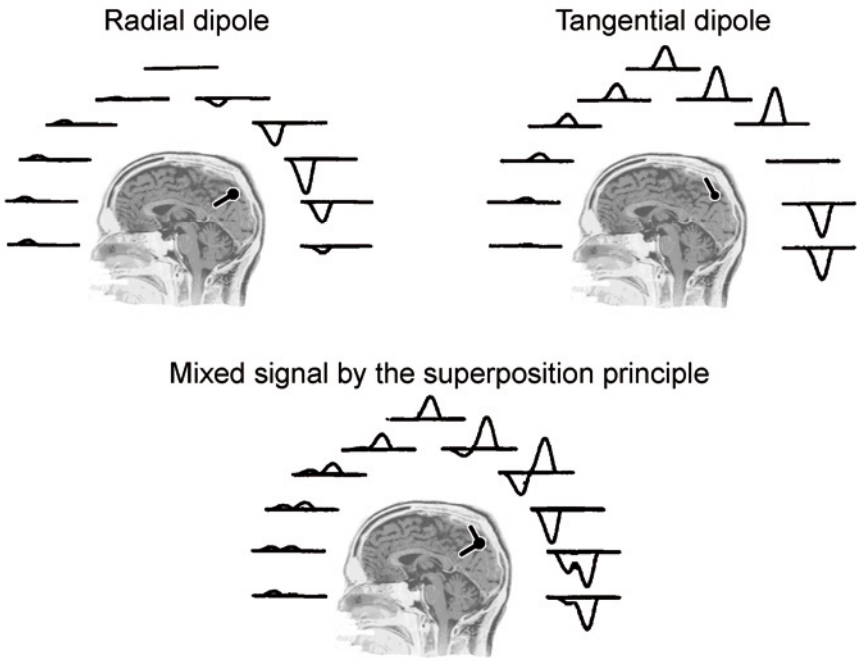


Figure 6. Top row show a section cut of different scalp topographies from two nearby patches of cortex with different orientation. The activation potential gradually increases over time and then decay. The two activation peaks are separated in time but overlap, resulting in a complex pattern due to the superposition principle.

The assumptions of traditional EEG have been criticized (Makeig, Debener, Onton & Delorme, 2004), especially when the signal of interest is complex and may be distributed over a network of brain areas. Therefore there have been several attempts to decompose the mixed signal into components that better reflect the activity of the underlieing brain sources. One of the methods that try to meet as many bioelectrical characteristics as possible is independent component analysis.

Independent Component Analysis (ICA)

In the case of EEG each channel represents one dimension of the collected data. Since each channel measures a mixture of electrical fields, the signals at two adjacent sensors are usually not independent of each other. That is, both channels measure the same electrical fields. Independent component analysis is a family of blind source separation methods that seeks to minimize the mutual information between different dimensions of the data. ICA exploits the central limit theorem in statistics that states that any linear mixture of two or more source activities is more normal (gaussian) than the original source activities. The ICA algorithms try to find dimensions in the data that maximize non-normality, and thereby contains the maximum amount of information (Delorme & Makeig, 2004). While this may be hard to visualize for a 128-channel EEG, which would contain 128 dimensions, the same principles apply for an example with two channels as in *Figure 7*. To find the most independent axis in a 128 dimension space is a very computationally demanding task, and in practice the ICA will not evaluate all possible combinations of axis. Rather, the ICA will search through the space by a training procedure (e.g. by examining gradients of kurtosis or measures of mutual information between axis projections) using random starting positions. This means that successive ICA procedures will give similar but not identical outputs.

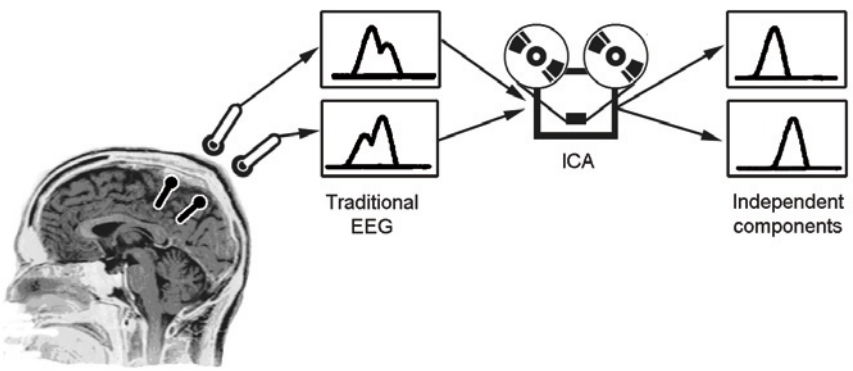


Figure 7. Two brain active areas are measured by two EEG channels. The dipoles are equally strong, but the distance between the measuring sensors make the EEG signals look different. The ICA then tries to find dimensions in the data that are maximally independent of each other.

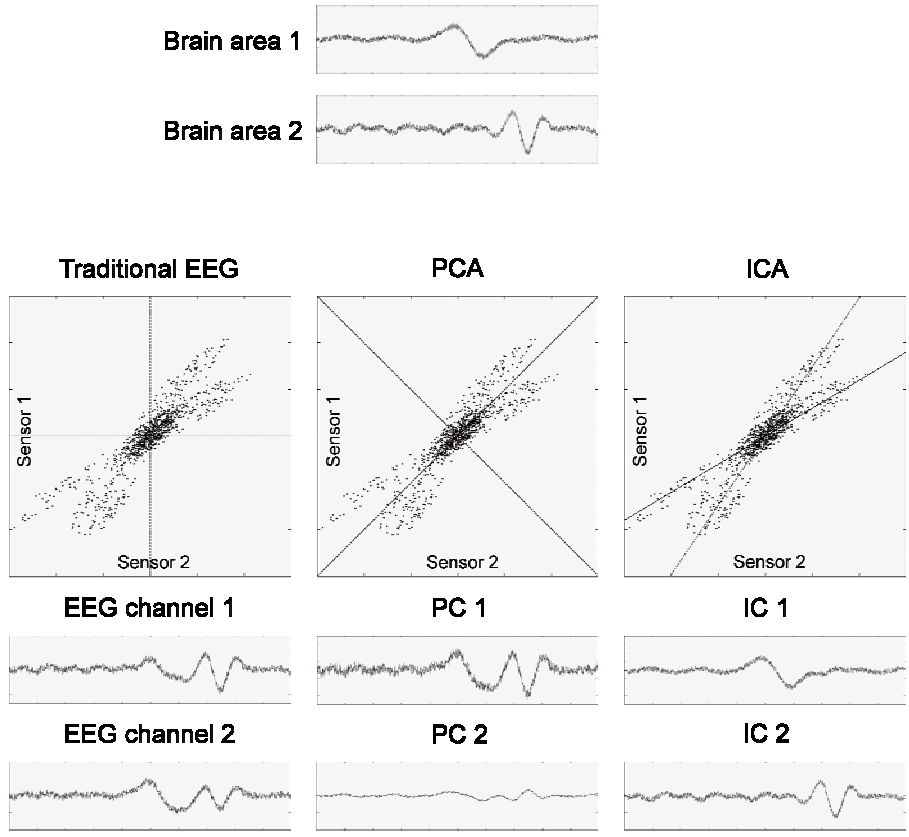


Figure 8. Illustration of traditional EEG, and PCA and ICA algorithms. Traditional EEG uses the measured signal without any axis rotation. PCA finds orthogonal axis with maximum variance. In ICA the oblique axis is rotated to make the projection of data points maximally independent. In this example IC 1 resembles Brain area 1 and IC 2 resembles Brain area 2 more than the EEG channels or principal components do.

A more familiar, but related, method to find underlieing dimensions in the data is principal component analysis (PCA). The PCA seek to determine few out of many dimensions that will explain the variability in the data. PCA would make each successive component (or dimension) account for as much as possible of the remaining variability that is uncorrelated with previously determined components. The uncorrelatedness of the components in PCA means that the dimensions have to be orthogonal (as shown in *Figure 8*), which is usually not a realistic assumption for biophysical data such as EEG. To find biologically

plausible sources, the PCA should be extended with a dimension rotation procedure. ICA can be viewed as such a procedure, where the PCA dimensions are obliquely rotated to find axes for which the projection of data is maximally independent of each other (also demonstrated in *Figure 8*).

Performing ICA decompositions is most appropriate when the sources are non-normal and lineraly mixed in the recorded signal without time delays. These conditions are met in EEG measurements, which make the ICA a suitable tool to separate brain processes and artifacts mixed in the EEG. Also, if the ICA sucessfully decomposes a localized brain process, the scalp topography of the ICA weights (which corresponds to the interrelationship between ICA dimensions and the scaling of the amplitude projected onto the scalp sensors) will be similar to the field projection of the underlying dipole on the scalp. As more sensors will give more information of the spatial characteristics of the dipole, it is recommended to have at least 64 sensors distributed over the scalp to estimate the dipole. The scalp signature of the ICA weights could thus be used to approximate the source localisation by a dipole fitting algorithm when using high density EEG (Makeig et al., 2004).

While the theoretical underpinnings of the dipole localization algorithms are reasonable, some practical issues makes is hard to get reliable information of the signal origin. First, the algorithms require as detailed information as possible about the anatomy of the brain and skull, and the EEG sensor positions. In the present studies there was no possibility to do MRI scans of the anatomy or 3-D scanning of electrode position (which is sometimes used in adult studies). Second, the infants do not provide as many trials as desired. This will result in less stable ICA decompositions and less dipole like scalp signatures. Despite these difficulties it might be worthwhile to perform dipole estimation, and there are some infant studies that have presented source localisation data (Richards, 2005; Reynolds & Richards, 2005; Johnson et al, 2005).

Notice that the time dimension is collapsed in *Figure 8*, which means that the activity time courses of signals should be (near) independent of each other to be decomposed into separate components. One limitation of the ICA is thus that it will not decompose different brain areas into separate components if their activities are synchronized in time. This means that several brain areas can be represented in the same

component and the scalp signature of the ICA weights will not resemble a single dipole field. Of course, this makes source localization harder. On the other hand, brain area activities that are synchronized in time often have a functional relationship and it would therefore be of significant interest to pool them together.

Another limitation of the ICA is that an axis can be rotated 180 degrees and still be as independent of the other axis as before. In practice this results in an inverted component activity since positive and negative polarities switch. Also, the amplitude of the component activity is not an absolute unit value. This is because the component activity does not have any spatial reference. However, if the component activity is backprojected to the scalp channels by the ICA channel weigths, the component activity at these locations have the right polarity and unit amplitude (usually mV in the case of EEG).

Perhaps the most difficult problem with ICA is the interpretation of components. In PCA the dimensions are ordered by the amount of variance the account for. This is not the case in ICA. Also, as different subjects differ in brain activity the decomposed components may not be very similar. Then, how can we correlate the independent components from two subjects to each other? There are several strategies, where the most common is to cluster components from all subjects with regard to special characteristics of the components (Makeig et al., 2004). Among these characteristics are scalp topography (which corresponds to source locations in dipoles with the same direction), frequency characteristics, and functional response to different stimuli. It is therefore important to design EEG studies that use ICA with regard to this, and the methods used in this thesis will be explained in later sections (Study II and Study III).

Cells in synchrony: rhythms and ERPs

EEG measurements in infants are notoriously difficult (Thierry, 2005). The first reason is that infants seldom sit passively for long periods, which results in many motion artefacts. The artefacts stem from muscle activation in the face and from the neck, and from changed contact between the sensors and the scalp. When the infant moves very much the EEG is so contaminated that long sections of the recordings have to be discarded. In other cases the artefactual signals can be ignored or

subtracted from the EEG. This can be achieved by ICA if the artefacts are not time-locked to the brain processes of interest.

The second reason for the difficulty of measuring EEG in infants is that they do not usually attend to presented stimuli for a long time. This is especially true when the stimuli is repetitive, which is usually the case. With adult subjects the scientist determines the length of the experiment, sometimes up to 45 minutes or more. With infant subjects the experiment stops when the infant is not interested any more or starts to fuss, which is usually after 2 – 10 minutes. So why does attention time matter, and why has the stimuli presentation have to be long and repetitive?

The reason is that changes in the EEG that is related to different stimuli are usually very small compared to the background EEG signals. These small changes can be detected if they are time-locked to a specific event in the stimuli. It is then possible to present the same stimuli several times and create an average of the signals. Signals that are not time-locked to the stimuli, such as background noise, will cancel out while signals that vary systematically with the stimuli will remain. How well the averaged signal reflects stimuli related signal versus background noise depend on the number of stimuli presentations. More trials give a better signal to noise ratio. Such averaged signals that are time-locked to the stimuli are called event related potentials (ERPs), and this is exemplified in *Figure 9*.

The stimuli presented in this thesis are often between 2 and 4 seconds long, and since infants are attentive approximately 5 minutes they get to see about 100 trials which should be divided into 3-4 conditions. Adult studies often exceed 200 trials in each trial before averaging. The difference in signal to noise ratio emphasis the need of signal separation techniques (such as ICA) in infant EEG studies.

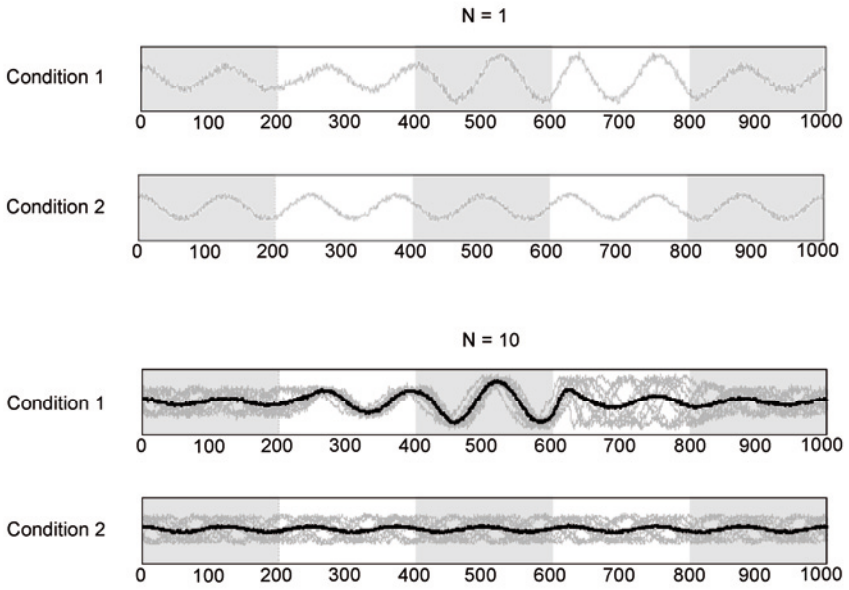


Figure 9. Example of signal averaging. The differences between the two conditions is not obvious after one stimuli presentation, but after 10 stimuli presentations the time intervals show different signal characteristics. The thick black line is the average of the ten grey signals. In condition 1 the signal is time-locked to the stimuli presented at time=0. At time=200 the phase of the signal is time-locked. At time=400 the amplitude of the signals increase. At time=600 the phase desynchronize but the amplitude remain high between trials. At time=800 the signals return to normal. In condition 2 the signals are not time-locked to the stimuli.

The averaging of the raw signal will discard much of the information in the trials (Makeig, Debener, Onton & Delorme, 2004). An example is the time interval between 600 and 800 in *Figure 9*. In this example the amplitude in each individual trial is high, but since the trials are not synchronized, the signals cancel out on the average. Also, if the signal vary in different ways at different frequencies it is even harder to interpret the ERP. A better understanding of the characteristics of the stimuli response is possible by analysing the event related spectral perturbations (ERSP) and inter trial coherency (ITC) of the signal. In this type of analysis a time/frequency decomposition is performed on each stimuli presentation (trial) and the power and phase spectrums are then averaged. An example of this is found in *Figure 10*.

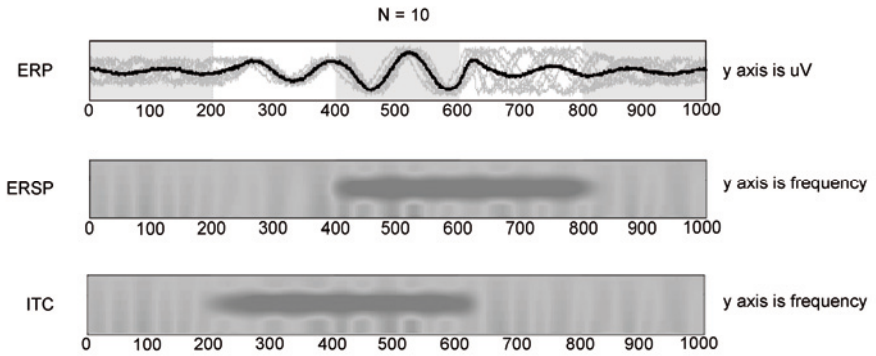


Figure 10. Artificial example of different ways to average signal characteristics. The event related potentials (ERP) averages the raw EEG signal. The event related spectral perturbations (ERSP) averages the amplitude of different frequencies. The inter trial coherency (ITC) averages the phase of different frequencies. In the ERSP and ITC plots the magnitude of the averaged signal is intensity coded (dark areas means increased magnitude compared to a baseline).

The idea to analyse separate frequency bands is also motivated by the fact that the human brain constantly generate electrical fields that oscillates with different frequencies. These rhythms are divided into alpha rhythms (8-12 Hz), beta rhythms (12-30 Hz), theta rhythms (4-7 Hz), gamma rhythms (26-100 Hz), delta rhythms (up to 3 Hz) and mu rhythms (9-14 Hz with individual variation). The rhythms can be linked to different cognitive processes by their functional response to stimuli and the localization of their sources (Kuhlman, 1980). One rhythm of particular interest to this thesis is the mu rhythm, which have been linked to mirror neuron activation.

The mu rhythm

The mu rhythm was discovered already in the 1950s by Gastaut & Bert (1954), long before the discovery of mirror neurons. They recorded EEG from subjects that observed cinematographic images (a movie) of different kinds. The results showed that a rhythm at approximately 10 Hz could be measured over central areas of the brain. However, when the observer saw moving people this rhythm disappeared. Later it was shown that this rhythm, now called “the mu rhythm”, also disappear during motor planning and performance (Kuhlman, 1980; Makeig et al., 2004).

The mu rhythm is sometimes thought of as a rhythm which appears when the brain is more or less passive and relaxed. However, in a recent review it is suggested that the mu rhythm is more than an idle rhythm (Pineda, 2005). The disappearance of the mu rhythm could reflect reduced activation in the mu rhythm generators. Although plausible, there is another possible explanation as follows: The mu rhythm is often considered to appear when sparsly activated brain cells are phaselocked and fire in synchrony (as in *Figure 10*, time 200-400). When the activity increase the phaselocking disappear, and the desynchronization of brain cells cancel out the electrical fields (as in *Figure 10*, time 600-800). Although it is impossible to tell which of these two possibilities is correct (using neuron population studies such as EEG) this thesis will adhere to the desynchronization terminology and use the term desynchronization interchangable with disappearance.

The mu rhythm interval overlaps with the alpha-band (8-12 Hz) that is commonly related to a strong posterior generator in the occipital lobe. The alpha waves are strongest when the eyes are closed or when there is no light in the room. Alpha waves are attenuated when the eyes are opened and receive visual motion. The differences between the mu rhythm and the traditional alpha rhythms are thus both in localization and in functional response (Oberman et al., 2005; Pineda, 2005).

The mu rhythm seems to originate in motor-sensory areas, as shown by several studies using different methodologies (Hari & Salmelin, 1997; Nishitani & Hari, 2000; Muthukumaraswamy & Johnson, 2004b). Since the mu rhythm originates in areas where mirror neurons have been localized and disappears both during action performance and action observation, it has been linked to MNS activity. Also, the rhythm is more desynchronized during observation of goal-directed actions compared to observation of non-goal-directed actions (Muthukumaraswamy & Johnson, 2004a). Finally, there is no other known neural substrate which is better explained by this functional response and topography (Oberman et al., 2005) than the MNS.

However, there is another rhythm that has been associated with the MNS as well. In several studies it have been shown that also the beta band (20-25 Hz) initially desynchronize when subjects observes goal directed actions (Hari & Salmelin, 1997; Nishitani & Hari, 2000; Muthukumaraswamy & Johnson, 2004b). The beta rhythm then in-

crease in a characteristic amplitude rebound. It has been argued that this rhythm could be a more stable MNS marker, with less individual variance. Unfortunately there are only studies linking infant rhythms to the adult mu rhythm, and no corresponding studies that relate the adult beta rebounds to infant data. The studies in this thesis will therefore focus on the slower mu rhythm in relation to the MNS.

The infant mu rhythm is still debateable, but there are some strong indicies that infant mu rhythms appear between 6 and 8 months of age (Stroganova, Orekhova & Posikera, 1999; Marshall, Bar-Haim & Fox, 2002). At this age the frequency is slower than in adults, approximately 6-8 Hz. The amplitude is also lower than in adults compared to surrounding frequencies. Both frequency and amplitude increase with age, and at 11 months the frequency is approximately 7-9 Hz. Although no longitudinal study has followed the development into adolescence it is thought that the frequency continues to increase until it reaches adult levels.

In *Figure 11* a rough overview of different mu rhythm intervals from a selection of studies is presented. The variability of the mu rhythm definition between studies could well reflect the intersubject variability. The exact interval of the mu rhythm in a study can thus only be established after a frequency analysis of the individual subjects. If the study requires a mu rhythm interval prior to this analysis, as in study II and III of this thesis, the interval should be appropriate for each age group and generous enough to account for intersubject variability. However, the exact border values will not be very important to the results, as long as they are within reasonable limits.

The differences between the infant and the adult mu rhythms are not well known. This is due to the fact that most studies adress the adult mu rhythms, and therefore much more is known about the adult rhythms. In the cited litterature the main difference between adults and infants appear to be the frequency at which the mu rhythm oscillates. The activation source and response to visual information seem to be similar in adults and infants. In this thesis it is therefore assumed that infants have mu rhythms that are analogous to the adult mu rhythms. The characteristics of the mu rhythm can then be used to design and refine a MNS analysis method as presented in study II and III.

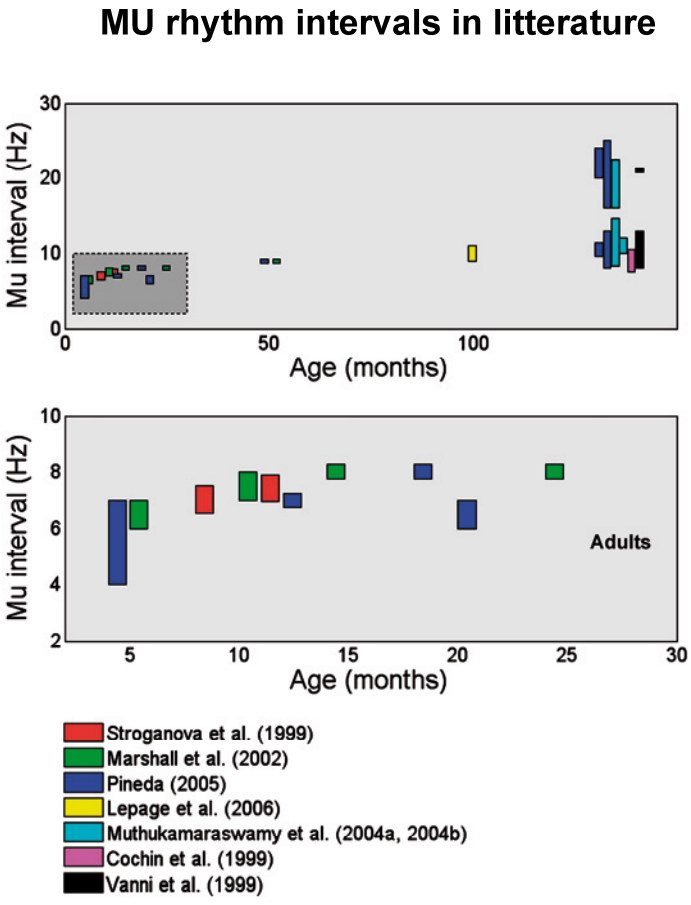


Figure 11. Overview of previous studies that assessed the mu rhythm, and the intervals used.

Methods

Subjects

All infant subjects in the studies were assigned by sending information letters to parents. The interested parents could then return a letter with a contact phone number, and an appointment could be made. The parents were informed about the experiment upon arrival at the lab and a written consensus was signed in accordance with the Helsinki Declaration. Adult subjects were also informed and signed the written consensus. All participants received a gift certificate of 100 Swedish kronor (approximately € 9). The experiment was approved by the Ethics committee at Uppsala University.

Table 1. Summary of the number of subjects in the different studies.

Study	Age group	Number of recorded subjects	Number of subjects in final analysis
Study I	~2 month	18	11
Study I	3 month	16	11
Study I	5 month	18	15
Study I	Adults	12	11
Study II	6 month	34	19
Study II	Adults	23	15
Study III	8 months	32	32 (23)

The number of participants in each study group is listed in Table 1. The subjects were matched with regard to gender. The subjects were pooled so that there were approximately as many males as females in each group. Some subjects were excluded from the studies due to fussing during the recording session, technical problems or insufficient data after artefact rejection. The number of subjects remaining in the statistical analysis is therefore also included in Table 1. Compared to most adult studies the percentage of excluded subjects is large. However, this is very common in infant studies and illustrates the fact that it is not possible to instruct preverbal infants to behave according to a study protocol.

General procedure

The session started with measurements of the subject's skull, and an appropriately sized 128-electrode EEG net (EGI Corp., Eugene, Oregon) were selected. The net was then applied on the skull of the subject and adjusted so that the reference electrode (placed at the vertex of the head) and the ear references were correctly placed. The placement of the EEG net took less than 2 minutes except in the cases when infants started to fuss. In these cases the infant was comforted and sung to until it lost attention of the net and the session could continue.

The infant was then positioned in front of the stimuli, which were presented on a monitor (study I and II) or in a live setting (study III). The 2-month-old infants were held by the parent over his/her shoulder so that the parent faced away from the monitor. This position gave good support to the infant's body and the infant would not lean on the net. The older infants sat in a special baby seat (Bumbo inc., SouthAfrica) that supported an upright sitting position. When the adult subjects were measured they sat in front of the monitor watching the stimuli. The parent of the infant and two experimenters were always in the room, making sure that the infant felt comfortable and attended to the stimuli. Also, the infant was recorded by a video camera placed on the top of the display monitor for later rejection of inattentive periods. During the whole session the recommendations (Picton et al., 2000) and measurement routines suggested by Johnson et al. (2001) were followed as closely as possible.

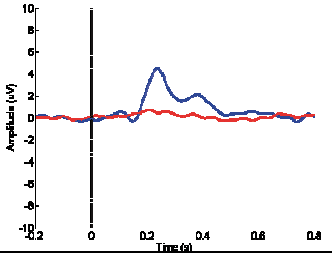
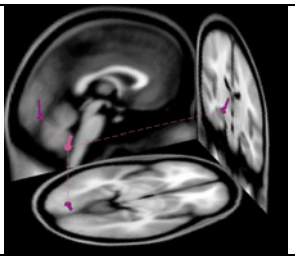
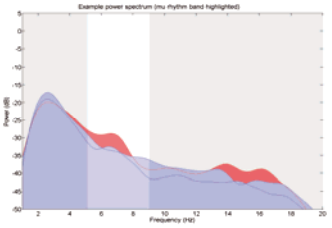
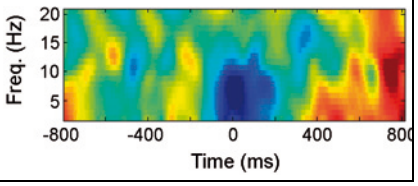
During stimuli presentation the brain electric potentials were recorded relative to the vertex, at 250 Hz. A hardware analogue filter (elliptical) bandpass filtered the signal from 0.1 to 100 Hz (EGI Netstation 3.5, Eugene, Or) during recording. After the experiment the data was transferred into the EEGLAB toolbox (Delorme and Makeig, 2004, version 4.512 for study I, version 5.1 for study II and III) in the Matlab environment. The video was inspected and longer intervals of inattention were excluded. The data was re-referenced to an average reference and notch filtered (45 to 55 Hz to remove line noise).

After this pre-processing the data was more specifically filtered and analysed according to the experimental design. More information of the post-processing is included in the sections of the individual studies.

Different kinds of EEG representations

The EEG data was analysed using different tools to help the interpretation of the signal. An overview of the EEG representations used and why we used them is given in Table 2.

Table 2. Overview of EEG representations used in study I – III.

What	When	Why	Example
ERP	Study I	A traditional EEG measure with high temporal resolution was used to capture the activation time-course in different brain regions.	
Head projection	Study I Study II	The headprojections give an overview of the spatial characteristics of the signal.	
3D dipole localization	Study II	The dipole fitting of sources gives an approximate origin of the signal, which could be used to valide that the signal originated in mirror neuron areas.	
Frequency spectrum	Study II Study III	Frequency analysis was performed on independent components to select the components that suppressed the mu rhythm in the action conditions.	
ERSP	Study II Study III	Time/frequency decompositions were performed to investigate the temporal characteristics of rhythms in more detail.	

Study I

All movement perception depends on sensitivity to motion. Most developmental studies have focused on general sensitivity to different types of motion, but not on the development of activity in different areas and how they relate to each other. It is well known that motion perception depends on a network of cortical areas, and that the area in the junction of the parietal, occipital and temporal lobe appear to have a key role (Zeki, 2004). This area is termed MT/MST. The present study asked both when and how cortical processing of visual motion develops in human infants. We used a traditional ERP design to identify patterns of neural activity in 2-, 3-, and 5-month-old infants and an adult group, when they watched stationary and rotating patterns of simple elements.

The analyses were focused on developmental changes and activation differences in regions that are activated by visual motion in adults. In adults the MT/MST area receives information from two visual pathways as illustrated in *Figure 2*. One pathway propagates from LGN to V1,V2 and finally to V5, the primary visual pathway (Sincich, Park, Wohlgemut, & Horton, 2004), and one subcortical pathway projects via superior colliculus (SC) and pulvinar to the MT+/V5 (ffytche, Guy, & Zeki, 1995; Buchner et al., 1997; Schoenfield et al., 2002; Callaway, 2005; Schneider & Kastner, 2005) or via LGN.

Design

The stimuli were presented on a calibrated CRT monitor that was placed 45-60 cm from the subject. The E-prime software (Psychology Software Tools Inc, 2002) was used to present the stimuli and synchronize the presentation with the EEG measurements.

Figure 12. Example of monitor displays. Each stimulus was presented approximately 3 seconds, with the first 0.8 to 1.25s always being stationary. In the motion condition the geometric objects started to move in circular trajectories at 60˚/s. Each condition was presented 64 times in random order with attentiongrabbers interleaved.

The stimuli consisted of a static background grid. In front of this grid ten geometric figures were evenly distributed over two imaginary circles with a radius of 20 mm and 60 mm respectively (see *Figure 12*). The stimuli were presented 128 times and each trial had duration of approximately 3 seconds. The trials were interleaved with various static pictures to sustain the attention of the subjects so the whole session took 6.4 minutes. In 64 trials the patterns started to rotate at 60˚/s after a random period of 0.8 – 1.25 seconds. In 32 of these trials the inner figures moved clockwise and the outer elements counterclockwise and in the other 32 trials the motions were reversed. The counter rotation of the inner and outer sets of geometric figures was chosen to avoid eye movements. The onset and duration of the static and moving intervals and attention grabbers were randomized so that an expectation response was avoided.

After the session, the EEG data were analyzed offline. One second of data from each trial was extracted, from 0.2s before motion onset until 0.8s after and corresponding time intervals were extracted from the static trials. The interval before time-lock was used for baseline correction. An artefact routine analyzed each channel separately and removed trials with an amplitude range of >120 uV in infants and >30 uV in adults before the average ERP was calculated for each subject. All subjects with less than 20 moving or stationary trials in any region of interest (ROI, see *Figure 13*) were excluded from further analysis.

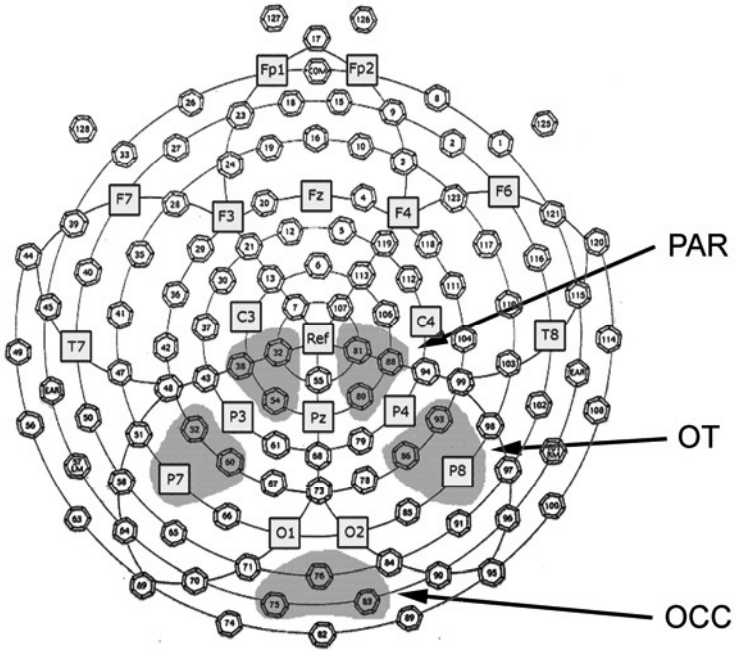


Figure 13. Schematic illustration of the 128 sensor locations. Grey areas show the regions of interest (ROI). These areas were used in the statistical analysis and are termed OCC (as in occipital), OT (as in occipitemporal) and PAR (as in parietal).

To fit the data in the statistical analysis, the ERP after timelock was divided into 40 ms periods. That gave 20 values for the stationary and 20 values for the motion conditions for each ROI, and the mean was calculated for each such period. This data was then fed into repeated measurement ANOVAs in the SPSS software. The independent variables were age, ROI, hemisphere, time, moving/stationary (Motion), and the dependent variable was ERP voltage. Sphericity was always tested and, if necessary, the SPSS correction was used. The adult group was only included in the tests of the separate ROIs.

Results

The results of the ANOVAs show both significant main effects and interaction effects. A table over the results of separate ANOVAs of the activations in the OT and PAR regions for the 2-month-olds, 3-month-olds, 5month-olds, and adults are found in the result section of study I. Also, the interactions between the OT and PAR regions for the 3-month-olds, 5-month-olds, and adults are found in the same sec-

tion. These tables are not presented here, as an overview of the activation time courses are presented in *Figure 14*.

The results show a complex pattern of significant effects and polarity switches. The ERPs for the moving and stationary stimuli in different age groups and ROIs are shown in *Figure 14* to give an overview of the spatio-temporal evolution of the signal. These group averages for the moving and the static condition were low pass filtered at 20 Hz for illustrational purposes.

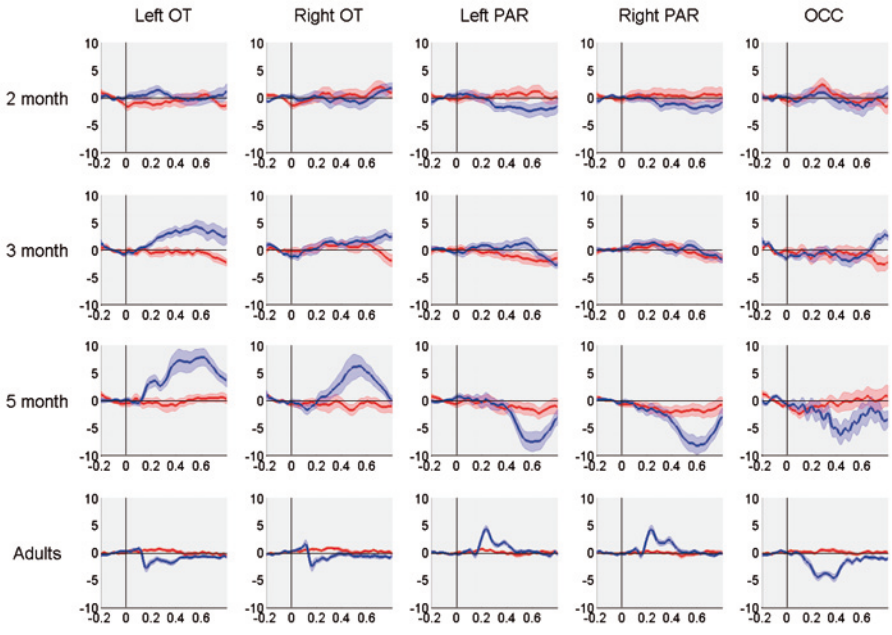


Figure 14. Grand mean ERP in different ROIs and different ages, lowpass filtered at 20 Hz. Blue lines indicate response to moving stimuli, and red lines are the stationary condition. The standard error between subjects is shown by faint blue/red colours. The vertical axis is amplitude (uV) and the horizontal axis is time in seconds. The vertical line at time 0 ms indicate onset of rotation, alternatively continued still picture.

In summary, the response of the 2-month-olds was weak and rather unfocused. The separate analyses of the OT and PAR regions gave a significant time dependent effects of motion, and the combined OT-PAR analysis showed a significant interaction between these two ROIs. The response to motion in OCC was absent for the 2- month-

olds. The response to motion in OCC for the 3-month-olds was both later and weaker than in the OT area. A distinct motion response was obtained for the 3-month-old infants in OT in the present study, but only for the left hemisphere (*Figure 14*).

The 5-month-old infants showed a strong bilateral response of motion in the OT region peaking at around 0.6 s. The response started earlier in the left than in the right hemisphere (see *Figure 14*), thus showing that the asymmetry found in the 3-month-olds is initially present for the 5-month-olds as well. Although the cortical response to visual motion was different in the 5-month-olds than in the adults, the behavioural correlates of smooth pursuit and motion perception are rather adult-like at this age. In the 5-month-olds the cortical activation in the PAR ROI was reversed relative to the 3-month-olds and the asymmetry had disappeared. In this sense the response at 5 months resembles the adult one.

Conclusions

The results show that dramatic changes take place in the cortical processing of visual motion between 2 and 5 months of age. While the activations for the 2-month-olds were barely measurable, the activations for the 5-month-olds were massive. The emerging patterns of activation were distinctly different for the different areas analyzed and the variation in the infant groups had a higher intersubject variability than the adult group. This can be expected in a period of dynamic change and is a function of biological variation in neural growth, maturation and differentiation.

This study show that the first processing of visual motion in the OT regions takes place in the left hemisphere and develops bilaterally between 3 and 5 months of age. During this period it is a successive involvement of visual areas. The absence of ERP in the OCC ROI at 2 months of age indicates that the subcortical pathway for visual motion develops ahead of the primary visual pathway.

Study II

If the maturation of the motion processing network is operative at 5 months, it is possible that also biological movement perception or action perception is functional. In study II a group of 6-month-olds were studied in an action-observation situation. The stimuli consisted of video presentations of static objects, moving objects, goal-directed reaches and non-goal-directed hand movements. Instead of using different regions of interest, as in study I, an ICA was used to decompose the signal into independent components and analyse components of interest. This approach was chosen since the signal was expected to be weaker than the signal in study I and possibly distributed over the scalp. Also, the functional response of the mu rhythm band could be used to select components that desynchronized in the action conditions compared to the static displays. Any differences between the two action conditions could then be considered to reflect activity from mirror neurons that were tuned to goal-directed actions.

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 mu desynchronization in adult humans (Hari & Salmelin, 1997; Oberman et al., 2005). The age group was selected since 6 months old infants usually master goal-directed reaches themselves at approximately 4-5 months of age (von Hofsten, 1979). All subjects were tested before recording and were found to reach successfully. Since the infants had only reached for about 1-2 months, any mirror neuron activity would suggest a strong developmental coupling between mirror neurons and motor neurons.

As the mu rhythm amplitude can be very low in 6-month-olds the data were analysed in two ways, both using slow wave ERPs and frequency analysis of the mu rhythm. These methods may also provide

complementary information (Babiloni et al., 1999). The ERP paradigm was chosen as the original mirror neuron studies show an increased activity in mirror neurons that peaks when the hand reaches the goal. While this peak can be expected to be small, it could possibly be detected by analysis of ICA decompositions.

Design

The stimuli were presented on a LCD monitor using the Windows Media Player. The top right section of the screen (5 x 5cm) was occluded by an optical sensor. This sensor synchronized the EEG with the stimuli by recognizing flash sequences that were hidden from the subjects' view.

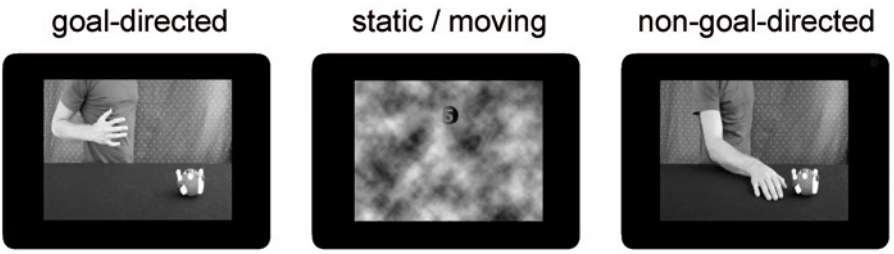


Figure 15. Example of stimuli displays. All pictures show the starting frame of each videoclip. In the goal-directed condition the hand reached for the object and picked it up. In the static condition the blue object remained stationary, and in the moving condition the object started to move in an S-shaped trajectory. In the non-goal-directed condition the hand rested next to the object and was then withdrawn.

Each videoclip started with a static period of 0.5-0.8s and had duration of 3.0-3.3s. In the goal-directed condition the hand touched the object exactly after 1 second after motion onset. This time-point is important since the original mirror neuron studies show a peak of activation when the hand reaches the goal. Of course this might not be the case in infants, where EEG latencies often are delayed compared to human adult latencies (also shown in study I) and presumably also compared to adult monkeys. Each condition was presented 32 times, which resulted in a total session time of 6.5 minutes. The order of conditions was randomized, but videoclips with dots and hands were always interleaved to capture the attention of the infants.

The recorded EEG was bandpass filtered at 0.5-30 Hz and artefact rejected by removing trials and/or channel with abnormal max absolute values, abnormal deviation or abnormal standard deviation (Junghöfer, Elbert, Tucker & Rockstroh, 2000). The EEG was then transformed to average reference and artefact rejected again. Next, an ICA algorithm was used to decompose the EEG into independent components, and components related to eye movements were subtracted from the EEG. A second ICA was performed using only the data in the 2-30 Hz band. This was done to prevent the ICA from separating different frequency bands into several components. Such a separation would remove low frequency information from components with mu rhythm properties and invalidate the use of ERPs in the subsequent analysis.

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 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. 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). A second criterion was that the static dot condition should have the highest mu power of all conditions. By using this strategy it is valid to compare the moving dot and action observation conditions with each other but not with the static condition.

Since the ICA decomposition cannot estimate the amplitude of the signal in the component activity, the data was also detrended (within each trial) and z-transformed (using all data points of the component, thereby including all conditions in the same transform). This was done to normalize each component's amplitudes.

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 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. 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 10 Hz 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. 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

The results of the frequency analysis showed similar patterns between the 6-month-olds and the adults. The static condition has the highest amplitudes, which is a logical consequence of the component selection procedure. All other conditions desynchronize in comparison, with the most desynchronization in the goal-directed condition for both groups. However, the only significant difference is between the goal-directed condition and non-goal-directed condition in the adult group (see *Figure 16*).

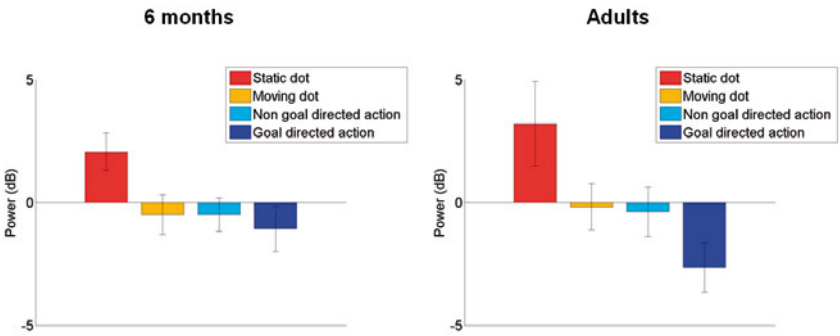


Figure 16. Mu rhythm power in the four conditions in decibel relative all conditions' mean power. Error bars indicate confidence intervals of 95%. Statistical tests were performed between all conditions except the static condition. In the adult group the goal directed action condition differs significantly 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.

The more detailed time/frequency analysis show that the significant difference between the goal-directed and non-goal-directed condition in the adult group is restricted to the mu-rhythm band and to a short time period after the hand touches the object (0.1 – 0.4s after touch). No significant desynchronization was found in the beta band, and no significant desynchronization was found in the infant group (see *Figure 17*).

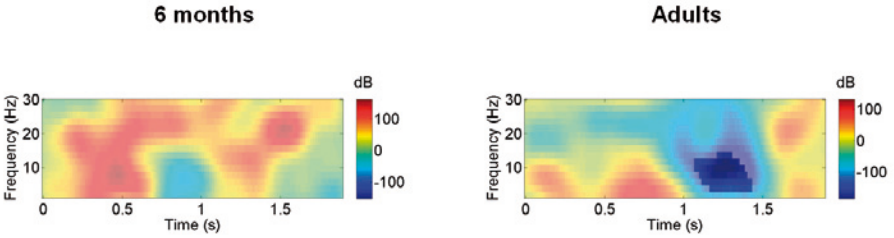


Figure 17. Time frequency spectrogram differences (goal directed – non goal directed conditions) showing desynchronization in blue colours. Pixel wise statistical probability maps are overlay and highlight significant areas ($p < 0.05$). Non significant values are shown in faint colours, and no significant values are found in the infant group. The color scale show decibel change from baseline (computed as the mean power from both conditions in each frequency band). The hand reaches the object at 1 second after timelock (Time = 1).

The ERP analysis show significant differences between conditions also in the infant group. This is possible since phase coherence be-

tween trials can generate a systematic peak in the evoked potential in either condition, even if the amplitude in each trial is low. The goal-directed condition show an activation peak just before the hand touches the object (within 0.5s before touch approximately until touch). This peak is significantly higher than the activation amplitude in all other conditions. Also, the moving dot condition show significant differences compared to a zero mean in the time interval 0.6 – 0.8s after motion onset. The activation time courses and significant differences are presented in *Figure 18*

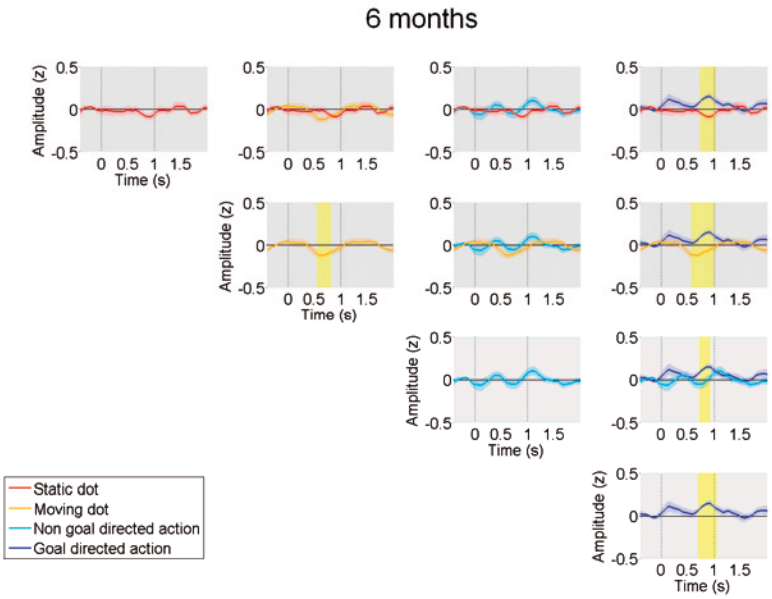


Figure 18. Infant ERPs with z-transformed amplitudes (μV) within subjects. Single ERP traces are tested sample wise against zero using t-tests, double ERP traces are tested against each other using paired t-tests. Significant intervals ($p < 0.05$) are highlighted in yellow. Shaded areas are standard 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).

The adult group shows significant differences in approximately the same time intervals, although with a little longer duration. The activation peak is also much more prominent than in the infant group. The activation time courses and significant differences are presented in

Figure 18. Furthermore, there are no significant differences between the motion condition and a zero mean.

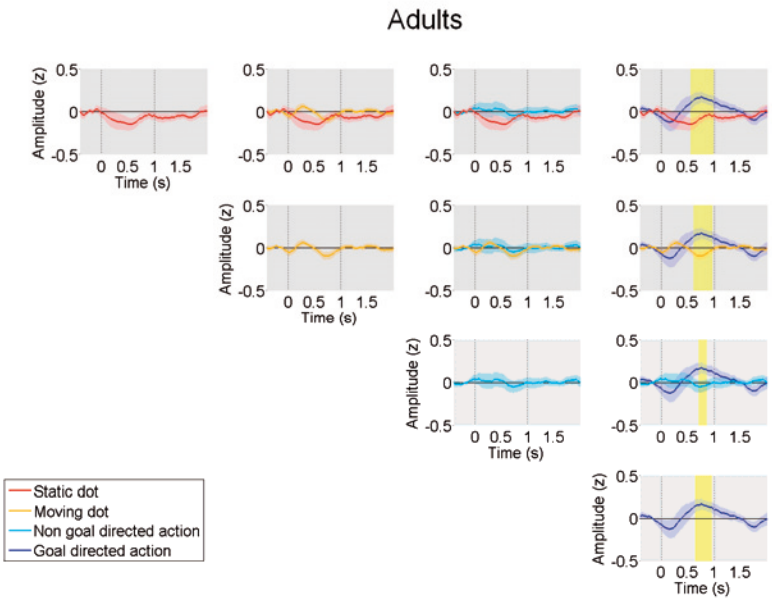


Figure 19. Adult ERPs with z-transformed amplitudes (μV) within subjects. Single ERP traces are tested sample wise against zero using t-tests, double ERP traces are tested against each other using paired t-tests. Significant intervals ($p < 0.05$) are highlighted in yellow. Shaded areas are standard 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).

The dipole fitting algorithm successfully localized all 16 components from the adult group and 15 components from the infant group with a residual variance less than 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 projected on an axial plane, is illustrated in *Figure 20*.

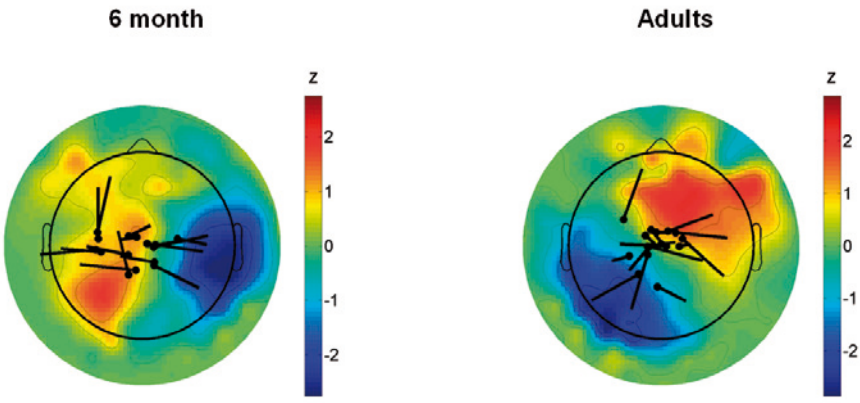


Figure 20. Mean topographic plots of the independent components' power projections (component weights, arbitrary unit). Black circles mark the axial projections of independent components' dipole localization (one per subject). Black lines show the projection of dipoles' directions with normalized length. The color scale shows the z-transformed component weights.

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). However, the mean Talairach coordinates does not specify the mean neural substrate and the individual coordinates are therefore presented in Table 3.

Table 3. Individual dipole location in Talairach coordinates

6 months		
X	Y	Z
10,5	-0,9	12,2
10,6	-17,6	4,2
-12,5	9	18
-5,9	-21	15,7
4,4	1,6	0,1
-13,1	-7	28,9
-40,9	13,5	31,2
11	-13,4	27,6
12,3	0,7	7,1
-5	8,9	28,8
32,1	7,4	27,9
-12,5	-24,9	19,3
-42,6	-3,6	2,8
-37,6	-5,5	3,7
-40,5	6,4	10,6

Adults		
X	Y	Z
-9,4	15	22,7
13,3	12,1	15,7
-20,8	-25	9,3
-4,5	11,6	-6,6
16,8	0,3	23,1
-3,3	-36,8	-1,5
19,5	6,6	14,3
-11,3	-1,4	-0,6
-14,3	9,3	9
6,3	13,8	20,9
4,8	-0,7	-4,5
0	1,7	10,7
-29,4	-9,7	-7,4
-12,2	-0,7	5
-12,4	-7	23
-34,5	22	-20,6

Conclusions

Taken together, this study investigated mirror neuron system activity in both adults and 6 month old infants. 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, activation 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.

From a methodological standpoint, the possibility to measure mirror neuron activity in infants using this method may open a wide range of developmental studies that can help in delineating the maturation of the human mirror neuron system. More studies are though needed both to validate the method and to replicate the findings.

Study III

Although study II showed ERP indices of mirror neuron activity in 6-month-olds, the mu rhythm supression was not significantly different between conditions in the infant group. Since the mu rhythm is the commonly used EEG marker of MNS activity, a natural step would be to investigate the mu rhythm in an older age group to establish the onset of mu rhythm desynchronization.

In study III a group of 8 months old infants was tested, since infants of this age usually generate measurable mu rhythms. The stimuli were presented in a reaching observation situation, thus the goal-directed action was of the same type as in study II. However, since previous studies have shown that the mu rhythm is reduced about 15-20% more when adult subjects view live actors compared to video presentation (Järviläinen, Schürmann, Avikainen & Hari, 2001) the actions were presented by a live model. The switch from video presentation to live actions was done to maximize MNS activation and associated mu rhythm perturbations.

Similar to study II, the signal was expected to be weak and masked by surrounding noise. ICA decompositions were therefore used to extract the signal of interest.

Design

The stimuli were presented by a lateral view of a live model that acted in front of the infant. The model sat on a chair next to a toy train on a short railway track placed on a table. Three conditions were presented: first, in the goal-directed condition the model grasped the train and placed it at the highest point of the railway track; second, in the non-goal-directed condition the model placed his open hand on the table; third, in the static condition the model sat passively for approximately 4 seconds (example shown in *Figure 21*). The EEG was synchronized

to the actions by electronical triggers hidden from the subjects' view when the hand touched the train or the table. In the static condition the EEG was triggered after approximately 1 second of passive sitting.

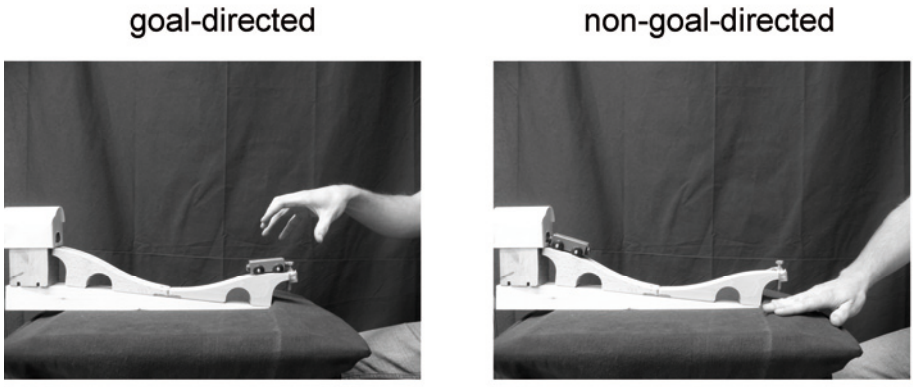


Figure 21. Example of model actions from infants' view. In the goal-directed condition the model reached for the toy train and placed it on top of the slope. When the train was on this more distal location from the model the non-goal-directed condition could be performed. In the non-goal-directed condition the model simply placed his open hand on the table. The EEG was synchronized to the actions by hidden triggers when the hand touched the train or the table. In the static condition the EEG was triggered after about 1 second of passive sitting.

The model never presented any condition if the infant was inattentive. When necessary the model ensured that the infant was attentive by sometimes talking to the infant between trials. During the trials the model was always silent to prevent MNS activation from speech. The conditions were presented repeatedly in a randomized order until the subject was no longer interested (between 10 – 49 presentations of each condition).

The recorded EEG was band pass filtered at 2 - 20 Hz to remove noise and to focus on the frequencies where most brain related signals appear. The data were segmented into trials from -1s to 1s after time-lock (touch of toy or table, or after approximately 1 second of resting). After artefact rejection, where bad trials and channels were excluded from the dataset as in study II, the EEG was transformed to average reference. A natural-gradient logistic infomax independent component analysis was then performed on the data (the Runica algorithm, Delorme and Makeig, 2004).

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 brain signals not related to the MNS. To reduce noise and to focus on components with mirror neuron properties a two step component selection procedure was performed as described below.

First we excluded components that reflected artefacts by the following criteria. Components with any abnormal ICA weight, >2.7 standard deviations of all weights within a component, were considered artefactual and were excluded. The value of 2.7 standard deviations was chosen after visual inspection of all components to retain components with dipole like scalp projections and to exclude components with channel pops or movement artefacts. Also, outlier values that could bias the subsequent frequency analysis were identified by the max absolute amplitudes within components. Trials with abnormal values (>3 sd) were removed. Components including less than 10 trials in any condition were then excluded.

Second, we selected components related to mu rhythm desynchronization from the remaining components. Frequency spectrums of the three conditions were extracted. The results were converted to dB values across a 1 Ohm reference load to simplify visual inspection of the power spectrums for quality estimation. Components with a mu rhythm 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. The mu rhythm frequency interval was based on previous studies of infant alpha rhythms (Stroganova, Orekhova & Posikera, 1999) and set to 5-9 Hz.

This two step procedure selected multiple components from each subject that showed mu desynchronization in the action conditions as compared to the static condition. As there was no distinction between the goal-directed and non-goal-directed condition at this stage it was possible to test them for statistical differences. In total 43 components with 10 – 49 trials from each condition stemming from 23 subjects were selected. Each subject contributed with between 1 and 5 components (mean = 1.87). The components that were not selected were sub-

tracted from the raw EEG to create datasets pruned from noise, artefacts and brain 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%).

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 sensitive analysis of the signal of interest. A time/frequency analysis using discrete wavelet transforms were performed on each channel's (or components's) conditions using the standard EEGLAB time-function which resulted in power maps with 200 time-points and 20 frequency bands. 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 I 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 pixelwize 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

Using the raw EEG data, point wise statistical tests (two tailed t-tests, n = 32) showed no significant desychronizations 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 22*.

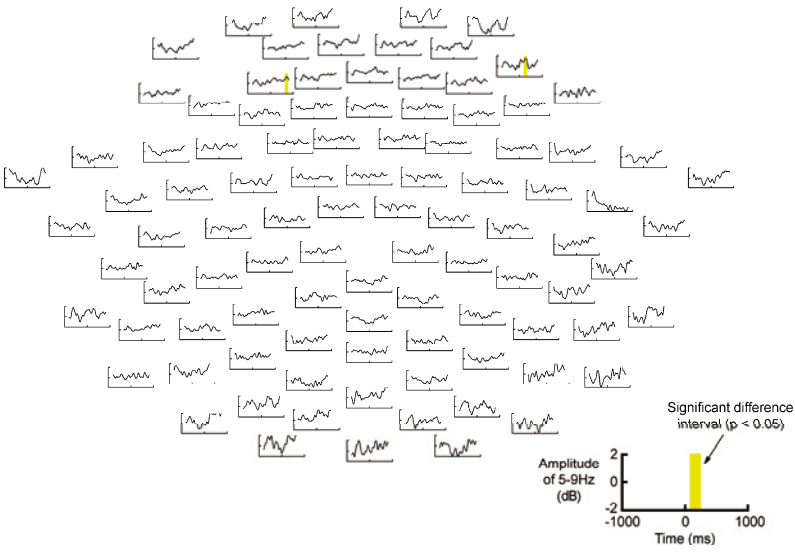


Figure 22. Amplitude differences of the 5-9 Hz frequency band between the goal-directed and non-goal-directed conditions in the raw datasets. Each curve map represents a channel and the layout describes the spatial relations of the sensors on the scalp (nose pointing upwards). Significant differences between conditions are marked with yellow intervals, and channels with significant desynchronization are shaded ($n = 32$, $p < 0.05$, adjusted for multiple testing by removing intervals with less than 10 significant p-values).

In the selected components' case the t-tests between the goal-directed and non-goal-directed conditions show a significant desynchronization of the mu rhythm band. The timing of the desynchronization in the goal-directed condition is approximately when the hand touches the object. A global power minimum of -1.6 dB was found approximately 10 ms after touch ($p=0.0007$). The channels with the largest difference between the goal-directed and the non-goal-directed movement conditions are located on the right frontal lobe as shown in *Figure 23* Two more channels with significant desychronization were located in the left frontal lobe (over prefrontal cortex) and finally two over the occipital lobe.

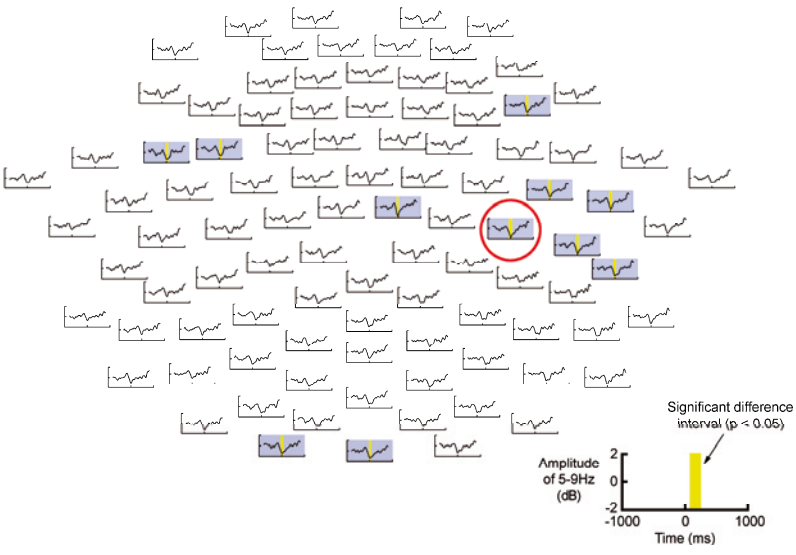


Figure 23. Amplitude differences of the 5-9 Hz frequency band between the goal-directed and non-goal-directed conditions in the pruned datasets containing only the selected independent component projections. Each curve represents a channel and the layout describes the spatial relations of the sensors on the scalp (nose pointing upwards). Significant differences between conditions are marked with yellow intervals, and channels with significant desynchronization are shaded ($n = 23$, $p < 0.05$, adjusted for multiple testing by removing intervals with less than 10 significant p-values). The channel with longest significant interval is marked with a red circle. This sensor is presented in more detail in Figure 24 and Figure 25

A magnified view of the most significant channel is presented in *Figure 24*, which also shows the amplitudes of 5-9 Hz of all conditions. The goal-directed condition desynchronizes at the time of touch and the static condition show a prominent peak in the latter half of the trial.

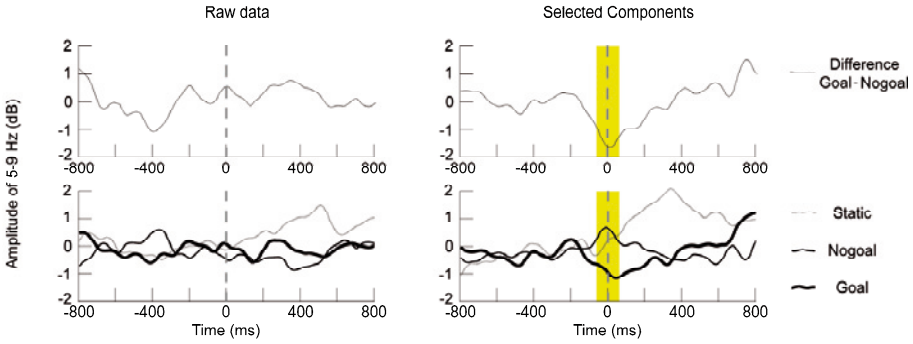


Figure 24. A presentation of the separate conditions in the most significant sensor (marked with a red circle in Figure 23). The left row show data from the raw datasets, and the right row show data from the pruned datasets that only contains the selected independent component projections. Dotted line indicates time of touch.

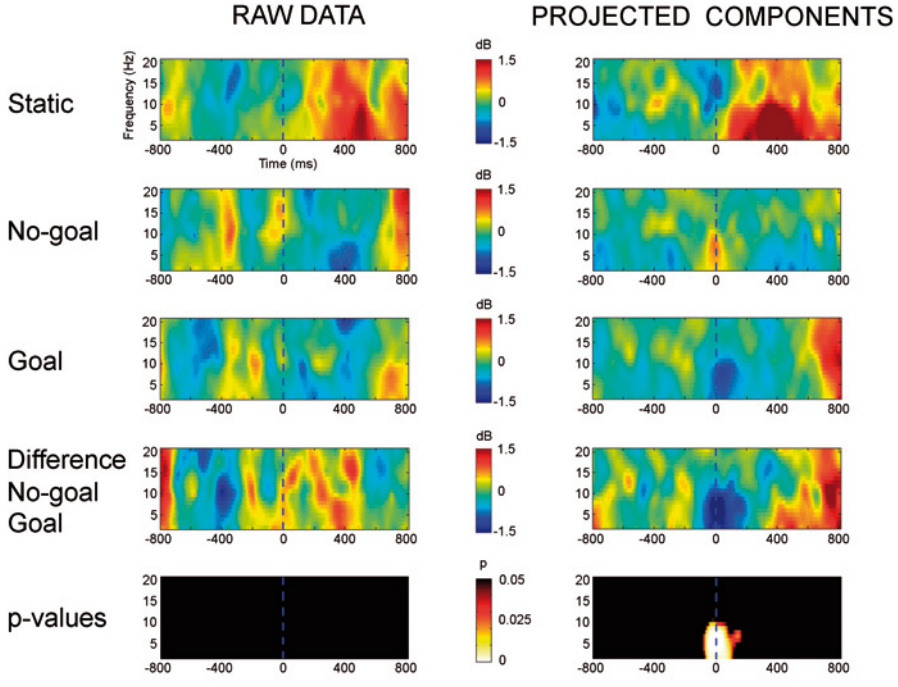


Figure 25. A presentation of the separate conditions in the most significant sensor (marked with a red circle in Figure 23) using the time/frequency decomposition returned by the EEGLAB timef function. The left row show data from the raw datasets, and the right row show data from the datasets that only contains the selected independent component projections. The bottom row show the point wise statistical t-tests' p-values (left plot n = 32, right plot n = 23), thresholded at alpha = 0.05 and adjusted for multiple comparisons (200x20 tests) by removing significant clusters smaller than 20 pixels. Dotted lines shows hand / object time of touch.

The detailed time / frequency analysis of the most significant sensor is presented in *Figure 25*. The difference between the goal-directed and non-goal-directed condition shows a global minimum of 1.6 dB at 10ms after the hand touch the object at 6.8 Hz. The t-test at this minimum results in $p = 0.0002$.

Conclusion

The results from the selected components clearly 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 are very similar (Muthukumaraswamy & Johnson, 2004b). This shows that 8-month-olds display somewhat adult like mu rhythm perturbations when observing goal-directed actions and that the reactivity of the mu rhythm is not lagging. This would imply that 8-month-olds have a relatively mature MNS.

The present results suggest that the development of the mirror neuron system could either precede or develop together with the emergence of various social skills. Perhaps most important, the method used could be applied in various developmental experiments that would further outline the maturation and characteristics of the mirror neuron system.

General discussion

This thesis concerns both neurophysiological and methodological issues. As a valid method is a prerequisite for a valid result the methodological discussion will preceed the neuropysiological discussion.

Methodological issues

The first study uses a fairly traditional and straightforward method. Conventional ERPs are calculated and the activation peaks are fed into an ANOVA. This kind of analysis is familiar to most researchers that do EEG studies. However, the methods used in study II and III are novel and there are several points worth discussing.

ICA is an approach that is used more and more frequently to separate signals into different categories. To perform an ICA there has to be enough datapoints from each subject, and the more datapoints the better the decomposition will turn out. The minimum number of datapoints is the squared number of channels, e.g. 128x128 in this thesis, and the recommended number of datapoints is the minimum number multiplied by a factor 20 for the EEG net used in this thesis (EEGLAB forum). All subjects in our samples exceeded the minimum number of datapoints, but hardly any subject had close to the recommended number of datapoints. It is a challenge to design EEG experiments that keep infants interested for a long period, and it is doubtful if it is possible to do better than a live situation. It is therefore of great importance to evaluate the quality of the ICA decomposition after analysis. In study II and III this was done by visual inspection of the scalp topographies, trial amplitudes and frequency spectrums from each independent component (not presented in the articles due to the large amount of information). It was also found that the standard deviation of the ICA weights were a good indicator of these three factors, but a more systematic evaluation of independent component quality estimation would be of great interest. For example, in study III a component was excluded if the max ICA weight deviated more than 2.7 standard

deviations from the others. This value was based on visual inspection, and a short test was performed after study III to assess the accuracy of this value. In this test two knowledgeable persons viewed the topographic plots of the ICA weights of all components with mu rhythm suppression, and categorized them as dipole-like or artefactual. A ROC curve could then be plotted for different exclusion criteria (0-10 standard deviations). The results from this test are illustrated in *Figure 26*. The test indicates that an exclusion threshold could be used, as the vertical dotted line fairly well separates dipole like and artefactual components. This is also indicated by the ROC curve which shows the balance between correct and incorrect categorized components by the threshold (true positive rate = 0.80, false positive rate = 0.03). The area under the curve (the AUC measure) was 0.95, and the threshold used in study III was located on the convex hull of the curve. This data justifies the use of the max standard deviation of ICA weights to categorize components as artefactual or dipole like. The value of 2.7 std was found to be relative conservative and it can be considered to be a reasonable threshold.

In study II and III the selected independent components were examined using visual inspection. All selected components were found to resemble stable decompositions, even though the recommended amount of EEG data could not be collected. After visual inspect the independent components were therefore considered to be a more sensitive measure than the EEG channel data.

The signal of interest was expected to be very weak. In the macaque brain about 25% of the motor neurons have mirror properties (Rizzolatti & Craighero, 2004), which suggest that the response should not be very strong. There are also MEG studies showing that goal-directed action observation suppress the mu rhythm only 15-17% more than non-goal-directed action observation (Hari, 2006). The signal was also expected to be mixed with other brain related signals, which would make it harder to detect in the raw EEG. 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. An efficient independent component selection procedure is therefore vital for the extraction of MNS activity from the EEG.

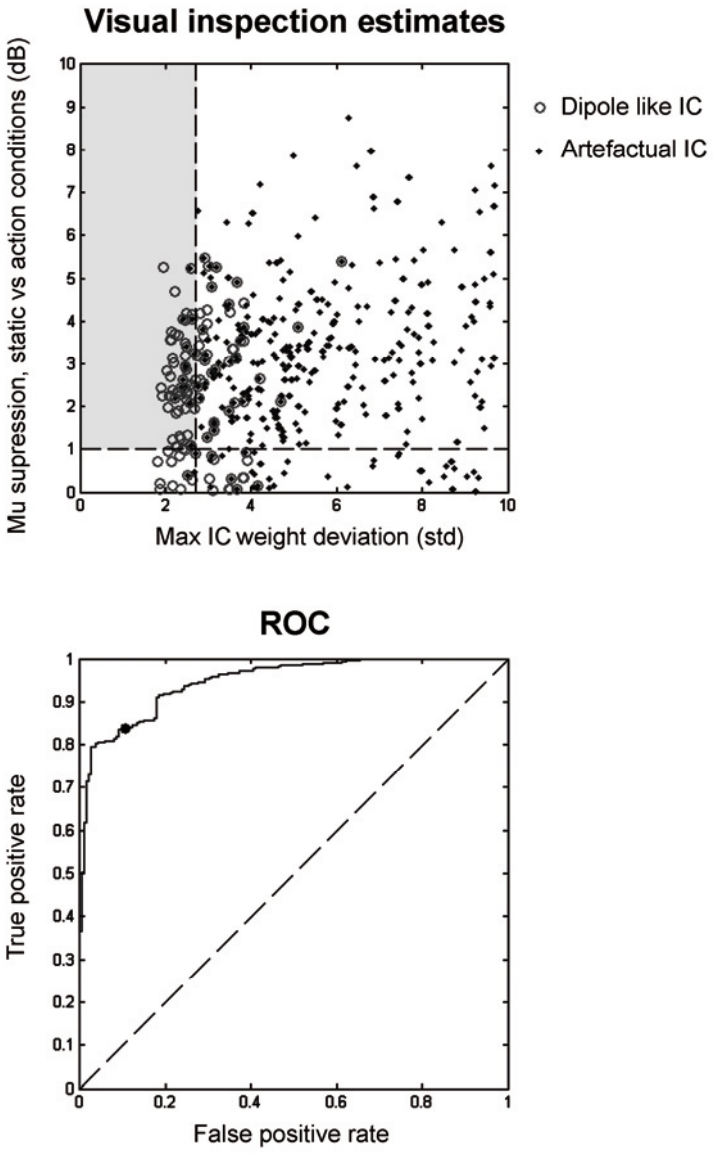


Figure 26. Categorization of independent components after visual inspection of the topographic ICA weights. In top plot the dotted lines represent exclusion criteria of components for study III, and only components appearing in the shaded region was included in the analysis. In the bottom plot the ROC curve for the max ICA weight deviation criterion is plotted. The dot indicate a threshold of 2.7, which was the exclusion criteria in study III.

Study II relies heavily on theoretical assumptions of ICA decomposition and the characteristics of the signal of interest. This is especially true in the component selection procedure because only one component was selected from each subject. A more appropriate method would allow more than one component from each subject. However, the choice of selecting one component was done based on several reasons. First, selecting a single component from each subject would not inflate the statistical effects by increasing the sample size. Second, it would avoid problems with how the statistics should deal with different sized contributions from each subject. Third, the risk of selecting components that were "false positives" was equal for all subjects. The independent component activation was normalized by transforming it to z-scores. This would compensate for the fact that ICA does not recover signal amplitudes and would ensure that each subject contributed equally to the statistical analysis. Normalizing this way is risky. It will be uncertain if we are normalizing signal or noise, and it will be impossible to tell how much variance each signal accounts for. In study II the component selection procedure assumed that the design of the experiment would make the MNS mu perturbations account for most variance in the EEG. However, this is not necessarily the case. The reason to believe that this still was a valid strategy comes from the converging evidence from the different types of analysis, and how the adult data resemble other studies.

In study III several components could be selected from each subject, which was considered an advantage compared to study II. The problems mentioned above were avoided by backprojecting the independent component activity to the scalp channels. This way the measured amplitudes could be compared between subjects. Also, every subject contributed with one signal each, so the statistics were not inflated. The backdraft of backprojecting is that the components will be mixed, which risk that some effects cancel out.

Both study II and III discard most of the signal during the component selection procedure. This is because both studies assumes that the infant MNS is functioning in a similar fashion as the adult MNS, and uses the characteristics of the mu response of static and action observation in adults. As the MNS hypothesis states that goal directed action will result in more mu rhythm desynchronization than just observing movements in general, the analysis can focus on independent components with mu rhythm properties and enhance the mu rhythm

signal. With this strategy most EEG information will be lost, 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 should not be related to the MNS (otherwise they would be included in the analysis). An issue of greater concern is about including components that are not related to MNS activity, or excluding 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.

Importantly, it should be mentioned that there were no significant effects when the components were selected randomly. The validation using random selection indicates that the selection procedure systematically picked out components with a functional reactivity to the stimuli.

Study II and III presents converging evidence that the MNS is activated in early infancy. At about 8 months of age the associated mu rhythms can be used as a marker of MNS activity. However, the MNS might even be innate (as discussed in the introduction section about facial imitation in newborns) and it may require different analysis strategies or measures to measure MNS activity in infants younger than 6 months. At least it would require a totally different experimental design, as the mu rhythm is very weak or absent in very young infants. Finally it should be noted that these novel methods can still be improved, and should be regarded as methodological starting points or references to evaluate improvements.

Neurophysiological issues

Study I bridges methodological and neurophysiological issues in that the questioned asked could not be answered without the methods presented here: how and when does motion sensitive areas in the brain develop? The reason is that brain imaging methods like fMRI, MEG and PET can hardly be used in infant studies due to loud noise, physical constraints or ethical considerations. The high density EEG does not suffer from these shortcomings and can answer the posed questions. Study I shows that dramatic changes take place in the cortical visual processes between 2 and 5 months of age, and the time courses of activation differentiate the investigated cortical areas.

One novel finding in study I is that the MT/MST appear to receive information without the involvement of primary visual areas in the 2-month-olds. The only known alternative pathway is the subcortical pathway via SC and pulvinar. The pathway via SC is suggested to be a phylogenetic old pathway, functioning for non-conscious fear (Morris, Öhman & Dolan, 1999) and fast moving stimuli (Buchner et al., 1997, ffytch, Guy & Zeki, 1995). Interestingly, this short latency pathway has been suggested to dominate the immature visual motion processing in newborn infants (Dubowitz, Mushin, De Vries, & Arden, 1986; Snyder, Hata, Brann, & Mills, 1990). The relative timing of the activations of MT/MST and primary visual areas gives an indication of the origins of the input to these areas. The results indicate that the subcortical pathway appears to be functioning in the youngest infant groups but not the cortical pathway.

Another novel finding in study I is that the motion reactivity start earlier in the left hemisphere and then spreads bilaterally. This was somewhat surprising since most cognitive functions are considered to activate both hemispheres initially and then, after some experience, one of the hemispheres begins to dominate. However, the finding may explain why children with unilateral congenital cataracts do not show impaired perception of global motion while those with bilateral cataracts do (Ellemberg, Lewis, Maurer & Brent, 2000). This would also suggest that the early visual motion processing in the right visual field is somehow connected to the MT/MST on the left hemisphere. This kind of transfer is known in adults (ffytche et al., 2000).

One interesting developmental change that was found in study I was that the polarities switch in the left parietal regions between 3 and 5 month of age. An even clearer polarity shift is seen between the 5-month-olds and the adults, where all polarities switch. While this might seem like a controversial issue, these kinds of polarity switches have been noted in other infant studies and are not considered to be of great concern. Different cognitive processes also switch at different ages. Why the polarity switches occur is still unknown, and requires further experiments to resolve.

In the 5-months group the activation emerges on the left side and spreads to a strong bilateral activation that peaks about 600 ms after motion onset. Although the cortical response is different in the 5-

month-olds than in adults, the behavioural correlates of smooth pursuit and motion perception are quite similar. It is possible that the bilateral activation at 5 months can be related to the development of visually guided reaching at this age (von Hofsten, 1979). The right side, which is activated by 5 months of age, is dominant in processing visuospatial information for reaching in adults. It could be argued that the maturation of the dorsal visual pathway (Gooddale and Milner, 1992) starts on the left side and proceeds to the right at about 5 months of age. This would open a window for visual-manual processing at 5 months of age, when most infants start reaching for moving objects.

The reaching skills of the infants are also important to study II and III. Since all infants in these studies were tested and found to reach successfully, there was a possibility that they could understand other persons' actions in terms of their own motor representations. This would correspond to mirror neuron activity, which was the target of study II and III.

Taken together, studies II and III indicate that MNS activity is present at 6-8 months of age. This is a new finding, since the infant MNS have never been assessed in this direct way before. One of the virtues of EEG is the high temporal resolution, which gives important information. For example, the significant differences were synchronized to the touch of the object. This relates well to the notion that mirror neurons are tuned to the goal of the action (Fadiga & Craighero, 2004; Rizzolatti & Craighero, 2004), and supports the hypothesis that it was the stimulus that elicited MNS activity. If there was no timing to the reaching of the object, the differences would have been more difficult to relate to the MNS.

Study II presented the first signs of MNS activity in infants. The finding was controversial since the method was new, and that slow wave ERPs had not been used to measure MNS activity even in adults. Since the writing of study II there is another study that show strikingly similar potential proportions and timing, which strengthens the claims made in study II. Van Shie and Bekkering (2007) measured slow wave ERPs in a task where an object was reached and transported. Their study also found that the slow wave ERPs was tuned to the goal of the action.

An interresting issue is how the ERP correlates to the mu rhythm perturbations. The ERP differences peaks just before the time of touch, while the mu rhythm differences appear just after the time of touch. This timing difference could indicate that there are two different processes that have been measured, but it could also be a single process with different time evolutions for different frequencies. To be certain, a functional dissociation between the ERP and the mu rhythm has to be demonstrated, but that is beyond the scope of this thesis.

Another important temporal aspect is that the timing of the infants' response was always similar to the adults'. Since there is no time lag, the infant MNS appear to be relatively functional from an early start. It also means that the subjects anticipate the goal, since all reactive responses should cause delays. This anticipation implies that the infants have some form of understanding of the observed action and its aim.

Since the 6-month-olds and 8-month-olds have only been reaching themselves for a few months, the findings in study II and III suggest a tight coupling between motor development and MNS development. The way in which the motor and mirror neurons co-develop is not clear from this thesis, and it is not possible to tell if mirror neurons in motor primitives could facilitate observation learning from combining these motor primitives into higher level action representations. However, without a tight coupling of motor and mirror development this kind of action learning would be impossible.

It is well known that many factors supress the mu rhythm. For example, eye-movements and motor activity during postural stabilization supress the mu rhythm. Other factors are fatigue and the attentional state of the infant (Cochin, Lejeune, Roux & Martineau, 1998). All these factors would make it more difficult to measure mu perturbations, but as the design made it possible to assume that they were not systematically different between conditions, they should not have affected the validity of the results.

It should also be mentioned that there were no significant differences in the beta frequency band. The beta band has been considered to be a part of the mu rhythm, and is considered to be a robust measure of MNS activity (Hari, 2006). Study II and III used the slower mu rhythm since only these have been investigated in infants before (but

not in relation to observed actions and the infant MNS). The absence of beta rhythm perturbations could be explained by the component selection procedures that selectively premiered components with the slower mu rhythm. Since the rhythms are independent of each other in time, the two different rhythms can not be expected to project into the same component.

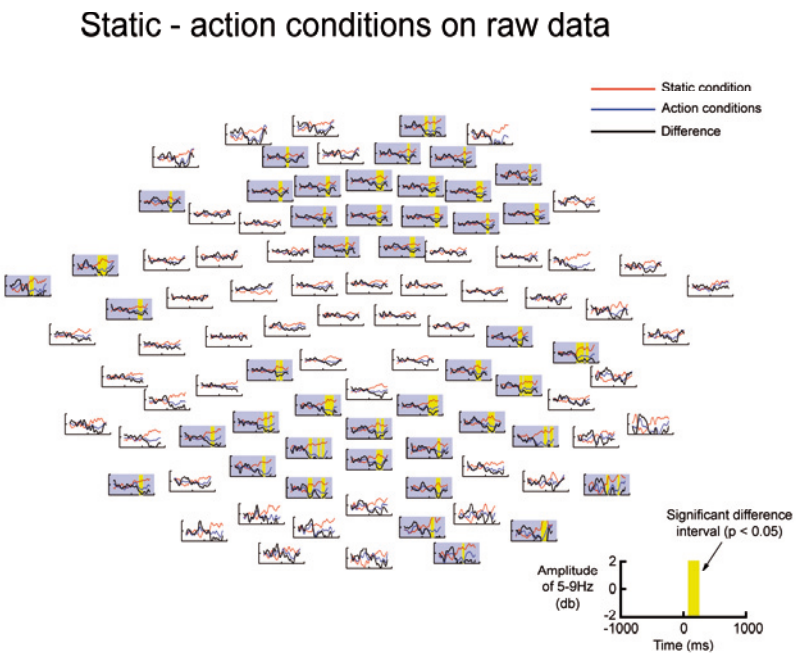


Figure 27. Amplitude differences of the 5-9 Hz frequency band between the static condition and action conditions (goal-directed condition and non-goal-directed condition pooled together) in the raw datasets. Each curve map represents a channel and the layout describes the spatial relations of the sensors on the scalp (nose pointing upwards). Significant differences between conditions are marked with yellow intervals, and channels with significant desynchronization are shaded ($n = 32$, $p < 0.05$, adjusted for multiple testing by removing intervals with less than 10 significant p-values).

Another important aspect of the component selection procedure is that comparisons between the static condition and action conditions cannot be made. This can only be done using the raw data. While the raw signal will contain more noise, the difference in signal will also be bigger. Such a test was performed using the raw data from study III but was not presented in the article since the test does not adress the

question of goal-directedness of actions. However, when making such an analysis, the results show strong mu rhythm differences in many sensors on the scalp (see *Figure 27*). The results presented in study III is thus evidence that the mu suppression during action observation is modulated by the goal-directedness of the action.

There appear to be a line of non-significant sensors approximately across the motor cortex in *Figure 27*. This could be the result of the electrical field projections of tangential dipoles. This is a plausible explanation since much of the motor cortex is buried within the central sulcus, and would thus generate tangential dipoles when activated.

Finally, the results of study II and III will need replications before any certainty about the infant MNS is achieved. These studies give the first evidence of infant MNS activity, but not necessarily the most important. The neurophysiological implications of this thesis will be extended further in the next section, where directions of future research are suggested.

Future directions

The main impact of this thesis is geared toward the future. The apparent possibility to measure MNS activity by the methods used in study II and III opens up a broad range of early MNS developmental studies, and the further work suggested here could also be seen as an implication of the methods presented in this thesis. The design of these experiments can both be replications of adult MNS studies and novel paradigms. It is tempting to discuss potential experiments in detail, but only the main directions will be mentioned below (not ordered in importance).

First, the mu rhythm correlates between action and perception in infants has yet to be established. In these studies the infant will not only observe an action, but also perform the action itself. There will be methodological challenges due to increased numbers of movement artefact, but these problems can hopefully be overcome by selecting suitable actions and intervals of analysis.

Second, we need to integrate the present method with other measures, such as EMG and eye-tracking. This will allow for both better artefact

rejection and behavioural correlates. EMG would be especially useful when infants perform actions themselves.

Third, there is a need for purely methodological studies on component selection. These studies would allow more time and resources for proper testing and evaluation of the methods used in study II and III.

Fourth, since the mu rhythm also consists of beta-rhythms (that are more robust as a measure of mirror neuron activity), these rhythms have to be investigated also in infants. This could be done in several ways, but the most promising would be to perform longitudinal studies similar to the ones by Stroganova, Orekhova & Posikera (1999) and Marshall, Bar-Haim & Fox (2002). In this set of studies also the performance conditions could be included.

Fifth, the motor co-development should be addressed. These studies would examine several different actions, some of which the infants already master and some not, and some that they learn to master during the course of the (longitudinal) study. These studies would also address whether MNS activity can precede the behaviour, and how the MNS facilitates action learning from observing others.

Sixth, is the human MNS innately different from other species'? The activation of the MNS during intransitive action observation has only been found in humans. However, it is still unknown if the infant MNS also parse intransitive actions. If not, when does intransitive action understanding appear and what other developmental changes occurs simultaneously? This knowledge could be important both when intransitive action understanding fails (and how it contributes to social skills) and in philosofical discussions. The skill of perceiving abstract actions have been associated with uniquely human traits such as language learning, imitation and theory of mind (Théoret & Pascual-Leone, 2002; Gallese et al., 2004). Without the knowledge of when and how the human MNS differ from other species it will be speculative to discuss on how the human MNS facilitates these skills.

Seven, different modalities of MNS triggering should be investigated. There are adult studies showing that the MNS does not rely on vision only (Keysers et al., 2003). These studies should be replicated in infant populations to validate the similarities between the adult and infant MNS. Besides, 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.

Eight, it is important to use different perspectives and different measures to fully understand a phenomenon. In this set of studies it would be beneficial to use other markers of MNS than the mu rhythm, such as measuring ERPs in previous mu rhythm designs.

This section shows that there are many studies that would contribute to our understanding of the MNS development. While some of them are more important than others, I believe it would be worthwhile to investigate the infant MNS thoroughly. If the MNS really lies at the core of human social functioning, such studies are of great importance for both the scientific community and for society at large.

Conclusions

Study I conform to other studies and validates that the infant motion perception processes mature rapidly between 2 and 5 months of age. This change in cortical organization is beginning to be measurable at about 3 months of age. Before that, at 2 months of age, the motion sensitive areas appear to receive input from the subcortical pathway that bypasses primary visual areas. Also, the latencies of activation were slower compared to adults, but decreased over age.

In study II a group of 6-month-olds observed videoclips with goal-directed reaches. The results show an increased activation compared to observation of hand movements without a goal object. This suggests that 6-month-olds can parse other people's actions, which in turn could mark MNS activity. The significant ERP measure was part of a novel method of analysis that extracts weak or masked MNS signals. The commonly used marker of MNS activity in adults, the mu rhythm, displayed tendencies but no significant differences.

Study III focused on the mu rhythm as it is an established marker of MNS activity. Since the mu rhythm reacts less to videoclips than to real actions, the infants observed a live model that presented goal-directed reaches and non-goal-directed hand movements. Also, the infants were somewhat older than in study II, 8 months old, and the method of analysis was improved. The results show a robust desynchronization when the infants observed the goal-directed actions, which is similar to the response in adults. We conclude that the MNS is functional already at 8 months of age, and that we have developed a method that makes it possible to measure MNS activity already in infancy.

It should be noted that this research needs to continue. Some methodological issues are still to be resolved or improved, and there are yet much more to investigate regarding the development of the MNS. The strength of this thesis is only in part the new results presented, in that

they increase our knowledge of the developing brain and provide a first EEG measure of the infant MNS. However, the most important part is that this thesis opens up a range of important MNS development studies. These studies can potentially help us outline the development of the normal as well as the dysfunctional MNS.

Summary in Swedish

Den här avhandlingen behandlar utvecklingen av spädbarns hjärnaktivitet i tre studier (I – III). För att mäta hjärnaktiviteten använde vi en sensor-mössa med 128 EEG-sensorer. Dessa sensorer kan mäta elektriska fält utanför huvudet som uppstår i aktiverade hjärnområden. Sensor-mössan sätts på som en vanlig barnmössa och barnen verkar inte bry sig om den när mössan väl är på. Barnen rekryterades genom att vi skickade ett informationsbrev till nyblivna föräldrar där vi beskrev våra projekt. De föräldrar som var intresserade av att vara med skickade tillbaka en svarstalong med sitt telefonnummer på, så att vi kunde ringa dem och komma överens om ett möte.

Studie I undersökte hur hjärnprocesser som bearbetar rörliga synintryck utvecklas. Vi lät barn i tre olika åldrar (2 månader, 3 månader och 5 månader) se roterande och stillastående videosekvenser. Resultaten visar att aktiveringen i rörelsekänsliga områden hos 2 månaders barn var knappt mätbar. Dessutom verkade det som att rörelseintrycken tog än annan väg till dessa områden än den som går via de primära synområdena på hjärnbarken. Hos 3 månaders barn märktes en tydlig aktivering som började på hjärnans vänstra sida, och som sedan spred sig till båda sidorna. Detta är ett intressant fynd eftersom hjärnprocesser ofta antas aktivera båda hjärnhalvorna först och sedan, efter större erfarenhet, domineras av ena hjärnhalvan. 5 månaders barn visade en kraftig aktivitet i båda hjärnhalvorna, vilket tyder på att det har skett en dramatisk förändring i sättet som barn tolkar rörelse med. Denna förändring sker samtidigt som barnens ögonrörelser och griprörelser förändras, och är något som vi vill relatera till varandra. Vi testade också vuxna försökspersoner, och i jämförelse med dessa var barnens aktivering i hjärnan långsammare, men blev snabbare och snabbare med åldern.

Studie II undersökte om det är möjligt att mäta spegelneuron-aktivitet hos spädbarn. Spegelneuron är hjärnceller som aktiveras både när man utför en handling och när man ser någon annan utföra samma hand-

ling. Denna koppling mellan sig själv och andra människor kan ligga till grund för många sociala färdigheter, såsom förståelse av andras handlingar, imitation, empati och språklig utveckling. Trots att spegelneuron är viktiga, inte minst för små barn som ska lära sig att tolka andra människor, är de inte undersökta med hjälp av hjärnaktivitetsmätningar. I studie II fick 6 månaders barn titta på videofilmer där en grip-handling utfördes. Resultaten visar en ökad aktivering när de såg dessa filmer än när de såg filmer där handen rörde sig utan mål. Detta tyder på att 6 månaders barn har en viss förståelse för andra människors handlingar, och är ett tecken på spegelneuronaktivitet. Måttet som användes var en del av en metodutveckling för att mäta svaga hjärnsignaler från spädbarn. Det vanliga måttet för spegelneuronaktivitet, my-rytmen, visade stora likheter med vuxna försökspersoner men inga statistiskt signifikanta effekter.

Studie III var inriktad på att undersöka my-rytmen, eftersom den är ett tillförlitligt mått på spegelneuron-aktivitet hos vuxna. Eftersom my-rytmen reagerar svagare på videofilmer än på verkliga handlingar så fick en person utföra griprörelsen och mållösa handrörelser live. Dessutom testades äldre barn än i studie II, 8 månader gamla, och metoden för att mäta de svaga signalerna var förbättrad. Resultaten visar tydligt att my-rytmen reagerar på nästan samma sätt som hos vuxna. Slutsatsen blir därför att spegelneuron-systemet är funktionellt när barn är 8 månader gamla, och att vi har utvecklat en metod som gör det möjligt att mäta spegelneuron-aktivitet redan hos spädbarn.

Det bör nämnas att forskningen i denna avhandling inte är avslutad. Dels innehåller mätmetoderna fortfarande förbättringsmöjligheter, och dels finns det fortfarande stora luckor i vår kunskap om utvecklingen av det viktiga spegelneuronsystemet. Styrkan i den här avhandligen ligger därför delvis i de nya resultat som presenteras, eftersom de ger en ökad förståelse för hur barns hjärnaktivitet utvecklas för olika tyder av synintryck. Den starkaste poängen med denna forskning är dock att den möjliggör många fler studier för att undersöka utvecklingen av spegelneuronsystemet hos spädbarn.

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Cortical processing of visual motion in young infants

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Abstract

High-density EEG was used to investigate the cortical processing of a rotating visual pattern in 2-, 3-, and 5-month-old infants and in adults. Motion induced ERP in the parietal and the temporal–occipital border regions (OT) was elicited at all ages. The ERP was discernable in the 2-months-olds, significant and unilateral in the 3-month-olds and significantly bilateral in the 5-month-olds and adults. The motion induced ERP in the primary visual area was absent in the 2-month-olds and later than in the OT area for the 3-month-olds indicating that information to OT may be supplied by the V1 bypass at these ages. The results are in agreement with behavioural and psychophysical data in infants.
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Keywords: Visual development; Infants; Visual motion; Extrastriate cortex; ERP

1. Introduction

Visual motion elicits activation in a complex and widespread neural network (Sunaert, Van Hecke, Marchal, & Orban, 1999). One area, the MT+ complex, is considered to have a key-role in this process (Zeki, 2004). It is activated by visual motion (Barton et al., 1996; Born & Bradley, 2005; Gruber, Muller, Keil, & Elbert, 1999; Probst, Plendl, Paulus, Wist, & Scherg, 1993; Sunaert et al., 1999; Tootell et al., 1995; Uusitalo, Virsu, Salenius, Näsänen, & Hari, 1997; Zeki, 1991), processes perceived motion direction, and is crucial for the control of smooth pursuit eye movements (Komatsu & Wurtz, 1988; Newsome, Wurz, & Komatsu, 1988; O'Driscoll et al., 1998). Patients with brain lesions that include the MT area have impaired motion perception (McLeod, Heywood, Driver, & Zihl, 1989; Schenk & Zihl, 1997; Zeki, 2004) and cannot perform smooth pursuit eye movements (Schoenfeld, Heinze, & Woldorff, 2002).

In adults, the signal input to the MT complex is realized by two parallel visual pathways: one that propagates from lateral geniculate nucleus (LGN) to V1,V2 and finally to V5, the primary visual pathway, and one that projects to the MT+/V5 via superior colliculus (SC) and pulvinar (Buchner et al., 1997; Callaway, 2005; Ffytche, Guy, & Zeki, 1995; Schneider & Kastner, 2005; Schoenfeld et al., 2002) or via LGN (Sincich, Park, Wohlgemuth, & Horton, 2004). The pathway via SC is suggested to be a phylogenetic old pathway, functioning for non-conscious fear (Morris, Öhman, & Dolan, 1999) and fast moving stimuli (Buchner et al., 1997; Ffytche et al., 1995). Interestingly, this short latency pathway has been suggested to dominate the immature visual motion processing in newborn infants (Atkinson, 2000; Dubowitz, Mushin, De Vries, & Arden, 1986; Snyder, Hata, Brann, & Mills, 1990). Martin et al. (1999), using functional MRI to study brain activation in young infants, found responses in subcortical structures when presenting flickering light to them. They concluded that the visual pathway for motion via SC is functioning in the neonate.

In addition to the activation of cells sensitive to coherent motion, (Sunaert et al., 1999), visual motion also activates cells sensitive to the temporal correlation of the stimuli, that is, flicker (Bach & Ullrich, 1994; Tootell et al., 1995; Spileers, Mangelschots, Maes, & Orban, 1996).

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The response to flickering light is present at birth (Vitova & Hrbek, 1970) and the sensitivity develops during infancy (Apkarian, 1993; Fiorentini & Trimarchi, 1992; Regal, 1980). In adults, several experiments with visual evoked potential, VEP, (Göpfert, Müller, & Simon, 1990; Kuba & Kubova, 1992; Kubová, Kuba, Spekreijse, & Blakemore, 1995; Schlykowa, van Dijk, & Ehrenstein, 1993) as an endpoint have shown that the response to flickering light strongly depends on adaptation (Herrmann, 2001; Kuba, Kubova, Kremlácek, & Langrova, 2007; Maurer & Bach, 2003; Schlykowa et al., 1993), and the choice of pattern parameters is critical if a genuine motion response will be induced. However, the distribution of cells sensitive to flicker is different from the distribution of motion sensitive cells. Earlier fMRI studies on adults have shown that the flicker response to visual motion is maximal in V1 while the motion-specific response is less prominent or even insignificant at this location (Sunaert et al., 1999). Sunaert et al. (1999) found that the flicker response continues to be strong in the ventral pathway but diminishes rapidly in the dorsal pathway. For instance, the response to flicker in the MT+ area was only 20–50% of the response in V1. In fact, the spatially different distributions of cells sensitive to coherent motion and to flicker give indications of the degree to which visual motion activates these two different kinds of cells in young infants.

The present study asked when cortical processing of visual motion develops in human infants and how the different parts of the visual cortex are activated. There is yet no brain imaging study that has answered these questions. The reason is that methods like PET, MEG and MRI are not generally accessible to a non clinical group of infants. Information about when the processing of visual motion begins to involve the cerebral cortex comes from behavioural studies and studies using VEP (Hamer & Norcia, 1994; Mason, Braddick, & Wattam-Bell, 2003; Wattam-Bell, 1991, 1992). For example, Braddick, Birtles, Wattam-Bell, and Atkinson (2005) studied motion direction sensitivity in young infants with VEP and concluded that between 5 and 18 weeks of age the response becomes progressively stronger. Considering that human infants younger than 6–8 weeks of age do not discriminate motion direction, and do not smoothly pursue small moving objects is another indication that the MT complex is not processing coherent motion before that age. Between 6 and 14 weeks of age infant's ability to discriminate motion direction (Atkinson, 2000; Braddick et al., 2005; Wattam-Bell, 1991), and to smoothly pursue moving objects (Aslin, 1981; Rosander & von Hofsten, 2002; von Hofsten & Rosander, 1997), improves rapidly. In a study of pattern motion integration in 2 to 5 month old infants, Dobkins, Fine, Hsueh, and Vitten (2004) concluded that at 2 months of age, cortical mechanisms process global coherent motion.

Questions related to how cortical processing of visual motion gets established, and especially how it differentially activates cells sensitive to the spatio-temporal (coherent motion) and temporal correlation (flicker) of the stimuli, can be made by analysis of the emerging spatio-temporal distributions of cortical activation over age. We used high-density EEG (EGI 128 Geodesic sensor net) in an ERP design to identify patterns of neural activity in 2-, 3- and 5-month-old infants and an adult group, when they watched stationary and rotating patterns of simple elements. The analyses were focused on changes occurring in the occipital–temporal border, the occipital and parietal regions as these are the ones activated by visual motion in adults. The way these cortical areas become increasingly involved with age provide information of how the visual pathways develop. The relationship between the activations of V1 and MT+, for instance, gives an indication of the degree to which visual motion activates flicker sensitive cells and cells sensitive to coherent motion. Furthermore, the relative timing of the activations of MT+ and V1 gives an indication of the origins of the input to these areas. For example, if the short latency visual pathway via the SC is functioning in the youngest infant groups, moving stimuli can be expected to activate MT+ before or without activation in the primary visual area.

Another set of questions relates to hemispheric asymmetries in the processing of visual motion. Such asymmetries have earlier been observed in adults and children for motion VEPs (Hollants-Gilhuijs, De Munck, Kubova, van Royen, & Spekreijse, 2000). O'Driscoll et al. (1998) found left-side response with PET in the temporal–occipital order area during smooth pursuit. Furthermore, in a study of attention to motion Pavlova, Birbaumer, and Sokolov (2006) found left hemisphere MEG response in the parieto–occipital region. Uusitalo et al. (1997) measured cortical responses to rotational stimuli in adults using MEG. In some of their subjects the responses to motion were only detected unilaterally.

2. Materials and methods

2.1. Subjects

Adult subjects and parents of the participating infants were informed about the experiment upon arrival at the lab. A written consensus was signed in accordance with the Helsinki Declaration. The experiment was approved by the Ethics committee at Uppsala University. A total of 52 infants and 12 adults participated. There were 18 full-term infants aged 6–9 weeks (''2-months''), 16 infants aged 9.5–14 weeks (''3-months'') and 18 infants aged 20-23 weeks old (''5-months''). They were healthy and had no visual problems. The adults were 25–30 years old and had normal vision. All parents and all adult subjects were right-handed.

2.2. Stimuli

The stimuli were designed in E-prime (Psychology Software Tools Inc., 2002). This program also synchronized the stimulus monitor with the EEG measurements. The stimuli consisted of an inner and an outer set of simple geometric figures positioned at the corners of two concentric pentagons on a static background grid (Fig. 1). The colour of the figure elements was the same for a specific stimulus but varied between them (Table 1). The elements of the inner pentagon were 14–17 mm in diameter and were posi-

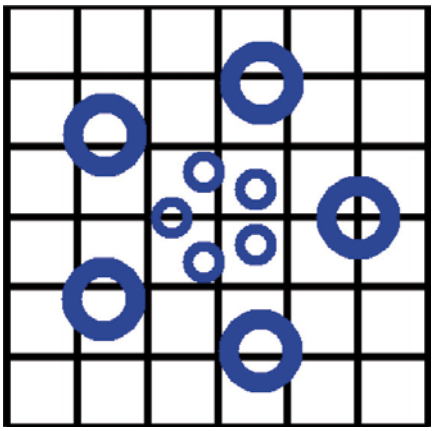

Fig. 1. The stimulus used had two pentagram sets of simple geometric figures oriented concentrically. The figures were coloured circles, crosses, or squares presented against a grid structure on greyish background.

Table 1
Average CIE parameters x, y (colour coordinates) and luminance (Y) for the stimulus, exemplified in Fig. 1

Colour	x	y	Y
White	0.276	0.304	89.1
Green	0.282	0.625	59.3
Magenta	0.272	0.147	27.0
Red	0.595	0.352	17.2
Blue	0.154	0.074	10
Black	0.285	0.338	0.4

tioned 20 mm from the centre of the pattern. The elements of the outer pentagon were 29–36 mm in diameter and were positioned 60 mm from the centre of the pattern. When the figures were set into motion, the inner and outer sets moved around the centre of the pattern in opposite directions at 60 deg/s. In terms of visual angle, the velocity of the inner elements was approximately 5 deg/s and the velocity of the outer elements 15 deg/s with 20 Hz motion frequency. In half the trials, the inner figures moved clockwise and the outer elements counter-clockwise and in the other half the motions were reversed. The counter rotation of the inner and outer sets of geometric figures in the motion stimuli were chosen in order to avoid eye movements.

The duration of every trial was 3 s. It always started with the stimulus pattern being stationary. On half the trials the patterns started to rotate after a random period of 0.8–1.25 s. The duration of motion was always 0.95 s. On half the trials the patterns remained stationary for the same period. The trial period was finished with showing a stationary colourful picture of an animal for 0.8–1.25 s. This was done to make the display more attractive. The onset and duration of the intermission picture as well as the onset of the static and moving parts of the pattern was randomized in such a way that an expectation response was avoided. Furthermore, the duty cycle is critical to avoid adaptation (Bach & Ullrich, 1994). Thus, the proportion of time with motion was 16%. (in one half the 3 s trials motion was presented for 0.95 s). This is similar to the proportion of time with motion in earlier studies. For instance, Hollants-Gilhuijs et al. (2000) presented motion 19% of the time in a study on children, Kubová et al. (1995) presented motion 17% of the time, and Schmolesky et al. (1998) presented motion 14% of the time. Consequently, no adaptation (Bach & Ullrich, 1994; Krekelberg, Boynton, & van Wezel, 2006) was supposed to occur, also when considering the size of the stimuli (Müller, Göpfert, Schlykowa, & Anke, 1990; Schellart, Trindade, Reits, Verbunt, & Spekreijse, 2004; Schlykowa et al., 1993; Sunaert et al., 1999). A whole session took 6.4 min, and included 64 static and 64 motion trials.

2.3. Procedure

An appropriately sized 128-electrode EEG net (EGI Corp., Eugene, Oregon) was applied on the skull of the subject and adjusted so that the reference electrode (vertex) and the ear references were correctly placed. The infant was then immediately positioned in front of the monitor at a distance of 0.45–0.50 m. At this distance the display, viewed binocularly, covered 40° visual angle horizontally and 28° vertically. The 2-month-old infants were held by the parent over his/her shoulder such that the parent faced away from the monitor. This position was found to give good support to the infant's body without making the infant lean on the net. The older infants sat in a special baby seat (the "Bumbo," SouthAfrica) that supported them in an upright sitting position and avoided leaning on the net. The light was dimmed during the experiment for the 3- and 5-month-old infants and was switched off for the 2-month-olds in order to make the surrounding less distracting. During the experiment, the face of the infant was recorded by a video camera placed on the top of the display monitor for later rejection 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 45 cm distance, watching the stimuli.

2.4. EEG measurements

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 3.5, Eugene, OR).

2.4.1. Data analysis

The recommendations (Picton et al., 2000) and measurement routines suggested by Johnson et al. (2001) were carefully followed. After the experiment, data was bandpass filtered 0.1–80 Hz, transferred to EEGLAB toolbox (version 4.512) in the Matlab environment (Delorme & Makeig, 2004), re-referenced to an average reference, and notch filtered (45–55 Hz to remove main voltage noise without smearing out high frequency artifacts). The video was inspected and extended intervals of inattention were excluded from further analysis. For the infants, we found that the lowest row of sensors in the neck seldom contacted the scalp, although the net was properly placed. These 21 sensors were excluded, as well as the most frontal ones (15 sensors), leaving 92 sensors to be analyzed. One second of data from each trial was extracted, 0.2 s before and 0.8 s after motion onset. Corresponding time intervals were extracted from the static trials. The resulting trials were base-line corrected using the interval before time-lock. An artifact routine analyzed each channel separately and removed trials with an amplitude range of $>120\ \mu V$ in infants and $>30\ \mu V$ in adults before the average ERP was calculated. If a subject had any region of interest, ROI, with less than 20 moving or stationary trials, the subject was excluded from further analysis. Group averages for the moving and the static condition were computed and low pass filtered at 20 Hz (Nyström, 2004).

2.4.2. Regions of interest (ROIs)

The regions of interest (ROI), i.e clusters of sensors (Fig. 2) were chosen to cover critical parts of the visual areas. The occipital ROI (OCC) covered the most medial-posterior–occipital part (sensors 75, 76 and 83), and the occipital–temporal (OT) border region covering the MT+ area was assigned to the sensor cluster that showed the highest ERP for motion in the study by Gruber et al. (1999) (52, 59, 60 and 86, 92, 93) . Finally, the sensors covering the parietal parts, (PAR), were chosen according to

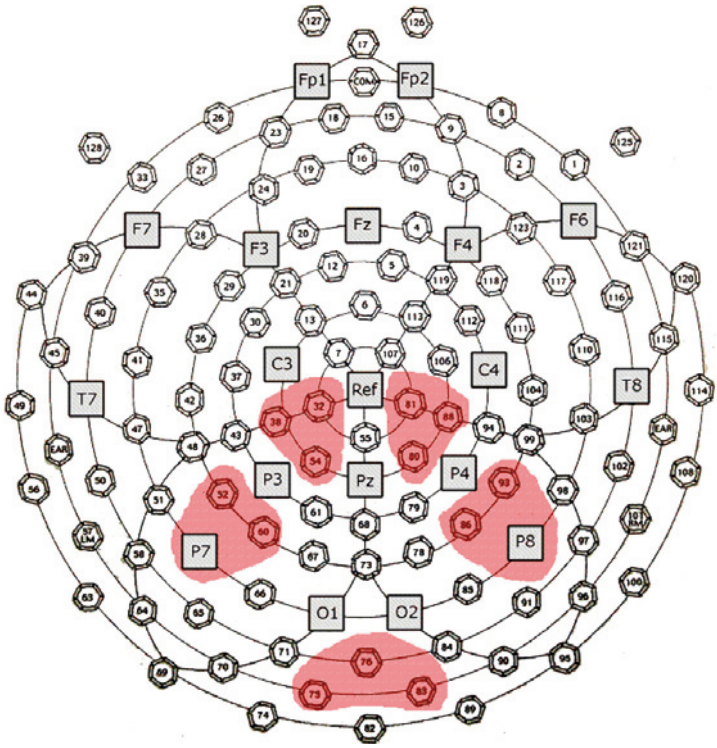

Fig. 2. Layout of the geodesic 128-sensor system (EGI System 200 Technical Manual, see www.egi.com). Ten to twenty electrode sites are indicated to allow for a comparison. Sensors 75, 76, 83 = OCC, 52, 59, 60 and 86, 92, 93 = OT, left and right respectively. Sensors 32, 38, 54 and 81, 88, 80 = PAR, left and right respectively.

Diana, Vilberg, and Reder (2005) (sensors 32, 38, 54 and 80, 81, 88). The activity in each ROI and hemisphere was averaged for the ERP calculation. The sensor clusters chosen for the analyses of the present results coincide with those in adults for which source analysis have been performed, a strategy that has been applied in other developmental studies, as when comparing ERP detected in face perception in infants (Halit, de Haan, & Johnson, 2003).

2.4.3. Statistics

Each individual ERP during the first 800 ms after motion onset was divided into 40 ms periods, and the mean was calculated for each period, thus giving 20 values for the stationary and 20 values for the motion conditions for each ROI. A set of GLM repeated measurement ANOVAs (one for each age level) were used to analyze the pattern of activation for the OT and PAR regions with motion (2), hemisphere (2) and time (20) as factors. In order to optimize detectability of activation in the V1 region, only one ROI was used to represent the activation of this area. Thus, the ANOVAs performed for OCC had only motion (2) and time (20) as factors. To analyze the motion related interactions between the OT and the PAR regions, ANOVAs including both these areas were conducted for each age group. In addition to these analyses, the effect of age was tested within each ROI. The independent variables were age, ROI, hemisphere, time after stimulus presentation (time), moving/stationary (motion), and the dependent variable was ERP voltage. Sphericity was always tested and, if necessary, the SPSS correction was used. The adult group was only included in the tests of the separate ROIs.

3. Results

3.1. Subjects

All infants accepted the sensor net very well and were interested in the stimuli. When the video films were inspected, no tracking eye movements or blinks were observed when the infant was attentive. In the group of 2-month-olds, 5 infants were excluded because of fussing and another 2 did not pass the artifact routine. Thus, a total of 11 subjects were analyzed. One subject was excluded in the 3-month-olds group because of fussing and 4 did not pass the artifact routine leaving 11 subjects to be analyzed. None of the 5-month-olds were excluded due to fussing and 3 did not pass the artifact routine leaving 15 subjects to be analyzed. In the adult group 11 out of 12 subjects passed the artifact procedure.

3.2. Scalp plots

Topographic head plots illustrating the differential activity changes in the temporal–occipital–parietal areas during

the stimulus presentation periods are shown in Fig. 3. The plots depict the average at each 100 ms period after stimulus onset. It can be observed that for the 2-month-olds, the response is small with a maximum at around 200 ms dominating on the left side. At 3 months the response is more wide spread and a peak is observed at around 500 to 600 ms, left side. In the 5-months the activation is clearly bilateral, starting on the left side. In adults it is also bilateral but with reversed polarity.

3.3. ERP distributions

The ERPs for the moving and stationary stimuli are shown in Fig. 4. As can be seen from Fig. 4, the time dependent effects of Motion change drastically with age. For the 2-month-olds they are just noticeable while they are quite dramatic for the 5-month-olds. All the 3 ROI's showed such age effects; OT ($F(38,646) = 5.309$, $p < 0.001$, $\eta^2 = 0.238$), PAR ($F(38,646) = 3.570$, $p < 0.001$, $\eta^2 = 0.174$), and OCC ($F(38,646) = 2.022$, $p < 0.001$, $\eta^2 = 0.106$). Below the time dependent effects for each age level are reported.

The results from the 2-month-olds showed just marginally significant differences in the evolvement of the ERP signal over time between the moving and stationary stimuli in the OT ($F(19,190) = 1.564$, $p = 0.07$, $\eta^2 = 0.14$) but not in the PAR ($F(19,190) = 1.501$, $p = 0.09$, $\eta^2 = 0.13$) regions. For the OT region, the peak amplitude occurred at 260 ms. In addition, there is an interaction between Region (OT and PAR) and motion ($F(19,190) = 2.173$, $p < 0.004$, $\eta^2 = 0.18$). As can be seen from Fig. 4, the effect of motion is positive for the OT region and negative for the PAR region.

For the 3-month-olds, there is a significant effect of motion ($F(19,190) = 3.348$, $p = 0.001$, $\eta^2 = 0.25$). This effect is different for the two hemispheres ($F(1,10) = 8.538$, $p = 0.015$, $\eta^2 = 0.46$). The motion ERP in the OT of the left hemisphere dominates. Furthermore, the peak on the left side appears earlier than that on the right side (550 and 760 ms, respectively). For the PAR region, there is a significant interaction between motion and hemisphere ($F(19,190) = 4.346$, $p = 0.001$, $\eta^2 = 0.30$). The activation at the PAR on the left hemisphere peaks at 550 ms but there is no significant activation on the right side. There is also a

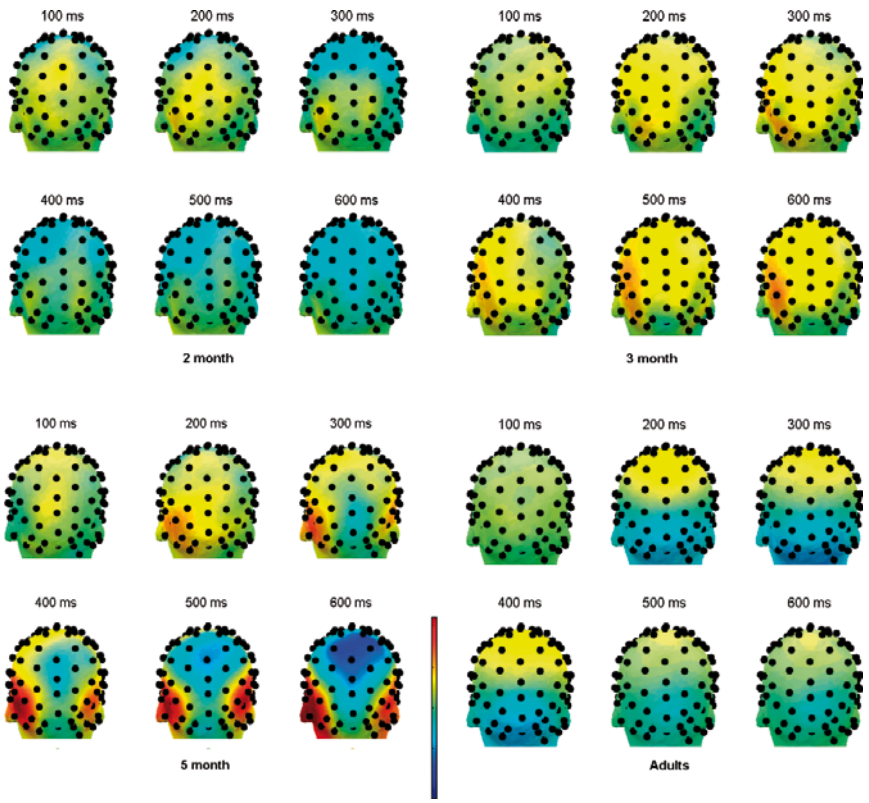

Fig. 3. Back view scalp plots in the infant groups at different time intervals (0–800 ms) after motion onset. Red indicates maximal positive response.

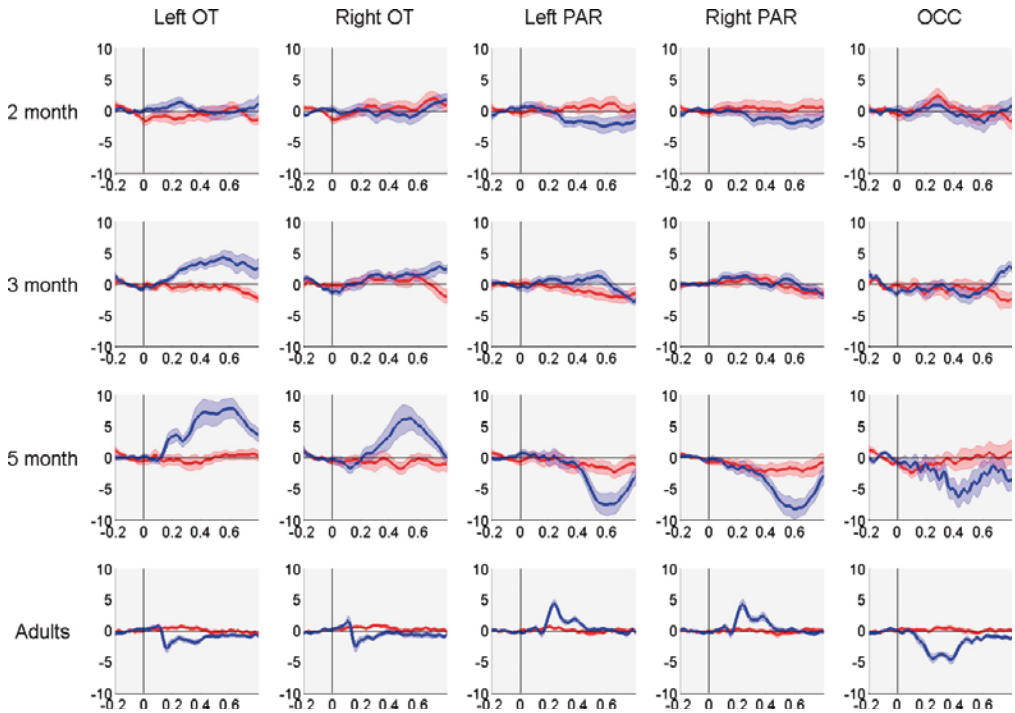

Fig. 4. Grand mean ERP in sensors 52, 59, 60 (OT,left side) and in 86, 92, 93 (OT,right side), 32,38,54 (PAR,left side), 80,81,88 (PAR, right side) and in sensors 75,76, 83 (OCC). Moving (blue) and stationary (red) stimuli are indicated, as well as the standard error between subjects (faint blue/red). The vertical axis is amplitude (μV) and the horizontal axis is time (s). The vertical line at time 0 ms indicate onset of rotation, alternatively continued still picture.

time dependent interaction between Region (OT and PAR) and motion ($F(19,190) = 2.401$, $p < 0.001$, $\eta^2 = 0.19$). Finally, for the OCC region, there is a marginal effect of motion ($F(19,190) = 1.559$, $p = 0.07$, $\eta^2 = 0.14$). This activation is quite late and peaks at 790 ms.

Fig. 4 shows that for the 5-month-olds, there is a strong effect of motion in the OT region ($F(19,266) = 8.738$, $p < 0.001$, $\eta^2 = 0.38$). There is a weak but significant effect of hemisphere ($F(19,266) = 1.715$, $p = 0.034$, $\eta^2 = 0.11$). The activation of the OT in the right hemisphere occurs later than in the left, but the peak activation does not show this difference (250/450/610 ms on the left and 540 ms on the right). Furthermore, there is a significant activation of the PAR region ($F(19,266) = 6.756$, $p < 0.0001$, $\eta^2 = 0.33$) but no effect of hemisphere. The ERP peaks at 600 ms for both sides. There is an interaction between ROI (OT and PAR) and motion ($F(19,266) = 19.01$, $p < 0.001$, $\eta^2 = 0.58$). The effect of motion is quite different for the two regions and it is dependent on time. In the OT region the effect is positive and in the PAR it is negative. Finally, there is a significant effect of motion in the OCC region ($F(19,266) = 3.417$, $p < 0.0001$, $\eta^2 = .2$) that peaks at 430 ms.

The ERPs of the adult group show a reversed polarity relative to the infants. There is a significant effect of motion in the OT region ($F(19,190) = 5.665$, $p = 0.001$, $\eta^2 = .36$) but no effect of hemisphere. The latencies of the peak activation are the same for both sides (165 ms). A time dependent effect of motion is also obtained in the PAR region ($F(19,190) = 14.22$, $p < 0.001$, $\eta^2 = .59$). The effect is similar for both hemispheres peaking at 240 ms. The ANOVA that included both the OT and the PAR regions show that the activations of motion was strongly dependent on Region ($F(19,190) = 23.25$, $p < 0.001$, $\eta^2 = 0.70$). The effect of motion is quite different for the two regions and is dependent on time. In the OT region the effect is negative and in the PAR it is positive. Finally, there is a significant activation from motion in the OCC region that peaks at 240 ms ($F(19,190) = 11.51$, $p < 0.001$, $\eta^2 = 0.54$).

4. Discussion

The present results show that dramatic changes take place in the cortical processing of visual motion between 2 and 5 months of age. While the activations for the 2-month-olds were just discernible, the activations for the

5-month-olds were massive. There is evidence that the stimulus motion used in the present study activated cells tuned to coherent motion as well as flicker: the objects moved with 20 Hz, inducing flicker response, and moved with 5 or 15 deg/s, inducing motion response. The arguments are as follows. From 2 months of age, infants track objects with smooth pursuit eye movements geared to the velocity of the stimulus with a lag of less than 100 ms and a gain adjusted to the stimulus velocity (Rosander & von Hofsten, 2002; von Hofsten & Rosander, 1997). This was at least valid for objects down to a size of 2.5° and for oscillations up to 0.4 Hz. Thus, the start of movement of the stimulus in Fig. 1 should activate motion sensitive cells in the infant's visual brain. In 50 ms the inner and outer stimulus elements move 0.25° and 0.77°, respectively, which corresponds to 5 and 15 deg/s. and is within the limits of resolution of the visual system. Clarke (1973) found that stimuli velocities close to 10 deg/s induced a VEP related to coherent motion (Clarke, 1973; Kuba et al., 2007). As the sensitivity for temporal flickering is almost adult-like for 2–5-month-old infants (Regal, 1980), the flicker in the present stimulus (20 Hz or less), should evoke cortical activation in all infant subjects. As discussed above (see Section 2) the activation of flicker vs motion was measured by Sunaert et al. (1999). Their stimulus size and velocity were in accordance with Clarke (1973), Müller et al. (1990) and Schlykowa et al. (1993), who measured VEP in adults. In conclusion, both types of motion, time (flickering) and spatio-temporal (object velocity) were observed attentively by the subjects.

The ERPs in the infant groups had a higher variation between individuals as compared to the adult group. This is just what is expected in a period of dynamic change and is a function of biological variation in neural growth, maturation and differentiation. Furthermore, the latencies of the ERP were considerably longer in infants as compared to adults. This makes sense considering the fact that that the latency for smooth pursuit onset is 0.6 s in 2-and 3 month olds (von Hofsten & Rosander, 1996) and 0.15 s in adults (Bahill & McDonald, 1983). Also the latency for saccades is around 0.5 s in infants (Gredebäck, Örnkloo, & von Hofsten, 2006), while it is 0.2 s in adults (Engel, Anderson, & Soechting, 1999). Another result that differs between infants and adults is the reversed polarity of the ERP. This has also been found for young children (Langrova, Kuba, Kreml'cek, Kubova, & Vit, 2006). However, a discussion of the neural background for such maturation requires further experiments,

4.1. The development of cortical activation to visual motion in the OT region

Although it was not possible to pinpoint the position of the MT+ area in any reliable way, the electrodes at the occipital-temporal (OT) border region showing the highest ERP response in Gruber et al. (1999) turned out to be very good indicators of cortical responses to visual motion in the infants studied. The results indicated that the response of the 2-month-olds is weak and rather unfocused. The separate analyses of the OT and PAR regions gave only marginally significant time dependent effects of motion, while the combined OT-PAR analysis showed a significant interaction between these two ROIs. The scalp plot also indicates that a response takes place (Fig. 3). Anatomically, histological studies by Flechsig (1901) and further reported by Burkhalter, Bernardo, and Charles (1993), Tootell and Taylor (1995), and Watson et al. (1993) support these findings. Flechsig (1901) found that the MT+ area (see Discussion in Watson et al., 1993) was myelinated at birth. Movshon, Rust, Kohn, Kiorpes, and Hawken (2004) measured receptive-field properties in infant macaques and found direction-sensitivity in the majority of MT cells at 1 week of age, (comparable to 1-month-old humans) although the neuronal dynamics was not adult-like.

Distinct cortical activation from visual motion was obtained for the 3-month-old infants in the present study, but only for the left hemisphere (Fig. 4). This unilateral activation was unexpected and there are several possible explanations for this result. If the input to the left hemisphere comes primary from the right eye at this age as suggested by LeGrand, Mondloch, Maurer, and Brent (2003), it would imply that the left eye does not provide any input to the visual cortex during, at least, the first 0.8 s after motion onset. This seems rather unlikely because visual smooth pursuit functions quite well over a large part of the visual field at this age with short onset latency (0.56 s) and high gain over the entire range of the trajectory (Rosander & von Hofsten, 2002; von Hofsten & Rosander, 1996, 1997). In those studies, infants were presented with horizontal motion covering 50° visual angle. If, on the other hand, the MT+ in the left hemisphere processes visual motion from both visual fields, it could explain why children with unilateral congenital cataracts, tested at 6 years of age do not show impaired perception of global motion while those with bilateral cataracts do (Ellemberg, Lewis, Maurer, & Brent, 2000). That explanation suggests that the early visual motion processing in the right visual field is somehow connected to the MT+ area on the left hemisphere. Such a transfer has been shown in adults (Ffytche, Howseman, Edwards, Sandeman, & Zeki, 2000).

The 5-month-old infants showed a strong bilateral response of motion in the OT region beginning at around 200 ms and peaking at around 600 ms. The response starts earlier in the left than in the right hemisphere (see Fig. 4), thus showing that the asymmetry found in the 3-month-olds persists to some extent for the 5-month-olds. Although the cortical response to visual motion was different in the 5-month-olds than in the adults, the behavioural correlates of smooth pursuit and motion perception are rather adult-like at this age. Another sensory quality processed by the MT+ area is binocular disparity (Born & Bradley, 2005). Psychophysical data on the development of binocular disparity

show that it also matures between 3 and 5 months of age (Birch & Held, 1983; Braddick, 1996; Gwiazda, Bauer, & Held, 1989).

In adults, rotation patterns with changing direction give rise to a strong response in the MT+ area (Morrone et al., 2000). Also Uusitalo et al. (1997) studied rotational stimuli using MEG. They found activation in the occipito–parietal–lateral region after 100–130 ms, with sources in the occipital lobe and in the pre-rolandic region. In some of their subjects activation was only detected unilaterally. Probst et al. (1993) determined dipole sources in the occipital–temporal–parietal region and in extrastriate areas peaking at 160–200 ms after motion stimuli, which is similar to the present study.

4.2. The development of activation to motion in the PAR region

In adults visual motion activates areas in the parietal region, downstream from MT+. To evaluate this activity in infants, sensors in the parietal region (PAR) were chosen according to Diana et al. (2005). Similar to Chugani's (1998) PET observation of maturation of the parietal region, an ERP in the left PAR was observed for the 3-month-olds. In contrast to this study, however, the response in the present study was only observed in left PAR region. A possible reason for this discrepancy is the difference in resolution between ERP and PET. While the present analysis included the first 0.8 s after motion onset, the PET technique average responses over much longer periods. Thus a right hemisphere response could be present but not within the time window analyzed.

In the 5-month-olds the cortical activation in the PAR ROI was reversed relative to the 3-month-olds and the asymmetry had disappeared. Thus, the response at 5 months resembles the adult one. Could the emergence of stronger right hemisphere OT and PAR activations at 5 months be related to the development of visually guided reaching at this age (von Hofsten, 1979)? The right side is dominant in processing visual–spatial information for reaching in adults (Farne et al., 2003; Hermsdörfer, Laimgruber, Kerkhoff, Mai, & Goldenberg, 1999; Jeannerod & Farne, 2003; Oreja-Guevara et al., 2004). Furthermore, Rizzo, Rotella, and Darling (1992) showed total loss of reaching in an adult patient with right side occipito–temporal brain lesion. It is possible that early human neural maturation of the dorsal visual pathway (Goodale & Milner, 1992) starts on the left side and proceeds to the right, thus opening a window for visual–manual processing at 5 months of age, when most infants start reaching for moving objects.

4.3. The qualities of cortical activation to visual motion

Visual motion activates both cells sensitive to the spatio-temporal (coherent motion) and temporal correlation (flicker) of the stimuli. The spatial scalp distribution of the measured cortical activation indicate how it is related to these different types of responses to motion. Sunaert et al. (1999) found that the response to flicker in the MT+ area was only 20–50% of the response in V1. In contrast, the response to coherent motion is weak in V1 and strong in MT+. This is the pattern observed in the present study. The response to motion in OCC was absent for the 2- month-olds and for the 3-month-olds it was both later and weaker in OCC than in the OT area. It is possible, of course, that the pattern of cortical activation from visual motion is different in infants than in adults, that is, that cells that later respond to coherent motion may respond to flicker and vice versa.

4.4. Visual pathways involved in the cortical response to motion

The absence of ERP in the OCC ROI at 2 months of age indicates that the SC pathway for visual motion develops ahead of the primary visual pathway. The interpretation of OKN data in infants agree with this conclusion. In newborns, it is driven by a subcortical system (Atkinson, 2000). The asymmetries between nasal and temporal direction found at 2–3 months of age indicate an emerging cortical path that functions at 5 months of age. (Norcia, Hamer, & Orel-Bixler, 1990; Wattam-Bell, 1991). Dubowitz et al. (1986) strongly suggest that the cortical processing of visual motion in the primary visual pathway starts at around 2 months. The higher demands on cell activity in the V1–V2 region are reflected in a reversal in BOLD at 8 weeks of age (Muramoto et al., 2002). In the present study only the first 0.8 s after motion onset was analyzed and it is possible that the un-responsiveness of the OCC for the 2-month-olds is related to the response latency of the different visual pathways. In other words, the primary visual pathway may just be too slow to be detected by our ERP analysis at this age. The developmental progression as reflected in ERP supports this conclusion. At 3 months, the motion evoked ERP in the OCC is only marginally significant, but more importantly, the differential response only evolves at the end of the measured time interval. In the 3-month olds the ERP latency in OT is much shorter than that in the OCC, which supports the hypothesis of a functioning subcortical pathway, as suggested by the Atkinson, Braddick and Wattam-Bell group.

5. Conclusion

It seems that the first level of visual motion processing in OT takes place in the left hemisphere and develops bilaterally between 3 and 5 months of age. During this period it is a gradual involvement of visual areas. For example our results strongly support that the maturation of the MT+ area results in the adult like smooth pursuit at 5 months of age.

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Paper II

Preliminary manuscript, do not quote without permission

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.

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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

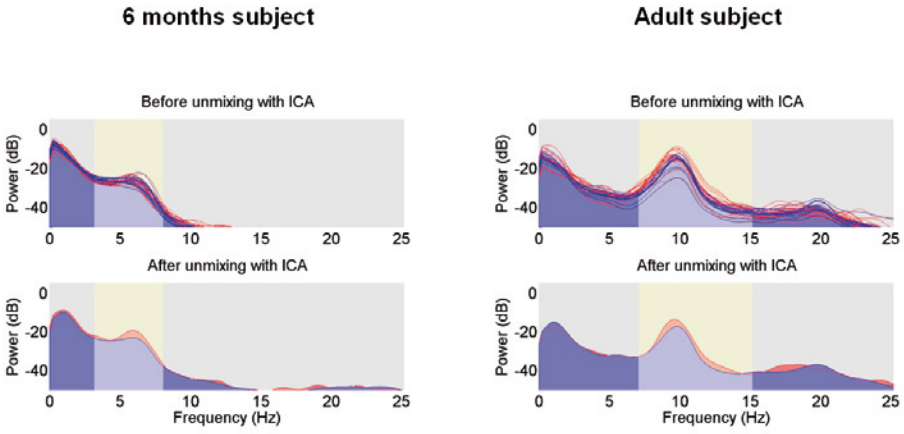

6 months subject
Adult subject
Before unmixing with ICA
After unmixing with ICA
Power (dB)
0
-20
-40
0
5
10
15
20
25
Frequency (Hz)

Figure 2

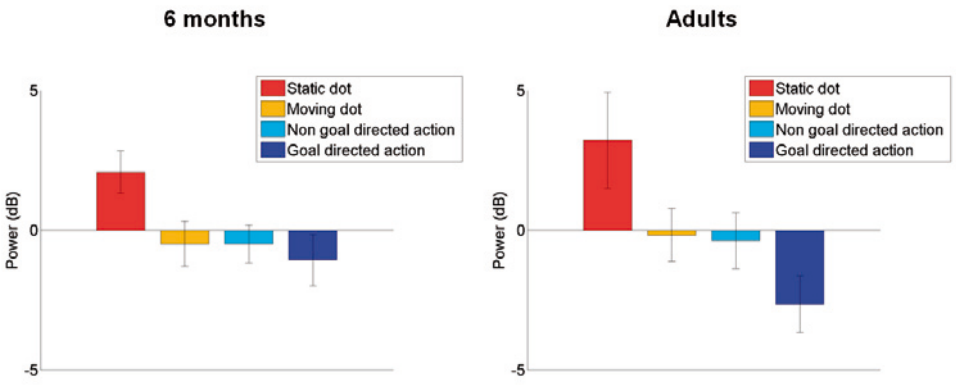

6 months
Adults
Static dot
Moving dot
Non goal directed action
Goal directed action
Power (dB)
5
0
-5

Figure 3

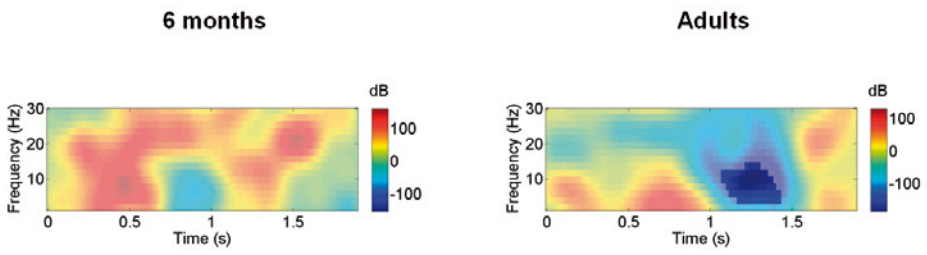

6 months
Adults
dB
100
0
-100
Frequency (Hz)
30
20
10
0
0.5
1
1.5
Time (s)

Figure 4

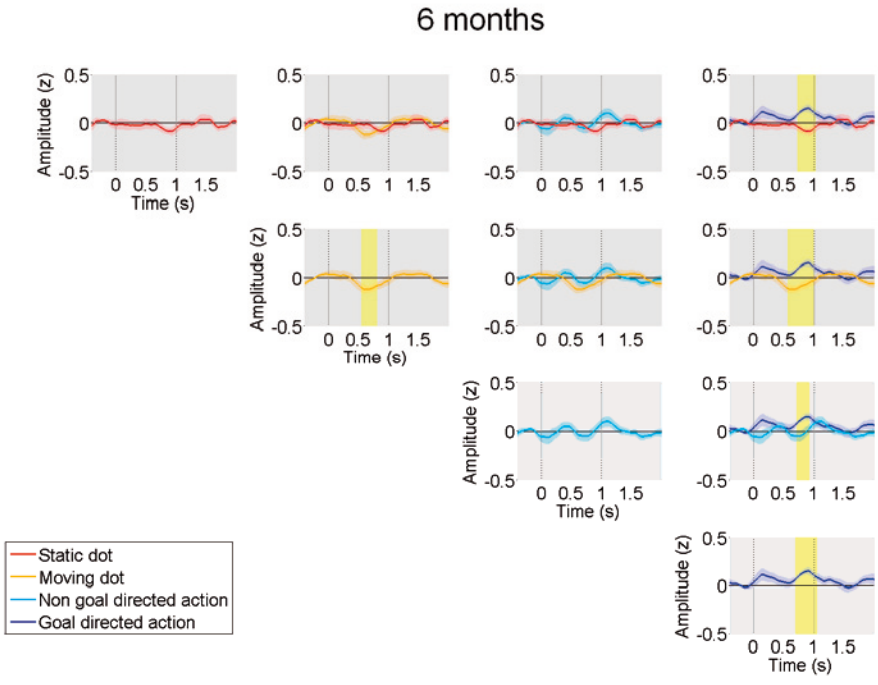

6 months
Amplitude (z)
0.5
0
-0.5
0 0.5 1 1.5
Time (s)
Static dot
Moving dot
Non goal directed action
Goal directed action

Figure 5

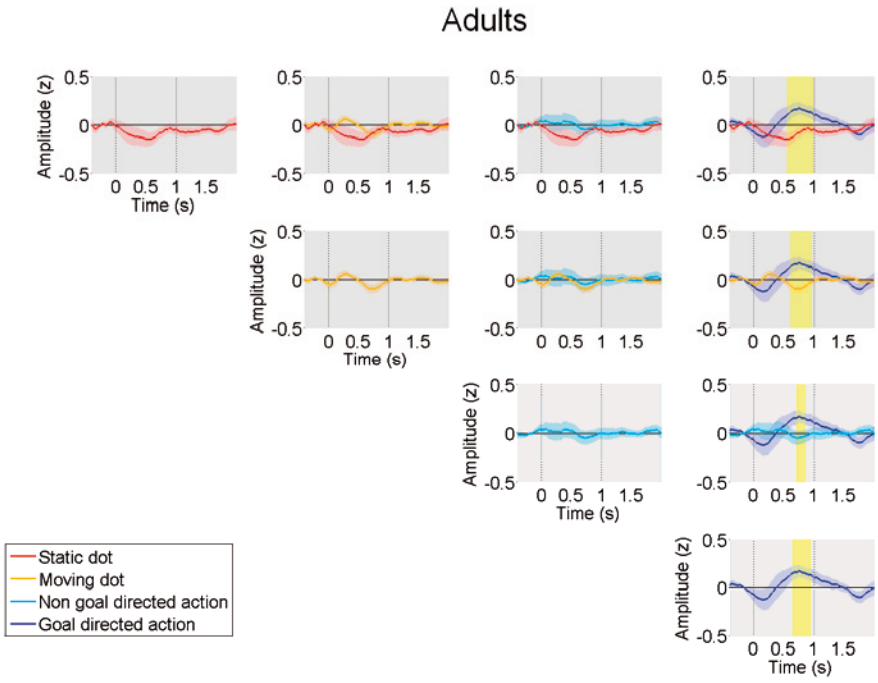
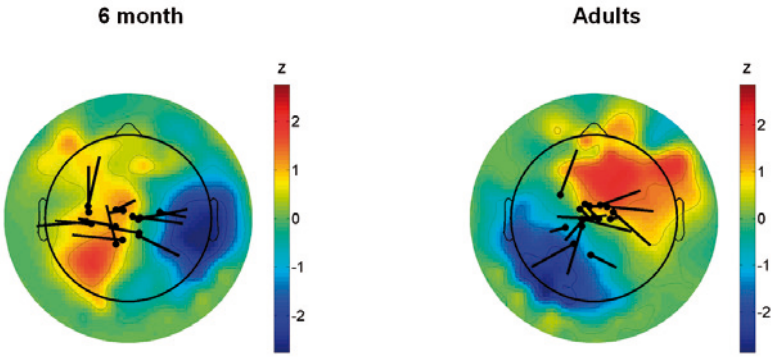

Adults
Amplitude (z)
0.5
0
-0.5
0 0.5 1 1.5
Time (s)
Static dot
Moving dot
Non goal directed action
Goal directed action

Figure 6



Paper III

Using mu rhythm perturbations to measure mirror neuron activity in infants

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Running title: Mu rhythms of the infant mirror neuron system

Abstract

The Mirror Neuron System hypothesis that observed actions are projected onto the observer's own action system gives an important role to development, because only action mastered by the observer can be mirrored. Yet, there are no direct measures of the infant MNS. This study measured MNS activity in infants using mu rhythm perturbations. The results show that the mu rhythm is more inhibited when 8-month-old infants observe a live goal-directed action than when they observe a spatially similar non-goal-directed movement. We thereby provide direct evidence that the mirror neuron system (MNS) is functioning at this age level. Importantly, the current method provides a means of performing infant studies using previous MNS paradigms. This will help delineating the maturing MNS and could be used to establish the significance of mirror neurons in social development.

Introduction

Mirror neurons are motor neurons activated both by the execution of a goal directed action and by the perception of someone else performing the same action (Rizzolatti et al., 1996; Fadiga & Craighero, 2004). The findings suggest 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). The mirror neurons thereby provide a unifying framework for human social cognition (Gallese et al., 2004), and have attracted attention from many research fields (Berthouze & Metta, 2005). However, the function of mirror neurons is still speculative and especially so for higher social skills where it is difficult to test their relationship with the MNS. One way to study the significance of MNS is to perform developmental studies. If this system is essential for social capabilities, it should develop in parallel with or ahead of them. Indirect behavioural evidence for MNS activity in infancy comes from studies of goal-directed eye movements (Falck-Ytter et al., 2006). They found that 12-month-old but not 6-month-old infants looked proactively at the goal of a hand transporting an object there. When the same object motion was shown without the hand, the eyes just tracked the object. Proactive looking at the goal is functional when subjects generate the action themselves but not when they just observe it. If, however, the observation of an action is projected onto the action system of the observer as the MNS hypothesis states, also the proactive looking should be a part of it. However, as the field of mirror neuron development expands there has been an increased demand for a more direct measure of the MNS development. Also, theoretical reviews of the field now explicitly requests empirical neurophysiological data to start to resolve speculations (Lepage & Théoret, 2007; Bertenthal & Longo, 2007; Kilner & Blakemore, 2007).

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 in all ages. MNS activity has been identified in adults using EEG by analysis of the mu rhythm (Pineda, 2005; Muthukumaraswamy et al., 2004a) which oscillates at approximately 9-13 Hz (related experiments has been performed by using MEG as well, e.g., Nishitani and Hari, 2000; Hari et al., 1998). These studies show that the mu rhythm desynchronizes when a subject performs an action as well as when the subject observes someone else perform the same action. The mu rhythm has also been used to identify MNS dysfunction in autistic subjects (Oberman et al., 2005), indicating its importance for social cognition. Mu rhythm desynchronization can therefore be considered being an established measure of MNS activity. Some infant alpha rhythms between 5-9 Hz show strong resemblances to the adult mu rhythm, and is suggested to be the infant mu rhythm (Stroganova et al., 1999). However, no study has previously shown significant mu rhythm perturbations associated with the MNS in infants.

One reason for this is probably that infant EEG is plagued by short recording sessions (due to short attention spans) and many motion 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 the ICA can be viewed as an oblique rotation method for PCA dimensions or

sphered raw data. The ICA is thus similar to principal component analysis (PCA), but whereas PCA aims at finding the factors that explains the most variance, the ICA aims at finding components that are most statistically independent of each other. Since the MNS in infants (measured by the desynchronizing mu rhythm) cannot be expected to account for a lot of variance but still have characteristic statistical properties the ICA appears to be a suitable tool. Unfortunately, the 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.

An important feature of mirror neurons is that they are tuned to goal directed actions. A well established finding is that the mu rhythm desynchronizes when adults and children above two years of age either perform or observe goal directed actions compared to non goal directed actions (Muthukumaraswamy & Johnson, 2004; Lepage & Théoret, 2006), and that the mu rhythm power increases when the subject observes a motionless scene. These results suggest a way to investigate MNS activity in infants by analyzing mu rhythm perturbations. 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 model. Then, the difference in mu rhythm activation between the baseline and the two movement conditions was used for the selection of EEG sources. 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 placing his hand on a table-top at the end. The difference between these two conditions was then analyzed. If the MNS is activated by the goal directed reaching movement,

then the mu rhythm should be more desynchronized than when viewing the simple displacement of the arm.

Methods

Participants

In the present study 36 healthy 8-month-old infants participated. The parents were given written information upon arrival to the lab. The parents signed a written consent form in accordance with the Helsinki Declaration. The experiment was approved by the Ethics committee at Uppsala University. 2 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 where the events were shown and a high density EEG net (128-channels) of the appropriate size was applied to the infant's head. The infants then observed from a lateral view a toy train, a short railway track placed on a table-top and a live male model sitting on a chair (see Figure 1). In the baseline condition the model sat passively. 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 a bit further away from the model . When the toy was in the new position the model could perform 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. The railway track was slanted toward the original starting position and when an electrical trigger was

released at the end of these events, the train returned to its starting position. 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 conditions 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 fuzz (between 10 – 49 presentations of each condition).

EEG recordings and processing

128 EEG channels was recorded using a high density geodesic net (EGI, Corp., Eugene, Oregon). Data was recoded at 250 Hz, with an analogue hardware band pass filter at 0.1 to 100 Hz. 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. A manual trigger hidden from the infants' view was used for this purpose. The resulting EEG recordings consisted of between 10 – 49 trials in each condition from every subject.

The data was transferred to the MATLAB v.7.2 environment and analyzed using the EEGLAB v5.03 toolbox and by following the guidelines as closely as possible. First, 29 of the outermost sensors were removed due to bad contact in most infants, although the net was properly placed. The continous 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 max-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 and 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 brain 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 stemming from channel pops or movement artefacts. Also, the max 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.

Second, we selected components related to mu rhythm desynchronization from the remaining components. Frequency spectrums 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 spectrums. 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 action 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 spectrums 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 spectrums used to select components is shown in figure 2 together with the mean spectrum of all selected components. In total 43 components with 10 – 49 trials from each condition stemming from 23 subjects were selected. Each subject contributed with between 1 and 5 components (mean = 1.87). The components that were not selected were subtracted

from the raw EEG to create datasets pruned from noise, artefacts and brain 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%).

Statistical analysis

First the statistical procedure was performed channelwise 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. A time/frequency analysis using discrete wavelet transforms were performed on each channels's (or components'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 timelock. However, as we were mainly interested in the 5-9Hz 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 I 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 pixelwize 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

Using the raw EEG data, point wise statistical tests (two tailed t-tests, n = 32) showed no significant desychronizations in any of the channels analyzed. The 5-9Hz amplitude difference between the goal-directed and non-goal-directed condition of each channel is shown in figure 2. The p-values are thresholded at alpha = 0.05 (adjusted for multiple testing by removing intervals with less than 10 significant p-values). 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.
In the selected components' case the t-tests between the goal-directed and non-goaldirected conditions show a significant desynchronization of the mu rhythm band for the goal-directed condition when the hand touches the object. A global power minimum of -1.6dB was found approximately 10 ms after timelock (p=0.0007). The channels with the largest difference between the goal-directed and the non-goal-directed movement conditions are located on the right frontal lobe as shown in figure 4. Two more channels with significant desychronization were located in the left frontal lobe (over prefrontal cortex) and finally two over the occipital lobe.

A magnified view of the most significant channel is presented in figure 5, which also shows the amplitudes of 5-9Hz of all conditions.

The most detailed time / frequency analysis of the most significant sensor is presented in figure 6. The difference between the goal-directed and non-goal-directed condition

shows a global minimum of 1.6dB at 10ms after the hand touch the object at 6.8Hz. The t-test at this minimum results in $p = 0.0002$.

Discussion

The results from the selected components clearly 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 are very similar (Muthukumaraswamy & Johnson, 2004b). This shows that 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 desychronization. This is probably a consequence of insufficiently filtered data and a mixture of too many ongoing neural processes. This is also illustrated by the fact 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 a lot of raw data and average. As it was impossible to gather more information from each infant (due to attention, fuzzying and fatigue) we believe that problems with mixed signals are best solved by using blind source separation techniques such as ICA.

In contrast to the raw data, the analysis of the selected components shows a high degree of consensus. Many adjacent channel representations show a significant desynchronization in the goal-directed condition compared to the non-goal-directed condition (figure 4). This desynchronization is most significant over the frontal right hemisphere; where we can expect projections from motor areas. Also, 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 as the adult MNS, and uses the characteristics of the mu response of static and action observation in adults. 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 properties and enhance the mu rhythm signal. 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 should not be related to the MNS (otherwise they would be included in the analysis). An issue of greater concern is about including components that are not related to MNS activity, or excluding 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. Also, 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 5 and 6) all conditions are shown. These plots show that the mu rhythm power is largest when the infant observes a passive model, that the mu power is lower when the infant observes a simple arm movement, and that the mu rhythm 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. We believe that this robust desynchronization represents the infant equivalence to the adult mu rhythm and that it is tightly linked to MNS activity. By reasoning just like previous adult mu rhythm studies ((Pineda, 2005; Muthukumaraswamy et al., 2004a; Nishitani and Hari, 2000; Hari et al., 1998; Oberman et al., 2005) and taking together the timing of desynchronization, the significant frequencies and the source locations we argue that the current study yields a valid measure of MNS activity in infants.

This study is the first to demonstrate mu rhythm desynchronization to observed actions in infants. While a previous study using a similar method failed to find significant mu rhythm desynchronizations to video presented actions in 6-month-olds (Nyström, in press) this study improved the method by using live actors, which appear to elicit stronger MNS activation (Järveläinen et al., 2001; Shimada and Hiraki, 2006). One important question is whether the MNS system is functioning also before 8 months of age. Although Nyström (in press) did not find evidence of consistent desynchronization of the mu rhythm at 6 months of age, an ERP analysis of the same data showed differential responses for goal-directed and non-goal-directed movements. The next natural step for the present set of studies is to determine when the MNS begin to function in the young infant.

When action dependent desynchronization of the mu rhythm emerges in development can easily be investigated using the current method. By relating the present results to behavioural studies we find that the result is in agreement with Falck-Ytter, Gredebäck & von Hofsten (2006). Other studies of social development have found that towards the end of the first year of life, infants rapidly develop social skills. 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 (Liszkowski, Carpenter & Tomasello, 2006; Tomasello, Carpenter, & Liszkowski, 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). The tendancy to imitate shows a marked developmental change shortly before one year of age (Tomasello, 2000). The present results suggest that the development of

the mirror neuron system either precedes or develops together with the emergence of these social functions. By using this neurophysiological measure it would be possible to investigate whether MNS activity precedes overt behaviour. In fact, this method makes a whole range of developmental studies possible to outline the maturation of the mirror neuron system.

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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 object.

Figure 2.

Power spectrums 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 "non-goal-directed action"). Top, example of mu rhythm power peak estimation in a single component. Bottom, mean of all selected components power spectrums.

Figure 3.

Amplitude differences of the 5-9Hz frequency band between the goal-directed and non-goal-directed conditions in the raw datasets. Each curve map 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 channels with significant desynchronization are shaded ($p < 0.05$).

Figure 4.

Amplitude differences of the 5-9Hz frequency band between the goal-directed 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 channels with significant desynchronization are shaded (left plot n = 32, right plot n = 23, both plots $p < 0.05$). The channel with longest significant interval is marked with a red circle. This sensor is presented in more detail in figure 5 and figure 6.

Figure 5.

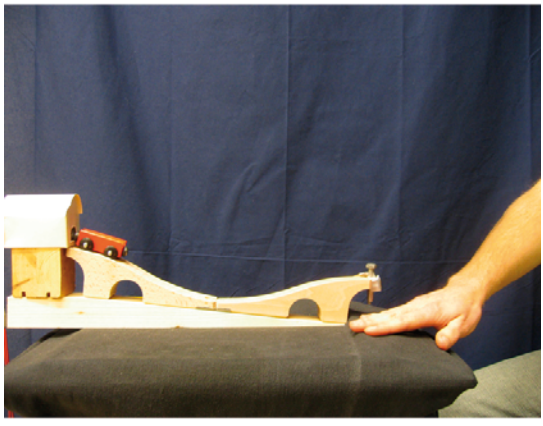
A presentation of the separate conditions in the most significant sensor (marked with a red circle in figure 4). The left row show data from the raw datasets, and the right row show data from the pruned datasets that only contains the selected independent component projections.

Figure 6.

A presentation of the separate conditions in the most significant sensor (marked with a red circle in figure 4) using the time/frequency decomposition returned by the EEGLAB timef function. The frequencies are truncated above 20Hz since these frequencies were filterred out before the ICA. The left row show 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 show 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 1

Non-goal-directed condition



Goal-directed condition

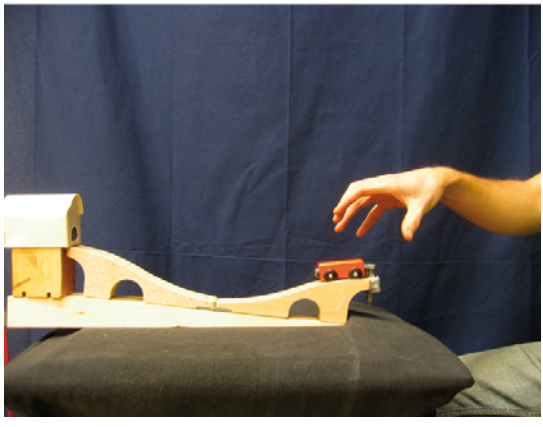


Figure 2

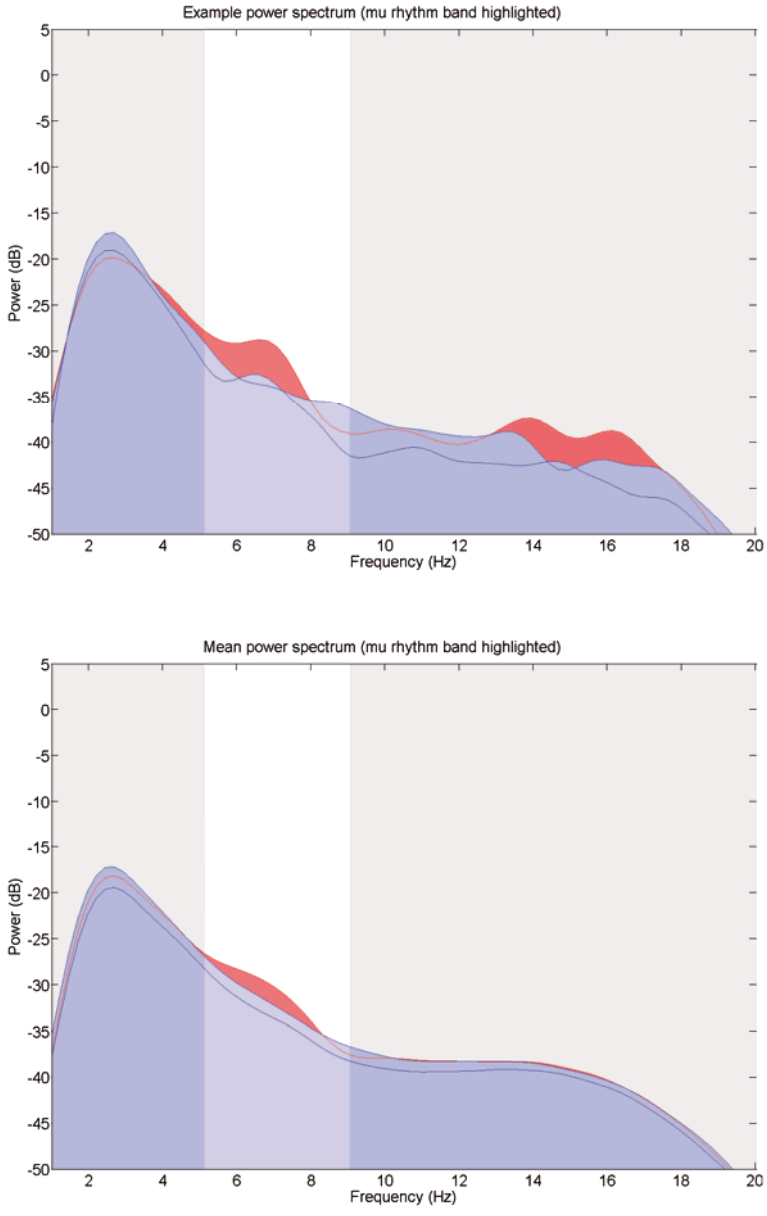

Example power spectrum (mu rhythm band highlighted)
Power (dB)
Frequency (Hz)
Mean power spectrum (mu rhythm band highlighted)
Power (dB)
Frequency (Hz)

Figure 3

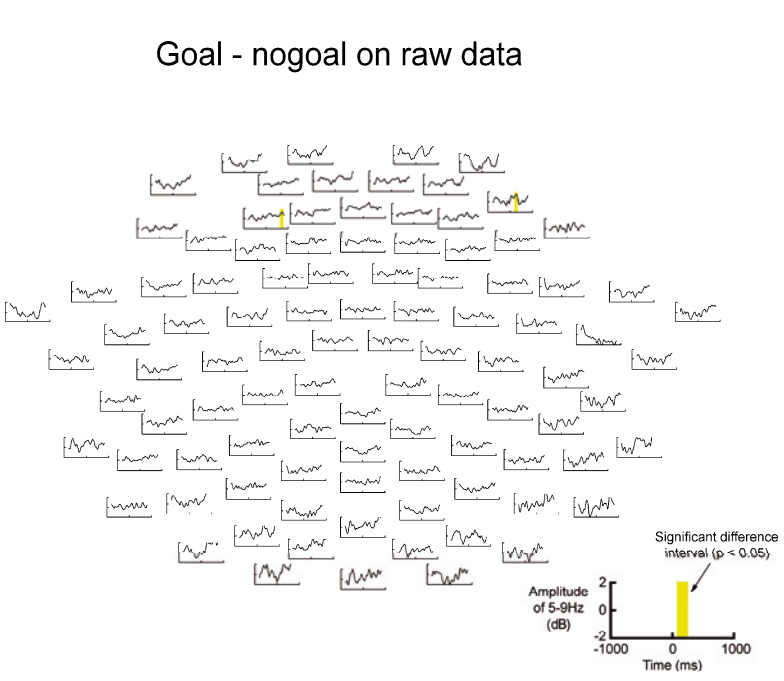

Goal - nogoal on raw data
Significant difference interval (p < 0.05)
Amplitude of 5-9Hz (dB)
2
0
-2
-1000
0
1000
Time (ms)

Figure 4

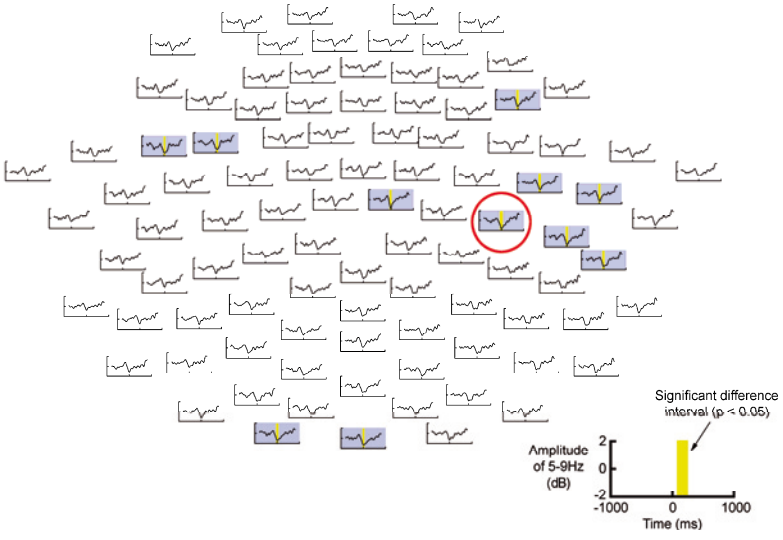

Goal - nogoal on selected components
Significant difference interval (p < 0.05)
Amplitude of 5-9Hz (dB)
2
0
-2
-1000
0
1000
Time (ms)

Figure 5

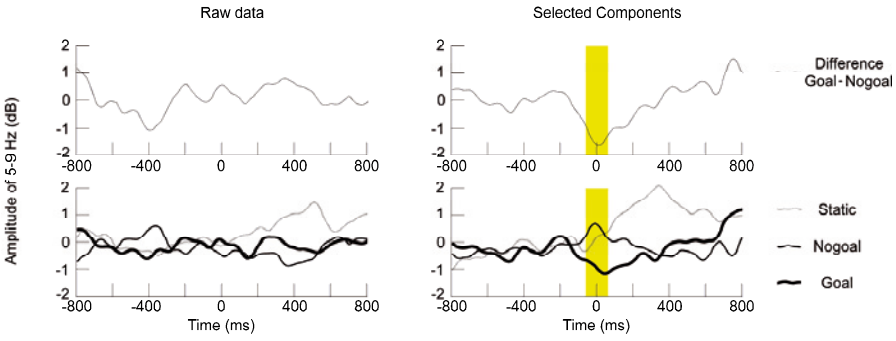
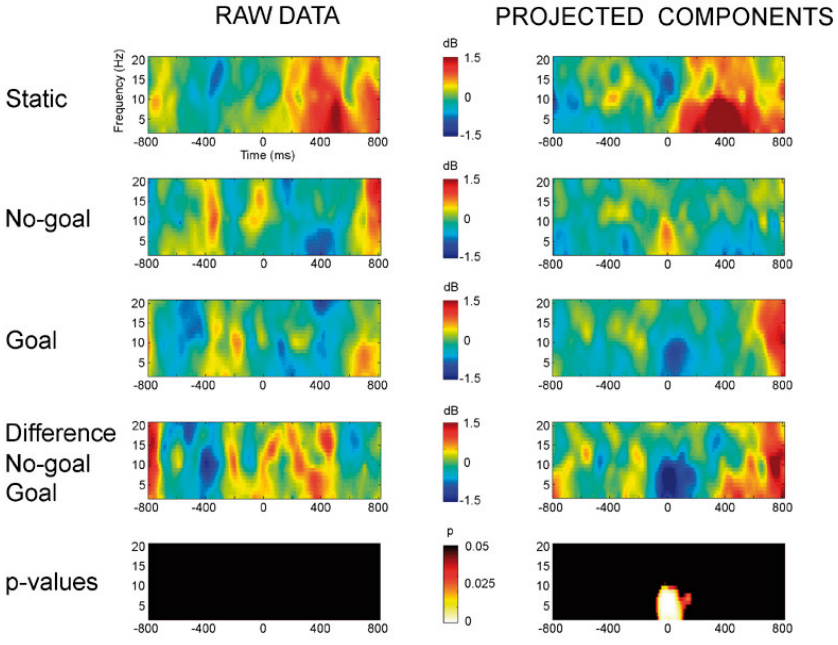

Raw data
Selected Components
Amplitude of 5-9 Hz (dB)
Time (ms)
Time (ms)
Difference
Goal - Nogoal
Static
Nogoal
Goal

Figure 6


RAW DATA
PROJECTED COMPONENTS
Static
No-goal
Goal
Difference
No-goal
Goal
p-values
Frequency (Hz)
Time (ms)
dB
p
20
15
10
5
-800
-400
0
400
800
1.5
0
-1.5
0.05
0.025