Infants’ mu suppression during the observation of real and mimicked goal-directed actions

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Highlights

18- to 30-month old infants show significant central mu suppression during the observation and execution of goal-directed actions.

Significant central mu suppression was also evident during the observation of intransitive hand movements.

Parietal suppression in the alpha/mu band may be driven by both neural mirroring and attentional mechanisms.

These attentional mechanisms may also influence central suppression during action observation.

No correlation was found between mu suppression and language abilities, but a better imitation performance was associated with weaker mu suppression.
Abstract

Since their discovery in the early 90’s, mirror neurons have been proposed to be related to many social-communicative abilities, such as imitation. However, research into the early manifestations of the putative neural mirroring system and its role in early social development is still inconclusive. In the current EEG study, mu suppression, generally thought to reflect activity in neural mirroring systems was investigated in 18- to 30-month olds during the observation of object manipulations as well as mimicked actions. EEG power data recorded from frontal, central, and parietal electrodes were analysed. As predicted, based on previous research, mu wave suppression was found over central electrodes during action observation and execution. In addition, a similar suppression was found during the observation of intransitive, mimicked hand movements. To a lesser extent, the results also showed mu suppression at parietal electrode sites, over all three conditions. Mu wave suppression during the observation of hand movements and during the execution of actions was significantly correlated with quality of imitation, but not with age or language level.

Key words: infants, mu suppression, imitation, action observation, neural mirroring system
1. INTRODUCTION

The discovery of macaque mirror neurons in the early 90’s (Di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; Rizzolatti, Fadiga, Gallese, & Fogassi, 1996), has inspired a wealth of research into the neurophysiological underpinnings of action understanding and related social behaviour, like imitation. Since then, many studies have been investigating the possibility of an analogous action observation/ action execution matching system in humans, mostly by using techniques such as transcranial magnetic stimulation (TMS; e.g., Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995), electroencephalography (EEG; e.g., Muthukumaraswamy, Johnson, & McNair, 2004), magnetoencephalography (MEG; Hari et al., 1998), and functional magnetic resonance imaging (fMRI; e.g., Buccino et al., 2001). These techniques are no direct measures of individual cell responses, but merely show an overlap in the activation of certain brain systems and/or regions during action observation and execution. Recently however, Mukamel and colleagues (Mukamel, Ekstrom, Kaplan, Iacoboni, & Fried, 2010) reported the first single cell study in humans providing direct evidence for the presence of neurons responding to both the observation and execution of grasping actions and facial expressions. Although this study confirms the presence of neurons with ‘mirror-like’ properties, it does not provide unequivocal evidence of a ‘human mirror neuron system’. On the other hand, the typical resonance behaviour of humans, both at behavioural (e.g., imitation) and physiological level (e.g., the unconscious and automatic facial muscle activity measured during the observation of emotional expressions, see for instance Dimberg, Thunberg, & Grunedal, 2002) is very likely to be supported by some neural circuitry, involved in observation-execution coordination (Frith & Frith, 2010; Hari & Kujala,
Therefore, and following Marshall and Meltzoff (2011), in the current paper we will refer to this circuitry with the more neutral term ‘neural mirroring systems’.

Involved in action observation and execution, these neural mirroring systems have been proposed to be related to imitation, which is a crucial skill in human development, learning, and socialization (Jeannerod, 1994). Imitation, whether inborn or not (see Anisfeld et al., 2001 for a brief overview of this discussion), seems to be present quite early in typically developing infants, certainly by 6 to 9 months of age (Collie & Hayne, 1999; Heimann, 2002; Learmonth, Lamberth, & Rovee-Collier, 2004; Meltzoff & Moore, 1977). If, as hypothesized, the neural mirroring system is necessary (but probably not sufficient) for imitation, then it should also be present and functional early in life. Therefore, to learn more about the functionality and purposes of this mirroring system and its role in human imitation, it is essential to investigate it in infancy and toddlerhood, where imitation plays a crucial role in development.

A quite commonly accepted measure of activity in the action observation/action execution matching system is suppression of the mu rhythm. The EEG mu rhythm, typically found in adults in the 8-13 Hz frequency range over central electrode sites, is reduced in amplitude when the person moves (Gastaut, Dongier, & Courtois, 1954). A similar mu rhythm desynchronization occurs when a person is observing others’ actions. Therefore, an attenuation or suppression in the mu frequency band, caused by a decrease in neural synchrony when neurons fire, is believed to be a measure of activity in the neural mirroring system (Muthukumaraswamy et al., 2004; Pineda, 2005). In infants, a central rhythm in the 6-9 Hz range was described that seemed to be analogous to the adult mu rhythm (Stroganova, Orekhova, & Posikera, 1999). This central rhythm was the focus of several recent studies indicating that it is similar to the adult mu rhythm, responding to both action observation and
execution, with a parallel topography (for a review of this research, see Marshall & Meltzoff, 2011). Following others, in this paper we will refer to this central rhythm as the infant mu rhythm (e.g., Marshall, Bar-Haim, & Fox, 2002; Marshall & Meltzoff, 2011). Others have also used the term ‘sensorimotor alpha’ to refer to this rhythm (e.g., Southgate, Johnson, El Karoui, & Csibra, 2010; Southgate, Johnson, Osborne, & Csibra, 2009).

At present, there are a number of studies that have explicitly focussed on mu suppression in infants. In 6-month-olds observing a video of a person reaching for an object, Nyström (2008) found an event-related potential (ERP) component similar to that reported in adults, which has been linked indirectly to mirror neuron activity, but there was no mu suppression. In a more recent experiment, he reported significant mu suppression in 8-month-olds watching a live model grasping and moving a toy train (Nyström, Ljunghammar, Rosander, & von Hofsten, 2011). Southgate and colleagues (2009, 2010) reported mu suppression in 9-month olds while they were observing grasping and while they were reaching themselves, but not during the observation of mimed grasping (no object present). Stapel, Hunnius, van Elk, and Bekkering (2010) reported a stronger mu suppression in 12-month-olds watching an unusual action compared to a usual goal-directed action (e.g., moving a phone to the mouth versus moving it to the ear). On the other hand, van Elk and colleagues (van Elk, van Schie, Hunnius, Vesper, & Bekkering, 2008) showed that mu suppression in 14- to 16-month olds was dependent on the amount of experience these infants had with the observed behaviour (crawling and walking). Regardless of their walking experience, infants with a longer crawling experience showed a greater desynchronization in the mu-frequency band when they watched crawling, compared to walking. Reid, Grigutsch, Striano, and Iacoboni (2011) found 14-month olds to show mu suppression when they were being imitated (which can be interpreted as the observation of known actions), but not when watching an adult performing complex movements, which were not part of the infants’ own motor
repertoire. Finally, Marshall, Young, and Meltzoff (2011) were the first to report mu suppression at different electrode positions during the observation and execution of an intentional action other than grasping. The 14-month old infants participating in their study showed suppression in the mu band at frontal, central, and parietal electrode sites during action observation, but only at central sites during action execution.

To our knowledge, most studies either seem to focus on younger infants (6 – 16 months) or school-aged children, adolescent, and/or adults, but not many studies have focused explicitly on the characteristics of mu suppression in toddlers and preschoolers. In 2004, Fecteau and colleagues reported mu suppression in a 36-month old girl drawing and watching an experimenter drawing (Fecteau et al., 2004). In a study of Meyer and colleagues (Meyer, Hunnius, van Elk, van Ede, & Bekkering, 2011), 3-year-olds played a joint action game, taking turns in pressing a button to make a frog character climb a ladder. They showed more mu suppression while observing a person pushing a button when they were involved in the game themselves, compared to observing two others persons playing the game. Unfortunately, no baseline was reported, so it is not clear whether or not the children showed mu suppression to the non-interactive condition as well. In somewhat older children (4- to 11-year-olds), Lepage and Théoret (2006) observed mu suppression during the observation and execution of a grasping movement.

So far, the results seem to add up to the following conclusions: from 8 to 9 months onwards, mu suppression is observed during the observation of object manipulation, but not of mimicked actions. 12-month-olds show stronger mu suppression if the object manipulations are unusual. By the age of 14 to 16 months, there seems to be mu suppression during the observation of an action (with or without objects), but only if that action is already a part of the infants’ motor repertoire. The amount of experience with an action seems to have
an effect on the magnitude of the mu suppression. Three-year-olds seem to show more mu suppression in an interactive compared to a non-interactive situation.

Although these initial findings provide some information about the modulation of the mu rhythm in early childhood, our knowledge is yet limited. While reviewing the available literature concerning mu suppression in infants and young children, Marshall and Meltzoff (2011) point out several limitations of the existing research and identify 5 open theoretical questions. Based on Marshall and Meltzoff’s and our own critical review of the literature the following issues seem to be of particular interest to the current study.

First, to be certain that a observation/execution matching system is involved, infants’ EEG should be measured during both action observation and action execution, instead of only the former. Until now, this has not always been the case. In addition, given the complex nature of human goal-directed behaviour and infants’ capabilities of imitating that behaviour, it is important to examine the EEG response to more elaborated actions than merely reaching or grasping. In the current study, we will try to expand the current knowledge by measuring mu suppression during both the observation and the execution of 5 more elaborated goal-directed actions.

Second, it is not yet clear whether the mu rhythm desynchronisation reflects a response to the observation of specific motor behaviour, or to the presence of goals. In monkeys, the sight of an agent mimicking an action or making intransitive (non-object-directed) gestures is ineffective to produce mirror neuron activity (Rizzolatti & Craighero, 2004). In adult humans, modulation of the motor cortex excitability is observed during the observation of transitive (object-directed) as well as intransitive or mimicked actions (e.g., Fadiga, Craighero, & Olivier, 2005; Fadiga et al., 1995; Maeda, Kleiner-Fisman, & Pascual-Leone, 2002). Nevertheless, young infants do not show mu suppression in response to
intransitive acts (mimed grasp; Southgate et al, 2010). Whether such a tendency is still present after the first year of life is to date unclarified. Therefore, one of the aims of our studies was to investigate the role of goal-directedness of actions for the mu rhythm desynchronization by including intransitive actions in our paradigm. More specifically, we added a second observation condition, where the hand movements were very similar to the ones used in the goal-directed actions, but without any objects present. In addition, to explore the possible contribution of a social cue to the EEG response, the experimenter made no eye contact during this condition.

Third, although the mu rhythm is defined as a central rhythm, it may be useful to explore the electrophysiological response to action observation and execution at other electrode sites as well. This will enhance our knowledge of the regional specificity of the response, allowing comparison with the adult literature. Therefore, we will not only report data from the central electrodes, but also from a set of frontal and parietal electrode positions.

Fourth, little is known about developmental changes in the infant’s mu rhythm response. In this study, we will investigate an age group where imitation plays a crucial role in the development of cognitive, communicative, and social skills: 18- to 30-months-olds. Although at an age where action understanding is evolving very rapidly, to our knowledge, EEG mu rhythm response to action observation and execution has not been studied before in this group.

And finally, although the human mirroring system has been theoretically linked to social-communicative abilities, the relation between both has rarely been investigated empirically. Therefore, we will also take into account the children’s imitative abilities and their language level, and explore possible correlations between those characteristics on the one hand, and central mu suppression on the other hand.
In summary, the current study was designed to examine the following research questions: 1) Do 18- to 30-month-olds show (central) mu suppression during the observation and execution of goal-directed actions, going beyond mere reaching or grasping. Based on previous research (e.g., Marshall et al, 2011), we hypothesize that this will indeed be the case. 2) Do 18- to 30-month-olds show (central) mu suppression during the observation of intransitive hand movements in a minimally social context? To our knowledge, the role of eye contact in eliciting mu suppression has not been studied before. It is therefore not possible to have specific predictions concerning the effects of this factor. Based on previous results concerning intransitive conditions (Southgate et al., 2010), we expect that – whether present or not – mu suppression in this condition will be less pronounced compared to the mu suppression observed during the observation of goal-directed actions. 3) Can we observe similar suppression in the mu frequency band over frontal and parietal electrodes? Based on Marshall and colleagues’ (2011) results, we may expect to find a suppression at these positions during action observation, but not action execution. 4) Are there, taking into account previous research, developmental changes in mu suppression? Marshall and colleagues (2011) tentatively compared the strength of the mu suppression found in their 14-months olds to that of 9-month-olds (Southgate et al, 2009) and of 4- to -11-year-olds (Lepage & Théoret, 2006). We will add our results to this preliminary comparison, and hypothesize that the size of the mu suppression during action observation and action imitation will be smaller than was found in 4- to 11-year-olds but somewhat larger than reported in 9- and 14-month-olds. 5) Is there a relation between the strength of the mu suppression and the level of social-communicative abilities such as language and imitation? Given the divergent theoretical opinions on this matter (for a recent discussion, see Gallese, Gernsbacher, Heyes, Hickok, & Iacoboni, 2011), we will perform exploratory analyses rather than testing a specific hypothesis.
2. MATERIAL AND METHODS

2.1 Participants

Thirty-five infants participated in the experiment. Prior to analysis, we excluded two infants due to insufficient cooperation throughout the experiment, two infants who refused to imitate, two infants because of technical malfunctions in the EEG system, and eleven infants of whom we obtained insufficient artefact-free data (40 sec/condition), partly due to excessive moving and/or talking during the experiment. Sufficient artefact–free data (at least 40 seconds for each condition and no excessive motor activity during baseline) were obtained for 19 infants (9 boys and 10 girls). Two children showed a mu suppression value outside the group mean ± 3 standard deviations interval, and were therefore excluded from further analyses. All participants were between 18 and 30 months old (mean age = 24.54 months, SD = 3.96 months). Characteristics of the participants are presented in Table 1. Hand preference was judged by parent report and by analyzing the video-recordings of the experiment. Twelve infants preferred using their right and five infants preferably used their left hand.

The participants were recruited through Flemish day-care centres and several advertisements on websites and in magazines. They were all healthy and developing normally.

2.2 Procedure

INSERT TABLE 1 ABOUT HERE
The experiment was carried out in a laboratory room at the university. Before participation of the infant, parental informed consent was obtained. After entering the experimental room, experimenter 1 handed the infant toys to play with while the general procedure was explained to the parent. Meanwhile, experimenter 2 prepared the EEG–cap. The infant was given ample time to get used to the experimenters and the experimental room. After the infant was acclimatized, the EEG–cap was fitted on its head while it was seated on its parent’s lap and watched a cartoon movie. A small amount of electrolytic conducting gel was inserted into each of the active electrodes after placement of the EEG–cap. A chest strap and a hairnet were used to hold the cap in place. The parent was instructed to avoid interacting with the infant during the test phase. During testing, the infants were seated on their parent’s lap and in front of a rectangular table. Experimenter 1 sat at the other side of the table facing the child. The stimuli were presented at a viewing distance of approximately 60 cm. A white blind between the infant and the experimenter moved up and down between the different conditions. In addition, a white screen was placed around the infant in order to minimize distracting environmental influences. The experiment was recorded with two cameras, one focusing on the experimenter and the other filming the infant. These videotapes were used for offline coding of the participants’ behavior (attention, vocalization and motor behavior).

The experiment consisted of 4 experimental conditions (with 5 different objects: a hippopotamus soft-toy, an egg-cup, a Pinnochio-like puppet, a car and a frog-loupe). During the object observation condition, the infants observed a moving object dangling on a rope, in front of the white curtain. Since the objects moved in a non goal-directed manner and the infant had no prior experience with the objects, this condition was used as a baseline condition. In the action observation condition, infants observed an action with each object and a white box (for example the egg-cup, starting from one side of the box, was playfully moved to the other side of the box, being bounced up and down once before and twice on top of the
box). In analogy with other studies (e.g., Nyström et al., 2010), we called these actions ‘goal-directed’ because the object always had a clear end position (either in or on the other side of the box), after which the presentation was repeated (or stopped). The actions were selected to be interesting for the children to imitate, without auditory effects. Prior to demonstration, the experimenter made eye-contact with the child and asked for the child’s attention (“name child, look!”). Each action was demonstrated six times; three times with the left hand and three times with the right hand. The starting hand was counterbalanced between the objects. Subsequently, the infants were asked to imitate the observed action during the action execution condition. The experimenter encouraged the infant (non-)verbally when necessary to imitate, in a non-specific way. For the hand movement condition, the infants observed the experimenter performing hand movements, identical to those used during the action observation condition but without the object and the white box (= mimicked actions). Contrary to the action observation condition, the experimenter did not make eye-contact with the child before or during the demonstration. Each hand movement was demonstrated six times.

The five objects were used for each infant. The experiment started with the object observation condition (baseline condition) for all five objects subsequently. Since the same 5 objects were used throughout the experiment, the baseline condition always had to be the first, in order to avoid memory effects (e.g., the object triggering the appropriate action in the infants’ memory). Then, for every object the infant went through the action observation, action execution, and hand movement condition. The order of the conditions was counterbalanced between subjects, with the constraint that the action execution condition always directly followed the action observation condition. The order of the five objects always remained the same. Each presentation (object movement, hand movement, action observation) lasted about 30 seconds per object. Children were given as much time as needed
for the imitation of the actions, usually this took no more than 40 seconds per object. The total experiment lasted about 15 to 20 minutes.

The EEG data were gathered during live actions. This is preferable over televised stimuli in young infants because the understanding of 2D representations is gradual and not complete in its development over the first years of life (Carver, Meltzoff, & Dawson, 2006), and since 2-year-olds imitate better from live compared to televised models (Nielsen, Simcock, & Jenkins, 2008).

After the experiment, the parents were debriefed and they received a small reward (gift card of a toy shop). They were also asked to fill in the Dutch version of the MacArthur-Bates Communicative Development Inventories (N-CDI, Zink & Lejaegere, 2002; original version Fenson et al, 1993). In the current paper we use the age equivalent for language comprehension and language production (in months).

2.3 EEG recording and analysis

2.3.1 EEG recording

Electrical brain activity was recorded using Brain Vision Recorder (Brain Products, 2007) and was registered with 28 active Ag/AgCl electrodes through an EEG–amplifier (QuickAmp, Brain Products GmbH, Munich, Germany), with a sample rate of 500 Hz. We used a child-friendly EEG-cap (EasyCap, Brain Products GmbH, Munich, Germany), in which 28 electrodes were embedded based on the international 10/20 method of electrode placement (Jasper, 1958) with an AFz ground electrode. A common average reference was used. Both vertical and horizontal eye movements were recorded (electro-oculogram, EOG) by 4 additional electrodes. Horizontal EOG (HEOG) was registered by placing the electrodes next to the eyes, at the outer canthi. Initially, we placed an electrode below the left eye for
monitoring vertical eye movements but many infants did not tolerate this electrode. Therefore, vertical EOG (VEOG) was calculated offline by comparing the activity of electrode Fp2 (above the eye) with the common reference. The inter-electrode impedance on all electrodes was considered acceptable at or below 10kΩ. The EEG was recorded with a time constant of 1 s, a low pass filter of 70 Hz, and a 50 Hz notch filter. During EEG recording, the experimenter pushed a button before every presentation, while the curtain was still down. This button sent a marker signal to the EEG equipment (integrated in the raw EEG data), while simultaneously activated a LED visible on both camera recordings. Afterwards, comparing the time intervals between the subsequent EEG markers and between the subsequent LED signals on tape allowed us to synchronize the EEG signal with the video recordings.

2.3.2. Offline coding and synchronizing

The videotapes were coded offline with The Observer XT 9.0. (Noldus Information Technology, 2009). Data of the three observation conditions (baseline, action observation, hand movement) were coded for the children’s attentiveness to the experimental demonstration (attentive versus non-attentive). Furthermore, in the action execution condition, we coded whether or not the child imitated the action presented during the action observation condition. Finally, over all four conditions, all vocalizations and instances where the child was moving were coded. All intervals with excessive motor movements and vocalizations were excluded from further analysis. Only those fragments in which the child was sitting still and quietly attending the demonstrations (during baseline, hand movement and action observation condition) or was actually imitating (during execution condition) were used in the subsequent analyses by allocating start and end codes. Since the EEG file and the video recordings were synchronized, these codes could easily be integrated in the EEG marker file, allowing us to link our observations (e.g., action observation condition, infant attentive, not moving or vocalizing) to all the EEG data points. In a second step (see also below), we
controlled for motion artifacts with Brain Vision Analyzer’s artifact rejection function. Obviously, it cannot be excluded that 18-30-month-olds move a little (e.g., fidgeting) but this way, we believe that the influence of possible movements was minimized. In addition, there were no significant correlations between the number of observed movements and vocalizations per condition of an infant and its observed mu suppression per condition (all $r < .35$ and all $p = >.15$).

2.3.3 Imitation quality

Based on the offline video recordings, the infants’ quality of imitation was coded. For every action, three criteria were formulated. For instance, for bouncing the egg-cup, the criteria were 1) bouncing at least once on the original side of the box, 2) bouncing at least twice on top of the box, and 3) moving the egg-cup to the other side of the box. For every object, children could obtain a score between 0 and 3, reflecting the number of criteria their imitation performance met. Children obtained a mean (over all 5 objects) imitation quality of 1.96 (SD = .39), indicating that their imitation performance met on average 2 out of 3 criteria, which is a reasonable level of detail. An independent coder double-coded 9 randomly chosen infants to assess inter-observer reliability. An excellent level of reliability was achieved with a Cronbach’s Alpha Coefficient of .94 (Cronbach, 1951).

2.3.4 EEG analyses

Brain Vision Analyzer (Brain Products, 2007) was used for offline analyses of the EEG data. We investigated the EEG data of the electrodes at positions F3, F4, C3, C4, P3, and P4. A high pass filter of 0.1 Hz, a low pass filter of 30 Hz and a 50-Hz notch filter were applied. Subsequently, the EEG data were corrected for horizontal and vertical eye movement using the Gratton and Coles algorithm (Gratton, Coles, & Donchin, 1983). Based on the start and end markers resulting from the video coding, the data of all five objects were included in
one interval per condition (mean length in seconds (SD) of baseline = 134.14 (37.05), action observation = 178.57 (15.18), action execution = 144.57 (53.43) and hand movement = 136.14 (16.58)). In a next step, these four segments were each divided in 2-second segments. Bad 2-second segments were removed with artifact correction using a maximal allowed voltage step of 100µV per sampling point and a maximal allowed absolute difference of 400 µV between two values in the segment. Only the infants with at least 20 artifact–free segments per condition (40 seconds) were included in further analyses. Fast Fourier Transforms (FFTs), with a Hanning window of 10%, were executed on the remaining segments, and the data segments were averaged. Following the procedure used in both child and adult studies (e.g. Lepage & Théoret, 2006; Muthukumaraswamy et al., 2004) we selected each child’s individual mu frequency band by calculating the 3 Hz-interval around the maximal power difference between the rest (baseline) and action execution (imitation) conditions, over the central electrodes. This maximal difference ranged between 5.37 and 9.77 Hz, with a mean of 7.84 Hz (SD = 1.13). This is in agreement with previously reported frequencies of the mu rhythm in this age range (Marshall et al., 2002; Stroganova et al., 1999).

In line with Marshall et al. (2011), mu wave suppression was calculated as a ratio of the mu wave power in the different conditions. Specifically, we calculated ([A - R]/R)*100 with A being the mu band power during the experimental conditions (action observation, action execution and hand movement) and R being mu power during the baseline condition (object movement condition) (Pfurtscheller & Lopes da Silva, 1999). A negative value indicates mu suppression, a positive value represents mu intensification and a zero value indicates no mu suppression, as compared to the baseline. Research questions 1 (is there central mu suppression during the observation and execution of goal-directed actions) and 3 (is there frontal and parietal suppression in the same frequency band during the same
conditions) are answered by means of repeated-measures ANOVA’s with region (frontal, central, parietal) as within-subjects factor, for both conditions separately (see also Marshall et al., 2011). The same was done for research question 2 (is there mu suppression during the new hand movement condition), and an additional repeated-measures ANOVA was performed with condition (action observation, action execution, hand movement) as within-subjects factor, taking into account central electrodes only.

3. RESULTS

The order in which the conditions (hand movement versus action observation/imitation) were presented had no effect on the mu suppression as measured on the central electrode positions (action observation t(15) = 1.990, p = n.s.; action execution t(15) = -.169, p = n.s.; and hand movement t(15) = 1.015, p = n.s.). Therefore, regardless of the order of presentation, the infants are treated as one group in the subsequent analyses.

3.1 Action execution

The repeated-measures ANOVA showed a significant main effect of region (F(2,15) = 17.006, p < .001). Follow-up contrasts showed significantly more mu suppression over the central electrode positions (M = -.41, SD = .29) compared to the frontal (M = -.13, SD = .36, p < .01), and parietal positions (M = -.17, SD = .22, p < .001). One sample t-tests showed mu suppression to be significantly different from zero over central (t(16) = -5.811, p < .001) and parietal sites (t(16) = -3.115, p < .01), but this was not the case for frontal electrode positions (t(18) = -1.448, p = n.s.). See Figure 1(a) for details.

3.2 Action observation
In the action observation condition, the repeated-measures ANOVA revealed no effect of region \( F(2,17) = 1.144, p = \text{n.s.} \). Mu suppression was significantly different from zero on central \( t(16) = -2.606, p < .05 \) and parietal \( t(16) = -3.713, p < .05 \), but not on frontal electrodes \( t(16) = -.964, p = \text{n.s.} \). See Figure 1(b) for details.

### 3.3 Hand movement

Similar to the action execution condition, in the hand movement condition the repeated-measures ANOVA showed a significant main effect of region \( F(2,15) = 9.145, p < .01 \). Again, mu suppression was stronger over central electrodes \( M = -.26, \text{SD} = .20 \) than over frontal \( M = -.08, \text{SD} = .19, p < .01 \) and parietal electrodes \( M = -.11, \text{SD} = .15, p < .01 \). Mu suppression was significantly different from zero over central \( t(16) = -5.324, p < .001 \) and parietal electrodes positions \( t(16) = -3.025, p < .01 \), but not over the frontal ones \( t(16) = -1.736, p = \text{n.s.} \). See Figure 1(c) for details.

In order to compare mu suppression in the different conditions, a second repeated-measures ANOVA was conducted, this time only taking into account the central mu wave suppression. The ANOVA showed a significant effect of condition \( F(2,15) = 5.822 \), with more suppression during action execution than during both action observation \( F(2,15) = 12.219, p < .01 \) and hand movement observation \( F(2,15) = 10.192, p < .01 \) and stronger mu suppression during the hand movement condition than during the action observation condition \( F(2,15) = 7.585, p < .05 \).

### 3.4 Relation with child characteristics

The correlations between central mu suppression in all three conditions on the one hand and the child’s age, comprehensive and expressive language level, and imitation quality score on the other hand were explored using Pearson’s correlations. Central mu suppression during the hand movement condition was significantly positively correlated with central mu
suppression during both action observation ($r = .516, p < .05$) and action execution ($r = .751, p < .01$), but the latter two were not significantly related ($r = .126, p = n.s.$). Age, language level and imitation quality were strongly intercorrelated (all but one $r > .550, p < .05$), and there was a significant positive correlation between imitation quality on the one hand and central mu suppression during hand movement ($r = .483, p < .05$) and action execution ($r = .586, p < .05$), but not action observation ($r = .285, p = n.s.$), on the other hand. See Table 2 for details.

### 3.5 Occipital alpha

Elevated attention or cognitive load is related to alpha suppression, which is most evident in occipital areas (Perry & Bentin, 2010). In order to ensure that what we were measuring at frontal, central, and parietal electrodes was mu and not alpha suppression, we analysed data from the electrode positioned at Oz. During both hand movement observation and action execution, the central suppression was significantly stronger than the suppression measured at Oz ($t(16) = -2.161, p < .05$ and $t(16) = -3.315, p < .01$, respectively), but this was not the case during action observation ($t(16) = 1.689, p = n.s.$). During the hand movement condition, the suppression measured over the parietal electrodes was significantly correlated with both central ($r = .626, p < .01$) and occipital suppression ($r = .641, p < .01$). Similar correlations were found in the action observation condition (central – parietal $r = .529, p < .05$ and parietal – occipital $r = .711, p < .01$). During the action execution, the central mu suppression correlated significantly with the frontal ($r = .496, p < .05$) and parietal suppression ($r = .780, p < .001$), but the activity at neither location correlated with the occipital electrode activity.

### 4. DISCUSSION
The current study investigated mu suppression of 18- to 30-month old infants during both observation and execution of actions on objects, as well as during the observation of non-goal directed hand movements. We tested a) whether 18- to 30-month old infants showed central mu suppression in response to the observation of actions on objects; b) if this mu suppression was also present during the observation of non-goal directed hand movements; c) if a suppression in the mu frequency band was also present over frontal and parietal electrode sites; d) whether the observed values fit in the idea of a developmental increase in mu suppression and e) whether there was relation between central mu suppression and child characteristics such as age, language level and imitation quality.

Concerning the first research question, we indeed observed significant mu suppression over central electrode sites during both action execution and the observation of more elaborate (as compared to reaching or grasping) goal-directed actions on objects. This is in line with previous research (see Marshall & Meltzoff, 2011, for a review), and extends the current evidence for an action observation/action execution matching system with the measurement of mu suppression over a longer time interval, and during the observation of longer and more complicated goal-directed actions.

To answer the second research question, we included an additional minimally social, non-goal-directed observation condition where no object was present, but only the hand movements were performed. During this condition, the infants showed significant mu suppression that was stronger than the suppression registered in the other observation condition. These results suggest that, similar to adults, 18- to 30-month-olds do show neural mirroring activity during the observation of intransitive hand movements, while this is not yet the case in younger infants (Southgate et al., 2009, 2010). Although some authors tentatively suggested that mu suppression may rather reflect the inference of action goals rather than a precise representation of motor movements (e.g., Csibra, 2007; Southgate et al., 2010), the
results of our hand movement condition suggest that movement itself is an important factor as well, independent from the action goal. This is also supported by the children’s imitation scores, where we observed that the children imitated many details that were not necessary to reach the action goal. In addition, in about half of the children, the mimicked hand movement condition preceded the actual action observation condition, and this presentation order did not have an effect on the children’s mu suppression during both conditions. This suggests that the children either responded to the presence of intransitive hand movements alone, or they were able to infer the presence of an object even though they had not yet seen the actual object. On the other hand, we must again consider the possibility that, due to the rather long time interval of measurement, other neurological processes were measured, and our results may not purely reflect neural mirroring functioning. Exploring this issue further by adding other conditions, possibly only showing the object in movement (without visible human action), or the action goal may be helpful to further clear out the means-versus-goal question. However, in the current study, piloting the paradigm showed that it was not feasible to add other conditions, because of the limited attention span and patience of 18- to 30-month-olds. Why the mu suppression during the observation of intransitive hand movements was actually stronger than that measured during action observation is not clear. We believe this effect is not caused by movements or motor planning, since analyses of our observation data revealed that we had to remove more intervals due to movement in the action observation than in the hand movement condition ($t(16) = -4.942, p < .001$). Future studies will show whether this effect can be replicated and which factors could be related to it.

Third, during both action execution and hand movement observation, mu suppression was stronger over the central electrode sites than over frontal and parietal sites. However, also parietal suppression in the mu frequency band was significantly different from zero. During action observation, suppression in the mu frequency band was equally strong over frontal,
central and parietal regions, which is consistent with previous studies (e.g., Marshall et al., 2011). Although mu suppression during action execution is commonly only observed or reported over central electrodes (e.g., Lepage & Théoret, 2006; Marshall et al., 2011; Oberman et al., 2007), some authors have suggested that a cluster of fronto-parietal electrodes may be more appropriate (Müller, Ball, Kristeva-Feige, Mergner, & Timmer, 2000; Southgate et al., 2009, 2010). At this point, it would be premature to conclude that a similar mu band suppression during action observation and execution over parietal sites reflects mirror neuron activity. Firstly, given the low spatial specificity of EEG measures, a similar EEG desynchronisation does not necessarily mean that the same neural processes are involved. Secondly, during both observation conditions, next to significant central mu suppression we also observed significant occipital suppression in the alpha frequency band. This may suggest the involvement of an attentional component during these conditions. Also, in both observation conditions, parietal suppression was significantly correlated with both central and occipital mu/alpha suppression. The parietal suppression during the observation conditions may therefore have been driven by both mirroring and attentional processes. On the other hand, in none of the conditions was central suppression significantly related to occipital suppression. In addition, in both the hand movement and action execution conditions, the central suppression was significantly stronger than the occipital suppression. It seems therefore highly unlikely that the central mu suppression was caused by occipital alpha suppression. The similar occipital suppression in the action observation condition may suggest that the attentional component was especially relevant in this condition, since the children were probably aware that they would have to imitate the observed action from the second or third object onwards, and may therefore have been extra attentive to the presentation.
Our fourth research question concerned possible developmental changes in infant mu suppression. In the current study, the calculation of the mu suppression values in analogy with previous work (Lepage & Théoret, 2006; Marshall et al., 2011; Southgate et al., 2009) allows for a very tentative comparison with the values obtained in those studies. Figure 2 respectively shows the mu suppression values for action execution and action observation reported by Southgate et al. (2009) in 9-month-olds, by Marshall et al. (2011) in 14-month-olds, found in the current study in 18- to 30-month-olds, and reported by Lepage & Théoret (2006) in 4- to 11-year-olds. As can be seen in Figure 2, there seems to be some developmental continuity, reflecting more pronounced mu suppression with increasing age. This observation may also confirm that a measurement of mu suppression during a longer time interval (but still time-locked to an event) may be comparable to the measurement of mu suppression during multiple short trials of for instance the observation of grasping, as is usually done.

Finally, we explored the correlations between mu suppression on the one hand, and the children’s age, receptive and expressive language and imitation quality on the other hand. In line with most previous studies involving adults as well as children (see Lepage & Théoret, 2007), we found no significant correlations between age and the degree of mu suppression during the observation conditions. The same was found for language age. On the one hand, this could be expected, since in typical infants language age is very strongly related to chronological age. On the other hand, if the neural mirroring system also plays a role in language development, as sometimes is suggested (Rizzolatti & Craighero, 2004), one may expect a meaningful relation between language level and mu suppression. It could be that the current sample was too small to detect these correlations, although it was large enough to detect significant correlations within child characteristics and within the mu suppression variables. In addition, it may be that our language measure was not sensitive enough. Since
the N-CDI’s are developed for children up to 30 months, several of the children in our sample reached a ceiling score. The possible relation between language and mu suppression could be further explored in a group of children with a more diverse language development, using different measures. Finally, we did find a significant correlation between the children’s imitation quality on the one hand, and mu suppression during the observation of hand movements and during action execution on the other hand. This correlation however had a positive value, indicating less (negative) mu suppression with increasing imitation scores. Although it may be argued that imitating more (non-functional) details may not necessarily reflect a better performance, the imitation score is positively related to both chronological and language age. This finding seems to argue against a straightforward, linear relation between imitation and the neural mirroring system. Mainly based on rTMS studies (Catmur, Walsh, & Heyes, 2009; Heiser, Iacoboni, Maeda, Marcus, & Mazziotta, 2003), several authors have suggested a strong and possibly causal relation between neural mirroring and imitation (see Gallese et al., 2011 for an overview). Bernier, Dawson, Webb, and Murias (2007) indeed found a significant correlation between imitation performance and mu suppression in both an autism and a control group. On the other hand, two later studies did not replicate this correlation (Fan, Decety, Yang, Liu, & Cheng, 2010; Oberman, Ramachandran, & Pineda, 2008). While the latter two studies used a mu suppression ratio score for the correlation analyses, Bernier et al. (2007) calculated a separate difference score for this purpose. In any case, it seems very useful to further investigate the relation between imitation and neural mirroring, using different neurophysiological techniques. Given the importance and quick development of imitation in early infancy, it may be especially relevant to study this topic at this early age.

During the collection and analysis of the current study’s data, we encountered some difficulties that may limit the results of the study. First, it was not possible to exclude all
movement and vocalisation artefacts from the data before analysing them. However, we followed three steps in order to minimize their effects. Before analysing, based on the off-screen coding of the videos, we excluded all intervals where movements and vocalisations were quite frequently or obviously occurring. Second, during the artefact rejection procedure, remaining movement artefacts that were not obvious on the video were removed. And finally, we examined the effect of the number of movements and vocalisations on the mu suppression per condition by calculating correlations. If a child was moving more in one condition than in another, we would expect more mu suppression in that condition for that child. This was not the case. Therefore, although it seems quite impossible to entirely prevent awake 18- to 30-month-olds from moving, we think we minimized the impact of movements and vocalisations on the results. A second possible limitation of the study is that there were at least two important differences between our action observation and our hand movement observation condition. During the hand movement condition, both the object and the eye contact with the examiner were missing, making it not only an intransitive but also minimally social condition. Adding one or two conditions with only one of these factors changing would have made a stronger study design, but given the limited attention span of children this age, we experienced in a pilot study that this was not possible. In addition, our results seem to suggest that neither the inclusion of an object (on which the action goal was performed), neither the eye-contact with the model was necessary to evoke mu suppression.

In summary, the current study adds to the rapidly growing literature on the neural basis of action understanding and execution by exploring several relevant questions. First, we measured brain activity while the children were watching and executing more elaborate actions on objects, as well as their mimicked equivalents, which has not been studied before. Second, we did not solely focus on central electrode positions, but we also reported results of frontal and parietal electrode sites. In addition, the age group included in this study, although
challenging for EEG-researchers, is of much interest because of their explosive development in the social domain, and their strong reliance on imitative learning. Our results indicate that 18- to 30-month-olds show significant mu suppression while watching actions of objects as well as their mimicked variants. During all three conditions, significant mu suppression was found over central and parietal electrode sites, supporting the presence of a functional action observation/action execution system in these children. In addition, during both observation conditions, the suppression measured over parietal electrodes sites was significantly correlated with both central mu suppression and occipital alpha suppression, suggesting that neural mirroring as well as attentional mechanisms may play a role during these conditions. Especially during the action observation condition, where occipital alpha suppression was as strong as the central suppression, visual attention and/or processing may have influenced the central mu/alpha suppression. Future research should further explore this potential relationship. No significant correlations with chronological or language age were found, which suggests that the current paradigm did not measure substantial developmental changes between 18 and 30 months. The inverse relation between mu suppression and imitation quality stresses the need for further research on this domain.

Future research may benefit from following up infants over their first years of life, in order to further explore the possible causal relation between the neural mirroring systems and imitation abilities. In particular, studying infants and toddlers with autism with the paradigm described in this paper may contribute to our understanding of the action observation/action execution system. Since they show a wide variability in imitation performance (see Vanvuchelen, Roeyers, & De Weerdt, 2011, for an overview) and since they have been found to exhibit deficits in mu suppression during action observation (e.g., Bernier et al., 2007; Oberman et al., 2005; Oberman et al., 2008; Oberman et al., 2012; Pineda et al., 2008), although not consistently (e.g., Fan et al., 2010; Raymaekers, Wiersema, & Roeyers, 2009),
studying mu suppression during action observation and execution in relation to imitation abilities in young children with autism may allow us to learn more about the specific connection and the hypothesized causal relation between neural mirroring and typical and atypical imitation development.
Acknowledgments

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Table 1. Subject characteristics (n = 17)

<table>
<thead>
<tr>
<th></th>
<th>M (SD)</th>
<th>Range</th>
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<tr>
<td>Chronological age (months)</td>
<td>24.54 (3.96)</td>
<td>18.50-30.60</td>
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<tr>
<td>Language age (months), n = 13</td>
<td></td>
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<tr>
<td>Expressive</td>
<td>22.46 (4.46)</td>
<td>17.00-30.00</td>
</tr>
<tr>
<td>Receptive</td>
<td>24.23 (4.17)</td>
<td>18.00-30.00</td>
</tr>
<tr>
<td>Gender ratio M:F</td>
<td>9:8</td>
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**Table 2.** Pearson’s correlations between child characteristics and central mu suppression

<table>
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<tr>
<th></th>
<th>AGE</th>
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<th>COMP</th>
<th>IMIT</th>
<th>HM</th>
<th>AO</th>
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<tr>
<td>EXP</td>
<td>.750**</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>COMP</td>
<td>.657*</td>
<td>.746**</td>
<td></td>
<td></td>
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<tr>
<td>IMIT</td>
<td>.547*</td>
<td>.550*</td>
<td>.446</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HM</td>
<td>.086</td>
<td>-.339</td>
<td>-.300</td>
<td>.483*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AO</td>
<td>-.147</td>
<td>-.329</td>
<td>-.048</td>
<td>.285</td>
<td>.516*</td>
<td></td>
</tr>
<tr>
<td>AE</td>
<td>.349</td>
<td>.096</td>
<td>.042</td>
<td>.586*</td>
<td>.751***</td>
<td>.126</td>
</tr>
</tbody>
</table>

Note. AGE = chronological age, EXP = expressive language level in months, COMP = language comprehension level in months, IMIT = imitation quality score, HM = mu suppression during hand movement condition, AO = action observation condition, AE = action execution condition, * = p<.05, ** = p<.01, ***=p<.001.
Figure 1. Mean mu suppression during (a) action execution, (b) action observation, and (c) hand movement observation conditions, over frontal, central, and parietal electrode locations. Error bars show ± 1 standard error. Significant differences from zero are indicated. * p < .05, ** p < .01, *** p < .001
Figure 2. Mu suppression values for action execution and action observation reported by Southgate et al. (2009) in 9-month-olds, by Marshall et al. (2011) in 14-month-olds, found in the current study in 18- to 30-month-olds, and reported by Lepage & Théoret (2006) in 4- to 11-year-olds.