EXPLORING NEURAL MIRRORING SYSTEMS IN TYPICALLY DEVELOPING CHILDREN AND IN CHILDREN WITH AUTISM SPECTRUM DISORDER

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Exploring neural mirroring systems in typically developing children and in children with autism spectrum disorder

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Ik wil dat mijn woorden
nu zeggen
wat ik je wil zeggen
in de hoop
door jou gehoord te worden
zoals ik wil dat je me hoort

(Pablo Neruda)

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Imitation and its underlying neurological mechanisms are well investigated topics in children with autism spectrum disorder (ASD). Nevertheless, the inconsistent results of imitation in ASD and the related neurological processes call for further exploration and research. In this first chapter, the theoretical background of this doctoral dissertation is provided. Furthermore, the aims and objectives of the research are outlined, followed by an overview of the chapters included in this dissertation.

DEFINING AUTISM SPECTRUM DISORDERS

According to the DSM-IV-TR, the concept of 'pervasive developmental disorders' (PDD) is subclassified in Rett's Disorder, Childhood Disintegrative Disorder, Autistic Disorder and Pervasive Developmental Disorder Not Otherwise Specified (American Psychiatric Association [APA], 2000). The latter three classifications are mostly referred to as 'Autism Spectrum Disorders' (ASD; Wing, 1997). ASD, a spectrum of neurodevelopmental disorders, is characterized by impairments in three domains: (1) social interaction, (2) verbal and non-verbal communication, and (3) a restricted repertoire of interests, activities, and behaviours (APA, 2000). Furthermore, ASD represents a broad variation in symptomatology, ranging from rather mild to very severe symptoms in the three areas of impairment (Wing, 1997). These impairments can fluctuate in gravity between individuals and within one individual over time (Charman, 2002). Recently, with the development of the DSM-5, new diagnostic criteria have been proposed concerning autism and related disorders. It is recommended to use one single category, i.e., ASD, which incorporates Childhood Disintegrative Disorder, Asperger's Disorder, Autistic Disorder, and Pervasive Developmental Disorder Not Otherwise Specified but excludes Rett's Disorder (APA, 2012). In this dissertation, the term Autism Spectrum Disorder (ASD) is used to refer to the broader umbrella of pervasive developmental disorders (PDD).

Autism spectrum disorders are not rare disorders. Research in the past suggested a rate of 30/10.000 for all forms of PDD, but recent studies suggest that the prevalence might be as high as 60/10.000 or even around 1% in the general population (Fombonne, 2009; Holtmann et al., 2011). Many explanations for this increased number have been discussed and investigated. Most evidence suggests that the increase in scientific research in the ASD domain leads to a change in diagnostic criteria of ASD reflected in a broadening of the definition (Wing & Potter, 2002). Furthermore, the recognition of ASD as a spectrum leads to an increase in the diagnoses of ASD which influences prevalence estimations (Charman, 2002). Additionally, other explanatory suggestions for the increased prevalence rates have been made such as growing awareness of the disorder in the society, existence of specialist institutions and the acknowledgement that ASD can co-occur with other disorders and can be diagnosed in individuals with average

intellectual abilities (Charman, 2002; Wing & Potter, 2002). Despite many explanations, the possibility of a true increase of ASD in the population cannot be ruled out (Wing & Potter, 2002). The overall ratio of males to females with ASD has traditionally been reported at approximately 3:1 to 4:1 (Volkmar, Szatmari, & Sparrow, 1993) with a higher incidence in males compared to females.

Because of the complexity and variability of symptoms within the autistic spectrum, multiple etiologies have been identified. The strongest evidence concerns the influence of genes with up to 90% heritability (Freitag, 2007). Based on several twin studies, the concordance rates for monozygotic twins is estimated between 60% and 90%, whereas in dizygotic twins it is assessed between 0% and 10% (Dawson et al., 2002). Several possible genes are investigated which indicates that ASD is a polygenetic disorder (Schroeder, Desrocher, Bebko, & Cappadocia, 2010). Related to the findings of genetic contributions in ASD, is the discovery of the 'broader autism phenotype' (BAP). The BAP reflects qualitatively similar brain and behavioural features of ASD which occur more often among relatives of autistic individuals (Losh & Piven, 2007). Learning more about this concept and about the characteristics of ASD has partially been accomplished by investigating relatives of individuals diagnosed with ASD, such as high-risk siblings of children with ASD due to their higher risk of developing ASD themselves (Ozonoff et al., 2011; Rogers, 2009). A unique strength of including high-risk siblings in ASD research is the opportunity to directly observe the relationship between a broad range of early behavioural markers of the disorder and later impairments typical of ASD (Rogers, 2009). In addition, many researchers found evidence for symptoms in several areas (such as motor development, communication, social and emotional development) being present between the ages of 12 and 24 months in infants who were later diagnosed with ASD (Rogers, 2009). Therefore, at-risk sibling studies can help exploring early manifestations and risk markers of ASD and its broader autism phenotype (Rogers, 2009). Recently, besides behavioural research, studies have started to use direct measurements of brain and cognitive functions in siblings and relatives of individuals with ASD to learn more about ASD and its BAP (Sucksmith, Roth, & Hoekstra, 2011).

To conclude, because there is good evidence that ASD is a multifactorial disorder, which is reflected in the clinical heterogeneity, non-genetic, environmental causes are also investigated in research concerning the etiology of ASD (see Rutter, 2005 for a

review). Furthermore, it seems tough to define only one etiological marker for ASD. Due to the variety in symptoms and severity between individuals with ASD, it is likely that individuals differ in etiologies and consequently that different brain areas are involved in the development of this developmental disorder (Charman, 2002; Schroeder et al., 2010).

IMITATION AND ITS ROLE IN ASD

Normal development of imitation

Imitation is often defined in multiple ways but a commonly used definition is 'the ability to replicate an observed novel action to achieve the same ends by using the same means'. This definition implicates that the imitator copies the observed means of the performer to achieve the same results (Tomasello, Carpenter, Call, Behne, & Moll, 2005). During successful imitation, the imitator uses the perceived actions to create his own matched behaviour (Marshall & Meltzoff, 2011). Imitation should be differentiated from emulation, mimicry, and stimulus enhancement as *imitation* requires not only copying the observed movements but also the understanding of the action goal (Elsner, 2007). *Emulation* is accomplishing the same goal without copying the actions or means to achieve that goal (Huang, Heyes, & Charman, 2002). *Mimicry* is the opposite as it is copying the model's behaviour without understanding the action goal (Tomasello, Kruger, & Ratner, 1993). Being attracted to the object manipulated by the model is called *stimulus enhancement* (Tomasello, Savage-Rumbaugh, & Kruger, 1993).

Although strongly debated (e.g., Anisfeld et al., 2001), in typically development, imitative responses seem to be innate or present at birth (Meltzoff & Moore, 1977; 1997). Starting a couple hours after birth, newborns can demonstrate facial imitation (e.g., tongue protrusion and mouth opening) (Heimann, 2002; Meltzoff & Moore, 1983). It is assumed that this behaviour is not purely reflexive but rather a goal-directed response in order to match with the observed action. Additionally, early imitation is specific as it is found that infants respond differentially on diverse demonstrated actions (Meltzoff & Moore, 1997). After six weeks, infants can imitate from memory, which

means that they can recall and produce actions on the basis of stored representations (Meltzoff & Moore, 2002). Furthermore, they can even imitate the duration of the modelled action (Meltzoff & Moore, 1994). Research revealed that from 9 months old onwards, infants can imitate certain simple actions with objects, both in immediate and deferred contexts (Meltzoff, 1988b). During imitation, the model is used as a source of information which influences infants' behaviour and at this age, they even realize that their own behaviour is imitated by others (Meltzoff, 1988b). Around the age of 1 year, infants show interest in imitation by treating the other as a biological mirror to discover what it means to be like the other person (Meltzoff & Moore, 1997). During the first two years of life, imitation abilities in infants improve and become increasingly flexible (Elsner, 2007). For example, 14-months olds use verbal and context information from the demonstration to adjust their own imitative behaviour (Carpenter, Akhtar, & Tomasello, 1998) and they can imitate irrational and substituted actions with objects (Meltzoff, 1988a). By the age of 18 months, imitation develops to a way of understanding others' intentions as infants at that age not only imitate what they observed, but complete the action that the model tended to perform (Meltzoff, 1995).

As the previous overview demonstrated, there is an evolution in imitation from bodily actions to actions on objects. Furthermore, infants demonstrate age-related changes in their imitation of different action types, from simple familiar actions over socially meaningful actions towards appropriate as well as inappropriate action imitation (Killen & Uzgiris, 1981). These findings concerning imitation and its development lead to the conclusion that imitation plays an important role in early learning of behavioural repertoire and social knowledge (Rogers, Young, Cook, Giolzetti, & Ozonoff, 2010). It is a way of communicating with others and of acquiring social skills (Cochin, Barthelemy, Roux, & Martineau, 2001).

Recently, neuropsychological processes of imitation have been reconciled with two important cognitive theories of imitation (Iacoboni, 2009). First, the ideomotor framework model assumes that action execution and action observation share a common representational basis (Iacoboni, 2009). Furthermore, imitation is based on the perceptual and motor experience of the imitator who uses this personal experience to automatically activate the representation of the actions necessary to achieve the same

action goal as the observed model. This latter principle can also be applied to the associative sequence learning model which is the second commonly used cognitive theory of imitation. The associative learning theory suggests associations between independent sensory and motor action representations created and linked by experience, which makes imitation possible (Iacoboni, 2009). Both cognitive theories support the important role of experience for imitation performance and provide evidence that imitation is based on an automatic activation of the stored motor presentations (Brass & Heyes, 2005). These two cognitive models of imitation are supported by the recent neuropsychological research on imitation, such as the neural mirroring theory, which will be discussed later in this introduction (Catmur, Mars, Rushworth, & Heyes, 2011).

It has been suggested that imitation is already present very early in life but changes and develops over time. Consequently, imitation is omnipresent during life and has various functions. In general, imitation plays a central role in social-communicative development and is a widely used tool for social learning (Elsner, 2007; Ogawa & Inui, 2012) and social–cognitive understanding (Gopnik & Meltzoff, 1993), helping the child developing an understanding of others' intentionality (Uzgiris, 1981). Children learn and acquire new behaviours by watching others using objects and by imitating those actions (Meltzoff & Moore, 1983). Imitation also serves as an identity function as infants try to identify with other people by imitating others' behaviour (Meltzoff & Moore, 2002). Moreover, through imitation, they try to understand the representations they have picked up by observing others. After this process, children will use imitation themselves in situations where they want to learn more about others' identity (Meltzoff & Moore, 2002). By using imitation, they try to identify and communicate with persons they have seen before to find out if they meet a familiar person or if they meet someone new for the first time (Bremner, 2002). This process is called the social–cognitive function of early imitation in infancy. Besides this important role, imitation also serves several other functions in the development of children. During the first years of life, imitation is the way by which the infant tries to continue its interaction with the mother (Kugiumutzakis, 1999). As infants grow older, imitation is used in order to make contact with other infants. In this view, imitation has a communicative function in interactions with mother and peers, which is a process mostly observed in pre-verbal children (Nadel, 2002).

Additionally, imitation is used as a cognitive tool to learn about the world and as an interpersonal tool to share experiences with others (Nielsen, Simcock, & Jenkins, 2008).

In general, we can conclude that imitation serves an important social-communicative and identity function suggesting that by imitation, self-other understanding can be developed. In light of these findings, it is suggested that impairments in imitation can cause several social-communicative deficits, for example observed in individuals with ASD (Williams, Whiten, Suddendorf, & Perrett; 2001).

Imitation in ASD

Impaired imitation has been included in the diagnostic criteria of the DSM-IV-TR (APA, 2000) as 'lack of social imitative play appropriate to developmental level', categorized as one of the ASD communication characteristics. Since the work of DeMyer and colleagues (1972), imitation in ASD has well been investigated but research leads to controversial findings (for an overview, see Charman & Baron-Cohen, 1994; Williams, Whiten, & Singh, 2004). However the majority of research points to the idea that imitation is impaired in young children with autism spectrum disorders in comparison with mental age-matched typically developing and developmentally delayed individuals (Rogers, Bennetto, McEvoy, & Pennington, 1996). The first review was by Rogers and Pennington (1991), who found evidence for bodily and action imitation deficits in ASD. Moreover, they suggested that this imitation deficit could cause other social-communicative impairments. A second review was performed by Smith and Bryson (1994). These authors concluded that the observed imitation problems in ASD are the consequences of perceptual organisation difficulties. Williams and colleagues (2004) concluded that children with ASD often show an imitation deficit. The size of this deficit is most apparent in younger age groups and is mostly characterized by difficulties in imitating non-meaningful gestures and non-meaningful object-oriented tasks compared to familiar or meaningful object-directed actions. Additionally, Charman and colleagues (1997) observed impaired imitation in 20-months old infants with ASD but this deficit was not present in school-aged children (Charman & Baron-Cohen, 1994). Furthermore, children with ASD can have problems with imitating unknown and unusual actions with a regular object (Smith & Bryson, 1994). In a study of Charman and colleagues (1997), 20-

months olds with ASD demonstrated less procedural imitation than the control group. Rogers, Hepburn, Stackhouse, and Wehner (2003) found in 34-months old infants with ASD a significant impairment in simple imitation skills such as oral-facial imitation and action imitation with objects. Roeyers, Van Oost, and Bothuyne (1998) found impaired gestural and procedural (i.e., action with objects) imitation in young children with ASD (mean age 57 months) compared to developmentally delayed children. Imitation deficits were also found in school-age children with ASD. For example, Green and colleagues (2002) found that children between 6 and 10 years old with ASD performed less accurate on gestural imitation than comparison groups. Fewer ASD studies have examined imitation skills in adolescence and adulthood, but found evidence for the persistence of imitation impairments in ASD. For example, Hobson and Lee (1999) found that adolescents with ASD failed in imitating the style of the performed action but were able in imitating the action goal. Adults with ASD demonstrated difficulties in imitating mirror images (Avikainen, Wohlschlager, Liuhanen, Hanninen, & Hari 2003) or showed impairments in hand and face imitation and imitation of meaningless movements (Bernier, Dawson, Webb, & Murias, 2007). In a prospective study conducted by Robins, Fein, Barton, and Green (2001), the absence of imitation was one of the most important predictors of a later ASD diagnosis in toddlers. Vanvuchelen, Roeyers, and De Weerdt (2011a) found in their study a significant predictive association between procedural imitation delay and ASD. As this overview demonstrates, imitation impairments in ASD have frequently been reported from infancy until adulthood.

Many explanations have been proposed trying to explain these imitation impairments in ASD. Some of them will be discussed in this section, however this overview is not conclusive as research on this topic is still inconsistent and obscure.

The model of Trevarthen and Aitken (2001) is based on the assumption that children with ASD are less motivated to interact in a social way which could predict poorer performance on imitation tasks. This explanation suggests that individuals with ASD are impaired in the use of imitation skills for social intentional behaviour (Ingersoll, Schreibman, & Tran, 2003). Findings of less joint attention behaviour during imitation in infants with ASD support the idea of less social interest and motivation to interact and to imitate others (Ingersoll, 2008). Recently, much attention goes to the social role of imitation in early development and a more social explanation for imitation problems in

ASD. For example, Hobson and Lee (1999) found less imitation of the style of the demonstrated actions and argued that individuals with ASD have difficulties in engaging with others through imitation. In addition, Dawson, Meltzoff, Osterling, Rinaldi, and Brown (1998) found in their study that children with ASD oriented less frequently to social stimuli and demonstrated less shared attention in comparison with developmentally matched children. This (weak) social attention could explain impairments in imitation in ASD. Previous possible behavioural explanations for imitation problems in ASD are based on selection mechanisms concerning 'what' to imitate, but it is possible that children with ASD do not know 'how' to imitate. This can be caused by a correspondence problem between the perception of an action performed by others and the execution of that similar action themselves. Imitation problems and other social-communicative difficulties in ASD could be based on an impaired formation of self-other representations (Williams et al., 2001). This self-other correspondence problem can be caused by impaired mirror neuron functioning (Williams et al., 2001). Mirror neurons, which are neurons activated during execution and observation of others' actions, may provide a way of identifying and developing awareness of self-other correspondence (Rogers et al., 2003). Additionally, imitation requires the transformation of visual input resulting from observing others to motor output matching this input, a process which could be accomplished by mirror neuron functioning (Heyes, 2001). The concept of mirror neurons will be discussed later as it is the main topic of this dissertation. Besides problems with self-other representations, other cognitive skills such as symbolic functioning (Baron-Cohen, 1988) or working memory (Rogers et al., 1996) are investigated as possible explanatory theories for imitation problems often observed in ASD. On a more perceptual-motor level, imitation in ASD can also be impeded by less motor skills, also known as the 'dyspraxia hypothesis' (Mostofsky et al., 2006). However, Williams and colleagues (2004) concluded that motor skill impairment cannot fully explain the imitation problems in ASD. Up till now, the motor theory and theory of the social factors are the most commonly used models to explain imitation impairments in ASD (Rogers et al., 2003). Furthermore, some researchers concluded that a possible explanation for imitation problems in ASD can be found in the role of emulation. Moreover, it is assumed that children with ASD depend more on emulation (i.e., copying only the goal) than on imitation (i.e., copying the mean/way and the goal) which could explain the different findings about imitation in

ASD (Rogers et al., 2010). In addition, Hamilton (2008) concluded that children with ASD have difficulties with mimicry of meaningless actions such as hand gestures or facial expressions, rather than with goal-directed emulation.

Arguments can be postulated for and against aforementioned hypotheses (for an overview, see Williams et al., 2004). However, it should be noted that previous hypotheses may be combined to create a comprehensive explanation for imitation impairments in ASD. Difficulties in 'what' to imitate and 'how' to imitate in ASD can be caused by different underlying mechanisms and processes. However, there are studies that are contradictory with the idea of an overall imitation deficit in ASD. Some of these studies mention that children with ASD have an intact ability to imitate object-oriented and goal-directed actions (for a review, see Hamilton, 2008). For example, Hamilton, Brindley, and Frith (2007) found that both autistic and typically developing children have the same tendency to imitate in a mirror way, to imitate an adult's goals and to imitate grasping. Furthermore, children with ASD could imitate complex goal-directed actions but were not able of imitating the style with which the actions were performed (Hobson & Lee, 1999). Other studies found good imitation of object-directed actions (Stone, Ousley, & Littleford, 1997) and good performance of explicit imitation tasks (Beadle-Brown & Whiten, 2004) in young children with ASD. Additionally, studies have demonstrated that sensory effects (e.g., actions producing light and sound) could facilitate imitation performance in ASD (Ingersoll et al., 2003). Finally, studies noted that individuals with ASD often demonstrate hyperimitation which means increased spontaneous imitation, reflected in symptoms such as echolalia and echopraxia (Spengler, Bird, & Brass, 2010). Although, various possibilities can be mentioned why several studies found no evidence for imitation impairment in ASD such as ceiling effects (Charman & Baron-Cohen, 1994; Ingersoll et al., 2003), previous results provide clear evidence against the idea of a global imitation impairment in children with ASD (for a review, see Hamilton, 2008; Vanvuchelen, Roeyers, & De Weerdt, 2011b). The review of Hamilton (2008) demonstrated that children with ASD may not show a global, simple imitation deficit of all actions but rather a more complex deficit limited to different action types. In addition, Williams and colleagues (2004) concluded that the imitation deficit in ASD is more a delay of the normal imitation development rather than a stable deficit. Furthermore, Young and colleagues (2011) concluded that infants with ASD demonstrate no quantitatively or qualitatively differences in the development of

imitation but are just delayed in the development of imitation compared to other comparison groups. According to this view, it is important to investigate imitation capacity in young children with ASD and to investigate if the age of acquisition of imitation skills in ASD differs from normal imitation development.

Furthermore, the heterogeneity of the ASD phenotype, the variability across imitation tasks and the inconsistency of the definition of imitation all impede the development of a clear view on imitation in ASD (Vanvuchelen, Roeyers, & De Weerdt, 2011c). Consequently, research concerning imitation in ASD is still debated and needs further exploration, especially in children under 4 years of age as there are only a few studies conducted in that age-period (Wu, Chiang, & Hou, 2011). As imitation plays in general an important role in social development and reciprocal social communication, it is suggested as one of the core symptom deficits in ASD (Williams et al., 2001). Therefore, it is important to investigate and to discover the underlying neurological imitation mechanisms functional in ASD (Rogers et al., 2010). The various findings regarding imitation in ASD lead to diverse proposed underlying processes and mechanisms. Recent development in imitation research explores the underlying neural mechanisms possibly involved in imitation and hypothesize that the inferior frontal gyrus is involved. This area involves Broca's area, which is the human homologue of area F5 in monkeys in which mirror neurons are present (Heyes, 2001). Consequently, the interest in discovering the neurobiology of imitation has been widely influenced by the discovery of mirror neurons in the macaque brain (Di Pellegrino, Fadiga, Fogassie, Gallese, & Rizzolatti, 1992). It has been suggested that imitation problems in ASD may be related to a dysfunction in the neural mirroring areas that provide a foundation for imitation and other behavioural functions (Williams et al., 2001).

MIRROR NEURONS

Mirror neurons in monkeys

Mirror neurons are a particular class of single-cell visuomotor neurons first discovered in the macaque brain in area F5 by Rizzolatti and colleagues (Di Pellegrino et

al., 1992; Rizzolatti, Fadiga, Gallese, & Fogassi, 1996). These neurons can be defined as 'monkey see–monkey do' neurons as they are active both during execution and during observation of goal-directed actions (e.g., grasping an object) performed by a conspecific or an experimenter (Carey, 1996). For example, a neuron which is active during reaching for a peanut was also active when the monkey observed someone else performing that identical action. In this light, mirror neurons mediate a common neural representation of action observation and action execution (Kanakogi & Itakura, 2010).

However, in the monkey brain, mirror neurons are active only when an object is manipulated by mouth or hand (Gallese, Fadiga, Fogassi, & Rizzolatti, 1996). Consequently, observing only an object without manipulator or non-object-directed actions (i.e., intransitive actions) is insufficient to produce mirror neuron activity in monkeys which suggests that the discharge of these neurons is related to coding the goal of the observed action (Rizzolatti & Graighero, 2004). Mirror neurons respond regardless the kind of visual stimuli, indifferent of hand orientation or regardless receiving a reward after performing the action (Rizzolatti & Graighero, 2004). Furthermore, the object to be grasped or the distance of which the monkey observes do not influence the degree of mirror neuron response (Iacoboni & Dapretto, 2006). Besides this visual generalizability, mirror neurons in monkeys also respond to abstract properties of observed actions. For example, the research of Umilta and colleagues (2001) revealed activity in monkey mirror neurons when the action was partly hidden behind a screen but with the restriction of prior knowledge of the presence of the object behind the screen. Furthermore, mirror neurons also code the intentions of the observed action. For example, mirror neurons discharged more when food was taken with the intention to eat compared to the action of taking food just for placing it in a box (Fogassi et al., 2005).

The majority of the monkey mirror neurons in area F5 are 'broadly congruent' which means that the observed action can deviate from the executed action but with the restriction to achieve the same goal (for example, grasping food with a hand or grasping food with a tool). 'Strictly congruent' mirror neurons respond only when the observed and executed action correspond both in terms of the goal of the action (e.g., grasping) and in terms of the style in which that action is executed (e.g., precision grip) (Rizzolatti & Graighero, 2004). Only a few neurons respond when there is no clear

relationship between the observed and executed action which are defined as 'non congruent' (Gallese et al., 1996).

Monkey mirror neurons are not only located in area F5. Other brain regions such as the superior temporal sulcus (STS), the inferior parietal lobule, and the ventral premotor cortex have mirror properties (Iacoboni, 2005). The STS is related to mirror neurons as it sends the input received during observation to the ventral premotor cortex and area F5 (Iacoboni, 2005). Although necessary for providing input, the STS has no motor properties (i.e., the STS is active during the observation of others' movements but not during the performance of one's own actions) which excludes this brain region from the core mirror neuron network in monkeys. Therefore, Rizzolatti and Graighero (2004) concluded that the core mirror neuron system in monkeys is formed by the ventral premotor cortex (which contains area F5) and the rostral part of the inferior parietal lobule.

As these neurons "mirror" observed actions into their own motor system without being necessarily performed, they are related to a cortical action observation/executing matching system (Pineda, 2005). Consequently, an important feature of these monkey mirror neurons is their involvement in action understanding. During observation of actions performed by others, mirror neurons are activated as if the observer is performing the action itself. By this direct sensory-motor mapping, visual stimuli are matched with stored information about the known result of the action to understand the actions performed by others due to the similarity between the two representations (Rizzolatti, Fogassi, & Gallese, 2001). This direct matching of a visual representation of the action with the neural experience based replication makes the link between mirror neurons and action recognition, action understanding and imitation possible (Gallese et al., 1996; Iacoboni, 2005). In this way, the observer is capable of recognizing the observed action, differentiating this action from other actions and using this information to imitate the observed action (Rizzolatti et al., 1996).

Mirror neurons in humans

Contradictory to direct, single-cell neuron studies in monkeys, indirect brain imaging and neurophysiological studies such as transcranial magnetic stimulation (TMS;

e.g., Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995), electroencephalography (EEG; e.g., Muthukumarasway, Johnson, & McNair, 2004), magnetoencephalography (MEG; Hari et al., 1998), and functional magnetic resonance imaging (fMRI; e.g., Buccino et al., 2001) suggest the presence of functional and structural comparable mirror neurons in humans. The existence of such an automatic and direct matching system has also been demonstrated by several behavioural measures such as gaze tracking (Falck-Ytter, Gredebäck, & von Hofsten, 2006). However, recently, direct evidence for the presence of mirror neurons in the human motor cortex was provided in the first single cell study of Mukamel and colleagues (Mukamel, Ekstrom, Kaplan, Iacoboni, & Fried, 2010).

Without overtly reproducing the action, when humans observe someone performing an action, mirror neurons and motor areas are activated as if the observer is executing the observed action himself. This matching is an implicit, automatic, and unconscious process by which the internal motor knowledge of the observer is automatically activated and attributed during action observation (Fogassi, 2011; Gallese, 2003). The idea of representing acts of their own and others in comparable modes is not unique to mirror neurons. The capacity of imitating others demands a similar link between perception and execution of actions (Nyström, Ljunghammar, Rosander, & von Hofsten, 2010).

The ‘core’ human mirror neurons are located in areas homologous to the monkey human mirror neuron system namely in the posterior part of the inferior frontal gyrus (human homologue of area F5 of the macaque), the ventral premotor cortex (including Broca’s area), as well as the rostral inferior parietal lobule and are somatotopically organized (i.e., different areas are activated dependent on the effector with intermediate activity during hand movement, ventral activity during oral action and dorsal activation during leg movement) (Buccino et al., 2001; Iacoboni, 2005). Human mirror neurons can be distinguished from monkey mirror neurons based on several characteristics. First, contradictory to monkey mirror neurons, human mirror neurons respond both during observation of transitive and intransitive, meaningless, non-goal-directed actions (Fadiga et al., 1995). Mirror neuron activity during observation of this latter represents motor resonance in which the observation activate stored action representations in the observer (Mukamel et al., 2010). Second, in humans, not only the

action itself but also the way in which the action is performed can activate mirror neurons (Gangitano, Mottaghy, & Pascual-Leone, 2001).

Human mirror neuron functioning has been theoretically (but strongly debated) related with several concepts such as action understanding (Rizzolatti & Craighero, 2004) and various social functions such as imitation (Iacoboni, 2005), empathy (Iacoboni & Dapretto, 2006), and language (Rizzolatti & Arbib, 1998).

Similar as in monkeys, human mirror neurons are related to action understanding through a direct representation of the observed action by activation of the same neural pathways as during execution (Debes, 2010). Another mechanism related to mirror neuron functioning is imitation. The supposed link between imitation and mirror neurons is the result of the requirement of a match between the observed and performed action during imitation and the finding that mirror neurons are active during observation as well as during execution of actions (Marshall & Meltzoff, 2011). Furthermore, several studies found an overlap between activated brain areas during imitation and mirror neuron regions in macaques which supports the link between imitation and mirror neuron activity (Iacoboni, 2005). According to Iacoboni (2005; 2009), the core circuitry of imitation consists of the superior temporal sulcus (STS) and two mirror neuron regions namely the inferior parietal lobule and the inferior frontal cortex. Moreover, in the STS, higher-order visual descriptions of the observed actions are coded and sent to the parietal mirror neuron areas for defining motor description of the imitated action. Subsequently, somatosensory information is transported from the parietal to the frontal mirror neuron areas, which are more focused on coding the imitated action goal. Finally, efferent copies of motor imitative commands are sent back to the STS to match the sensory predictions of the imitative motor plans with the visual descriptions of the observed actions. Finally, a good match leads to imitation. Imitative learning and social mirroring, two different forms of imitation, are based on previously described circuitry and additional neural regions (Iacoboni & Dapretto, 2006). Empathy is the ability to map others' feelings into its own system and can be accomplished by imitating facial and bodily expressions (Leslie, Johnson-Frey, & Grafton, 2004). Assuming a possible link between imitation, action understanding and mirror neuron activity, the hypothesis of a neural mirror substrate involved in empathy seems inevitable (Iacoboni & Dapretto, 2006). However, research revealed the involvement of additional brain

systems during empathy such as the limbic system which is connected with the mirror neuron areas through the insula (Augustine, 1996). The link between language and mirror neurons is anatomically supported. Moreover, area F5 is the anatomically monkey homologue of Broca's area in the human brain which is a cortical area related with language development (Rizzolatti & Arbib, 1998). Mirror neurons make it possible to understand actions performed by others by being the mediating factor between observer and performer, which can also be applied to the process of communication (Iacoboni, 2009).

Human neural mirroring regions have 2 important functions. First, mirror neurons serve a social function through their relationship with imitation (Iacoboni et al., 1999) and action observation (Buccino et al., 2001). This leads to the assumption of a broken mirror functioning in individuals with ASD, which will be elucidated further in this dissertation. A second function of neural mirroring regions is controlling motor actions. Mirror neurons are presented in our motor system which explains their role in performing flexible, visual guided motor actions such as goal-directed hand movements (Buxbaum, Kyle, & Menon, 2005). Profound research on the human mirror neuron functioning revealed several other characteristics. To cause activity in the mirror neurons, the observed action needs to be part of the own motor experience (Buccino et al., 2004). In addition, human mirror activation is bigger during the observation of familiar actions compared to unfamiliar actions. In the study performed by Calvo-Merino, Glaser, Grezer, Passingham, and Haggard (2005), fMRI data demonstrated stronger mirror neuron activity when dancers observed their own dance style compared to the observation of an unfamiliar style. They concluded that human mirror neuron activity can be modulated by the degree of familiarity of the observed action. Furthermore, human mirror neurons are more strongly activated when the observed actions are modelled by humans, rather than executed by other species such as dogs or monkeys (Buccino et al., 2004). Finally, Oberman, McCleery, Ramachandran, and Pineda (2007) discovered that human mirror neurons are responsive during the observation of both biological (e.g., hand movement) and artificial movements (e.g., movement of a robotic arm). Additionally, reactivity of mirror neurons during the observation of biological or artificial movements is bigger when the entire body is moving than only individual parts (Francuz & Zapala, 2011). Therefore, in the research paradigm applied in

this doctoral research, the experimenter who modelled the movements was entirely visible for the participants.

As the mirror neuron hypothesis postulates that humans understand other people's actions in term of mapping them into one's own motor programs, it is expected that observed actions are not fully understood until infants dominate those motor actions themselves. If this hypothesis is valid, it is expected that the development of infants' understanding of others' actions progresses equally with their own motor development (Nyström et al., 2010). Recently infant studies found that similar brain processes were activated when infants executed and observed actions from 6-months old onwards with several developmental changes noticeable as infants grow older (for a review, see Marshall & Meltzoff, 2011). Assuming that a functional mirror-like system could be present at birth is based on the imitative abilities observed in newborns (Meltzoff & Moore, 1977) and the idea that imitation requires a link between perception and action mediated by mirror neurons (Wohlschlager & Bekkering, 2002). Therefore it is important to investigate mirror neuron functioning in infancy.

Several studies found evidence for a sensorimotor matching system in infants as early as 6 months of age. For example, Nyström (2008) was the first to demonstrate a direct event-related potential (ERP) measure of mirror neuron activity in 6-months old infants. Shimada and Hiraki (2006) found a significant difference in mirror neuron activity during live action observation compared to object observation in 6- to 7-months old infants using near-infrared spectroscopy (NIRS). The study by Nyström and colleagues (2010) found that when 8-months olds observed goal-directed actions, mirror neurons responded more strongly than when they observed non-goal-directed actions similar as in adults indicated by mu rhythm desynchronization, which supports the hypothesis of neural mirroring activity in infants. Southgate, Johnson, Osborne, and Csibra (2009) reported overlapping neural activity during the execution and observation of others' grasping actions in 9-months olds reflected in EEG measurements of changes in the sensorimotor alpha band activity. Moreover, this motor activation started once the action could be anticipated and was driven by infants' understanding of the goal of the observed action (Southgate, Johnson, El Karoui, & Csibra, 2010). Other studies demonstrated the flexibility of the neural mirroring system and the influence of experience on its functioning. For example, van Elk, van Schie, Hunnius, Vesper, and

Bekkering (2008) found stronger mirror neuron activity reflected in stronger mu- and beta-desynchronizations when the observed action was closely related to one's own action experience and motor repertoire in 14- to 16-months old infants. Infants' own experience influenced their perception of others, suggesting that action execution and action observation are related already early in life (van Elk et al., 2008). Even more, 14-months old infants demonstrated mu suppressed mirror neuron activity during dyadic interactions but not during the observation of complex adults' movements which were not fully established within their own motor repertoire (Reid, Striano, & Iacoboni, 2011). Additionally, Meyer, Hunnius, van Elk, van Ede, and Bekkering (2011) found mirror neuron activity in 3-years old children during action observation in a joint action as indicated by attenuated sensorimotor mu- and beta-power. Finally, Lepage and Théoret (2006) found EEG evidence for activity in mirror neuron areas during the execution and observation of hand grasping movements in school-aged children (between 52 and 133 months).

Aforementioned studies suggest the presence of functional mirror neuron activity in infants as early as 6 months old. In this light, observation and execution can be possibly directly related from early infancy onwards and be refined during development. However, when and how this matching process exactly develops is not discovered yet.

Mu rhythm suppression

In monkeys, mirror neuron activity is directly registered by using implanted electrodes. In humans, other indirect measures are used to analyse mirror neuron functioning. One commonly used method, which is also child friendly and used in the research of this doctoral dissertation, is measuring oscillatory activity in electroencephalogram (EEG) recordings (Ramachandran & Oberman, 2006). Gastaut and Bert (1954) were the first who discovered a desynchronization of EEG oscillations in adults who observed boxing actions performed by others. EEG synchronization reflects deactivation while EEG desynchronization is related to activated cortical regions (Pineda, 2005). Therefore, activity in mirror neurons is reflected by desynchronization of the sensorimotor mu rhythm, which is different from the regular alpha frequency rhythm although both rhythms occur within the same frequency band (8-13Hz). However, the

classical alpha rhythm is related to visual processing modulated by occipital networks and responsive to open eyes, whereas sensorimotor processing in frontoparietal networks is reflected in mu rhythm not affected by opening or closing the eyes (Berchicci et al., 2011). Furthermore, both rhythms differ in source localization, power, spatial and bilateral distribution, and in functional sensitivity (Pineda, 2005). Both rhythms are supposed to be present early in development but are dynamic and flexible with changing characteristics even in adulthood (Pineda, 2005).

Several arguments can be used for associating the desynchronization of the mu rhythm with activity in the mirror neurons. First, at rest sensorimotor neurons spontaneously fire in synchrony leading to a large amplitude of the EEG mu wave. During execution, sensorimotor neurons are desynchronized, which decreases the power of the mu band oscillations, also called 'mu wave suppression' (Lepage & Théoret, 2007). This attenuation of the mu frequency band during action execution also occurs during action observation. Therefore, mu wave suppression, typically recorded from sensorimotor cortex, indicates activity in the underlying neurons, displaying active processing during motor movement and action observation (Pineda, Allison, & Vankov, 2000). Second, the mu rhythm is a sensorimotor rhythm consisting of several frequencies and with various origins both in parietal sensory areas and in sensorimotor areas, consistent with the mirror neuron locations (Pineda, 2005). Furthermore, Muthukumaraswamy and colleagues (2004) hypothesized that mirror neuron areas were the only regions active during action observation as bodily movement could not account for the presence of mu wave suppression. In infants, a topographically and functionally similar mu rhythm has been observed but at a lower frequency range and with a lower amplitude (Stroganova, Orekhova, & Posikera, 1999). The mu frequency range in infants (estimated between 6 and 9Hz) gradually increases with age to the adult frequency range, between 8 and 13Hz (Berchicci et al., 2011; Marshall & Meltzoff, 2011). Therefore, several authors suggested that analysis of EEG mu frequency band oscillations may be a useful and non-invasive method for monitoring mirror neuron functioning (Muthukumaraswamy et al., 2004; Ramachandran & Oberman, 2006).

Mirror neurons, imitation, and ASD – The broken mirror theory

Given the assumed relationship between mirror neuron functioning, imitation and other social-communicative abilities often impaired in individuals with ASD, it is hypothesized that dysfunctional mirror neuron functioning causes ASD symptoms. Moreover, abnormal functioning of mirror neurons should result in the inability to activate a motor representation of an observed action. As a consequence of this impaired matching process, social-communicative functions such as imitation can be affected (Gallese, Gernsbacher, Heyes, Hickok, & Iacoboni, 2011). Recently, neuropsychological research on ASD and imitation had paid a lot of attention to this so called 'broken mirror theory' of ASD (Ramachandran & Oberman, 2006). This theory postulates a three-way relationship between ASD, imitation, and impaired mirror neuron functioning and claims that broken mirror neurons cause imitation impairments in ASD.

However, the literature on this theory is not unanimous. Some researchers found evidence for broken mirror functioning in infants, adolescents, and adults with ASD. For example, the EEG study of Martineau, Cochin, Magne, and Barthelemy (2008) revealed impaired activation in the mirror neuron areas during video action observation in ASD children, whereas the matched control group demonstrated EEG desynchronization during action observation. Additionally, Dapretto and colleagues (2006) found in their fMRI study support for dysfunctional mirror neuron functioning which was negatively correlated with symptom severity in children with ASD. Martineau, Adersson, Barthélémy, Cottier, and Destrieux (2010) found atypical activation of different cerebral areas, including the neural mirroring network, during the observation but not during the imitation of hand movements in high-functioning young adults with ASD. These results were confirmed in the study of Bernier and colleagues (2007) where mu suppression was found in high-functioning adults with ASD only during action imitation but not during action observation. This impaired action observation/execution matching system in ASD was related to the quality of behavioural imitation which was impaired in the ASD group compared to the TD group. Théoret and colleagues (2005) found that the observation of finger movements away from the observer (i.e., egocentric view) did not activate the sensorimotor mirror neurons in high-functioning adults diagnosed with ASD. However, neural mirroring activity was present during the observation of hand

movements directed towards the ASD observer which suggests that the neural mirroring response depends on the directional context in which the action is executed. The authors suggested that a self-consciousness deficit causes impaired self-other representations. Oberman and colleagues (2005) performed an EEG study with high-functional individuals with ASD ranging in age from 6 to 47 years old. This study supported the broken mirror theory of ASD as the ASD individuals showed only significant mirror neuron activity during self-executed hand movements but not during the observation of the hand movements. The study of Williams and colleagues (2006) found less mirror neuron activation of the somatosensory cortex during imitation in the ASD group compared to the control group. However, they suggested that this impaired neural mirroring during imitation in ASD is only one part of a broader neural network related to imitation. Additionally, it is observed that mu suppression appears to increase with age, both in individuals with ASD and in control groups. This reflects a general developmental process, indicating improved mirror neuron functioning in both groups (Oberman et al., 2012).

The aforementioned (not exhaustive) overview represents evidence for impaired neural mirroring in individuals with ASD. However, as already mentioned, many researchers found no evidence for broken mirror functioning in individuals with ASD and suggest that the theory of broken mirrors as the cause for ASD impairments is premature (Southgate & Hamilton, 2008). Some of them argue that the intact imitation abilities and the adequate representation of others often observed in individuals with ASD are in favour of some functional mirror neuron activity in ASD (e.g., Bird, Leighton, Press, & Heyes, 2007; Hamilton et al., 2007; Hobson & Lee, 1999; Williams et al., 2004). For example, Raymaekers, Wiersema, and Roeyers (2009) found in their study with high functioning children between 8 and 13 years old similar significant mirror neuron activity during the observation and execution of hand movements as observed in typically developing peers. These results were in line with the study of Hamilton and colleagues (2007) where children with ASD were not impaired in imitation performance which provides evidence against a global impaired mirror neuron functioning in ASD. These authors suggested the existence of multiple brain regions related to different imitation types. Fan, Decety, Yang, Liu, and Cheng (2010) investigated neural mirroring activity in young adults with ASD during the observation and execution of hand actions. Results

revealed that the ASD group, similar as the control group, demonstrated significant mirror neuron activity during hand action observation and this activity was positively correlated with communication performance which may indicate the symptom severity in ASD. However, despite the intact neural mirroring, the ASD group could not imitate the observed actions. Therefore the authors concluded that neural mirroring is intact to a certain degree in individuals with ASD. Avikainen, Kulomäki, and Hari (1999) found no differences between the adult ASD group and the control group concerning precentral motor cortex activity during action observation. They concluded that the impaired mindreading and imitation capacities often observed in individuals with ASD are not the result of a deficit in action recognition mediated by impaired mirror neuron functioning. However, some studies found adequate mirror neuron functioning in ASD but under specific conditions. For example, Oberman, Ramachandran, and Pineda (2008) discovered in their study that mirror neuron areas in children with ASD responded to the observation of hand actions but only when the hand was familiar. This suggests that mirror neuron functioning in ASD is sensitive to the degree of familiarity of the presented stimuli.

As the previous overview demonstrates, the role of mirror neurons in the symptomatology of ASD is still debated.

RESEARCH OBJECTIVES AND OUTLINE OF THIS DISSERTATION

The diverse results in the literature concerning imitation and mirror neuron functioning in ASD call for further research and exploration of these two topics. Therefore, this doctoral research focused on investigating imitation abilities and mirror neuron functioning in typically developing infants and additionally in infants diagnosed with ASD. Furthermore, due to the genetic alliance of children diagnosed with ASD with their younger siblings reflected in the 'broader autism phenotype', this latter group was included as well. Therefore, this doctoral thesis had 3 main goals. Firstly, we tried to get insight in neural mirroring in typically developing infants and the development of an adequate paradigm to investigate this neural mechanism in infants. We wanted to investigate whether neural mirroring responses in typically developing infants differ in a

televised setting compared to a live setting. Secondly, we aimed to investigate neural mirroring in children at risk for ASD (i.e., high-risk siblings) and in children diagnosed with ASD to learn more about neural mirroring in ASD and in its BAP. Finally, we explored imitation in high-risk siblings and in toddlers with ASD. In the following chapters, five empirical studies will be presented addressing the aforementioned research goals.

In *Chapter 2* we tested whether neural mirroring activity in typically developing infants between 18 and 36 months old differ during the observation of live versus televised actions. Therefore, central mu suppression, as an index for neural mirroring activity, was measured through EEG recordings during the observation and execution of goal-directed actions and during the observation of hand movements, presented either live or on television.

Chapter 3 supplements the previous study by investigating infants' mu suppression during the observation of real and mimicked goal-directed actions. In this EEG study, mu suppression in 18- to 30-months old infants recorded from frontal, central and parietal electrodes was investigated during imitation and observation of goal-directed actions and during the observation of mimicked, hand movement actions.

Chapter 4 presents the result of neural mirroring in children at risk for ASD (i.e., high-risk siblings) to learn more about ASD and its broader phenotype. Central mu suppression was investigated in 18- to 36-months old high-risk siblings compared to low-risk control infants during observation and imitation tasks.

In *Chapter 5* neural mirroring in children with ASD was explored. Therefore, children between 24 and 48 months old diagnosed with ASD were tested compared to typically developing children during an EEG study. Central mu suppression was measured during the observation of goal-directed actions and hand movements and during action imitation.

Chapter 6 explored imitation in high-risk siblings and toddlers with ASD between 48 and 69 months old. Procedural and bodily imitation performance was compared between high-risk siblings, toddlers diagnosed with ASD and low-risk toddlers without any family history of ASD. Additionally, correlations between imitation performance and autism severity were examined.

Finally, in *Chapter 7*, a summary and discussion of the most important findings are provided. Furthermore, limitations and implications for future research and practice are given.

It should be noted that the chapters in this dissertation correspond to individual manuscripts, which are submitted for publication or are under editorial review. Chapters may therefore partially overlap as each manuscript should be able to stand on its own.

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CHAPTER 2

NEURAL MIRRORING DURING THE OBSERVATION OF LIVE AND TELEVISED ACTIONS IN INFANTS[1]

ABSTRACT

Previous infant studies investigated neural mirroring during the observation of live or televised actions. However, both methods have their (dis)advantages and studies using one of these methods are not always directly comparable. Therefore, present study directly compared neural mirroring activity in a televised setting with a live setting in infants between 18 and 36 months old. Central mu rhythm suppression was measured through EEG recordings during the observation and imitation of the same goal-directed and mimicked actions presented either on television or live. Results revealed significant mu suppression during action imitation in both settings but stronger mu suppression was observed in the live setting during this condition. Significant mu suppression during the observation of goal-directed actions and mimicked actions was only observed in the live setting. This study revealed a different influence of televised and live actions on neural mirroring activity in infants and it is recommended to use live actions to investigate neural mirroring in young children.

[1] Based on Ruysschaert, L., Warreyn, P., Wiersema, J.R., & Roeyers, H. (2012). *Neural mirroring during the observation of live and televised actions in infants.* Manuscript submitted for publication.

INTRODUCTION

Mirror neurons, discovered in the macaque brain, are active both during execution and observation of actions (Di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992). Activity in this action observation/execution matching system has been measured by using the mu rhythm derived from electroencephalography (EEG) recordings (Arnstein, Cui, Keysers, Maurits, & Gazzola, 2011). In adults, the power of the mu band oscillations, typically recorded from the sensorimotor cortex is suppressed both during action execution and observation (Gastaut, Dongier, & Courtois, 1954; Muthukumaraswamy, Johnson, & McNair, 2004). Research findings suggest that a mu rhythm similar as in adults with an identical topography but at a lower frequency range can be recorded in children (Cochin, Barthelemy, Roux, & Martineau, 2001).

Previous infant studies investigated neural mirroring during the observation of live (e.g., Nyström, Ljunghammar, Rosander, & van Hofsten, 2010; Southgate, Johnson, Osborne, & Csibra, 2009) or televised actions (e.g., Nyström, 2008; van Elk, van Schie, Hunnius, Vesper, & Bekkering, 2008). The majority of the researchers used live stimuli reasoning that live stimuli provide a more realistic view on brain processing because these stimuli are efficiently processed due to their similarity with the real world (Carver, Meltzoff, & Dawson, 2006). However, investigating neural mirroring in a live setting is often hampered by motor movement and/or vocalization of the participant which can increase the presence of artifacts in the brain imaging data (Junghofer, Elbert, Tucker, & Rockstroh, 2000). Some arguments can be postulated why it can be useful to use televised stimuli in infant studies concerning neural mirroring. Firstly, a methodological advantage of televised stimuli is the identical manner of presenting stimuli which makes it a direct and repeatable research setting (Barr, Muentener, Garcia, Fujimoto, & Chavez, 2007). Furthermore, televised presentation makes it possible to control more for motor planning or inhibited reaching which often occur in live observation studies (Järveläinen, Schurmann, Avikainen, & Hari, 2001). Finally, as Nyström (2008) discussed, adult research often uses televised stimuli to investigate neural mirroring (e.g., Oberman et al., 2005) which makes it interesting to compare these results with infant studies. However, it is suggested that infants do not process virtual 2D-televised stimuli in the same way as real 3D-live stimuli because they do not always seem to understand the

relationship between these two different kind of stimuli (Carver et al., 2006). These findings lead to the hypothesis that neural mirroring activity will be less pronounced using televised stimuli (Shimada & Hiraki, 2006). Therefore, investigating neural mirroring responses to televised compared to live stimuli in young infants seems interesting to learn more about the sensitivity of the infant neural mirroring.

To our knowledge, no studies so far have been conducted that directly compare infants neural mirroring responses to televised and live stimuli. Therefore, the present study aimed to investigate infants' EEG mu wave suppression during the observation and execution of goal-directed and mimicked televised actions compared to the presentation of the identical actions in a live setting in infants between 18 and 36 months old. Given the findings of Shimada and Hiraki (2006) who found stronger mu suppression during live actions in infants, we may expect to find differences in neural mirroring activity in televised versus live conditions.

MATERIAL AND METHODS

Participants

The initial sample consisted of 68 infants who were allocated to either the live or the televised setting. Prior to analyses, 34 tested infants were excluded due to insufficient artifact free data (TV: n = 15; live: n = 13), insufficient or no cooperation of the infant (TV: n = 2; live: n = 2) or technical problems with the EEG equipment or recording (live: n = 2) which resulted in an inability to obtain clear EEG data for these infants. The final sample consisted of 34 infants (15 boys and 19 girls), between 18 and 36 months old (M = 26.44; SD = 3.96) with 16 participants in the televised setting and 18 participants in the live setting. Both groups did not significantly differ on chronological age and gender; $F(1,32) = 2.40$, $p = .131$ for age and $\chi^2(1) = 0.54$; $p = .464$ for gender. All infants were healthy and developing normally. Participants in the current study were recruited through Flemish day-care centres and magazine or website advertisements. Characteristics of the participants are presented in Table 1. Handedness was determined by parent report or by analysing the video–recordings of the experiment.

Table 1. *Subject characteristics*

	Live setting (*n* = 18)	TV setting (*n* = 16)	
Chronological age (months)			
Mean (*SD*)	25.47 (4.35)	27.54 (3.28)	$F(1,32) = 2.40$
Age Range	19.80-35.30	20.20-30.70	
Gender ratio M : F	9 : 9	6 : 10	$\chi^2(1) = 0.54$
Handedness (R : L : ambi)	12 : 5 : 1	12 : 4 : 0	

General procedure

The experiment took place in a quiet laboratory room at Ghent University after obtaining parental informed consent. The EEG data were collected when the infant was alert and attending to the stimuli. In order to let the child get used to the environment and experimenter, the experiment started with a short free play moment with some attractive toys. Experimenter 1 (also the demonstrator of the actions during test phase) played with the child, while experimenter 2 prepared the appropriate EEG cap. Meanwhile, the procedure was explained to the parent. When the child was feeling comfortable, the parent was asked to sit at the table together with the child. Subsequently, the experimenters placed the EEG cap on the child's head while the child was watching a popular cartoon movie. Electrolytic conducting gel was applied with a syringe at each active electrode on the EEG-cap. During testing, each infant was seated on his/her caregiver's lap who was instructed to minimize interaction with the child. In the live setting, experimenter 1 sat at the other side of the table facing the child. In the televised (TV) setting, a computer monitor was put on the table in front of the child. In both settings, the same stimuli were presented either live or on television at a viewing distance of approximately 60 cm. White curtains surrounded the testing area to minimize distracting environmental influences; a white screen was placed around the infant and a white drop-curtain moved up and down between the different conditions (in the live setting). Two video cameras recorded the whole experiment; one focusing on experimenter 1 in the live setting or the monitor in the televised setting and the other

on the side profile of the child in both settings. Participants' behaviour (attention, vocalization, motor movement, and imitation) was coded offline on the basis of these video recordings.

EEG data were collected during 4 experimental conditions with 5 different objects (i.e., a hippopotamus soft-toy, an egg-cup, a Pinocchio-like puppet, a car, and a frog loupe) which were presented live during the live setting. (1) The experiment always started with the observation of a moving object in a non-goal-directed manner, dangling on a rope with the experimenter hidden behind a curtain out of the infant's view. Because the infants had no prior experience with the objects, this *object observation condition* was used as a baseline condition to which every subsequent condition was compared. (2) During the *action observation condition*, participants observed the experimenter performing a simple goal-directed action with each object and a white box (for example, car is picked up, driven on top of the box, and is released at the edge of the box, so it falls (carefully) down at the other side of the box). These actions were 'goal-directed' because the object had a clear end position. Before demonstration, the experimenter asked the attention of the infant by saying 'look' and making eye-contact with the infant. Each action was demonstrated three times from the left side of the box and three times from the right side. The starting hand was counterbalanced between the different objects. (3) After observing the demonstrated actions, infants were encouraged (non-)verbally in a non-specific way to imitate the observed action during the *action imitation condition*. (4) During the fourth condition, the experimenter demonstrated hand movements identical to those used during the action observation condition but now without the object (i.e., mimicked actions). During this *hand movement condition*, the hand movements were executed without direct reference or gaze towards the participant which made the condition less social. Subjects were expected to observe these actions but not to imitate them. Each hand movement was also demonstrated three times with the left hand and three times with the right hand.

During the televised setting, the live actions of the object observation, action observation and hand movement conditions were replaced by the same actions but pre-recorded on video. The imitation condition was always in vivo, regardless of the setting. The experiment always started with the object observation condition (baseline condition) in both settings. Afterwards, the action observation, action imitation, and

hand movement condition were presented for each object. The order in which these three conditions were presented to the participants was counterbalanced across subjects, with the requirement that the action observation condition always preceded the action imitation condition. The five objects were always presented in the same order. Each demonstrated action (object movement, action observation, and hand movement) lasted about (live setting) and exactly (TV setting) 30 seconds per object. During action imitation, participants were given as much time as needed to perform the actions. The entire experiment lasted about 20 minutes. Afterwards, parents were debriefed and received a reward/gift card for participation.

EEG data acquisition

Brain Vision Recorder (Brain Products, 2007) was used to record electrical brain activity to an average reference from 32 active Ag/AgCl electrodes through an EEG-amplifier (QuickAmp) with a sample rate of 500 Hz. EEG data were recorded with 1 s time constant, a low pass filter of 70 Hz and a notch filter of 50 Hz. Electrodes were placed according to the international 10-20 system (Jasper, 1958) embedded in a child-friendly stretch EEG-cap with a ground electrode placed at AFz (Easycap, Brain Products, GmbH, Munich, Germany). Electro-oculogram (EOG), both vertical and horizontal, were recorded by 3 additional electrodes. Horizontal EOG electrodes (HEOG) were applied next to the eyes, at the outer canthi. A vertical electro-oculogram (VEOG) was performed with an electrode above the eye, at position Fp2, compared with the common reference. Initially, we used an electrode positioned below the left eye for monitoring the vertical eye movement but many infants did not tolerate this electrode. However, in comparison with the data including these electrode, results showed no significant difference concerning the use of the common reference. An inter-electrode impedance of all electrodes at or below 10kΩ was considered acceptable. Synchronization of the EEG signal with both camera recordings was done by pushing a button before the start of each condition. This button sent a marker signal to the EEG recording and simultaneously emitted a LED light signal visible on both cameras. The time intervals between the markers on these 2 recording systems were compared afterwards which allowed synchronization of the EEG data with the video recordings of the child's behaviour.

Offline behaviour coding

The behaviours on the camera recordings were coded offline with The Observer XT 9.0. (Noldus Information Technology, 2009) by ascribing start and stop codes to the child's attentive behaviour, vocalizations, motor movements, imitation behaviour, and the different experimental conditions. The fragments where the child was sitting still and quietly observed the demonstrations (during the object movement, hand movement, and action observation condition) or was actually imitating (during the imitation condition) were used for further analysis. Intervals with excessive motor movements and vocalizations were excluded beforehand to minimize contamination of the EEG signal. In addition, artifact rejection was performed in the subsequent Brain Vision Analyzer analyses to control for artifacts. This insured that differences in terms of mu suppression between conditions could not be explained by overall differences between conditions in motor and vocalization behaviour of the infant.

One observer, who was blind for the setting in which the child was tested, coded quality of imitation of the participants during the action imitation condition. The coding was based on three different criteria per object. For example, for driving the car, it was coded if (1) the child drove the car on the side of the box, (2) followed by driving on top of the box, and (3) finally dropped it at the other side of the box. Score 1 was given for every criteria the infant met. Afterwards, a quality of imitation score was calculated by taking the mean of the best scores for each object with a maximum of 3 per object. In this sample, participants in the televised setting obtained a mean score of 1.80 (*SD* = .43) and a mean score of 2.04 (*SD* = .38) for the live setting, indicating that overall the imitation performance of the infants met (almost) 2 of the 3 criteria in both settings. An independent coder double-coded 25% randomly chosen videos to assess inter–observer reliability. An excellent level of reliability was achieved with a Cronbach's Alpha Coefficient of .94 (Cronbach, 1951).

EEG data processing

We used Brain Vision Analyzer (Brain Products, 2007) for offline analyses of the recorded EEG data. Data recorded from electrodes C3 and C4 were further investigated

because mu rhythm is defined as oscillations measured over the sensorimotor cortex (Marshall, Young, & Meltzoff, 2011; Muthukumaraswamy et al., 2004). The recorded EEG data were first inspected visually offline to eliminate bad recordings. In addition, bad channels were excluded before re-referencing to prevent spreading of bad data. The remaining channels were re-referenced to an average reference. Afterwards, the EEG-signal was filtered with a high pass filter of 0.1 Hz, a low pass filter of 30 Hz, and a 50 Hz notch filter. Subsequently, the EEG data were corrected for horizontal and vertical eye movements using the Gratton and Coles algorithm (Gratton, Coles, & Donchin, 1983). The remaining EEG data were further segmented to separate data per experimental condition and afterwards divided in 1-s epochs with 50% overlap. Bad segments were removed with artifact rejection using a maximal allowed voltage step of 100 μV per sampling point; a maximal allowed absolute difference of 400 μV between two values in the segment and an activity of 0 μV during maximum 100 milliseconds. In this way, an average of 229.24 segments (*SD* = 86.00) remained. Fast Fourier Transforms (FFTs), with a Hanning window of 10%, were performed on the remaining segments and the resulting magnitudes were averaged for each condition. Similar as in analogous experiments (e.g., Lepage and Théoret, 2006; Muthukumaraswamy et al., 2004), the individual mu rhythm bandwidths were conducted by subtracting the baseline condition from the imitation condition for each subject individually. In this way, we controlled for the differences in spectral power that could be caused by mere presentation of visual stimuli. In addition, the 3-Hz interval around the maximal power difference of this subtraction over the central electrodes was calculated. This procedure was selected because it enables the precise definition of the frequency band that is modulated by the execution of actions in each individual subject. Contrasting the baseline and the imitation condition results in a clearer individual mu rhythm (Muthukumaraswamy et al., 2004). The mean peak in the live group was 8.1 Hz (*SD* = 0.75) and 8.1 Hz (*SD* = 0.60) for the televised group. This is in agreement with previous studies on mu/alpha rhythm frequencies in infants (Marshall, Bar-Haim, & Fox, 2002; Stroganova, Orekhova, & Posikera, 1999).

Following the procedure used by Oberman and colleagues (2005) and Raymaekers, Wiersema, and Roeyers (2009), mu wave suppression was calculated as a ratio of the mu wave power in the different conditions. Specifically, we calculated the

individual mu power during the experimental conditions (the action observation, action imitation, and hand movement condition) relative to the mu power in the baseline condition (the object movement condition). A ratio was used to control for the individual variability in absolute EEG power due to individual differences such as electrode impedance or scalp thickness. Given the non-normal distribution of ratio data, a log transform was computed for each ratio. A negative value indicates mu suppression, a positive value represents mu augmentation, and a zero value indicates no mu suppression, as compared to the baseline.

RESULTS

Counterbalancing of the order of the presented conditions (the action observation condition/action imitation condition versus the hand movement condition) in both settings (live setting and TV setting) had no effect on the mu suppression as measured at the central (C3 and C4) electrode positions, all $-.47 < t(16) < 1.33$, all $p > .05$ and all $-.67 < t(14) < .29$, all $p > .05$ respectively. Therefore, the order of presentation of the conditions was not further included as a factor in the analyses.

A 3x2x2 repeated-measures ANOVA was conducted with condition (hand movement observation, action observation, and action imitation) and hemisphere (C3 and C4) as within-subjects factors and setting (live setting vs televised setting) as between-subjects factor. Results revealed a significant main effect of setting with, $F(1,32) = 7.75$, $p = .009$ and a significant main effect of condition, $F(2,31) = 7.56$, $p = .002$. Follow-up contrasts demonstrated significantly more mu suppression during the action imitation condition ($M = -.31$, $SD = .44$) compared to the hand movement condition ($M = -.09$, $SD = .23$) and the action observation condition ($M = -.01$, $SD = .16$) with $F(1,32) = 12.41$, $p = .001$ and $F(1,32) = 15.60$, $p < .001$ respectively, and significantly stronger mu suppression during hand movement observation compared to action observation, $F(1,32) = 5.17$, $p = .030$. No main effect of hemisphere was found with $F(1,32) = .24$, $p = .627$. Furthermore, no significant 2- and 3-way interactions were found (all $p > .05$).

We tested whether the mean values per condition in the live setting, calculated over central electrode positions (i.e., assembled over positions C3 and C4), significantly

differed from zero. The one-sample t-tests revealed significant mu suppression during the hand movement, the action observation as well as during the action imitation condition, $t(17) = -3.64$, $p = .002$; $t(17) = -3.54$, $p = .002$; and $t(17) = -3.23$, $p = .005$, respectively.

For the televised setting, only mu suppression during action imitation differed significantly from zero, $t(15) = -2.58$, $p = .021$. No mu suppression was found during the hand movement condition and the action observation condition, $t(15) = 1.36$, $p = .195$ and $t(15) = 1.70$, $p = .111$ respectively. Table 2 shows an overview of the means and standard deviations of the central mu wave power during the live and televised setting.

Table 2. *Mean (M) and standard deviation (SD) of mu power values for both settings at C3 and C4 separately and assembled during each condition*

		Live setting (n = 18)		TV setting (n = 16)	
		M	*SD*	*M*	*SD*
C3	Hand movement	-.26**	.36	.08*	.15
	Action observation	-.15**	.18	.12*	.16
	Action imitation	-.42*	.63	-.23°	.46
C4	Hand movement	-.16*	.29	.00	.15
	Action observation	-.03	.21	.04	.23
	Action imitation	-.38**	.46	-.19*	.31
C	Hand movement	-.21**	.24	.04	.12
	Action observation	-.09**	.11	.08	.18
	Action imitation	-.40**	.52	-.21*	.32

Note. C3 = mu suppression at electrode position C3; C4 = mu suppression at electrode position C4; C = mean central mu suppression assembled over electrode position C3 and C4.
°$p < .10$; *$p < .05$; **$p < .01$.

Additionally, activity recorded from an occipital electrode (Oz) was investigated to evaluate if the observed central suppression was related to the mu rhythm and not to other possibly overlapping activity such as posterior alpha activity. During action imitation in both settings, no significant suppression was found at electrode Oz in the frequency band under investigation, $t(33) = -1.09$, $p = .282$. When central mu suppression was compared to occipital activity, we found significant more central suppression ($M = -.31$, $SD = .44$) compared to occipital suppression in the same frequency band ($M = -.07$, $SD = .37$), $t(33) = -2.39$, $p = .023$. These results indicate that the observed mu suppression was specific to the central electrode positions and was not the result of occipital activity.

DISCUSSION

To our knowledge, this is the first study investigating neural mirroring during observation and imitation of televised goal-directed and mimicked actions compared to the same actions presented live in infants. As Marshall and Meltzoff (2010) suggested, we included both observation and execution conditions.

As expected, given the motor properties of the mu wave (Lepage & Théoret, 2007), the infants in both settings showed significant mu suppression during the action imitation condition. However, only during the live presentation, the infants demonstrated significant mu suppression during both observation conditions. The finding of significant mu suppression during both observation and imitation in the live setting suggests the presence of a functional action observation/execution matching system in infants between 18 and 36 months old (Marshall & Meltzoff, 2010). The occurrence of mu wave suppression during the observation of live non-goal-directed hand movements, suggests that the observation of live motor movements alone is sufficient to provoke mu suppression in infants between 18 and 36 months old (Oberman et al., 2005). In contrast, the mere observation of televised hand movements and goal-directed actions was insufficient to evoke mu suppression, which differed significantly with the live setting observation conditions.

The results of the current study suggest different neural mirroring activation during the observation of televised and live actions in infants. Since the children in the present study showed more attention to the televised stimuli than to the live stimuli ($t(31) = -5.48$, $p < .001$), less mu suppression in the televised setting cannot be explained by different attentive behaviour dependent on the setting. Neither was neural mirroring activation in the live setting the result of motor activity during observation, since no significant differences were found between the two settings concerning motor activity during both observation conditions (all $t(31) < 1.7$, all $p > .05$). In the present study, although the quality of imitation of the live presented actions was slightly better than the televised actions, the difference was not significant ($t(32) = 1.75$, $p = .090$), which suggests that infants understood the imitation tasks both when it was presented on television or live. This is in line with the findings of Barr and Hayne (1999) that infants from 18-months old onwards can imitate televised modelled actions. Furthermore, the same tasks were used during the televised as well as during the live setting which excludes a potentially different impact of tasks on neural mirroring activation in both settings. Therefore, we can conclude that the infants responded neurologically different to the observation of televised compared to live actions probably due to a different visual experience with 2D stimulus presentation in contrast to real 3D object presentation (Shimada & Hiraki, 2006). Additionally, research revealed that infants under 3 years of age find it difficult to symbolize 2D scale models as 3D real objects (DeLoache, 2000).

Although the findings of the present study are in agreement with previous research (e.g., Järveläinen et al., 2001; Shimada & Hiraki, 2006), some limitations need to be considered. First, the differences between the two settings could be due to the variation in the duration of the live demonstrations in contrast to the pre-recorded televised demonstrations. It seems inevitable that during live demonstrations, the experimenter unconsciously adapted the demonstration time to each individual participant, contingent upon its behaviour, whereas the duration of the pre-recorded televised demonstrations was not dependent on the child's behaviour. However, it should be noted that in the present study, only the fragments where the child was attentive to the demonstrations during live as well as during televised conditions were used for further analyses. Secondly, especially during the live setting, inhibited

movement or motor planning can cause significant neural mirroring activity during the observation tasks. By excluding the fragments with too many motor movements and vocalizations beforehand during video coding and by using a profound artefact rejection during the EEG analyses, we tried to control for these artefacts. However, we could not control for all of it which makes it possible that this can differ between the two settings with different neural mirroring activity as result.

To our knowledge, this is the first study that directly compared neural mirroring activity during the observation of hand movements and goal-directed actions in a televised setting with the same actions in a live setting in infants between 18 and 36 months old. Therefore, these findings need to be replicated in future studies with larger sample sizes.

To conclude, our study revealed less mu suppression during goal-directed action observation and hand movement observation when stimuli were shown on television in comparison with the observation of live actions in infants between 18 and 36 months old. These findings clearly indicate a different sensorimotor processing of televised compared to live presented actions in infancy and imply the importance of using live actions to investigate neural mirroring activity in infancy. Apparently, live movements have a higher ecological validity than televised actions. This result can be taken into account in the design of adequate paradigms to investigate neural mirroring activity in infancy.

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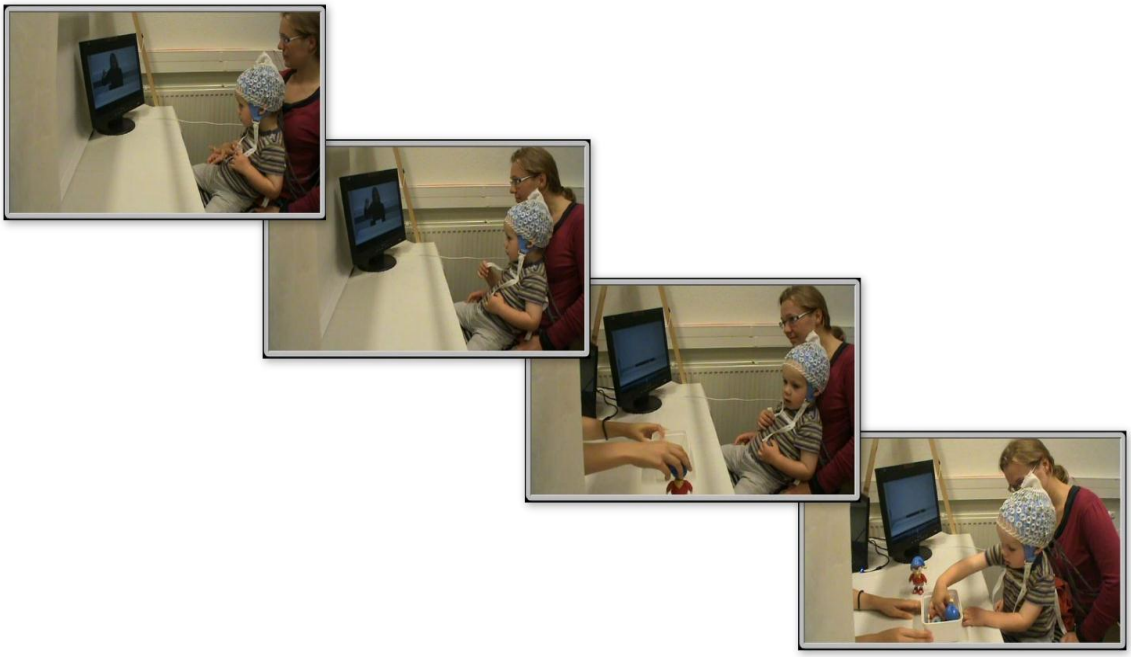


A boy who is observing hand movements (without objects) and goal-directed actions (with objects) presented on television to receive afterwards the objects while being encouraged to imitate the observed goal-directed actions

CHAPTER 3

INFANTS' MU SUPPRESSION DURING THE OBSERVATION OF REAL AND MIMICKED GOAL-DIRECTED ACTIONS[1]

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-months 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.

[1] Based on Warreyn, P., Ruysschaert, L., Wiersema, J.R., Handl, A., Pattyn, G., & Roeyers, H. (in press). Infants' mu suppression during the observation of real and mimicked goal-directed actions. *Developmental Science.*

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, 2009; Marshall & Meltzoff, 2011). 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-months 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-months 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-months 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-months 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-months 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-months 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-months 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 pre-schoolers. In 2004, Fecteau and colleagues reported mu suppression in a 36-months 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 other 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-months 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 an 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 desynchronization 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-months 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-months 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-months 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-months 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.

MATERIAL AND METHODS

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 value ± 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 analysing 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.

Table 1. *Subject characteristics (n = 17)*

	M (*SD*)	Range
Chronological age (months)	24.54 (3.96)	18.50-30.60
Language age (months), *n* = 13		
Expressive	22.46 (4.46)	17.00-30.00
Receptive	24.23 (4.17)	18.00-30.00
Gender ratio M : F	9 : 8	

Procedure

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' behaviour (attention, vocalization, and motor behaviour).

The experiment consisted of 4 experimental conditions (with 5 different objects: a hippopotamus soft-toy, an egg-cup, a Pinocchio-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).

EEG recording and analysis

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.

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- to 30-months 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 $p > .15$).

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).

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 100 μV 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 and colleagues (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.

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 $t(15) = 1.99$, $p = .065$; action execution $t(15) = -.17$, $p = .868$; and hand movement $t(15) = 1.02$, $p = .326$.

Therefore, regardless of the order of presentation, the infants are treated as one group in the subsequent analyses.

Action execution

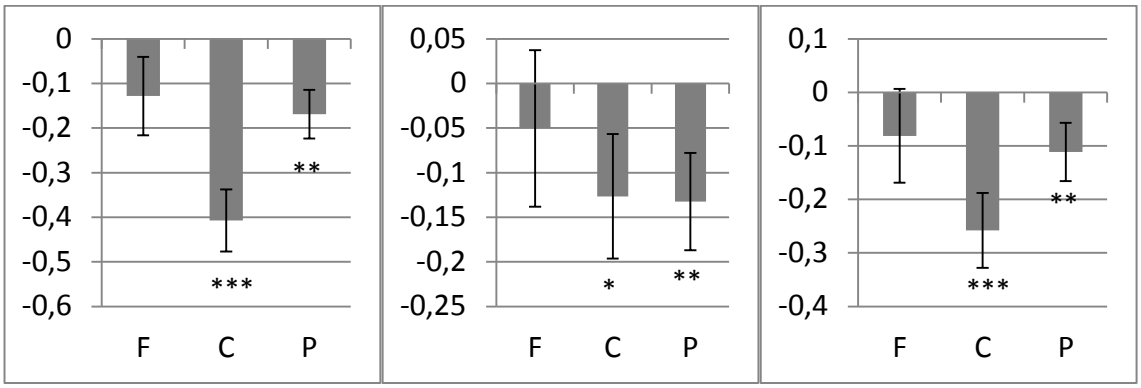
The repeated-measures ANOVA showed a significant main effect of region ($F(2,15) = 17.01$, $p < .001$). Follow-up contrasts showed significantly more mu suppression over the central electrode positions ($M = -.41$, $SD = .29$) compared to the frontal ($M = -.13$, $SD = .36$, $p = .003$), and parietal positions ($M = -.17$, $SD = .22$, $p < .001$). One sample t-tests showed mu suppression to be significantly different from zero over central ($t(16) = -5.81$, $p < .001$) and parietal sites ($t(16) = -3.12$, $p = .007$), but this was not the case for frontal electrode positions ($t(16) = -1.45$, $p = .167$). See Figure 1(a) for details.

Action observation

In the action observation condition, the repeated-measures ANOVA revealed no effect of region ($F(2,15) = 1.14$, $p = .345$). Mu suppression was significantly different from zero on central ($t(16) = -2.61$, $p = .019$) and parietal ($t(16) = -3.71$, $p = .002$), but not on frontal electrodes ($t(16) = -0.96$, $p = .349$). See Figure 1(b) for details.

Hand movement

Similar to the action execution condition, in the hand movement condition the repeated-measures ANOVA showed a significant main effect of region ($F(2,15) = 9.15$, $p = .003$). Again, mu suppression was stronger over central electrodes ($M = -.26$, $SD = .20$) than over frontal ($M = -.08$, $SD = .19$, $p = .005$), and parietal electrodes ($M = -.11$, $SD = .15$, $p = .001$). Mu suppression was significantly different from zero over central ($t(16) = -5.32$, $p < .001$) and parietal electrodes positions ($t(16) = -3.03$, $p = .008$), but not over the frontal ones ($t(16) = -1.74$, $p = .102$). See Figure 1(c) for details.



a. action execution **b.** action observation **c.** hand movement observation

Figure 1. *Mean mu suppression during (a) action execution, (b) action observation, and (c) hand movement 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.*

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 ($F(2,15) = 5.82$, $p = .013$), with more suppression during action execution than during both action observation ($F(1,16) = 12.22$, $p = .003$), and hand movement observation ($F(1,16) = 10.19$, $p = .006$) and stronger mu suppression during the hand movement condition than during the action observation condition ($F(1,16) = 7.59$, $p = .014$).

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 ($r = .516$, $p = .034$) and action execution ($r = .751$, $p = .001$), but the latter two were not significantly related ($r = .126$, $p = .629$). 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 = .050) and action execution (r = .586, p = .013), but not action observation (r = .285, p = .268), on the other hand. See Table 2 for details.

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) = -2.16, p = .046 and t(16) = -3.32, p = .004, respectively), but this was not the case during action observation (t(16) = 1.69, p = .111). During the hand movement condition, the suppression measured over the parietal electrodes was significantly correlated with both central (r = .626, p = .007) and occipital suppression (r = .641, p = .006). Similar correlations were found in the action observation condition (central – parietal r = .529, p = .029 and parietal – occipital r = .711, p = .001). During the action execution, the central mu suppression correlated significantly with the frontal (r = .496, p = .043) and parietal suppression (r = .780, p < .001), but the activity at neither location correlated with the occipital electrode activity.

Table 2. *Pearson's correlations between child characteristics and central mu suppression*

	AGE	EXP	COMP	IMIT	HM	AO
EXP	.750**					
COMP	.657*	.746**				
IMIT	.547*	.550*	.446			
HM	.086	-.339	-.300	.483*		
AO	-.147	-.329	-.048	.285	.516*	
AE	.349	.096	.042	.586*	.751***	.126

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.
*$p \leq .05$, **$p < .01$, ***$p \leq .001$.

DISCUSSION

The current study investigated mu suppression of 18- to 30-months 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-months 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 a 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-months 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-months 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 ($t(16)= -4.94$, $p < .001$). 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., 2005), 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 desynchronization

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. 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 and colleagues (2009) in 9-months olds, by Marshall and colleagues (2011) in 14-months olds, found in the current study in 18- to 30-months olds, and reported by Lepage and 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 (Zink & Lejaegere, 2002; original version Fenson et al., 1993) 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 and colleagues (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.

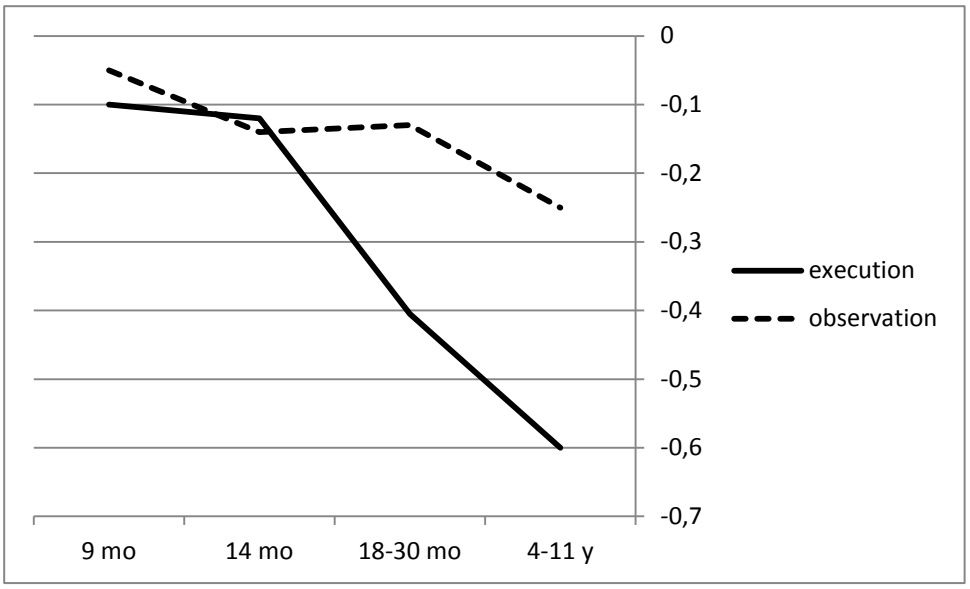


Figure 2. *Mu suppression values for action execution and action observation reported by Southgate et al. (2009) in 9-months olds, by Marshall et al. (2011) in 14-months olds, found in the current study in 18- to 30-months olds, and reported by Lepage & Théoret (2006) in 4- to 11-year-olds.*

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 vocalization 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 vocalizations 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 vocalizations 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-months olds from moving, we think we minimized the impact of movements and vocalizations 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-months 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 electrode 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.

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An infant who is observing moving objects dangling on a rope during the object observation condition and who is imitating the observed action during the action execution condition

CHAPTER 4

NEURAL MIRRORING IN HIGH-RISK SIBLINGS[1]

ABSTRACT

Investigating younger siblings of children with autism spectrum disorder (ASD) can expand the knowledge on ASD and its broader autism phenotype (BAP). This study aimed to investigate neural mirroring during imitation and observation tasks in high-risk siblings between 18 and 36 months old. Results revealed equally strong central mu suppression in the EEG during action observation and execution in a high-risk sibling group compared to low-risk infants. Quality of imitation correlated marginally significant with mu suppression during action imitation. Mu suppression was stronger in girls than in boys during hand movement observation and action imitation. Results of the present study do not support the hypothesis of impaired neural mirroring as a distinctive neurophysiological characteristic of the BAP.

[1] Based on Ruysschaert, L., Warreyn, P., Wiersema, J.R., & Roeyers, H. (2012). *Neural mirroring in high-risk siblings.* Manuscript submitted for publication.

INTRODUCTION

Autism Spectrum Disorder (ASD) represents a broad variation in symptomatology, ranging from rather mild to very severe symptoms in three separate domains: (a) impairments in social interaction, (b) communication, and (c) restricted and repetitive patterns of interest or behaviours (Wing, 1993). ASD includes Asperger's Disorder, Autistic Disorder, and Pervasive Developmental Disorder Not Otherwise Specified (Wing, 1997). To date, the etiology of ASD is not fully known, but the role of genetics is believed to be significant. Based on several twin studies, the concordance rates for monozygotic twins is estimated between 60% and 90%, whereas this is only 0% to 10% for dizygotic twins (Dawson et al., 2002). These results suggest the influence of multiple interacting genes in the development of different characteristics of ASD (Rutter, 2005). Even more, younger siblings of children with ASD have a higher risk of developing ASD themselves (i.e., high-risk siblings), which additionally supports the idea of genetic underlying mechanisms in the development of ASD (Ozonoff et al., 2011). The genetic contribution in ASD can be expressed in milder qualitatively similar brain and behavioural characteristics that have been referred to as the 'broader autism phenotype' (BAP). This phenotype includes repetitive and stereotyped behaviours mostly together with either social or communicative impairments (for an overview see Sucksmith, Roth, & Hoekstra, 2011). These features occur more often in first-degree relatives of persons with ASD (Rogers, 2009). Although there is some consensus about these milder characteristics, the exact limits of the BAP are still unclear and there is still no consensus about the exact definition of the BAP in infancy (Rogers, 2009). Learning more about the characteristics of this BAP may result in a better insight concerning the genes and characteristics related to ASD (Losh & Piven, 2007).

In the interest of documenting early manifestations and characteristics of ASD and its BAP, several researchers investigated high-risk siblings of children with ASD (Rogers, 2009). By investigating siblings, understanding the nature of the BAP in early development can influence diagnostic criteria and the possibility of early intervention of children at risk for autism. Recently, studies have started to use direct measurements of brain and cognitive functions in siblings and relatives of individuals with ASD to learn more about the BAP (Sucksmith, Roth, & Hoekstra, 2011). For example, Elsabbagh and

colleagues (2009) found differences in eye gaze processing and baseline resting electroencephalography (EEG) responses in unaffected infant siblings compared to a control group. These findings were consistent with the research of Dalton, Nacewicz, Alexander, and Davidson (2007) who found differences in gaze fixation and brain functioning during face processing in a sibling group. These findings in unaffected siblings support the idea of unique characteristics of the BAP. The advantage of using sensitive neuroimaging methods such as EEG/event related potential (ERP) measurements is that they could reveal more underlying neurological processes which are not always directly manifested in overt behaviour (Elsabbagh & Johnson, 2010).

Recently, in light of learning more about the underlying mechanisms and processes of ASD, there has been considerable attention for the broken mirror theory of autism (Southgate & Hamilton, 2008). The discovery of mirror neurons in the 90's in the macaque brain (Di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992) and the support for a similar neural mirroring mechanism in humans (for a review, see Keysers & Fadiga, 2008) strengthened the interest in the underlying neurobiological processes in ASD. In humans, analysis of suppression of the EEG mu wave rhythm is a commonly used method to study neural mirroring (Muthukumaraswamy, Johnson, & McNair, 2004; Raymaekers, Wiersema, & Roeyers, 2009). At rest, sensorimotor neurons spontaneously fire in synchrony leading to a large amplitude of the EEG mu band oscillations typically recorded in adults in the 8-13 Hz frequency range. During motor activation, sensorimotor neurons are desynchronized, which decreases the power of the mu band oscillations, also named 'mu wave suppression' (Gastaut, Dongier, & Courtois, 1954). Additionally, similar mu wave suppression is present during the observation of actions performed by others (Gastaut & Bert, 1954). Therefore, mu suppression during execution as well as during observation of actions, typically recorded from sensorimotor cortex, has been argued to indicate activity in the mirror neurons (Muthukumaraswamy et al., 2004; Pineda, 2005). A mu rhythm similar as in adults with an identical topography but at a lower frequency range (between 6 and 9 Hz) is observed in infants as well (Marshall & Meltzoff, 2010; Stroganova, Orekhova, & Posikera, 1999).

Mirror neuron functioning has theoretically been related to action understanding (Rizzolatti & Craighero, 2004) as well as to various social–cognitive functions such as imitation (Iacoboni, 2005), theory of mind (Iacoboni & Dapretto, 2006), language (Rizzolatti & Craighero, 2004), and empathy (Decety & Meyer, 2008). These social–

cognitive functions are often impaired in individuals with ASD. Early developmental impairment of mirror neurons has been considered as a possible cause of these social–cognitive deficits (Rogers, Hepburn, Stackhouse, & Wehner, 2003). However, findings concerning the role of mirror neurons in the development of ASD are still unclear and controversial. Some support for impaired mu suppression in autism was found in adults and children (e.g., Martineau, Cochin, Magne, & Barthelemy, 2008; Oberman et al., 2005), however other studies found no evidence of impaired neural mirroring in ASD (e.g., Fan, Decety, Yang, Liu, & Cheng, 2010; Raymaekers et al., 2009). Additionally, some studies revealed some nuanced results (for an overview, see Gallese, Gernsbacher, Heyes, Hickok, & Iacoboni, 2011). For example, Oberman, Ramachandran, and Pineda (2008) found in their study that children with ASD showed mirror neuron activity during the observation of hand actions but only when the actions were performed by a familiar hand.

As studies about neural mirroring, especially in children and adults with ASD, are still inconsistent, more research is needed to understand the exact role of mirror neurons in individuals with ASD, beginning in early infancy. Because some researchers suggest that dysfunctional neural mirroring can result in several ASD symptoms (Ramachandran & Oberman, 2006), it is important to learn more about the development of this early neural mirroring. Until now, the research on neural mirroring in very young infants with ASD is limited. One possible explanation for this restricted amount of research is the fact that ASD is mostly diagnosed only from 2 or 3 years of age onwards (Elsabbagh & Johnson, 2010). Additionally, parents of young infants recently diagnosed with ASD are often confronted with stress and uncertainties after their child has been diagnosed (Twoy, Connolly, & Novak, 2007). It is often quite (emotionally) difficult for parents with young children recently diagnosed with ASD to participate in scientific brain imaging research. Therefore, research concerning neural mirroring in very young infants diagnosed with ASD is rather scarce.

To our knowledge, so far no studies have been conducted investigating neural mirroring in younger high-risk siblings of children with ASD. Investigating the role of mirror neurons in high-risk siblings may provide additional phenotypic information about ASD and may additionally help to define the BAP in early infancy. Therefore, this

study aimed to investigate neural mirroring in a group of children between 18 and 36 months all of whom have an older brother/sister diagnosed with ASD (i.e., high-risk siblings) compared with matched infants without a family history of ASD (i.e., low-risk infants). As Marshall and Meltzoff (2010) suggested, we included both observation and execution conditions and used mu suppression as indicator of activity in the mirror neurons (Muthukumaraswamy et al., 2004). The present study has the following research questions. (1) Do high-risk siblings (age 18-36 months old) show central mu suppression during the observation and execution of goal-directed actions compared to matched low-risk control infants? Due to the genetic relatedness with their older brother/sister with ASD and the hypothesis of possible impaired neural mirroring in ASD, we expect less mu suppression during action observation in siblings compared with the matched low-risk control group. Furthermore, we may expect similar mu suppression during action execution in both groups as there is no evidence for impaired areas in sensorimotor cortex in ASD (Bernier, Dawson, Webb, & Murias, 2007). (2) Do high-risk siblings and low-risk control infants (age 18-36 months old) show central mu suppression during the observation of mimicked (non-goal-directed) actions? Until know, it is unclear if the presence of motor movements alone is sufficient to provoke neural mirroring activity in infants. Southgate, Johnson, El Karoui, and Csibra (2010) found that 9-months old infants did not show mu suppression during the observation of mimicked actions and hypothesized that this unfamiliar mimicked action was not interpreted as goal-directed. However, research in adults revealed that the mere observation of intransitive actions evoked mirror neuron activity (e.g., Maeda, Kleiner-Fisman, & Pascual-Leone, 2002). To date, it is unclear when this transition takes place. Based on the research of Southgate and colleagues (2010) in infants, we expect less mu suppression during this observation condition compared to the goal-directed observation condition in both participant groups. (3) Is mu suppression related to other developmental child features in both groups? As neural mirroring has theoretically been related with several social-cognitive functions (Gallese et al., 2011), we will explore possible correlations between mu suppression and language and imitation performance. Furthermore, as high-risk siblings are genetically related with their relative(s) with ASD, we will explore correlations between mu suppression and scores on screeners for ASD.

MATERIAL AND METHODS

Participants

Initially, 24 unaffected siblings (high-risk sibs) and 35 matched control subjects (low-risk group) participated in the study. However, prior to analyses, 29 infants were tested but excluded due to insufficient or no cooperation of the infant (high-risk sibs: n = 3; low-risk group: n = 2), insufficient artifact-free data (high-risk sibs: n = 9; low-risk group: n = 13) or technical problems with the EEG equipment or recording (low-risk group: n = 2). Therefore, the final sample was composed of 12 high-risk siblings and 18 low-risk control infants between 18 and 36 months old (mean age = 25.67, SD = 4.61). Both groups did not significantly differ for gender ($\chi^2(1) = .81$, $p = .367$) or for age ($F(1,28) = 1.20$, $p = .284$). Although the high-risk sibs group scored slightly higher on the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003; Dutch translation by Warreyn, Raymaekers, & Roeyers, 2004) and the Modified Checklist for Autism in Toddlers (M-CHAT; Robins, Fein, Barton, & Green, 2001; Dutch translation by Dereu, Meirsschaut, Warreyn, & Roeyers, 2006) which screen for autism (M = 9.75, SD = 10.75; M = 2.00, SD = 3.89 respectively) compared to the low-risk group (M = 5.92, SD = 3.99; M = 0.31, SD = 1.01 respectively), the differences were not significant, $t(18) = -0.97$, $p = .362$ and $t(22) = -1.21$, $p = .264$. Both groups of participants completed the same experimental tasks. Characteristics of the participants are summarized in Table 1. Information about handedness was gathered through parent report.

Infant siblings were either enrolled in a larger ongoing longitudinal study or were specifically recruited for this study with the help of the Parent Association For Autism in Flanders. All siblings had at least one older infant formally diagnosed with ASD but met no clinical ASD diagnosis themselves. Control subjects were recruited through Flemish day-care centres and several magazine or website advertisements. Parental informed, signed consent was required for each participant.

Table 1. *Subject characteristics*

	Low-risk group (*n* = 18)	High-risk sibs group (*n* = 12)
Chronological age (months)		
Mean (*SD*)	25.47 (4.35)	27.05 (3.00)
Age Range	19.80-35.30	22.70-33.40
Language mean age (months)		
Receptive (*SD*)	25.14 (3.84)	25.38 (4.81)
Expressive (*SD*)	22.86 (4.22)	25.25 (4.23)
Gender ratio M : F	9 : 9	8 : 4
Handedness (R : L : ambi)	10 : 4 : 4	10 : 1 : 1
M-CHAT mean (SD)	0.31 (1.01)	2.00 (3.89)
SCQ mean (SD)	5.92 (3.99)	9.75 (10.75)

Note. Low-risk group = control group; High-risk sibs group = younger siblings of children with ASD; M-CHAT = total score on the Modified Checklist for Autism in Toddlers (M-CHAT; Robins et al., 2001; Dutch translation by Dereu et al., 2006); SCQ = total score on the Social Communication Questionnaire (SCQ; Rutter et al., 2003; Dutch translation by Warreyn et al., 2004).

General procedure

Children were tested in a quiet laboratory room at Ghent University. The EEG data were collected when the infant was alert and attending to the objects and experimenter. In order to let the child get used to the environment and experimenter, the experiment started with a short free play moment with some attractive toys. Experimenter 1 (also the demonstrator of the actions during test phase) played with the child, while experimenter 2 prepared the appropriate EEG cap. Meanwhile, the procedure was explained to the parent. After the placement of the electrodes in the appropriate EEG cap, the parent was asked to sit at the table together with the child. To maximize attention and to minimize movement, each child was seated on its parent's lap throughout the test phase. Subsequently, the experimenters placed the EEG cap on the child's head while the child was watching a popular cartoon movie. Once the EEG cap was in place, electrolytic conducting gel was applied with a syringe at each active electrode site in order to obtain a good EEG signal. The testing room was surrounded with white curtains to minimize visually distracting environmental influences. A white

roller blind was attached on a wooden frame and went up and down between the different conditions. The stimuli and movements were demonstrated at a viewing distance of approximately 60cm. Parents were instructed to be as quiet as possible in order not to distract the child during the EEG recording period. The experiment was video-recorded by 2 cameras (one focusing on experimenter 1 and one focusing on the child) in order to code the child's behaviour offline.

EEG imitation and observation paradigm

EEG data were collected during 4 conditions with 5 different objects: (1) *Object observation condition*: Each testing phase started with the demonstration of a dangling object, moving back and forth in a non-goal-directed way. The experimenter was hidden behind the white curtain during this condition. This observation condition was used as a baseline condition based on the assumption that the subjects had no prior experience with the objects. Each following condition was compared with this baseline condition. (2) *Action observation condition*: The experimenter demonstrated a simple goal-directed action (with an observable end-state) with each object and a white box (e.g., Pinocchio was picked up and put into the box, on its back). To ensure that the subject was attentive to the demonstration, the experimenter said: 'look <name child>' and made eye-contact with the child. In order to obtain enough artifact-free EEG data, each action was demonstrated three times with the left hand and three times with the right hand. The starting side was counterbalanced between the objects. (3) *Action imitation condition*: After modelling the action, the experimenter handed the objects to the infant who was asked to imitate the observed actions. Infants were encouraged (non)-verbally in a non-specific way to imitate and were given as much time as needed to perform the actions themselves. (4) *Hand movement condition*: Mimicked actions were demonstrated during the fourth condition. The experimenter performed hand movements, which were identical as those during the action observation condition but now without the objects and without direct reference of gaze towards the child which makes this condition less social. Subjects were expected only to observe those actions, not to imitate them. Similar as during the action observation condition, the hand movements were demonstrated 3 times with the left hand en 3 times with the right hand.

Each experimental session started with the object observation condition (baseline condition) for all 5 objects subsequently. The order of the other three conditions was counterbalanced across subjects, with the restriction that the action imitation condition always followed the action observation condition so that the participants first observed what they had to imitate. The order of the objects remained the same for each participant. Each demonstrated action lasted about 30 seconds per object which resulted in a total duration of about 20 minutes for the entire experiment. After the EEG recording, the parents were debriefed and received a gift card as reward for their participation. Finally, the parents were asked to fill in the Dutch version of the MacArthur-Bates Communicative Development Inventories (N-CDI;e Zink & Lejaegere, 2002; original version Fenson et al., 1993), the Social Communication Questionnaire (SCQ; Rutter et al., 2003; Dutch translation by Warreyn et al., 2004) and the Modified Checklist for Autism in Toddlers (M-CHAT; Robins et al., 2001; Dutch translation by Dereu et al., 2006) at home.

EEG data recording

The EEG was recorded relative to an average reference from 32 active Ag/AgCl electrodes placed according to the international 10-20 system (Jasper, 1958) embedded in a child-friendly stretch EEG-cap with a ground electrode placed at AFz (Easycap, Brain Products, GmbH, Munich, Germany). Data recording took place with Brain Vision Recorder (Brain Products, 2007) with the use of an EEG-amplifier (QuickAmp) with a sample rate of 500 Hz, 1 s time constant, a low pass filter of 70 Hz and a notch filter of 50 Hz. Horizontal electro-oculogram (HEOG) electrodes were placed at the left and right outer canthi of the eyes. Vertical EOG was calculated by comparing the recording of an electrode above the eye, at position Fp2, with the common reference. Initially VEOG was computed by comparing Fp2 with an electrode placed below the left eye, but many infants did not tolerate this. After comparing data with this electrode and the common reference method, no significant differences occurred. Inter–electrode impedance was measured and confirmed to be below 10 kΩ for all electrodes. EEG recordings and video recordings were synchronized by pushing a button at the beginning of each condition. This button sent a marker to the recorded EEG signal and simultaneously emitted a LED

light which was visible on both cameras. Synchronization was possible by comparing the time intervals between the different markers on both recording systems.

Behaviour coding

The camera recordings were coded offline with The Observer XT 9.0. (Noldus Information Technology, 2009). The subject's behaviour was coded by ascribing start and stop codes to each condition generally and more precisely to attentive behaviour in the observation conditions, imitative behaviour in the action imitation condition and vocalization and motor movements within each experimental condition. Further analyses were based on the fragments where the child was quietly attending the demonstrations (during object observation, action observation, and hand movement) and was actually imitating during the action imitation condition. During this coding, fragments with too much motor and vocalization codes were excluded in order to minimize contamination of the EEG data. Obviously, it was impossible to exclude all those fragments but further investigation revealed no significant influences of the number of movements and vocalizations on the data (all $-.26 < r < .26$, all $p > .05$). An additional exclusion of motor movements and vocalizations was performed by applying an artifact rejection procedure during the EEG analyses.

Quality of imitative behaviour of each infant was coded by an observer who was blind for group membership. Therefore, three criteria were assigned for each action. The child received score 1 for every criterion he/she met. Afterwards, the mean of the best scores for each object was calculated which reflected the total quality of imitation score per child with a maximum of 3. In the sibling group, the mean score was 2.31 (*SD* = .28) whereas in the control group, the mean score was 2.04 (*SD* = .38) which implies that both groups imitated reasonably well. Inter-observer reliability was based on double-coding of 25% randomly chosen video recordings by an independent coder. This resulted in a Cronbach's Alpha Coefficient of .94 reflecting an excellent level of reliability (Cronbach, 1951).

EEG data analysis

Brain Vision Analyzer (Brain Products, 2007) was used for offline analyses of the recorded raw data. Based on the assumption that mu rhythm is defined as oscillations measured over the sensorimotor cortex (Marshall, Young, & Meltzoff, 2011; Muthukumaraswamy et al., 2004), EEG power data recorded from electrode positions C3 and C4 were further investigated. The raw EEG data were first inspected visually to eliminate contaminated signal due to artefact influences. Afterwards, EEG was re-referenced to an average reference with exclusion of the most disturbed electrode channels. EEG data were filtered with a high pass filter of 0.1 Hz, a low pass of 30 Hz, and a 50 Hz notch-filter. Correction for horizontal and vertical eye movement was obtained by using the Gratton and Coles algorithm (Gratton, Coles, & Donchin, 1983). Remaining data were segmented in 1s-epochs with 50% overlap. Bad segments were removed with artefact rejection using a maximal allowed voltage step of 100µV per sampling point; a maximal allowed absolute difference of 400µV between two values in the segment and an activity of 0µV during maximum 100 milliseconds. In this way, an average of 236.77 segments (*SD* = 84.60) per infant per condition was left. Finally, Fast Fourier Transform was performed on the remaining segments with a Hanning window of 10% and averaged for each experimental condition. The mu frequency band of interest was conducted by subtracting the baseline condition from the action imitation condition for each subject individually as has been performed in previous studies (e.g., Lepage & Théoret, 2006; Muthukumaraswamy et al., 2004). Furthermore, the individual mu frequency band was selected by calculating the 3-Hz interval around the highest peak value of that subtraction at the central electrode positions. The mean of the highest peak value was 8.2 Hz (*SD* = .88) in the total sample which is in agreement with previous studies on mu/alpha rhythm frequencies in infants (Marshall, Bar-Haim, & Fox, 2002; Stroganova et al., 1999).

Mu suppression was calculated following the procedure of Oberman and colleagues (2005). To control for variability due to individual differences (e.g., scalp thickness or electrode impedance), we used a ratio to calculate the relative power for each condition. We calculated the ratio of the power during respectively the action observation condition, the hand movement condition, and the action imitation condition relative to the power during the object observation condition (baseline condition).

Subsequently, the log transform of the ratio was calculated since the ratio data were non-normally distributed. As a result, a negative value represents mu suppression, a positive value indicates mu enhancement whereas a value of zero indicates no suppression.

RESULTS

First, we tested if the order of the presentation of the different conditions influenced mu suppression in both groups. An independent sample t-test revealed no influence of counterbalancing in both the low-risk group, all $-.47 < t(16) < 1.33$, all $p > .05$ and the high-risk sibs group, all $-.47 < t(10) < 1.47$, all $p > .05$. Therefore, the order of presentation of the conditions was not further included as factor in the analyses.

Mu wave suppression

A 3x2x2 repeated-measures ANOVA was conducted with condition (hand movement observation, action observation, and action imitation) and hemisphere (C3 and C4) as within-subjects factors and group (high-risk sibs and low-risk infants) as between-subjects factor. A significant main effect of condition was found, $F(2,27) = 4.84$, $p = .016$. Follow-up contrasts showed significantly more mu suppression during the action imitation condition ($M = -.36$, $SD = .42$) compared to the hand movement condition ($M = -.22$, $SD = .22$) and the action observation condition ($M = -.09$, $SD = .19$) with $F(1,28) = 4.28$, $p = .048$ and $F(1,28) = 9.01$, $p = .006$ respectively, and significantly stronger mu suppression during hand movement observation compared to action observation, $F(1,28) = 7.85$, $p = .009$. Results showed no significant main effect of hemisphere and group, $F(1,28) = .87$, $p = .358$ and $F(1,28) = .04$, $p = .849$ respectively. Furthermore, no significant interaction effect between condition and group for mu suppression, $F(2,27) = .45$, $p = .641$, no significant interaction effect for group by hemisphere $F(1,28) = .31$, $p = .584$, for hemisphere by condition $F(2,27) = .44$, $p = .650$, and no significant 3-way interaction effect between condition, hemisphere and group, $F(2,27) = .22$, $p = .806$ were found.

A one-sample t-test in the low-risk control group showed significant central mu wave suppression (i.e., mu suppression assembled over positions C3 and C4) during all three conditions, with $t(17) = -3.64$, $p = .002$ during the hand movement condition, $t(17) = -3.54$, $p = .002$ during the action observation condition and $t(17) = -3.23$, $p = .005$ during the action imitation condition.

The high-risk sibling group demonstrated significant mu suppression during the hand movement condition and the action imitation condition, $t(11) = -4.51$, $p = .001$ and $t(11) = -4.88$, $p = .000$ respectively. Mu suppression during action observation was not significant, $t(11) = -1.27$, $p = .229$. The means and standard deviations of mu suppression at electrode positions C3 and C4 separately and assembled in both participant groups are presented in Table 2.

Table 2. *Mu suppression for both groups at C3 and C4 separately and assembled during each condition*

		Low-risk group (n = 18)			High risk sibs group (n = 12)		
		M (SD)	*t*(17)	*p*	*M (SD)*	*t*(11)	*p*
C3	HM	-.26 (.36) **	-3.03	.008	-.25 (.13) ***	-6.47	.000
	AO	-.15 (.18) **	-3.45	.003	-.11 (.30)	-1.30	.222
	AI	-.42 (.63) *	-2.83	.012	-.32 (.17) ***	-6.33	.000
C4	HM	-.16 (.29) *	-2.29	.035	-.22 (.27) *	-2.80	.017
	AO	-.03 (.21)	-0.52	.607	-.09 (.27)	-1.12	.287
	AI	-.38 (.46) **	-3.46	.003	-.31 (.41) *	-2.58	.026
C	HM	-.21 (.24) **	-3.64	.002	-.24 (.18) ***	-4.51	.001
	AO	-.09 (.11) **	-3.54	.002	-.10 (.27)	-1.27	.229
	AI	-.40 (.52) **	-3.23	.005	-.31 (.22) ***	-4.88	.000

Note. Low-risk group = control group; High-risk sibs group = younger siblings of children with ASD; C3 = mu suppression at electrode position C3; C4 = mu suppression at electrode position C4; C = mean central mu suppression assembled over electrode position C3 and C4; HM = mu suppression during the hand movement condition, AO = mu suppression during the action observation condition; AI = mu suppression during the action imitation condition.
*$p < .05$; **$p < .01$; ***$p \leq .001$.

To assure that the observed central suppression was related to the mu rhythm and not to other overlapping activity such as posterior alpha activity, additional electrode activity (recorded from an occipital electrode at position Oz) was investigated. No significant suppression was found at Oz during action imitation in the frequency band under investigation, $t(17) = -1.05$, $p = .309$. Therefore, we can conclude that the observed mu suppression was specific to the central electrode positions and was not the result of occipital activity.

Relation between mu wave suppression and other developmental child features

Pearson's correlation coefficients and Spearman rho coefficients were calculated to investigate relations between mu wave suppression at the central electrode positions during all three conditions on the one hand and chronological age, receptive and expressive language age equivalents, quality of imitation, M-CHAT, and SCQ-scores on the other hand. An independent sample t-test was conducted to investigate the relation between mu suppression and gender.

In the control group, quality of imitation correlated marginally significant with mu wave suppression during action imitation with a medium positive correlation of $r = .42$, $p = .082$ and receptive language tended to correlate significantly with mu wave suppression during action observation with a medium negative correlation of $r = -.50$, $p = .066$. Finally, chronological age correlated marginally significant with central mu suppression during action imitation with a medium correlation of $r = .41$, $p = .094$.

In the sibling group, no significant correlations were found between the child characteristics and central mu wave suppression. For chronological age, language, and SCQ-scores, the magnitude of the correlations was medium ranging from $-.58 < r < .40$, all $p > .05$. Correlations with quality of imitation and M-CHAT scores were small ranging from $-.39 < \rho < .14$, all $p > .05$. A medium negative correlation (although not significant) was found between expressive language and central mu suppression during action imitation ($r = -.57$, $p = .138$). See Table 3 for details.

Table 3. *Correlations between central mu suppression and child characteristics in the low-risk control group and the high-risk sibling group*

	Low-risk group (n = 18)						High-risk sibs group (n = 12)					
	Age (r)	REC (r)	EXP (r)	QUA (r)	M-CHAT (ρ)	SCQ (r)	Age (r)	REC (r)	EXP (r)	QUA (ρ)	M-CHAT (ρ)	SCQ (r)
CHM	.322	-.115	-.020	.263	.118	.145	.010	-.249	-.280	-.271	.136	.390
CAO	-.085	-.504°	-.355	-.021	-.141	.104	.243	.228	.269	.018	-.382	.117
CAI	.406°	.249	.295	.421°	.353	.154	-.279	-.227	-.573	-.175	.082	.245

Note. Low-risk group = control group; High-risk sibs group = younger siblings of children with ASD; CHM = mu suppression at central electrode positions during the hand movement condition; CAO = mu suppression at central electrode positions during the action observation condition; CAI = mu suppression at central electrode positions during the action imitation condition; REC = receptive language age equivalent; EXP = expressive language age equivalent; QUA = quality of imitation; M-CHAT = total score on the Modified Checklist for Autism in Toddlers (M-CHAT; Robins et al., 2001; Dutch translation by Dereu et al., 2006); SCQ = total score on the Social Communication Questionnaire (SCQ; Rutter et al., 2003; Dutch translation by Warreyn et al., 2004).
°$p < .10$.

Furthermore, correlations were explored within the total sample, regardless of group membership. Quality of imitation correlated marginally significant with central mu suppression during the action imitation condition with a small correlation of $r = .31$, $p = .095$. Chronological age, receptive language, SCQ scores (all $-.15 < r < 31$, all $p > .05$), expressive language, and M-CHAT scores (all $-.22 < \rho < .24$, all $p > .05$) were not significantly correlated with central mu suppression in all three conditions reflected in rather small correlation coefficients. Table 4 shows all correlations between mu suppression at the central electrode positions and the different child characteristics in the total sample.

Table 4. *Correlations between central mu suppression and child characteristics in the total sample*

	Age (r)	REC (r)	EXP (ρ)	QUA (r)	M-CHAT (ρ)	SCQ (r)
CHM	.225	-.145	-.036	.130	.049	.236
CAO	.066	-.046	-.088	-.047	-.211	.189
CAI	.305	.111	.076	.310°	.234	.185

Note. CHM = mu suppression at central electrode positions during the hand movement condition; CAO = mu suppression at central electrode positions during the action observation condition; CAI = mu suppression at central electrode positions during the action imitation condition; REC = receptive language age equivalent; EXP = expressive language age equivalent; QUA = quality of imitation; M-CHAT = total score on the Modified Checklist for Autism in Toddlers (M-CHAT; Robins et al., 2001; Dutch translation by Dereu et al., 2006); SCQ = total score on the Social Communication Questionnaire (SCQ; Rutter et al., 2003; Dutch translation by Warreyn et al., 2004).
°$p < .10$.

An independent sample t-test revealed significant differences of gender regarding mu suppression during the hand movement condition, $t(28) = 3.15$, $p = .004$, and the action imitation condition, $t(28) = 2.75$, $p = .010$, in the total group with significantly more mu suppression in girls (hand movement condition: $M = -.34$, $SD = .19$; action imitation condition: $M = -.58$, $SD = .47$) than in boys (hand movement condition: $M = -.12$, $SD = .19$; action imitation condition: $M = -.20$, $SD = .31$).

DISCUSSION

To date, most of the research investigating neural mirroring in individuals with ASD has focused on childhood (e.g., Hamilton, Brindley, & Frith, 2007; Martineau et al., 2008; Raymaekers et al., 2009) to learn more about the underlying neurological processes in ASD. Research in early infancy can expand the knowledge of the exact role of mirror neurons in the development of ASD, but is difficult to conduct and consequently rather scarce. Therefore, investigating the activity of mirror neurons in young high-risk siblings may provide additional phenotypic information about the underlying mechanisms of ASD and its BAP. To these ends, the present study aimed to investigate neural mirroring in young high-risk siblings between 18 and 36 months old. Therefore, an EEG study was conducted to evaluate mu suppression during observation and imitation of goal-directed actions as well as during the observation of non-goal-directed hand movements in infant siblings of children with ASD compared with matched control infants. Furthermore, we investigated whether mirror neuron activity was related to several developmental child features in both participant groups.

Overall no group differences concerning neural mirroring were found between the low-risk control group and the high-risk sibling group. Both high-risk and low-risk infants showed central mu wave suppression during the hand movement, action observation, and action imitation condition. These results suggest the presence of an action observation/action execution matching system in high-risk siblings of children with ASD. However, mu wave suppression during the action observation condition in the sibling group was not significant. This may be due to the small sample size although this could not explain the significant mu suppression during the hand movement and the action imitation condition. The occurrence of significant mu suppression in the low-risk control group during the observation and execution condition is in line with previous infant research about the presence of an action observation/action execution matching system in infants (Marshall & Meltzoff, 2010). In addition, the occurrence of mu wave suppression during the observation of non-goal-directed hand movements in both groups, suggests that the presence of motor movements alone are sufficient to provoke neural mirroring activity in infants between 18 and 36 months old, similar as in adults (Maeda et al., 2002). This is contradictory with other studies such as the research of

Southgate and colleagues (2010) who found that in 9-months old infants, activation of mirror neurons only occurred when the infants could interpret the observed action as goal-directed based on familiarity with the action. The lack of differences between both participant groups cannot be explained by a lack of statistical power as the observed mu suppression in both groups is significant, except for mu suppression during the action observation condition in the high-risk sibling group.

The presence of activity in the neural mirroring systems in high-risk siblings could lead to the conclusion that despite their genetic alliance with their older brother/sister with ASD, high-risk siblings demonstrate the same mirror neuron activity as low-risk infants. This genetic reliability is reflected in the higher scores on the ASD-screeners M-CHAT (Robins et al., 2001; Dutch translation by Dereu et al., 2006) and SCQ (Rutter et al., 2003; Dutch translation by Warreyn et al., 2004) in the high-risk sibs group compared to the low-risk control group. Our study demonstrated that abnormal or deficient neural mirroring is not a clear distinctive characteristic of the BAP. To our knowledge, no previous research investigated neural mirroring in this high-risk group. Therefore these findings need to be replicated with larger sample sizes to make more profound conclusions.

Additionally, correlations between mu suppression and child characteristics (such as chronological age, gender, language, quality of imitation, and scores on the M-CHAT and SCQ) were investigated. Except for a trend with mu suppression during action imitation in the control group, we found no significant correlations between age and mu activation in both groups which is in line with previous research (e.g., Lepage & Théoret, 2006; Oberman et al., 2005). However findings concerning the relation between mu suppression and age in ASD are contradictory (Oberman et al., 2012). More specifically, it may be that the age range of 1.5 years in the participant groups tested in this study was too limited to find age-related differences. For example, Oberman and colleagues (2012) investigated the correlation between age and mu suppression in individuals with ASD in childhood and adolescence, ranging from 6 to 17 years. They found a general developmental negative correlation between mu suppression and age, both in typically developing individuals and in persons with ASD. Measuring neural mirroring from infancy until adulthood at different moments in the same group of participants can give more information about stability or development of mu wave activity during action

observation and execution. Therefore, additional research is needed to compare the same group of children with, without or at risk for ASD over different time periods during observation and imitation tasks.

Furthermore, the total group showed significantly different mu suppression in boys compared with girls during hand movement observation and action imitation with more mu suppression in girls. The finding of more mu suppression in girls compared to boys is in line with the findings in adult research. Cheng and colleagues found evidence for stronger mu suppression during observation of hand actions in females compared to males (Cheng et al., 2008; Cheng, Tzeng, Decety, Imada, & Hsieh, 2006). Their explanation is that women often show stronger empathy which can occur during observation of other's action reflecting in stronger activation of their action observation/action execution matching system (Cheng et al., 2006). As it is assumed that infants can demonstrate empathic behaviours (Rieffe, Ketelaar, & Wiefferink, 2010) with empathic markers already present at 8- and 10-months of age (Roth-Hanania, Davidov, & Zahn-Waxler, 2011), it is possible that infant girls are more empathic with the observer during observation of non-goal-directed actions and during action execution. However, it should be noted that gender differences in empathic behaviour are not consistently found in infancy. Some studies found support for gender differences with more empathy in girls compared to boys (e.g., Knafo, Zahn-Waxler, Van Hulle, Robinson, & Rhee, 2008) while other studies did not find evidence for this gender difference (e.g., Roth-Hanania et al., 2011). Furthermore, it is assumed that gender differences in empathy may become more pronounced and stable as infants grow older (Roth-Hanania et al., 2011). To conclude, findings about gender differences and mu suppression are scarce, especially in infancy and during imitation tasks. Future studies need to focus on the influence of gender on mu wave activity in infants during different tasks, including both observation and imitation, and whether there is a link with empathy differences between girls and boys.

Concerning language, no significant correlations in the total sample with mu suppression were found. More specifically, our control sample demonstrated only a marginally significant correlation between receptive language and mu suppression during action observation. The absence of significant correlations between language and mu suppression during other conditions in both groups is contradictory with the assumption that language and mirror neuron activity are strongly related (Rizzolatti &

Craighero, 2004). However, it should be noted that 4 infants of the low-risk group and 3 of the high-risk group in the current study achieved a maximum score on the N-CDI (Zink & Lejaegere, 2002; original version Fenson et al., 1993) due to the age limit of 30 months of this questionnaire which could have influenced the correlations. Therefore, future research should consider this restriction by using appropriate instruments in a more heterogeneous and bigger sample to detect possible correlations between language and mu wave suppression. This suggestion for future research can also be made concerning the lack of correlations between quality of imitation and mu suppression. Only a nearly significant correlation was found between mu suppression during action imitation and quality of imitation in the control group and in the total sample.

Both groups separately and the total sample demonstrated no significant correlations between the scores on the M-CHAT (Robins et al., 2001; Dutch translation by Dereu et al., 2006) and the SCQ (Rutter et al., 2003; Dutch translation by Warreyn et al., 2004) on the one hand and central mu suppression during all conditions on the other hand. Although the high-risk sibs group scored higher on the M-CHAT and the SCQ compared to the low-risk control group, no significant correlations were found with mu suppression. Therefore, the present study suggests that weaker mu suppression is not a clear distinctive characteristic of the BAP.

However, a critical remark should be mentioned concerning the interpretation of correlations. It is well established that distribution of correlations is related to the sample size of the investigated group (Kareev, Lieberman, & Lev, 1997). Therefore, the absence of correlations or significance should be interpreted carefully in our study given the rather small sample sizes. It is possible that medium or strong correlations are not significant in the current study due to these small sample sizes.

Although, to our knowledge, this is the first study investigating neural mirroring in high-risk siblings of infants with ASD, some limitations need to be mentioned. The results should be evaluated cautiously for several reasons. First, our finding of no differences in mirror neuron activity between high-risk siblings and low-risk young infants must be interpreted carefully. If the broken mirror hypothesis of ASD should be correct, we would expect impaired neural mirroring as primary deficit in very young infants at risk for ASD. However, it might be that vulnerabilities in siblings are rather subtle and less present in preschool age, but may increase and become more noticeable

as infants grow older (Warren et al., 2012). As Rogers (2009) postulated, a group difference or lack of difference is only one step in the analyses. It could be of interest to follow these group of infants up to later age periods, definitely in the light of developing ASD or not. Furthermore, it should be taken into account that findings from studies with unaffected infant siblings could also be influenced by variability in expression of risk markers. For that reason, results from sibling studies should be interpreted carefully and generalizability should be handled cautiously. Secondly, this study used a comparison group of low-risk infants with no older brother or sister diagnosed with ASD. As Yirmiya and Ozonoff (2007) claimed, until now it is unclear which comparison groups we should use to discover specific and unique characteristics in siblings of children with ASD. It is possible that environmental factors, such as family stress or parenting behaviour, affect siblings of children with ASD in a different manner than in the comparison group (Warren et al., 2012). Therefore, using a clinical control group can be useful because in this way confounding variables as result of the presence of a child with special needs in those families can be excluded as possible explanatory factor (Sucksmith et al., 2011). A final critical remark is the possible occurrence of covert movement which could cause mu suppression itself. By excluding the fragments with too many motor movements and vocalizations beforehand during video coding and by using a profound artifact rejection, we tried to control for those artifacts. Additionally, as reported previously, no significant effect of the number of movements and vocalizations on the data was found. However, we could not control for motor planning or inhibited reaching during observation conditions which could cause mu suppression.

With this study, we wanted to make a contribution to the existing research about neural mirroring in ASD and more specifically siblings at risk for ASD. The inclusion of both observation and execution conditions is a strength of this study (see Marshall & Meltzoff, 2010 for a critical review). However, future work is needed to expand this topic. Investigating neural mirroring in bigger samples, with accurately matched control groups could contribute to the identification of early neurological differences associated with ASD and its BAP.

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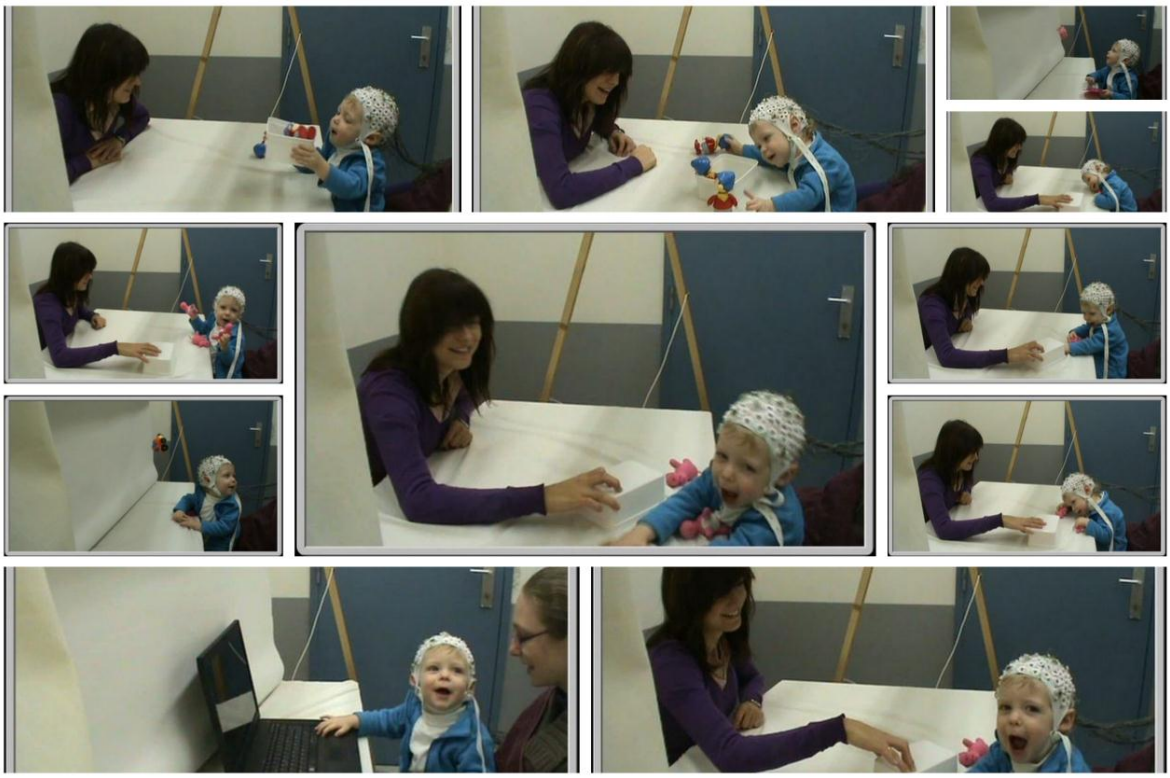

A little boy who is having fun during the experiment

CHAPTER 5

EXPLORING THE ROLE OF NEURAL MIRRORING IN CHILDREN WITH AUTISM SPECTRUM DISORDER[1]

ABSTRACT

Investigating the underlying neural mechanisms of autism spectrum disorder (ASD) has recently been influenced by the discovery of mirror neurons. These neurons, active during both observation and execution of actions, are thought to play a crucial role in imitation and other social-communicative skills which are often impaired in ASD. In the current EEG study, we investigated neural mirroring in children with ASD between the age of 24 and 48 months and age-matched typically developing children, during observation of actions and hand movements and during action execution. Results revealed no significant group differences with significant central mu suppression in the ASD children and control children during both execution and observation of goal-directed actions and during observation of hand movements. Furthermore, no significant correlations between mu suppression on the one hand and quality of imitation, age, and SCQ scores on the other hand were found. These findings challenge the 'broken mirror' hypothesis of ASD, suggesting that impaired neural mirroring is not a distinctive feature of ASD.

[1] Based on Ruysschaert, L., Warreyn, P., Wiersema, J.R., Metin, B., Oostra, A., & Roeyers, H. (2012). *Exploring the role of neural mirroring in children with autism spectrum disorder.* Manuscript submitted for publication.

INTRODUCTION

Pervasive Developmental Disorders (PDDs), a spectrum of neurodevelopmental disorders, are characterized by impairment in three domains: social interaction, language and communication, and the presence of restricted, repetitive behaviours. According to the DSM-IV-TR, the concept of PDD is subclassified in Rett's Disorder, Childhood Disintegrative Disorder, Asperger's Disorder, Autistic Disorder, and Pervasive Developmental Disorder Not Otherwise Specified (American Psychiatric Association, 2000). The latter three classifications are often referred to as 'Autism Spectrum Disorders' (ASDs; Wing, 1997). ASD represents a broad variation in symptomatology, ranging from rather mild to very severe symptoms in the three areas of impairment (Wing, 1997).

ASD has been characterized by various social-communicative dysfunctions (Williams, Whiten, & Singh, 2004). One frequently reported characteristic of ASD is imitation impairment which has been included in the diagnostic criteria of the DSM-IV-TR (American Psychiatric Association, 2000) as 'lack of social imitative play appropriate to developmental level', categorized as one of the ASD communication characteristics. This ASD deficit, first reported by DeMyer and colleagues (DeMyer et al., 1972), is well documented (for a review, see Williams et al., 2004). However, the heterogeneity of the ASD symptomatology, the variability across imitation tasks in research and the inconsistency of the definition of imitation all impede the development of a clear view on imitation in ASD (Vanvuchelen, Roeyers, & De Weerdt, 2011). Consequently, research on imitation in ASD is still debated and needs further exploration.

Because the presence of imitation is necessary for a normal social-cognitive development (Meltzoff & Decety, 2003), it has been suggested that social-communicative symptoms in ASD could be the result of an imitation impairment which reflects a neurological deficiency (Rogers & Pennington, 1991). Despite the difficulty finding the underlying neural basis of ASD, one commonly used explanation for imitation impairment in ASD is the inability to map the perception of others into the observer's own system (Williams et al., 2004). This self-other mapping requires a match between observation and execution by which the motor knowledge of the observer is used to

understand the observed action. This process is driven by 'an action observation/action execution matching system' (Gallese, Fadiga, Fogassi, Gallese, & Rizzolatti, 1996).

The interest in the neurobiological mechanism of this matching system is recently increased by the discovery of the mirror neurons. Mirror neurons were initially detected in area F5 of the macaque premotor cortex (Rizzolatti & Craighero, 2004). These neurons, distinguishable from other motor neurons, discharge when the monkey executes an action as well as when it observes another individual (human or monkey) performing a similar action (Di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992). The core idea is that the observation of an action leads to the activation of parts of the same cortical neural network that is active during action execution. Due to this neural mirroring, it is possible to accomplish automatic execution as well as simulation of the observed actions. Impaired neural mirroring could lead to impaired self-other representations (Williams, Whiten, Suddendorf, & Perrett, 2001) and has been proposed to mediate the social and communicative deficits that characterize ASD (Oberman, Ramachandran, & Pineda, 2008).

Indirect measures of the brain activity in humans using several non–invasive neurophysiological and brain imaging studies (e.g., Buccino et al., 2001; Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995; Hari et al., 1998) and behavioural measures such as gaze tracking (e.g., Falck-Ytter, Gredebäck, & von Hofsten, 2006) revealed the occurrence of a similar observation/execution matching system in humans. Direct evidence for the presence of mirror neurons in the human motor cortex was provided in the first single cell study of Mukamel and colleagues (Mukamel, Ekstrom, Kaplan, Iacoboni, & Fried, 2010). One commonly used method and non-invasive way of investigating human neural mirroring is analysing electroencephalographic (EEG) mu rhythm band oscillations (Muthukumarasway, Johnson, & McNair, 2004; Raymaekers, Wiersema, & Roeyers, 2009). More specifically, resting motor neurons show spontaneous synchronization leading to a large amplitude of the EEG mu wave typically recorded in the 8-13 Hz frequency range in adults. Attenuation of mu rhythm reflects an increased activity level of these neurons and is also called 'mu wave suppression' (Gastaut, Dongier, & Courtois, 1954). Similar mu wave suppression has been observed during the observation of actions performed by others as well as during motor activation (Gastaut & Bert, 1954). Therefore, suppression of the mu wave rhythm typically recorded over the sensorimotor

cortex has been argued to indicate a selective reflection of mirror neuron activity (Pineda, Allison, & Vankov, 2000). A mu rhythm at a lower frequency range (between 6 and 9 Hz) with similar properties as the adult mu rhythm has been discovered in infants (Stroganova, Orekhova, & Posikera, 1999).

The discovery of mirror neurons and the pivotal role of imitation both in typical and atypical development have led to the hypothesis of dysfunctional mirror neurons in ASD (Williams et al., 2001). This dysfunction is likely to result in imitation and social-communicative deficits often present in ASD (Fan, Decety, Yang, Liu, & Cheng, 2010; Williams et al., 2001). This hypothesis has been tested frequently but so far, evidence for the so called 'broken mirror theory of autism' seems inconsistent (Southgate & Hamilton, 2008). Several research findings support the idea of impaired mirror neuron functioning in ASD in adults (e.g., Bernier, Dawson, Webb, & Murias 2007) and children. For example in the study of Oberman and colleagues (2005), individuals with ASD between 6 and 47 years old showed significant mirror neuron activity during self-performed hand movements, but not during movement observation. These findings support the idea of broken mirror neurons in ASD which was also the case in the study of Martineau, Cochin, Magne, and Barthelemy (2008), were 5-year-old autistic children showed no mu suppression during action observation. Additionally, Dapretto and colleagues (2006) found in their fMRI study support for dysfunctional neural mirroring mechanisms during both imitation and observation of emotional expressions in ASD children. Impaired mirror neuron functioning in this study was negatively correlated with symptom severity in children with ASD which may influence social deficits often observed in ASD. On the other hand, Oberman and colleagues (2008) measured significant mirror neuron activity during action observation in individuals with ASD under specific conditions such as the use of a familiar hand model. In addition, Raymaekers and colleagues (2009) found equally strong mirror neuron activity during both self-performed and observed hand movements in children between 8 and 13 years with high functioning autism compared to the control group. Similarly, also Fan and colleagues (2010) found in their study that individuals with ASD showed mu suppression similar to the control group during the observation of hand actions. Hence, to date, there is insufficient support for the broken mirror theory of autism (see Gallese, Gernsbacher,

Heyes, Hickok, & Iacoboni, 2011 for an overview of this discussion; Southgate & Hamilton, 2008).

The discordant conclusions call for more research to understand the exact relationship between neural mirroring and imitation in individuals with ASD, particularly in young children. Infancy and early childhood seem to be an ideal period to study the relationship between imitation and neural mirroring since imitation has been observed early in development (Meltzoff & Moore, 1977) and because imitation is a crucial skill in human social-communicative development (Rogers & Pennington, 1991). Furthermore, research suggested that already early in life, someone's own action experience is closely related to neural mirroring activity (e.g., van Elk, van Schie, Hunnius, Vesper, & Bekkering, 2008). Therefore, the link between imitation and neural mirroring in ASD does not indicate a simple causal relationship (Southgate, Gergely, & Csibra, 2009). Consequently, investigating young children diagnosed with ASD can help to learn more about neural mirroring and its relationship with imitation in individuals with ASD.

Therefore, this study aimed to explore neural mirroring in young children with ASD between 24 and 48 months old and in age-matched typically developing controls. We used mu suppression as indicator of activity in the mirror neurons during the observation of goal-directed actions and non-goal-directed mimicked hand movements and during action imitation. The present study examined following research questions: (1) Do children with ASD (age 24-48 months old) show central mu suppression during the observation of goal-directed actions compared to a matched control group of typically developing children? According to the broken mirror hypothesis in ASD (e.g., Dapretto et al., 2006; Oberman et al., 2005), we may expect a lack of or diminished mu suppression during the action observation condition in the ASD group. (2) Do ASD children and typically developing children (age 24-48 months old) show central mu suppression during the observation of mimicked (non-goal-directed) actions? To date, only a few studies investigated neural mirroring activity during hand movement observation in typically developing children (e.g., Southgate, Johnson, El Karoui, & Csibra, 2010) but not in children diagnosed with ASD. However, in line with the idea of impaired mirror neuron functioning in ASD, we may expect less mu suppression during this observation condition in the ASD group. (3) Do children with ASD (age 24-48 months old) show central mu suppression during the execution of goal-directed actions

compared to a matched control group of typically developing children? We may expect (equally strong) suppression of mu oscillations during action execution in both groups as there is no evidence for impaired areas in sensorimotor cortex in ASD (Bernier et al., 2007). (4) Is mu suppression related with several child characteristics such as quality of imitation and chronological age? We may expect significant correlations with imitation performance, as neural mirroring has theoretically been related with this ability (see Gallese et al., 2011 for a discussion).

MATERIAL AND METHODS

Participants

The total initial sample consisted of 35 children with ASD and 42 control children. From this original sample of participants with ASD (ASD group) and control children (TD group), 17 participants with ASD and 23 typically developing children were excluded prior to analyses due to no cooperation (ASD: n = 4; TD: n = 2), insufficient artifact free data (ASD: n = 13; TD: n = 19) or technical problems with the EEG equipment (TD: n = 2). As a result, the final sample for further analyses was composed of 18 ASD children and 19 typically developing children (mean age = 41.94, SD = 13.80). The groups were matched on chronological age, $F(1,35) = 0.06$, $p = .808$. The ASD group scored significantly higher on the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003; Dutch translation by Warreyn, Raymaekers, & Roeyers, 2004) than the TD group ($t(26) = -5.16$, $p < .001$). Table 1 presents the characteristics of the participants. Information about handedness was gathered through parent report.

ASD subjects were recruited through Belgian Government certified University Clinics for Developmental Disorders and multiple treatment centres for developmental disorders. All ASD participants were examined and formally diagnosed independently by a qualified multidisciplinary team of specialists who were all familiar with ASD. One of the tests included in the diagnostic protocol was the Autism Diagnostic Observation Schedule (ADOS; Lord, Rutter, DiLavore, & Risi, 1999). Except for two participants, the diagnosis was confirmed with the ADOS as the ASD children scored above the cut-off for

ASD. Control subjects were recruited through Flemish day-care centres and several magazine or website advertisements. For each participant, parental informed, signed consent was required.

Table 1. *Subject characteristics*

	ASD group (*n* = 18)	TD group (*n* = 19)
Chronological age (months)		
Mean (*SD*)	42.52 (13.72)	41.39 (14.23)
Age Range	25.90-60.00	25.20-58.70
Language mean age (months)		
Receptive (*SD*)	39.85 (12.89)	45.63 (16.72)
Expressive (*SD*)	38.77 (14.47)	44.94 (18.62)
SCQ mean (SD)	13.42 (4.68)	5.00 (3.95)
Gender ratio M : F	14 : 4	8 : 11
Handedness (R : L : ambi)	15 : 1 : 2	12 : 5 : 2

Note. ASD group = children diagnosed with ASD; TD group = typically developing group; SCQ = total score on the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003; Dutch translation by Warreyn et al., 2004).

General procedure

Children were tested in a quiet laboratory room at Ghent University. The EEG data were collected when the participant was alert and attending to the objects and experimenter 1 who demonstrated the actions. The experiment started with a short free play moment with some attractive toys in order to let the child get used to the environment and experimenters. Experimenter 1 (also the demonstrator of the actions during the test phase) played with the child, while experimenter 2 prepared the appropriate EEG cap. Meanwhile, the procedure was explained to the parent. After the placement of all the electrodes in the appropriate EEG cap, the parent was asked to sit at the table together with his/her child. To maximize attention and to minimize movement, each child was seated on its parent's lap throughout the entire test phase. Subsequently, the experimenters placed the EEG cap on the child's head while the child was watching a popular cartoon movie. Once the EEG cap was in place, electrolytic

conducting gel was applied with a syringe at each active electrode site in order to obtain a good EEG signal. White curtains surrounded the laboratory room to minimize visually distracting environmental influences. A white roller blind, attached on a wooden frame, went up and down between the different conditions. The objects and actions were demonstrated at a viewing distance of approximately 60 cm. Parents were asked to be as quiet as possible in order not to distract the child during the EEG recording period. The experiment was videotaped by 2 cameras (one focusing on experimenter 1 and one focusing on the participant) in order to code the child's behaviour afterwards.

EEG imitation and observation paradigm

EEG data were collected during 4 experimental conditions with 5 different objects: (1) *Object observation condition*: Each testing phase started with the presentation of a dangling object, moving back and forth in a non-goal-directed way. During this condition, the experimenter was hidden behind the white curtain at the other side of the table. This observation condition was used as a baseline condition based on the assumption that the subjects had no prior experience with the objects. Each following experimental condition was compared with this baseline condition. (2) *Action observation condition*: The experimenter demonstrated a simple goal-directed action (with an observable end-state) with each object and a white box (e.g., the loupe is taken and brought in a wave-movement to the other side of the box). The experimenter said: 'look <name child>' and made eye-contact with the child to ensure that the subject was attentive to the demonstration. In order to obtain enough EEG data, each action was demonstrated three times with the left hand and three times with the right hand. The starting hand was counterbalanced between the different objects. (3) *Action imitation condition*: After modelling the action, the objects were handed to the child who was asked to imitate the observed actions. Participants were encouraged (non)-verbally in a non-specific way to imitate and were given as much time as needed to perform the actions themselves. (4) *Hand movement condition*: Mimicked actions were demonstrated during this fourth condition. The experimenter executed hand movements identical as those during the action observation condition but now without the objects and without direct reference of gaze towards the child which makes this condition less social. Subjects were expected only to observe those actions, not to

imitate them. Similar as during the action observation condition, the hand movements were demonstrated 3 times with the left hand en 3 times with the right hand.

Each experimental session started with the object observation condition (baseline condition) for all 5 objects subsequently. The order of the other three experimental conditions was counterbalanced across subjects, with the limitation that the action imitation condition always followed the action observation condition so that the participants first observed what they had to imitate. The order in which the objects were presented remained the same for each participant. Each demonstrated action lasted about 30 seconds per object and resulted in a total duration of about 20 minutes for the entire session. After the EEG recording and the test phase, the parents were debriefed and received a gift card as reward for their participation. Finally, the parents were 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) and the Social Communication Questionnaire (SCQ; Rutter et al., 2003; Dutch translation by Warreyn et al., 2004) at home.

EEG data recording

EEG data were recorded relative to an average reference from 32 active Ag/AgCl electrodes placed according to the international 10-20 system (Jasper, 1958) embedded in a child-friendly stretch EEG-cap with a ground electrode placed at AFz (Easycap, Brain Products, GmbH, Munich, Germany). EEG was recorded by the use of an EEG-amplifier (QuickAmp) with a sample rate of 500 Hz, 1 s time constant, a low pass filter of 70 Hz, and a notch filter of 50 Hz. Horizontal electro-oculogram (HEOG) electrodes were placed at the left and right outer canthi of the eyes. Vertical EOG was calculated by comparing the recording of an electrode above the eye, at position Fp2, with the common reference. Initially VEOG was computed by comparing Fp2 with an electrode placed below the left eye, but many children did not tolerate this additional electrode. After comparing data with this electrode and the common reference method, no significant differences occurred between these two calculations. Inter–electrode impedance was measured and confirmed to be below 10 kΩ for all electrodes. To synchronize the EEG recordings and video recordings, a button was pushed at the beginning of each

experimental condition. This button sent a marker to the recorded EEG signal and simultaneously emitted a LED light which was visible on both video-cameras. Consequently, synchronization was possible by comparing the time intervals between the different markers on both recording systems.

Offline behaviour coding

After the experiment, the video recordings were coded offline with The Observer XT 9.0. (Noldus Information Technology, 2009). First, the subject's behaviour was coded by ascribing start and stop codes to each experimental condition and followed by coding attentive behaviour in the observation conditions, imitative behaviour in the action imitation condition, and vocalization and motor movements within each experimental condition. Further analyses were only based on the sections where the child was quietly attending the demonstrations (during the object observation, action observation, and hand movement condition) and was actually imitating during the action imitation condition. During this behaviour coding, fragments with too much motor and/or vocalization codes were excluded in order to minimize contamination of the EEG data. Obviously, it was impossible to exclude all these segments. Therefore, an additional exclusion of motor movements and vocalizations was performed afterwards through the artifact rejection procedure during the EEG analyses.

Furthermore, quality of imitative behaviour of each participant was coded by an observer who was blind for group membership. Three criteria were assigned for each performed action. The child received score 1 for every criterion he/she met. Afterwards, the mean of the best scores for each object was calculated which reflected the total quality of imitation score per child with a maximum score of 3. In the ASD group, the mean score was 2.34 (*SD* = .45) whereas in the control group, the mean score was 2.44 (*SD* = .39) which implies that both groups imitated 2 of the 3 criteria correct. A t-test revealed that the ASD group showed an equal performance as the TD group ($t(35) = .72$, $p = .476$) concerning quality of imitation. To asses inter-observer reliability, an independent coder double-coded 25% randomly selected videos which resulted in a Cronbach's Alpha Coefficient of .94 (Cronbach, 1951).

EEG data processing

Brain Vision Analyzer (Brain Products, 2007) was used for offline analyses of the recorded raw EEG data. Based on the assumption that mu rhythm is measured over the sensorimotor cortex (Marshall, Young, & Meltzoff, 2011; Muthukumaraswamy et al., 2004), EEG power data recorded from electrode positions C3 and C4 were further investigated. First, the raw EEG data were visually inspected to exclude contaminated signals due to artefact influences. Afterwards, EEG was re-referenced to the average reference with exclusion of the most disturbed electrode channels. EEG data were filtered with a high pass filter of 0.1 Hz, a low pass of 30 Hz, and a 50 Hz notch-filter. Furthermore, the Gratton and Coles algorithm correction was applied to correct for horizontal and vertical eye movements (Gratton, Coles, & Donchin, 1983). The remaining data were segmented in 1s-epochs with 50 % overlap. Bad segments were removed with artifact rejection using a maximal allowed voltage step of 100 µV per sampling point, a maximal allowed absolute difference of 400 µV between two values in the segment, and an activity of 0 µV during maximum 100 milliseconds. In this way, an average of 226.45 segments (*SD* = 100.66) per child per condition was left. Finally, Fast Fourier Transform was performed on the remaining segments with a Hanning window of 10 % and averaged for each experimental condition. The mu frequency band of interest was conducted by subtracting the baseline condition from the action imitation condition for each subject individually as has been performed in previous studies (e.g., Lepage & Théoret, 2006; Muthukumaraswamy et al., 2004). Furthermore, the individual mu frequency band was selected by calculating the 3-Hz interval around the highest peak value of that subtraction at the central electrode positions. The mean of the highest peak value was 8.58 Hz (*SD* = .67) in the total sample which is in agreement with previous studies on mu/alpha rhythm frequencies in infants (Marshall, Bar-Haim, & Fox, 2002; Stroganova et al., 1999).

The procedure of Oberman and colleagues (2005) was used to calculate the mu suppression values. To control for variability due to possible individual differences (e.g., scalp thickness or electrode impedance), we used a ratio to estimate the relative power for each condition. More specifically, the ratio of the power during the action observation condition, the hand movement condition, and the action imitation condition

respectively relative to the power during the object observation condition (baseline condition) was calculated. Since the ratio data were non-normally distributed, the log transform of each ratio was estimated. This resulted in a value representing mu suppression (i.e., a negative value), mu enhancement (i.e., a positive value) or no suppression (i.e., a value of zero).

RESULTS

Independent sample t-tests, to investigate if the order of condition presentation influenced mu wave activity, revealed no significant influence of counterbalancing in both groups, all .68 < $t(16)$ < .79, all p > .05 for the ASD group and .27 < $t(17)$ < .96, all p > .05 for the TD group. Therefore, the order of presentation of the conditions was not further included as a factor in the analyses.

Mu suppression

An overall 3x2x2 repeated-measures ANOVA was conducted with condition (hand movement, action observation, and action imitation) and hemisphere (C3 and C4) as within-subjects factors and group (TD and ASD) as between-subjects factor. Results revealed no significant main effects of group $F(1,35) = 1.38$, $p = .248$, condition $F(2,34) = 1.59$, $p = .218$ or hemisphere $F(1,35) = .99$, $p = .326$ and no significant interaction effects, with $F(2,34) = 1.59$, $p = .219$ for condition by group, $F(2,34) = 1.68$, $p = .202$ for hemisphere by condition and $F(1,35) = 1.36$, $p = .252$ for hemisphere by group. No significant 3-way interaction effect was found between condition, hemisphere and group, $F(2,34) = .61$, $p = .550$.

Furthermore, one sample t-tests revealed central mu suppression (i.e., mu suppression assembled over electrode positions C3 and C4) during the hand movement condition, action observation, and action imitation condition in both the ASD group and the TD group, with $t(17) = -3.99$, $p = .001$; $t(17) = -4.29$, $p < .001$; and $t(17) = -3.71$, $p = .002$ respectively for the ASD group and $t(18) = -4.02$, $p = .001$; $t(18) = -3.55$, $p = .002$; and $t(18) = -2.37$, $p = .029$ respectively for the TD group. The means and standard

deviations of the mu suppression at electrode positions C3 and C4 separately and averaged as overall central mu wave activity are presented in Table 2.

Table 2. *Mu suppression for both groups at each electrode position separately and assembled during each condition*

		ASD group (*n* = 18)		TD group (*n* = 19)	
		M (SD)	*t*(17)	*M (SD)*	*t*(18)
C3	HM	-.24 (.22) ***	-4.50	-.23 (.31) **	-3.17
	AO	-.24 (.19) ***	-5.19	-.16 (.19) ***	-3.78
	AI	-.24 (.37) *	-2.76	-.28 (.55) *	-2.19
C4	HM	-.20 (.33) *	-2.61	-.12 (.25) *	-2.08
	AO	-.23 (.32) **	-3.01	.01 (.16)	0.15
	AI	-.31 (.38) **	-3.49	-.23 (.45) *	-2.21
C	HM	-.22 (.23) ***	-3.99	-.17 (.19) ***	-4.02
	AO	-.23 (.23) ***	-4.29	-.08 (.10) **	-3.55
	AI	-.28 (.32) **	-3.71	-.25 (.46) *	-2.37

Note. ASD group = children diagnosed with ASD; TD group = typically developing group; C3 = mu suppression at electrode position C3; C4 = mu suppression at electrode position C4; C = mean central mu suppression assembled over electrode position C3 and C4; HM = mu suppression during the hand movement condition; AO = mu suppression during the action observation condition; AI = mu suppression during the action imitation condition.
*$p \leq .05$; **$p < .01$; ***$p \leq .001$.

Additional analyses of electrode activity recorded from an occipital electrode (Oz) were conducted to assure that the observed central suppression was related to the mu rhythm and not to posterior alpha activity. The total sample showed no mu suppression at Oz during action imitation in the frequency band under investigation, $M = .01$, $SD = .42$; $t(32) = 0.14$, $p = .889$. Furthermore, during action imitation, a paired sample t-test revealed significantly stronger central suppression ($M = -.26$, $SD = .39$) compared to occipital suppression, $t(32) = 2.84$, $p = .008$. Therefore, we can conclude that the observed mu suppression was specific to the central electrode positions and was not the result of overlapping occipital activity.

Relationship between mu suppression and imitation, chronological age and SCQ scores

Pearson's correlation coefficients were calculated to investigate the relations between central mu wave suppression on the one hand and quality of imitation, chronological age, and SCQ-scores on the other hand. Both the ASD group and the TD group showed no significant correlation between central mu suppression and quality of imitation with small correlations ranging from -.03 < *r* < .21, all *p* > .05 in the ASD group and -.07 < *r* < .04, all *p* > .05 in the TD group. In the ASD group, age correlated significantly with central mu suppression during the hand movement condition with a medium correlation of *r* = -.54, *p* = .020. Furthermore, both groups demonstrated no significant correlations with chronological age, reflected in small correlations of all -.23 < *r* < -.01, *p* > .05 in the ASD group and all -.01 < *r* < .09, *p* > .05 in the TD group. Central mu suppression during action observation in the TD group correlated marginally significant with SCQ scores with a medium correlation of *r* = -.44, *p* = .088. Central mu suppression during the other conditions in the TD group and during all conditions in the ASD group did not correlate significantly with SCQ scores, with small correlations between -.07 < *r* < .23, *p* > .05. See Table 3 for details.

Table 3. *Overview of Pearson correlations between central mu suppression and imitation, chronological age and SCQ scores*

	ASD group (*n* = 18)			TD group (*n* = 19)		
	Quality imitation (*r*)	CA (*r*)	SCQ (*r*)	Quality imitation (*r*)	CA (*r*)	SCQ (*r*)
CHM	-.020	-.541*	-.061	-.068	-.008	.090
CAO	.201	-.220	-.032	-.030	.078	-.440°
CAI	-.016	-.007	.111	.037	.085	.226

Note. ASD group = children diagnosed with ASD; TD group = typically developing group; CHM = mu suppression at central electrode positions during the hand movement condition; CAO = mu suppression at central electrode positions during the action observation condition; CAI = mu suppression at central electrode positions during the action imitation condition; CA = chronological age; SCQ = total score on the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003; Dutch translation by Warreyn et al., 2004).
°*p* < .10; **p* < .05.

DISCUSSION

The aim of the current study was to investigate neural mirroring during imitation and observation tasks in children diagnosed with ASD compared with chronological age-matched control children, all between 24 and 48 months old. Following the idea of broken mirrors in ASD, we expected less mu suppression during the observation conditions in the ASD group compared to the control group. As there is no evidence for impaired areas in sensorimotor cortex in ASD (Bernier et al., 2007), we expected (equally strong) mu suppression during the action imitation condition in both groups.

Concerning our first two research questions, results revealed significant central mu suppression in both groups during the observation of goal-directed actions and hand movements. The occurrence of mu wave suppression during the observation of non-goal-directed hand movements in both groups, suggests that the observation of motor movements alone without objects is sufficient to induce neural mirroring activity in children with and without ASD (Maeda, Kleiner-Fisman, & Pascual-Leone, 2002).

With respect to the third research question, both groups showed significant mu suppression during action imitation, as expected. Additionally, no differences were found between both groups regarding overall neural mirroring activity. The absence of a difference between groups regarding central mu wave activity is in line with the idea of an intact action observation/action execution matching system in children diagnosed with ASD and argues against the broken mirror hypothesis of ASD (Hamilton, Brindley, & Frith, 2007; Marshall & Melzoff, 2010; Oberman et al., 2008; Southgate & Hamilton, 2008). These results suggest that impaired mirror neuron functioning is unlikely to be the cause of ASD impairments.

To answer the fourth research question, Pearson's correlation coefficients were calculated and revealed that central mu suppression in both groups was not correlated with quality of imitation. However, if mirror neuron activity is related to imitation abilities as hypothesized (Rizzolatti & Craighero, 2004), we would expect significant correlations between these ability and neural mirroring in the present study. However, it is possible that our sample was not diverse enough to detect possible correlations. This can also be an explanation for the lack of significant correlations with chronological age.

Only the ASD group showed a significant correlation between age and central mu suppression during the hand movement condition. It is possible that the age range of 2 years within our participant group may be too small to detect significant correlations between age and mu suppression in the TD group and in the other conditions in the ASD group. Additional research is needed to replicate mirror neuron activity during observation and imitation tasks, over different time periods in individuals with ASD. This could give more information about stability or evolution of neural mirroring in ASD. Except for a trend between central mu suppression during action observation and scores on the SCQ in the TD group, no significant correlations were present in both groups. Although the ASD group scored significantly higher on the SCQ compared to the TD group, mu suppression did not correlate significantly with SCQ-scores in this clinical group.

Some limitations of this study can be mentioned. A first critical remark concerns the sample of the current study. Our sample of ASD participants excluded children with a severe developmental delay, which makes this sample not completely representative for the general ASD population. However, this study wanted to investigate neural mirroring in children with ASD, independently from developmental delay. Additionally, the sample size was rather small. It is possible that the small and medium correlations are related to the sample size of the investigated groups (Kareev, Lieberman, & Lev, 1997). Therefore, this study needs to be replicated with larger samples. Secondly, simple imitation tasks were used with clear instructions by which the participants were explicitly asked to imitate. However, it would be interesting to investigate neural mirroring in ASD during automatic imitation, without clear or explicit instructions. Additionally, imitation requires more than only mapping of observed visual information to execute motor output (Southgate & Hamilton, 2008). Other cognitive processes such as motor control or visual analyses are needed to perform correct imitative behaviour (Tessari & Rumiati, 2004). Therefore, it would be interesting to include different types of imitation tasks in future research about neural mirroring in ASD. In this way, it could be investigated if mirror neurons or other processes respond differently depending on the task variability. Finally, although the direction of the relation between neural mirroring and imitation is not clear (Southgate & Hamilton, 2008), we did not take into account if the children diagnosed with ASD followed therapy or intervention programs outside the

research project which could have influenced their imitation abilities because the present study revealed no significant difference between our ASD group and control group regarding quality of imitation performance. Additionally, it should be noted that the 2 children diagnosed with ASD who scored below the cut-off for ASD on the ADOS (Lord et al., 1999), demonstrated similar mu suppression as the other ASD children.

To conclude, as the 'broken mirror theory' of ASD is still debated, more research is needed in young children to understand the exact relationship between neural mirroring and imitation in ASD. Therefore, the present study investigated neural mirroring in young children diagnosed with ASD during the observation of goal-directed actions and hand movements and during action execution. Results revealed no evidence of impaired imitation and dysfunctional neural mirroring in ASD and can be added to the growing literature that challenges this broken mirror theory (e.g., Hamilton et al., 2007; Raymaekers et al., 2009).

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A boy who is observing the experimenter during the action observation condition and during the hand movement condition respectively

CHAPTER 6

EXPLORING IMITATION IN HIGH-RISK SIBLINGS AND TODDLERS WITH AUTISM SPECTRUM DISORDERS[1]

ABSTRACT

Imitation plays a fundamental role in social-communicative development and has widely been investigated in toddlers with autism spectrum disorders (ASD). However, findings about imitation in ASD are still debated so further exploration of this concept is needed. One way to explore imitation in ASD and in its broader phenotype (BAP) is investigating brothers/sisters of infants diagnosed with ASD. This study compared procedural and bodily imitation in siblings of children with ASD (Sibs-ASD group) with imitation performance in toddlers diagnosed with ASD (ASD group) and in toddlers without a family history of ASD (low-risk group) between 48 and 69 months old. Furthermore, it was examined whether imitation abilities were related to autism severity. Results revealed a significantly lower bodily imitation performance of the ASD compared to the Sibs-ASD group and the low-risk group. The Sibs-ASD group did not differ significantly from the low-risk group on bodily imitation performance. There were no significant group differences concerning procedural imitation. Autism severity only correlated significantly with procedural imitation in the Sibs-ASD group. Bodily and procedural imitation performance interrelated significantly in the ASD group and in the low-risk group but not in the Sibs-ASD group. The current research suggests that procedural imitation develops differently in ASD and its BAP.

[1] Based on Ruysschaert, L., Warreyn, P., & Roeyers, H. (2012). *Exploring imitation in high-risk siblings and toddlers with autism spectrum disorders.* Manuscript submitted for publication.

INTRODUCTION

Imitation plays an important role early in social-cognitive development (Ogawa & Inui, 2012). A commonly used description is 'the ability to replicate an observed novel action to achieve the same ends by using the same means' (Sevlever & Gillis, 2010; Tomasello, Carpenter, Call, Behne, & Moll, 2005). In other words, the imitator copies the observed means of the performer to achieve the same results (Tomasello et al., 2005). Although strongly debated (e.g., Anisfeld et al., 2001), several studies report that newborns can demonstrate facial imitation (e.g., tongue protrusion) already a couple hours after birth (Meltzoff & Moore, 1977). Meltzoff and Moore (2002) found evidence for imitation from memory after a delay of 24-hours in six-weeks old infants. Object imitation and deferred imitation were observed in 9-months old infants (Meltzoff, 1988). Additionally, around that age, infants start to recognize when they are being imitated by other individuals (Meltzoff, 1988). During the second year of life, imitation in children increases progressively (Young et al., 2011) and contributes to the development and learning of several social and behavioural characteristics (Rogers, Young, Cook, Giolzetti, & Ozonoff, 2010). Moreover, through imitation children spontaneously learn new skills which cannot always be taught by direct instructions of their parents (Vanvuchelen & Vochten, 2011). Therefore, a commonly accepted idea is that imitation plays a central role in social-communicative development and in social learning (Elsner, 2007; Ogawa & Inui, 2012). Furthermore, imitation makes social-cognitive understanding possible (Gopnik & Meltzoff, 1993) by helping the child developing the understanding of others' intentions (Uzgiris, 1981). Additionally, imitation is related to general cognitive and mental development. For example, research revealed that both procedural (i.e., object imitation) and bodily (i.e., imitation of facial and gestural expressions) imitation performance is predictive of later language skills (Carpenter, Akhtar, & Tomasello, 1998; Charman, 2003).

In general, we can conclude that imitation serves an important social-communicative and identity function suggesting that by imitation self-other understanding can be developed. In the light of these findings, it is assumed that impairments in imitation can lead to several social-communicative deficits often observed in developmental disorders such as autism spectrum disorder (Williams, Whiten, & Singh, 2004).

Imitation in autism spectrum disorder (ASD) has well been investigated since the work of DeMyer and colleagues (1972). The majority of studies found evidence for imitation deficits in object (e.g., Stone, Ousley, & Littleford, 1997), bodily (e.g., Roeyers, Van Oost, & Bothuyne, 1998; Stone et al., 1997), vocal (e.g., Sigman & Ungerer, 1984), or oral-facial (Rogers, Hepburn, Stackhouse, & Wehner, 2003) tasks in ASD compared to other groups. Furthermore, it is consistently found that infants with ASD score better on object imitation compared to bodily imitation tasks (e.g., Ingersoll & Meyer, 2011; Zachor, Ilanit, & Ben Itzchak, 2010). Additionally, Vanvuchelen, Roeyers, and De Weerdt (2011b) found that procedural imitation delay was the only significant predictor of developing later ASD. However, it has been suggested that this pattern is not unique to autism as it is also observed in typically developing and in developmentally delayed children (Stone et al., 1997). Williams and colleagues (2004) found that children with ASD often show an imitation deficit which is most apparent below the age of 4 and is mostly characterized by difficulties in imitating non-meaningful gestures and non-meaningful object-oriented tasks. Furthermore, research revealed that imitation in ASD is related to the overall developmental level (Rogers et al., 2003). Consequently, developmental abilities are important in the exploration and interpretation of imitation capacities in ASD with better imitation performance related to higher scores on mental and developmental tests (Wu, Chiang, & Hou, 2011). However, findings concerning imitation in ASD are controversial (for an overview, see Charman & Baron-Cohen, 1994; Williams et al., 2004). Imitation abilities in individuals diagnosed with ASD are not always described in a similar way due to the various definitions of imitation (Sevlever & Gillis, 2010) and the complexity and variability of symptoms within the autistic spectrum (Levy, Mandell, & Schultz, 2009). Consequently, research concerning imitation in ASD is still debated and needs further exploration.

As imitation plays in general an important role in reciprocal social communication, it is suggested that impaired imitation is one of the earliest signs of ASD and one of the core symptom deficits in ASD (Robins, Fein, Barton, & Green, 2001). This idea received much attention through the review of Rogers and Pennington (1991). In their paper, the authors postulated that impaired imitation in ASD can cause a cascade of several other social-developmental problems. Following the work of Rogers and Pennington (1991), Smith and Bryson (1994) reviewed another 15 studies and concluded

that imitation impairment in ASD is a secondary consequence of primary problems with perceptual functioning of movements which can cause abnormal action representations. This idea was supported by several studies that found no evidence for an overall imitation deficit in ASD (e.g., Beadle-Brown & Whiten, 2004; Stone et al., 1997). The review of Hamilton (2008) demonstrated that children with ASD may not show a global, simple imitation deficit of all actions but rather a more complex deficit limited to different action types. Additionally, Williams and colleagues (2004) and Stone and colleagues (1997) concluded that the ASD imitation deficit is a delay of normal development and of acquiring imitation skills rather than a stable deficit. This idea was also confirmed by several intervention studies which suggests that imitation abilities in ASD can improve through treatment. For example, Ben-Itzchak and Zachor (2007) found in their intervention study that children with ASD showed an improvement in imitation after behavioural intervention in a structured setting. Even children with severe ASD symptoms or lower cognitive abilities showed progress in imitation after teaching strategies. Due to its predictive relation, many behavioural interventions focus on imitation to facilitate the acquisition of other social-communicative behaviours such as language and play (Charman et al., 2003; Stone et al., 1997). Furthermore, imitation as important factor for early learning and social-communicative development can be taught by intervention in infants with ASD which can contribute to an overall positive outcome in ASD (Ben-Itzchak & Zachor, 2007).

Because findings about imitation in ASD are still debated and inconclusive, further exploration of this concept is needed (Wu et al., 2011). One possible way to learn more about the early development of imitation in ASD is to include high-risk siblings of children diagnosed with ASD. Investigating this group of infants is interesting to learn more about the concept of the 'broader autism phenotype' (BAP) which reflects various behavioural and brain characteristics qualitatively similar to those associated with ASD but milder than the diagnostic criteria of ASD (Rogers, 2009). This phenotype includes several developmental communication abnormalities and social difficulties which occur more often in first-degree relatives of infants with ASD (Ozonoff et al., 2011; Sucksmith, Roth, & Hoekstra, 2011). Some consensus about these features has been achieved but the exact boundaries of the BAP and the possible variation of these characteristics during development are still under discussion (Rogers, 2009). Therefore,

infant sibling studies can contribute in defining this BAP. This can influence the development of more accurate diagnostic criteria and early intervention strategies of children at risk for autism (Losh & Piven, 2007; Rogers, 2009). Similar difficulties as seen in ASD have been observed in previous sibling research (for an overview, see Yirmiya & Ozonoff, 2007). For example, problems with joint attention behaviour (Toth, Dawson, Meltzoff, Greenson, & Fein, 2007), motor functioning (Iverson & Wozniak, 2007), speech and communication (Toth et al., 2007), reduced affective responses (Cassel et al., 2007) and diminished eye gaze during social interactions (Merin, Young, Ozonoff, & Rogers, 2007) have been reported. Toth and colleagues (2007) found no significant differences in deferred and immediate imitation between unaffected siblings and control infants. Consequently, some researchers found a variable pattern with some impairments in social-communicative development and language together with intact skills on several other domains in unaffected siblings (Toth et al., 2007). However, to our knowledge, no research has been conducted directly comparing imitation performance in high-risk siblings with toddlers diagnosed with ASD.

In summary, findings of imitation in ASD are still inconsistent. Imitation serves an important function for later social learning and social-communicative abilities in early development (Elsner, 2007) and is assumed to be one of the core deficits in ASD (Williams et al., 2004). Therefore, it seems interesting to investigate this skill in unaffected young siblings (i.e., siblings at risk for ASD but without having the diagnosis of ASD themselves) to learn more about imitation in the BAP. This could lead to better insight concerning imitation development in the BAP early in development, independently from having the diagnosis of ASD. Exploring imitation skills in unaffected siblings could help to define the characteristics of this BAP in toddlerhood. Therefore, the present study aimed to investigate both bodily and procedural imitation in high-risk siblings of children with ASD compared with toddlers diagnosed with ASD and low-risk toddlers with no family history of ASD. In addition, it was investigated whether those two imitation types were interrelated in each participant group and whether they were related with autism severity. The present study used chronological age- and gender-matched toddlers as a control group. Matching on age and gender rules out the chance of misleadingly high estimates of the ASD performance.

MATERIAL AND METHODS

Participants

The sample consisted of 15 siblings of children with ASD (Sibs-ASD group), 19 toddlers with a diagnosis of ASD (ASD group) and 16 children with no family history of ASD (low-risk group). All children were between 48.20 and 68.60 months old (*M* = 55.57, *SD* = 4.89) and the total sample consisted of 32 boys and 18 girls. High-risk siblings were either enrolled in a larger ongoing longitudinal study of early social-communicative skills at Ghent University or were recruited through the Parent Association For Autism in Flanders. All siblings had at least one older brother or sister formally diagnosed with ASD but met no clinical ASD diagnosis themselves at the moment of testing. ASD subjects were recruited through Belgian Government certified University Clinics for Developmental Disorders and Autism and multiple treatment centres for developmental disorders. Assignment of the participants to the ASD group was based on a formal diagnosis made by a qualified and independent multidisciplinary team of specialists who were all familiar with autism spectrum disorders. The clinical diagnosis in our ASD group was confirmed in 68.4 % of the participants by the ADOS-G (Lord, Rutter, DiLavore, & Risi, 2003). All toddlers from the low-risk control group were recruited through Flemish day-care centres and magazine or website advertisements. Assignment of the participants to the low-risk group was based on a negative score on both the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003; Dutch translation by Warreyn, Raymaekers, & Roeyers, 2004) which screens for autism and on the ADOS-G scoring algorithm (Lord et al., 2003). All the participants in the high-risk sibling group scored negatively on the SCQ (Rutter et al., 2003; Dutch translation by Warreyn et al., 2004) and 11 of the 15 high-risk siblings scored negative on the ADOS-G (Lord et al., 2003).

Parental informed, signed consent was required for each subject and parents were asked to complete a questionnaire concerning socio-demographic information. Based on that questionnaire, the Hollingshead index score (Hollingshead, 1975) was calculated as measure of the socio-economic status (SES) with a mean social status in the total sample ranging from 20.50 to 64.50 (*M* = 47.22, *SD* = 12.06) which is an average

social status score (Cirino et al., 2002). The mean developmental index (MSEL; Mullen, 1995) for the current total sample was 100.40 (*SD* = 18.32) and the mean mental age in months was 54.98 (*SD* = 7.29). One-way analysis of variance (ANOVA) and a Pearson's chi-square test revealed no significant differences in age, SES, and gender between the three groups of participants, $F(2,47) = 0.09$, $p = .919$ for age, $F(2,35) = 0.73$, $p = .489$ for SES, and $\chi^2(2) = 4.19$, $p = .123$ for gender. The groups differed significantly on mental age, $F(2,41) = 15.95$, $p < .001$ and on the ADOS severity score, $F(2,47) = 8.23$, $p = .001$. Characteristics of the participants are summarized in Table 1.

Measures

Autism Diagnostic Observation Schedule-Generic (ADOS-G; Lord et al., 2003).

The Autism Diagnostic Observation Schedule-Generic is a semi-structured, interactive observation scale designed to measure social-communicative functioning and the severity of ASD symptoms in individuals who have or may have ASD. Module 2 (for infants who are using short sentences but no fluent speech yet) was used to evaluate all the individuals in the current study. Scoring results in two cut-offs, one for autism and one for ASD. Gotham, Risi, Pickles, and Lord (2007) developed a new scoring algorithm which results in an autism severity score ranging from 1 to 10 (Gotham, Pickles, & Lord, 2009). This revised algorithm was used in the present study to assess ASD symptom severity.

Mullen Scales of Early Learning (MSEL; Mullen, 1995).

The MSEL is a standardized developmental test evaluating the cognitive functioning of infants between the age of 0 and 68 months. The MSEL results in an Early Learning Composite score based on the sum of the subscale T-scores. Age equivalents were calculated for the subscales of interest. In the current study, subscales Fine Motor, Visual Reception, Expressive and Receptive Language were administered. The subscale 'Gross Motor' was excluded because this subscale only covers the age 0-33 months, which is younger than the tested participants of the current sample. The mean mental age was formed in this study by calculating the mean of the age equivalents on the four residual subscales.

Preschool Imitation and Praxis Scale (PIPS; Vanvuchelen, Roeyers, & De Weerdt, 2011c).

This standardized multidimensional imitation test assesses the accuracy of imitation in preschool children between 12 and 59 months of age. The test is composed of 30 items measuring both procedural (i.e., object imitation) and bodily imitation (i.e., facial and gestural imitation, without objects). The tasks were selected to cover a broad range of imitation processes and to avoid spontaneous performance of the actions. Furthermore, items are divided to assess meaningful and non-meaningful bodily imitation as well as goal-directed and non-goal-directed procedural imitation. The child's imitation accuracy is reflected in the total PIPS score. Furthermore, imitation age equivalents (AEs) from the PIPS scores, based on a normative sample of 654 typically developing children, can be calculated (Vanvuchelen, 2009).

General procedure

Participants and their parents were asked to come to the university lab where they were individually tested in a quiet room by one experimenter. During the experiment, one parent was allowed to stay in the test room, seated behind the child, but was asked to be as quiet as possible during the test phase. The other parent or other people present observed the experiment trough video-cameras in an adjacent room. The test laboratory (4m x 7m) was surrounded by curtains to minimize visual distraction and contained a small carpet with some toys, a small and a large table, several chairs and a highchair. During the administration of the ADOS-G (Lord et al., 2003), the participant and experimenter were sitting at a child friendly table with small chairs. The child was allowed to explore the toys lying on the ground near the table. The MSEL (Mullen, 1995) and PIPS (Vanvuchelen et al., 2011c) were administrated while the child was sitting in a highchair at a table opposite to the experimenter. Each experiment started with the free play task of the ADOS-G (Lord et al., 2003) to give the child enough time to familiarize with the experimenter and the environment, followed by the ADOS-G administration, the MSEL and the PIPS. Regarding the imitation test, actions were only modelled when the toddler was attentive to the model. During the warm-up play, the experiment procedure was described to the parent. All tasks were videotaped for later review and coding. A break between the different measures was provided when needed.

Table 1. *Subject characteristics*

	Sibs-ASD group				ASD group				Low-risk group				
	M	*SD*	*Range*	*n*	*M*	*SD*	*Range*	*n*	*M*	*SD*	*Range*	*n*	
CA	55.49	6.51	48-20-68.60	15	55.92	4.52	49.30-61.80	19	55.23	3.69	49.60-61.90	16	$F(2,47) = 0.09$
MA	58.50[b]	3.52	52.50-64.25	11	48.97[a]	6.87	35.75-61.75	17	58.94[b]	5.22	48.25-70.75	16	$F(2,41) = 15.95^{***}$
ADOS severity	2.33[d]	1.99	1-6	15	4.32[c]	1.97	1-8	19	1.94[d]	1.61	1-6	16	$F(2,47) = 8.23^{***}$
SES	47.32	11.94	24.50-64.50	14	43.91	14.39	20.50-60.50	11	49.92	10.17	29.50-63.00	13	$F(2,35) = 0.73$
Gender ratio M : F	11 : 4				14 : 5				7 : 9				

Note. Sibs-ASD group = siblings of children with ASD diagnosis; ASD group = children diagnosed with ASD; Low-risk group = control toddlers with no family history of ASD; CA = chronological age in months; MA = mental age in months; ADOS severity = symptom severity score measured with the Autism Diagnostic Observation Schedule module 2; SES = socio-economic status measured by the Hollingshead index score (Hollingshead, 1975).
Superscripts indicate significant differences on mental age and ADOS severity between the groups with a < b ($p < .001$) and c > d ($p < .01$).
$^{***}p \leq .001$.

RESULTS

Kolmogorov–Smirnov tests indicated a non-normal distribution of the variables bodily and procedural imitation with $D(50) = .16$, $p = .004$ and $D(50) = .26$, $p < .001$ respectively. Therefore Spearman rho correlation coefficients were calculated to explore a possible relationship between mental age and the dependent measures (i.e., procedural and bodily imitation score). A significant correlation was found between mental age and bodily imitation performance in the low-risk group ($\rho = .63$, $p = .009$). Furthermore, mental age tended to correlate significantly with bodily imitation in the ASD group ($\rho = .43$, $p = .087$). Therefore, following analyses were conducted with mental age as covariate.

A multivariate analysis (MANOVA) with group (Sibs-ASD, ASD, and Low-risk) as between-subjects factors, mental age as covariate and bodily and procedural imitation as dependent variables was conducted. Results revealed a nearly significant effect of group, with $F(4,78) = 2.15$, $p = .082$. Tests of between-subjects effects revealed a significant group difference concerning bodily imitation, $F(2,40) = 4.50$, $p = .017$ but not concerning procedural imitation, $F(2,40) = .65$, $p = .528$. The group differences regarding bodily imitation were explored in more detail.

Independent sample t-tests revealed that the ASD group ($M = 40.26$, $SD = 5.24$) scored significantly lower on bodily imitation compared to the low-risk group ($M = 47.44$, $SD = 3.86$), with $t(33) = 4.53$, $p < .001$ and compared to the Sibs-ASD group ($M = 46.73$, $SD = 4.95$), $t(32) = -3.66$, $p = .001$. The Sibs-ASD group demonstrated no significant difference on bodily imitation score compared to the low risk group, $t(29) = .44$, $p = .661$. The means and standard deviations of the scores on bodily and procedural imitation in the three groups of participants are presented in Table 2.

Furthermore, the relationships between imitation scores and ASD symptomatology measured by the ADOS severity algorithm were explored. Correlations were calculated using a nonparametric Spearman rho coefficient because the Kolmogorov–Smirnov test indicated a non-normal distribution of the ADOS severity variable $D(50) = .25$, $p < .001$. In the Sibs-ASD group, the ADOS severity score was significantly correlated with procedural imitation, with a large correlation of $\rho = .60$, $p =$

.017. Procedural imitation did not correlate significantly with ADOS severity score in this participant group, with a small negative correlation of ρ = -.28, p = .310. The ASD group demonstrated no significant small correlations between imitation score and ADOS severity with ρ = .09, p = .712 for bodily imitation and ρ = -.14, p = .556 for procedural imitation. Similarly, in the low-risk group, both bodily and procedural imitation performance did not correlate significantly with ADOS severity with small correlations of ρ = .08, p = .757 and ρ = -.11, p = .689 respectively. See Table 3 for an overview of the correlations.

Finally, Spearman rho coefficients were calculated to mutually compare bodily and procedural imitation performance in all three groups. No significant correlation was found between bodily and procedural imitation in the Sibs-ASD group with a small negative correlation of ρ = -.27, p = .333. Bodily imitation was significantly correlated with procedural imitation in the ASD group and the low-risk group with a medium positive correlation of ρ = .581, p = .009 for the ASD group and ρ = .513, p = .042 for the low-risk group. See Table 3 for details.

Table 2. *The means and standard deviations of the scores on bodily and procedural imitation in the three groups of participants*

	Group	*M*	*SD*
Bodily imitation	Sibs-ASD	46.73[b]	4.95
	ASD	40.26[a]	5.24
	Low-risk	47.44[b]	3.86
Procedural imitation	Sibs-ASD	19.40	1.55
	ASD	17.68	2.87
	Low-risk	19.44	1.21

Note. Sibs-ASD = siblings of children with ASD diagnosis; ASD = children diagnosed with ASD; Low-risk = control toddlers with no family history of ASD.
Superscripts indicate significant differences on imitation performance between the groups with a < b ($p \leq$ .001).

Table 3. *Overview of the Spearman correlations between ADOS-G scores and imitation scores and between bodily and procedural imitation performance mutually*

Group	Imitation score	ADOS severity score	Bodily imitation
Sibs-ASD group	Bodily imitation	-.281	-.269
	Procedural imitation	.604*	
ASD group	Bodily imitation	.091	.581**
	Procedural imitation	-.144	
Low-risk group	Bodily imitation	.084	.513*
	Procedural imitation	-.108	

Note. Sibs-ASD group = siblings of children with ASD diagnosis; ASD group = children diagnosed with ASD; Low-risk group = control toddlers with no family history of ASD; ADOS severity = total symptom severity score measured with the Autism Diagnostic Observation Schedule module 2.
*$p < .05$; **$p < .01$.

DISCUSSION

This study investigated imitation skills in high-risk siblings of children with ASD, compared with an ASD group and matched control toddlers without family history of ASD. The bodily and procedural imitation scores were compared between the three groups of participants. Results revealed significant group differences in bodily imitation with a significantly lower score in the ASD group compared to both the low-risk and Sibs-ASD group. The groups did not differ on procedural imitation scores which arguments against a general imitation deficit in ASD (Beadle-Brown & White, 2004). The majority of research found impaired performance on both imitation forms in children with ASD compared to typically developing children (e.g., Stone et al., 1997) and to developmentally delayed children (e.g., Roeyers et al., 1998; Stone et al., 1997). Vanvuchelen and colleagues (2011b) found bodily and procedural imitation problems in pre-schoolers with ASD but further analyses revealed that only procedural imitation performance was predictive of an ASD diagnosis.

Thus our ASD group demonstrated adequate procedural imitation performance which involves an object. However, imitating facial and gestural movements was

impaired compared to the Sibs-ASD and the low-risk group. Consistent with previous research, our results suggest that bodily imitation is more difficult for individuals with ASD than procedural imitation (e.g., Stone et al., 1997; Zachor et al., 2010). Despite their shared genetic material with their older brother/sister diagnosed with ASD, the high-risk sibling group showed equal imitation skills as the low-risk group on bodily and procedural imitation which is in line with the study of Toth and colleagues (2007). It should be noted that we investigated unaffected siblings, not diagnosed with ASD at the moment of testing. It is possible that some vulnerable factors in high-risk siblings are less present at younger age, but may become more noticeable as infants grow older (Warren et al., 2012). Therefore, it is important to follow this group of infants up to later age periods to investigate their imitation abilities.

Furthermore, correlations between ASD symptom severity and imitation abilities were explored to clear out if imitation can be a primary symptom in ASD and in the BAP. The Sibs-ASD group showed a significant correlation between procedural imitation skills and ADOS severity score. However, this correlation was positive, indicating the more ADOS symptoms, the better procedural imitation performance. This correlation seems illogical and should definitely be further investigated. No significant correlation between ADOS severity score and bodily imitation performance was found. Further research is needed to investigate the imitation skills in this high-risk group making more distinctions in imitation tasks with variation in meaning (e.g., meaningful goal-directed and non-meaningful non-goal-directed actions), type (e.g., object, body, vocal, facial) or consequences of the action (e.g., actions with and without sensory effect). Furthermore, ADOS severity score was not significantly correlated with imitation performance in the ASD group and in the low risk group which is contradictory with previous studies that found a strong correlation between imitation and autism severity. However, we did not control for developmental age as previous studies did, which may have influenced our findings (e.g., Rogers et al., 2003). Furthermore, imitation in ASD is characterized by individual variability (McDuffie et al., 2007). Further research is needed to investigate whether different imitation types in ASD are related with ASD symptom severity taking into account the individual variance in imitation performance in ASD.

Additionally, correlations between bodily and procedural imitation in all groups were explored. The Sibs-ASD group demonstrated no significant correlation between

bodily and procedural imitation performance. This suggests that both imitation forms develop separately in this high-risk group. Performance on both imitation types were significantly correlated in the low-risk group as well as in the ASD group. However, in the ASD group, a difference was found between bodily and procedural imitation capacities in favour of the latter one (e.g., Stone et al., 1997; Zachor et al., 2010). The finding of this correlation in the ASD group suggests that intervention focussing on one type of imitation (i.e., bodily or procedural) could influence the other type of imitation. However, some studies found that the imitation type where the intervention was focused on, improved more than the other type (Ingersoll & Meyer, 2011). This correlation and the relationship with intervention need further exploration. More specifically, future research is definitely needed to explore the findings in the high-risk group. Although procedural imitation is significantly correlated with ASD severity, bodily and procedural imitation are not significantly interrelated within this group. It should be interesting to investigate the relationship between both imitation forms and the scales of the MSEL (1995) and to compare this relationship between the different groups. It is possible that different developmental abilities are differently related with bodily and procedural imitation dependent on group membership.

Some limitations of this study can be mentioned. A first critical remark is the small sample size of each group which can limit our power for detecting significant results and limits generalizability of the findings. The three groups of participants were accurately matched on age, SES and gender, but the sample of ASD children contained no individuals with severe developmental delay, which makes the sample not wholly representative for the total ASD population. Additionally, the ADOS-G classification was negative in nearly one third of the toddlers in the ASD group. However, it is important to take into account that the sensitivity of the ADOS-G may vary across different centres and examiners and due to other factors (Gotham et al., 2007). Therefore, assignment of the participants in the present study was based on formal diagnoses for the ASD group. Supplementary, performing the analyses without the ASD participants with a negative ADOS-G score did not change the results. To conclude, bigger and more specific samples could reveal more about the distinctive features of imitation in ASD and its broader phenotype. Secondly, intervention information of the ASD group was missing. It is possible that some children followed therapy or intervention programs outside the

research project which could have influenced their imitation performance. Therefore, replications of the results with more participants tested in various contexts with different and counterbalanced imitation tasks in various matched clinical and comparison groups could offer more support. Finally, imitation tasks took place in a structured setting where the child's response was directly evoked by the experimenter. Imitation responses in ASD can differ depending on the interactive context with different attention demands and different opportunities for social interaction with the experimenter (McDuffie et al., 2007). The current study should be replicated in other contexts such as interactive play or during observational learning to investigate if imitation responses of the participants differ depending on different contextual factors.

In summary, we found weaker bodily imitation performance in the ASD toddler group compared to the Sibs-ASD group and the low-risk group. Furthermore, the groups did not differ on procedural imitation scores. Both imitation types were significantly interrelated in the ASD and the low-risk group but not in the Sibs-ASD group. Autism severity was only significantly correlated with procedural imitation in the Sibs-ASD group. These findings suggest that procedural imitation in the Sibs-ASD group and the ASD group develops differently from bodily imitation. It is possible that the BAP is characterized by a variable profile concerning imitation performance. Further research is needed with representative, bigger samples tested on diverse imitation tasks in different contexts.

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A girl who is performing 3 bodily imitation tasks

A girl who is performing 3 procedural imitation tasks

CHAPTER 7

GENERAL DISCUSSION

The present doctoral dissertation aimed to investigate imitation and neural mirroring in typically developing infants, infants with a diagnosis of autism spectrum disorder (ASD), and infants at risk for ASD (i.e., high-risk siblings). To this end, five empirical studies were conducted. This final chapter encloses a summary and a discussion of the main findings. Additionally, limitations are discussed and future directions for research are described. In conclusion, practical implications and recommendations are outlined.

RECAPITULATION OF THE RESEARCH GOALS AND MAIN FINDINGS

Imitation serves various social-communicative and cognitive functions in development (Ogawa & Inui, 2012). Although imitation and its underlying neurological processes in ASD have been well investigated, uniformity of the findings is still lacking (e.g., Jones, 2007; Paulus, Hunnius, Vissers, & Bekkering, 2011). Research concerning the underlying neural mechanisms of ASD and imitation has recently been influenced by the discovery of mirror neurons, which appear to be active both during action observation and action execution (Di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; Rizzolatti, Fadiga, Gallese, & Fogassi, 1996). Williams, Whiten, Suddendorf, and Perrett (2001) were the first researchers who postulated the idea that imitation problems often present in individuals with ASD could be caused by an impaired action observation/action execution matching system and impaired neural mirroring. Moreover, the dysfunctional matching between observation and execution of actions leads to difficulties to convert other's representations into one's own which makes imitation difficult (Williams, Whiten, & Singh, 2004). From then on, many other researchers investigated neural mirroring and its relationship with imitation in ASD (e.g., Hamilton, Brindley, & Frith, 2007; Oberman et al., 2005; Raymaekers, Wiersema, & Roeyers, 2009). The present doctoral thesis aimed to extend this existing research by exploring imitation and neural mirroring systems in typically developing children, in children with ASD, and in children at risk for ASD (i.e., high-risk siblings).

The first research goal of this doctoral thesis (*Chapter 2*) was to investigate whether neural mirroring responses in typically developing infants differ in a televised setting compared to a live setting. Therefore, central mu suppression, as an indication for neural mirroring activity, was measured through EEG recordings during the observation and imitation of the same goal-directed and mimicked actions presented either on television or live in 18-to 36-months olds. We found significant mu suppression during the observation of live goal-directed and mimicked actions which was not found during the observation of both these actions on a television screen. Additionally, mu suppression during the imitation of live actions was stronger than during the imitation of the televised actions. These results suggest a different impact of the televised versus the live presented actions on neural mirroring activity in typically developing infants and

imply the use of live actions in the design of paradigms to investigate neural mirroring in infants. Live actions seem to be more ecologically valid in the research on neural mirroring in infants. Therefore, the paradigm we used in the next empirical studies and chapters of this doctoral dissertation took these findings into account and included only live actions. In addition, we investigated mu suppression in typically developing infants between 18 and 30 months old during the observation and imitation of goal-directed actions and during the observation of mimicked actions (*Chapter 3*). Results revealed stronger mu suppression on the central electrodes during hand movement observation and imitation than over frontal and parietal sites. Mu suppression was equally strong during action observation over frontal, central, and parietal electrode sites. This research extends the existing evidence of an action observation/execution matching system in typically developing infants as during all three conditions, significant mu suppression was found over central and parietal electrode sites.

Our second research goal (*Chapter 4 and 5*) was to explore neural mirroring in children at risk for ASD (i.e., high-risk siblings) and in children diagnosed with ASD. In *Chapter 4*, neural mirroring was investigated in high-risk siblings between 18 and 36 months old during observation and imitation tasks compared with a low-risk control group. No difference was found concerning neural mirroring between the high-risk sibling group and the low-risk control group. Both groups showed equally strong central mu suppression during the observation and imitation tasks. These results do not support the hypothesis of impaired neural mirroring as a distinctive neurophysiological characteristic of the broader autism phenotype. The EEG study reported in *Chapter 5* investigated neural mirroring in children with ASD between the ages of 24 and 48 months compared with a typically developing control group. Both groups demonstrated significant central mu suppression during the observation and execution of goal-directed actions and during the observation of hand movements. The lack of differences concerning neural mirroring between both groups challenges the broken mirror theory of ASD, suggesting that impaired neural mirroring is not a distinctive feature of ASD.

Finally, our third research goal was to examine procedural and bodily imitation performance in high-risk siblings compared to toddlers with ASD and low-risk toddlers without a family history of ASD, all between 48 and 69 months old (*Chapter 6*). The toddlers with ASD performed significantly lower on bodily imitation compared to the

high-risk sibling group and the low-risk group. The high-risk siblings did not differ from the low-risk group with respect to bodily imitation. Furthermore, procedural imitation performance did not differ between the three groups. Additionally, it was examined whether imitation performance was related with ASD severity. In our study, ASD severity only correlated significantly with procedural imitation in the high-risk sibling group. Finally, bodily and procedural imitation performance interrelated significantly in the ASD group and in the low-risk group but not in the high-risk sibling group. This research suggests that procedural imitation develops differently in ASD and in its broader phenotype (BAP).

COVERING CONCLUSIONS AND DISCUSSION

Top-down versus bottom-up vision of imitation

This doctoral dissertation mainly focused on the relationship between neural mirroring and imitation by which mirror neurons evoke an automatic understanding of the observed action due to their functional role of action and intention understanding (Chapter 2, 3, 4, and 5). However research demonstrated different patterns of relationships between social-communicative abilities and different imitation types which suggests that imitation is more than just a singular concept influenced by different processes and mechanisms (Ingersoll & Meyer, 2011). Additionally, in imitation research, it is important to consider the distinction between imitation as an automatic process on the one hand and imitation as mechanism for social learning, mediated by cognitive processes on the other hand (Southgate, Gergely, & Csibra, 2009). The latter one requires more complex processes during the decision to execute similar as the model whereas automatic imitation is an unconscious matching process between individuals. The imitation tasks that were applied in this doctoral dissertation were complex imitation forms, demanding cognitive processes. Therefore, investigating imitation and its underlying neurological mechanisms should take this differentiation into account.

Two main arguments have been mentioned against the idea that imitation is solely based on mirror neuron activity. First, monkey mirror neurons only respond to

object-directed actions whereas human mirror neurons respond to both object- and non-object-directed actions (Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995). Second, imitation in humans, defined as copying a novel action by understanding the performer's intention, is more than the basic imitation capacities observed in monkeys. Monkeys demonstrate more observational learning and a form of emulation, i.e., copying the goal but not the motor part to reach the goal by behaving in the same way as conspecifics (Gallese, Gernsbacher, Heyas, Hickok, & Iacoboni, 2011; Wohlschläger & Bekkering, 2002). In this case, monkeys use the observed visual input to perform an analogous action by which the mirror neurons make them ready to imitate. These findings lead to the suggestion that mirror neurons can be related to imitation however these neurons are not sufficient to explain imitation abilities. Other top-down visual, motor and cognitive processes may be active during imitation and may regulate imitation (Gallese et al., 2011). Consequently, an alternative hypothesis could be that the impaired imitation often found in ASD is the result of an impaired modulation of the processes to imitate caused by deficits in several top-down processes, rather than the result of single bottom-up neurological mechanisms. An imbalance of these higher-order processes can cause the ASD impairments (Hamilton, 2008). Support for this idea comes from research that found difficulties in imitation-inhibition tasks in individuals with ASD, suggesting problems with mechanisms needed to control imitation (Spengler, Bird, & Brass, 2010). Additionally, functional and behavioural research in typically developing individuals revealed that during imitation different neural mechanisms are activated, dependent on the content of the action or the context. More specifically, new, meaningless direct action imitation requires activation of areas belonging to the dorsal stream whereas meaningful, familiar action imitation is associated with areas in the ventral stream as semantically stored knowledge is needed (Rumiati et al., 2005). This provides evidence against only a simple direct match between action observation and imitation. Imitation performance in infants with and without ASD should therefore be approached from a broad view, taking into account not only the underlying neurological mechanisms as the only explanatory factors, but also other top-down processes which could influence the imitation performance. Consequently, the top-down vision should be combined with the bottom-up vision. The top-down vision looks at the behavioural characteristics of ASD to understand the cognitive impairments. The bottom-up vision investigates the underlying neurological processes of those cognitive functions (Williams, 2008). Both visions are

linked and should be used together in the research on imitation in ASD instead of focusing on only one vision and excluding the other vision.

Imitation and neural mirroring : What about the ontogeny ?

Despite the debate about the exact role of mirror neurons in imitation, there is evidence that neural mirroring mechanisms are related to imitation (see Williams et al., 2001 for a review). However, the direction and the characteristics of the relationship between these two processes are still debated. More precisely, it is still unclear when and what the exact influence is of mirror neurons during imitative processes (Gallese et al., 2011). Until now, the controversy about the ontogeny of mirror neurons is still unresolved. Little is known whether mirror neuron functioning is the result of learning processes or whether these neurons are genetically pre-programmed ('innate') to some degree (Del Guidice, Manera, & Keysers, 2009). Recently, Ferrari and colleagues (2012) investigated EEG recordings in newborn macaques from 1- to 7-days old during the observation and execution of facial expressions. These macaques demonstrated suppression of 5-6 Hz EEG activity during the observation and execution of biological communicative expressions but not during the observation of non-biological stimuli, which suggests that some mechanisms of the mirror neuron system are already active during the first days after birth. To date, Shimada and Hiraki (2006) showed the earliest evidence of neural mirroring in humans. In their study, 6-months old infants demonstrated the presence of a matching system between action execution and observation using near infrared spectroscopy.

Some researchers suggest that neural mirroring is an innate process based on the assumption that newborns can imitate (Lepage & Théoret, 2007; Meltzoff & Moore, 1977). Because newborns do not have the opportunity to observe the same actions in others, neonatal imitation could be the result of an inherited mirroring mechanism transforming the observed visual action into the infant's own motor pattern (Jones, 2009). However, the finding of neonatal imitation is controversial as research suggests that neonates only match tongue protrusion and that this is more a non-specific arousal mechanism, rather than specific imitation (Jones, 2009; Ray & Heyes, 2011). Thus,

research concerning neonatal imitation does not provide compelling evidence that neural mirroring is an innate process.

The genetically inherited capacity to match observed actions with executed actions is also explained by the adaptation theory which acknowledges both genetic and experiential contributions (Heyes, 2009). This theory postulates that action observation/execution matching is innate and that sensory and motor experiences can only trigger this innate process. Contradictory to this theory, Catmur, Walsh, and Heyes (2007) concluded from their study that mirror neurons become functional as a result of correlated sensorimotor experience with action observation and execution. In this light, human mirror neuron functioning is both the result and the process of social interaction and learning by which neural mirroring develops gradually through exposure to performed actions. By learning to correlate observation and execution of actions in the sociocultural world, people develop and influence their mirror neuron functioning. These authors do not support the idea of an innate mirror neuron functioning in humans as the plasticity of neural mirroring is demonstrated by reversed mirror neuron responses as a result of incompatible sensorimotor training (e.g., Catmur et al., 2007). Additionally, if sensorimotor experience is both the result and the source of social interactions, developmental disorders such as ASD, characterized by social impairments, will be related with impaired mirror neuron functioning. This sensorimotor learning hypothesis can be used as an explanation for reduced mirror neuron functioning often found in ASD as the deficits in social interactions can limit these individuals to have sensorimotor experience with observed and executed actions resulting in impaired mirror neuron functioning (Catmur et al., 2007). Beside this sensorimotor experience, motor and sensory experience can also influence the development of neural mirroring. By performing actions and by seeing actions, mirror neurons can become functional (Gallese, Rochat, Cossu, & Sinigaglia, 2009). This flexibility of the mirror neurons has also been demonstrated by the possibility of controlling the automatic mapping process. It is observed that mirror neurons are active during execution but inhibited during observation of actions performed by others which is the result of controlling the automatic mapping responses (e.g., Mukamel, Ekstrom, Kaplan, Iacoboni, & Fried, 2010). This inhibition indicates the possibility to control the shared representations and to make a self-other distinction (Brass, Ruby, & Spengler, 2009).

Instead of investigating which of both aforementioned theories is the right one, maybe both theories can be used together. It is possible that there exists some underlying genetic predisposition in newborns to display mirror neuron activity (for example visible in imitation capacities reported after birth; Meltzoff & Moore, 1977) but that by learning and experience as infants develop, mirror neuron functioning becomes more delineated, fine-tuned and specific. According to this view, the development of mirror neuron functioning can be seen as a process and a product whereby the rudimentary mirror neurons are modulated through motor experience and visual learning during observation of the own actions and actions performed by others (Marshall & Meltzoff, 2011). For example, van Elk, van Schie, Hunnius, Vesper, and Bekkering (2008) found that the neural mirroring in 14- to 16-months old infants during the observation of walking or crawling was related to their own personal experience with these motor movements.

More neurophysiological research combined with behavioural research is needed to resolve this controversial and speculative debate and to learn more about the bidirectional influences between brain and behaviour and additionally about the directionality of the relationship between neural mirroring and imitation (Kanakogi & Itakura, 2010; Marshall & Meltzoff, 2011). Linking neurological and behavioural deficits related to ASD can lead to the development of profound theories explaining ASD and its impairments.

One mirror neuron system or different neural mirroring systems?

Iacoboni (2005) suggested the presence of a 'mirror neuron system' in humans formed by the posterior inferior frontal gyrus, the ventral premotor cortex, as well as the rostral inferior parietal lobule. Research revealed that goal-directed imitation (Iacoboni et al., 1999; Koski et al., 2002), motor planning (Johnson-Frey, Newman-Norlund, & Grafton, 2005) and observation of hand movements (Lotze et al., 2006) all rely on those particular brain regions. However, the neurons present in these areas are only one sort of neurons with mirror properties as the idea of one single human mirror neuron system is strongly debated (e.g., Heyes, 2009). Research revealed that the abilities mediated by mirror neurons differ according to the location of the mirror

neurons (Fabbri-Destro & Rizzolatti, 2008). For example, mirror neurons situated in the insula and the rostral cingulate are related to empathy (Wicker et al., 2003). Thus, various regions that process motor and sensory information can have different mirror properties and can be related with diverse functions and abilities (Catmur, Mars, Rushworth, & Heyes, 2011). Therefore, instead of one single mirror system, the possibility was raised that multiple cells with mirror properties and diverse neural mirroring mechanisms are present in the human brain which enables integration and differentiation of perceptual and motor information coming from actions executed by self and others (Mukamel et al., 2010). This idea is in line with the 'Associative Sequence Learning' (ASL) theory which suggests that any neuron can obtain mirror properties through association learning between observation and execution of an action in a contingent manner (Heyes, 2009). Sensory and motor representations of one action are connected. This connection can be accomplished by being imitated, during the observation of others' actions or during engaging in synchronous interactions with others (Catmur et al., 2011).

Marshall and Meltzoff (2011) supported this vision and suggested the use of the term 'neural mirroring systems' which supports the idea of a human neural circuitry instead of one single system as present in monkeys. During this dissertation, we used this term in order not to narrow our vision to one single system in humans. However, in the studies reported in this doctoral thesis (Chapter 2, 4, and 5), mu suppression was investigated only from central electrode sites (more particularly C3 and C4) based on the assumption that mu rhythm is measured over the sensorimotor cortex (Marshall, Young, & Meltzoff, 2011; Muthukumaraswamy, Johnson, & McNair, 2004). An additional argument for considering only the recordings from central electrodes is that including more electrodes that do not overlap with neural mirroring areas can cause disturbing noise to the recorded data (Nyström, 2008), which was also the case in our research. In this doctoral project, additional analyses were performed to assure that the observed central suppression was related to the mu rhythm and not to posterior overlapping alpha activity caused by visual attention or cognitive load (Chapter 2, 3, 4, and 5). However, as Marshall and Meltzoff (2011) discussed, it could be interesting to investigate the scalp topography during observation and execution measured by a range of electrodes across the scalp but with the constraint to control that the measured EEG suppression is mu rhythm suppression and not the result of other processes and/or

overlapping activity. This could also teach more about the whole neural mirror circuitry and the specific functions of each area. This was only conducted in Chapter 3 where we found that central mirror neuron activity was stronger compared to frontal and parietal activity during imitation and hand movement observation. During the observation of goal-directed actions, neural mirroring activity was equally strong over central, frontal, and parietal electrode sites. This suggests that neural mirroring activity can be observed at different electrode sites. Therefore, a thorough investigation of different areas with mirror neuron properties during different tasks can reveal more about the specific functions of those neural mirroring areas. It should be clear that in humans, there is more evidence for different neural mirroring areas than for the presence of just one single mirror system as observed in monkeys.

Lateralization

Lateralization during imitation has been investigated in several EEG studies. Analyses of our data revealed no significant lateralization effect in typically developing infants, high-risk siblings and children diagnosed with ASD which supports the idea of bilateral activation of the mu rhythm during both observation and execution tasks in infants with, without and at-risk for ASD. In general, inconsistent results are reported in infant studies. For instance, in de study of Southgate, Johnson, Osborne, and Csibra (2009), infants demonstrated bilateral mu rhythm activity during action execution and left-hemisphere dominance during action observation. However, Marshall and colleagues (2010) found bilateral activity during both observation and execution tasks which is in line with our findings. This inconsistency is also found in adult literature. For example, Muthukumaraswamy and colleagues (2004) found bilateral mu suppression during the observation of goal-directed actions whereas Perry and Bentin (2009) found mu suppression over the hemisphere contralateral to the observed hand.

In ASD research, although abnormal brain lateralization is an interesting topic, findings are inconsistent mostly suggesting atypical functional brain asymmetries in ASD (Stroganova et al., 2007), which is not supported in our research. In typically developing infants and children, left-hemispheric predominance is often reported, related to right-handedness (Stroganova, Pushina, Orekhova, Posikera, & Tsetlin, 2004), whereas left-

handed persons show a contralateral, more symmetric mu rhythm response (Stancak & Pfurtscheller, 1996). Although the majority of the participants in our research were right-handed (i.e., 69%), the normal left-hemispheric predominance was not found. In literature, manual motor imitation tasks have been associated with left hemisphere dominance of the mu rhythm (Dawson, Warrenburg, & Fuller, 1985). Adversely, right hemisphere dominance was explored during tasks requiring body awareness and visual-spatial discrimination (Perry & Bentin, 2009). Therefore, the bilateral activity found in this doctoral dissertation can probably be explained by the used tasks in our studies. The observation and imitation tasks involved a manual motor component and required visual-spatial discrimination as the observer was asked to imitate in a non-mirror way and to perform exactly the modelled actions. It is assumed that lateralization effects can be dependent on the number of electrodes used in data collection (Francuz & Zapala, 2011) or changes according to development from infancy to adulthood (Crone et al., 1998). This should be taken into account when interpreting lateralization effects.

Broader autism phenotype

The broader autism phenotype (BAP) entails that ASD characteristics are often observed in brothers/sisters of children diagnosed with ASD. The research of our doctoral dissertation investigated younger unaffected siblings to consider if characteristics concerning imitation and neural mirroring similar as in the ASD group could be observed. We found no evidence for the presence of similar behavioural (i.e., imitation) and brain (i.e., neural mirroring) characteristics in our sibling groups. The unaffected siblings demonstrated equal neural mirroring activity as the typically developing group during observation and imitation tasks (Chapter 4). Additionally, they did not differ on procedural imitation compared to ASD toddlers and a low-risk group and on bodily imitation compared to the low-risk control group (Chapter 6). This suggests that both imitation and neural mirroring are not distinctive characteristics of the BAP. Investigating these behavioural and brain functioning in younger siblings in children with ASD will probably not reveal more about the development of ASD in this group of infants. However, it should be noted that procedural and bodily imitation performance were not interrelated and that procedural imitation was correlated with

ASD symptom severity in this high-risk group, which was not the case in the low-risk group and in toddlers diagnosed with ASD. Although no significant group effects were found, these results indicate that these unaffected siblings slightly differ in imitation abilities. This should definitely be further explored in more detail.

Broken mirror model or EP-M model for imitation in ASD?

To conclude, the exact role of mirror neurons in ASD is still unclear and raises a lot of debate. However, our results suggest that children at risk for ASD (i.e., high-risk siblings) and children with ASD are capable of using the same neural mirroring mechanisms as low-risk children during action observation and during goal-directed imitation tasks which does not support the idea of the broken mirror theory. In addition, infants with ASD demonstrated an equal performance on goal-directed object imitation (Chapter 5) and procedural imitation (Chapter 6) compared to a typically developing control group but scored lower on bodily imitation (Chapter 6). Furthermore, all the children with ASD who participated in this doctoral research had a formal diagnosis of ASD and except for two participants in Chapter 5 and 31.6 % in Chapter 6, the diagnosis was confirmed with the Autism Diagnostic Observation Schedule (ADOS; Lord, Rutter, DiLavore, & Risi, 2003) as the ASD children scored above the cut-off for ASD (Chapter 5). This means that the diagnostically observed social-communicative dysfunctions in the infants with ASD cannot be explained by impaired mirror neuron functioning. Additionally, the broken mirror theory of ASD includes the impairment of one single and total neural mirroring system. However, behavioural studies demonstrated various findings about imitation impairment in ASD which are not in accordance with one single broken mirror theory but rather support a multiregional mirror neuron network (Wan, Demaine, Zipse, Norton, & Schlaug, 2010).

With this idea in mind, Hamilton (2008) proposed an alternative model for the broken mirror hypothesis. The new model tries to explain the occurrence of different types of imitation behaviour in ASD which is in accordance with our research findings. The 'EP-M' model consists of 3 routes, one indirect *Emulation* (i.e., using the visual information of the observed action to infer the goal or meaning of the action) and *Planning* route (i.e., planning an action based on that goal) and one direct *Mimicking* route (i.e., an immediate association between the visual representation of the observed

action and the motor representation). By partitioning this process, activation of different regions can explain different imitation outcomes in ASD. Moreover, this model for ASD suggests an intact EP route to emulate an observed action if the goal of the action is clear and an abnormal M route which reduces spontaneous imitation of facial expressions and meaningless gestures. The assumptions of this theory are confirmed in our findings of intact procedural and impaired gestural imitation performance in children with ASD compared to typically developing toddlers (Chapter 6). Maybe this model of Hamilton (2008) is a better fitting model for our results and findings than the broken mirror model.

LIMITATIONS AND IDEAS FOR FUTURE RESEARCH

Several limitations of the studies were formulated in each of the previous chapters of this dissertation. In the current section, more general limitations of this research project are summarized and suggestions for future research are given.

A first limitation of this doctoral thesis refers to the samples used in the different studies. Overall, because EEG research in young infants is difficult to perform, relatively small sample sizes were used which could reduce the statistical power to find group differences. However this could not explain the occurrence of significant mu suppression as found for example in Chapter 4. The rather small sample sizes could also influence the distribution of correlations (Kareev, Lieberman, & Lev, 1997). It is possible that medium or strong correlations are not significant in this doctoral research due to these small sample sizes.

Another weakness of our study concerning the sample is the use of only high-functioning ASD infants (Chapter 5 and 6) which makes generalizability over the total ASD population difficult. A significant proportion of individuals diagnosed with ASD are lower-functioning persons. However, performing research and in particular neurological methods with this latter population is quite challenging due to several reasons. Firstly, brain imaging studies (such as EEG) demand some skills of the participant such as attention, comprehension of the instructions, being calm and sitting still or cooperative behaviour. These skills are often impaired in low-functioning individuals with ASD. Secondly, research with low-functioning individuals with ASD requires comparison with

matched control participants. This means cognitively impaired individuals to exclude cognitive capacities as possible explanation for the findings. However, recruiting this comparison group and performing brain imaging studies with these groups is rather difficult. More research is definitely needed, with bigger and more appropriate sample sizes, compared with profoundly matched control groups.

Finally, it is possible that a great number of the participating families were more likely to refer themselves to the sibling study if they already had some concerns about their infant (Ozonoff et al., 2009). However the results of our study show that even those siblings demonstrate no impaired neural mirroring (Chapter 4) and imitation performance (Chapter 6). Furthermore, we labelled this group as 'unaffected' siblings as they had no ASD diagnosis themselves at the moment of testing, which is the focus of most studies investigating characteristics of the BAP (Sucksmith, Roth, & Hoekstra, 2011). Concerning the high-risk sibling participants, we did not take into account the difference between simplex and multiplex families. A simplex family has only one child with an ASD whereas a multiplex family has more than one child with an ASD (Schwichtenberg, Young, Sigman, Hutman, & Ozonoff, 2010). It is suggested that ASD features are more assembled in multiplex families compared to simplex families (e.g., Constantino, Zhang, Frazier, Abbacchi, & Law, 2010). Further research needs to investigate the possible difference in mirror neuron activity by including siblings from simplex families compared to siblings from multiplex families. It is possible that this family affectedness has its impact on the genetic and neurodevelopmental constitution in ASD and its BAP.

Studies investigating imitation capacities in infants (with and without ASD) differ in the methodologies they use. For example, imitation tasks can be accompanied by different phrases and suggestions like "do this" or "your turn" (Wu, Chiang, & Hou, 2011). In the studies discussed in this dissertation, the latter phrase was used. The child was not provided with a cue, which is the case when the experimenter says "please do this". By saying "your turn", the suggestions towards the participant are reduced (Wu et al., 2011). However, using the instruction of "your turn" entails a subtle social cue to derive which part of the action needs to be copied. In contrast, the use of these more 'objective' phrases can affect the imitation performance of the participants. In our research, it is possible that the younger infants did not understand what was expected

of them (which we observed occasionally during the experiments). When this was the case, the experimenter encouraged the participant more directly to imitate. However, this was a distinctive element between the different participants which could have influenced the results of the imitation tasks conducted in the studies of this dissertation. Therefore, automatic imitation tasks, where the participants are not instructed to imitate, are a more direct measure of the observation/execution matching system because during these tasks cognitive processes are minimized (Gallese et al., 2011). In the future, research concerning neural mirroring during imitation could use automatic imitation tasks instead of instructed imitation tasks to discover the underlying action observation/execution matching system.

Furthermore, the neurophysiological method used in the research of this doctoral thesis is electroencephalography (EEG) because it is a child friendly, non-invasive method which demands only minimal restrictions on the normal behaviour of the participant (Stapel, Hunnius, van Elk, & Bekkering, 2010). However, the low spatial resolution of EEG makes it difficult to exactly differentiate activity in the core premotor neural mirroring areas (i.e., posterior inferior frontal gyrus, the ventral premotor cortex, as well as the rostral inferior parietal lobule) from activity in the broader network of mechanisms with mirror properties (Oberman et al., 2005). Therefore future research should take this into account by using techniques with higher spatial resolution such as functional Magnetic Resonance Imaging (fMRI) or high-resolution EEG to clarify the unresolved issues as the integration of both methods can shed further light on neurological mechanisms (Arnstein, Cui, Keysers, Maurits, & Gazzola, 2011). Learning more about the underlying neurological risk factors and processes can create a better understanding of the behavioural characteristics of ASD (Oberman et al., 2005; Raymaekers et al., 2009). However, there is still a need for better techniques to investigate those processes in very little infants and low-functioning individuals who are impaired in skills often required to participate or cooperate in brain imaging studies (such as being attentive, no talking, ..).

Additional possibly disturbing factors should be taken into account during brain imaging studies. For example, a possible way to control for latent muscle movement during EEG recordings is the use of electromyography or of an eye tracking system to control for attentive processes and/or eye movements (Marschall & Meltzoff, 2011). It

should be considered to use these techniques during brain imaging studies in infancy and toddlerhood. Additionally, concentration and attention to the presentation of the stimuli is important during neurological research. In the studies of this dissertation (Chapter 2, 3, 4, and 5), attentive behaviour was evaluated by a coder using The Observer XT 9.0. (Noldus Information Technology, 2009). Each participant was instructed to focus on the experimenter and the modelled actions, but it could be argued that this coding is more or less subjective. A more profound and exact method to control for attention is including an additional attention task during observation (for example a counting task) which does not draw the attention away from the stimuli processing. This additional control task could be used in a televised paradigm, but is probably more difficult to include in live paradigms. However, future research investigating neural mirroring responses during observation tasks should consider the use of an objective and exact estimation of the attentive behaviour of the participant such as the use of eye-tracking measurements. Additionally, as it is instructive to connect behavioural, cognitive and neuropsychological findings about neural mirroring and imitation in infancy, future research on neural mirroring should take the top-down influences from social, cognitive, perceptual, and contextual factors into account instead of only focussing on the neurological processes (Marshall & Meltzoff, 2011). Therefore, neurological measurement techniques should be complemented with several cognitive tasks, questionnaires, and other behavioural methods and additionally overlook the context in which the measures took place as this can influence the results as well.

Research in ASD is often confronted with some challenges. A general but mayor difficulty is the heterogeneity of ASD. Multiple causes, symptoms, severity and descriptions are associated with the disorder which makes it unlikely that only one model could explain the diversity of the symptoms and causes of ASD (Dawson, 2008; Wing, 1997). Furthermore, ASD often occurs with co-morbid disorders (e.g., chromosomal disorder, intellectual impairment,..) which impedes generalizability (Leyfer et al., 2006; Sucksmith et al., 2011). Due to this heterogeneity of ASD, many models are proposed to explain the core characteristics of ASD (Schroeder, Desrocher, Bebko, & Cappadocia, 2010). One of those models is the mirror neuron system model, which was the focus of this dissertation. However, it should be noted that this model is related to other models such as the theory of mind model (Schroeder et al., 2010). These different

explanatory models try to explain distinctive predictions of brain processes involved in the development of ASD and cannot be easily separated from each other. The mirror neuron system model is a recently developed model and needs further exploration to be evaluated and to be included in the domain of possible explanatory models of ASD (Schroeder et al., 2010). Additionally, as our research found no evidence of impaired mirror neuron functioning during observation and imitation tasks in children with ASD (Chapter 5), other models should be taken into account. As individuals with ASD often demonstrate problems with abilities such as empathy, theory of mind, and other social-communicative functions, other neural models and systems such as the superior temporal cortex, the amygdala, the limbic system, and the insula, suggested to be part of the broader neural network of these abilities, should be investigated to help explaining the neural basis of ASD (Carr, Iacoboni, Dubeau, Mazziotta, Lenzi, 2003; Siegal, & Varley, 2002). It is possible that weaker connectivity between the neural mirroring network and other brain areas can explain the ASD symptomatology as the evidence for only impaired mirror neuron functioning as single cause for ASD is lacking.

Finally, if mirror neurons play a substantial role in abilities (such as imitation, language, empathy,…) which can be impaired in some disorders, it seems relevant to know which mechanisms can be adjusted or treated to minimize the impairments. As imitation skills develop as individuals grow older, it seems important to conduct imitation studies over time, this is, following up a same group of participants on different test moments over time from childhood until adulthood. It is possible that observed relationships disappear as infants grow older or it is possible that absent relationships become present only at a certain age or developmental level. Furthermore, it seems interesting to compare infant siblings who develop ASD with infant siblings who do not and to explore their mirror neuron functioning and imitation performance at early age in comparison with later development. Therefore, longitudinal imitation studies with children with or at risk for ASD can be of high importance, also concerning the role of experience on neural mirroring activity with stronger activity related with more experience (Ingersoll & Meyer, 2011; van Elk et al., 2008). This could expand the knowledge on early deficits and their influence on developing ASD. Furthermore, this can have important implications on diagnosis and the development of intervention strategies. Another argument for longitudinal studies on this research topic is the

suggestion of developmental changes of the brain in general and of the electroencephalography (EEG) mu desynchronization in particular (Mukherjee et al., 2001; Oberman et al., 2012). Moreover, the adult EEG mu wave occurs in the alpha frequency range between 8 and 13 Hz. In infants, mu wave activity occurs at a lower frequency range, between 6 and 9 Hz (Marshall & Meltzoff, 2011). This rhythm in infants has topographically and functionally similar characteristics as the adult mu wave. However, research revealed developmental changes of this infant rhythm as they grow older with an increase in frequency and amplitude until it gets to the adult frequency range (Marshall, Bar-Haim, & Fox, 2002; Stroganova, Orekhova, & Posikera, 1999). Additionally, it has been suggested that an increasing developmental change is present in the magnitude of the mu wave desynchronization from infancy until adulthood which is partly found in Chapter 3 of this doctoral thesis (Marshall & Meltzoff, 2011). These developmental changes in EEG measurement could be an additional argument to perform longitudinal research on neural mirroring from infancy until adulthood.

PRACTICAL IMPLICATIONS

Some practical implications can be drawn based on the findings and conclusions of this doctoral dissertation.

This doctoral research confirmed that toddlers with ASD performed significantly lower on bodily imitation compared to the high-risk sibling group and the low-risk group (Chapter 6). These results implicate and endorse that imitation as part of intervention strategies for ASD seems meaningful. However, interventions focusing on improving imitation skills in ASD need to consider that the amount of generalization of the acquired skills is mediated by different contexts. For example, Ben-Itzchak and Zachor (2007) found that children with ASD showed better imitation performance after a behavioural intervention in a structured setting. Research suggests that learning imitation skills in an appropriate context together with a high motivation of the individual with ASD lead to higher generalizability of the acquired imitation skills (Ingersoll & Schreibman, 2006). Therefore, different intervention techniques need to be used in different contexts to teach imitation skills. Furthermore bodily and procedural

imitation were interrelated in the ASD group and the low-risk group (Chapter 6). This suggests that intervention focussing on one type of imitation (i.e., bodily or procedural) during intervention and therapy could influence the other type of imitation. Thus, as imitation serves a considerable role in early learning and social-communicative development, it should be an important part of intervention paradigms in infants with ASD to create an overall positive outcome in ASD (Ben-Itzchak & Zachor, 2007).

Contradictory to the confirmation in our research of the usefulness of teaching imitation skills in children diagnosed with ASD, our results found no support for impaired mirror neuron functioning in ASD (Chapter 5). Consequently, we cannot support the suggestion of using (impaired) mirror neuron functioning to diagnose and treat ASD. Interventions, that receive much interest lately, such as neurofeedback (i.e., creating self-regulation of someone's own cortical activity through trial and error and visual feedback of the own cortical processes; Holtmann et al., 2011) or correcting chemical imbalances (for example stimulating the release of neuromodulators which effects mirror neuron activity; Ramachandran & Oberman, 2006) do not find support in our results. Although it has been suggested to use such biological intervention techniques additionally to traditional behavioural therapies, finding no unambiguous evidence for broken mirror neurons in ASD suggests that the results concerning neural mirroring in ASD must be carefully interpreted before developing therapeutic and intervention strategies. Additionally, our results did not support the focus on motor functioning in the treatment of ASD based on the rationale that a better motor knowledge can also influence a better social knowledge and consequently more adjusted behaviour based on the mirror neuron principle (Rizzolatti, Fabbri-Destro, & Cattaneo, 2009). As we found no impaired neural mirroring in ASD (Chapter 5) and in high-risk siblings (Chapter 4), focusing on (pre-emptive) improving this matching mechanism to adjust the social-communicative impairments of ASD related to this process seems ineffective. Consequently, our research suggests that neural mirroring functioning is not a diagnostic powerful tool meaning that it is not useful to focus diagnostic and intervention techniques in ASD on this matching process and that it is not supported to consider impaired neural mirroring as biomarker for ASD or its BAP.

Further research is needed to explore which brain mechanisms are related to what skills and processes so intervention could be adapted to the needs of each

individual with ASD to create the best possible outcome. Therefore, as evidence for impaired neural mirroring as single cause for ASD is lacking from our research, further research is definitely needed taking into account other brain areas related to the neural mirroring network. Additionally, it can be assumed that we found no differences between the ASD group and the low-risk group concerning neural mirroring because their mirror neuron functioning had been influenced by sensorimotor experiences as the participants were already between 2 and 4 years old (Chapter 5). Therefore, it is definitely interesting to investigate neural mirroring in younger infants with or at risk for ASD, using appropriate techniques to discover the initial mirror neuron functioning without too much modulation through sensorimotor experiences or before participating in intervention sessions. Finally, as already suggested, longitudinal research concerning neural mirroring and imitation should definitely be taken into account to compare early and later mirror neuron functioning and imitation capacities in ASD and its BAP.

CONCLUSION

Although the interest in the underlying neurological processes of ASD and imitation in ASD has been well investigated, uniformity of the findings is still lacking. Extending the research on imitation and the neural mirroring in typically developing and in children with ASD and in high-risk siblings was the main goal of this doctoral thesis. Overall, we found significant neural mirroring activity during action observation, hand movement observation and action imitation in typically developing infants. More profound investigation of the used paradigm to investigate neural mirroring in young children revealed a different impact of televised and live actions on neural mirroring activity in infants. Consequently, using live actions to investigate neural mirroring in young children seems to be the best fitting paradigm. Furthermore, our results challenged the theory of broken mirrors in ASD and did not find evidence for impaired neural mirroring in siblings at risk for ASD. Thus results of this doctoral dissertation did not support the idea of impaired neural mirroring as a distinctive characteristic and primary deficit of ASD and its broader phenotype. Additionally, our final study found no evidence of a global imitation deficit in ASD as the toddlers with ASD demonstrated significantly lower bodily but not procedural imitation performance compared to high-

risk siblings and low-risk toddlers. The high-risk siblings did not differ concerning imitation capacities compared to low-risk toddlers. The studies in this doctoral project revealed no global impaired goal-directed imitation performance, both behavioural and neurological in children with ASD. These findings do not support the idea of a straightforward broken mirror theory of ASD as the only explanatory model. We rather suggest that a combination of different models (including the neural mirroring model) has more explanatory power to explain the heterogeneous symptomatology in ASD.

This doctoral thesis aimed to add to the existing literature on the neural basis of action observation and action execution in young children with or without ASD or at risk for ASD. However, many questions remain unsolved and future research is needed to discover what contribution neural mirroring might make to the development of ASD and its BAP taking the findings and critical reflections of this doctoral dissertation into account.

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NEDERLANDSTALIGE SAMENVATTING

Autismespectrumstoornissen (ASS), een spectrum van neurologische ontwikkelingsstoornissen, worden gekenmerkt door kwalitatieve tekorten op vlak van sociale interactie, communicatie en repetitieve, stereotiepe gedragingen (American Psychiatric Association [APA], 2000). ASS representeren een brede variatie in symptomatologie, gaande van mild tot ernstige symptomen op deze 3 domeinen (Wing, 1997). Door de complexiteit en de variabiliteit van de symptomen binnen het ASS-spectrum, worden verschillende oorzaken voor de ontwikkeling van deze stoornis aangehaald (Schroeder, Desrocher, Bebko, & Cappadocia, 2010).

Uit onderzoek blijkt dan ook dat ASS multifactoriële, sterk genetisch bepaalde ontwikkelingsstoornissen zijn (Rutter, 2005). De genetische bijdrage in de ontwikkeling van ASS kan tot uiting komen in mildere, kwalitatief gelijkende ASS-kenmerken, ook wel het 'breder autisme fenotype' genoemd (BAF). Deze kenmerken komen vaker voor bij familieleden van individuen met ASS. Bijgevolg kan onderzoek met jongere broertjes/zusjes van kinderen met ASS (d.i., siblings) helpen bij het definiëren van dit BAF omwille van hun hoger risico op het ontwikkelen van ASS (Ozonoff et al., 2011; Rogers, 2009). Onderzoek naar de precieze oorzaak van ASS wordt recent sterk gekenmerkt door neurobiologische bevindingen. Naast gedragsonderzoek kan beeldvormingsonderzoek meer indirecte, onderliggende kwetsbaarheden aantonen die niet altijd onmiddellijk zichtbaar zijn in openlijk gedrag (Elsabbagh & Johnson, 2010).

Recent wordt dan ook heel wat aandacht besteed aan de rol van spiegelneuronen in de ontwikkeling van ASS. Spiegelneuronen, eerst ontdekt in de makaak aap, zijn visuomotorische neuronen die actief zijn wanneer een actie wordt uitgevoerd alsook wanneer deze actie wordt geobserveerd bij anderen (Di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; Rizzolatti, Fadiga, Gallese, & Fogassi, 1996). Deze neuronen worden theoretisch gelinkt aan verschillende sociaal-communicatieve vaardigheden, zoals imitatie (Iacoboni, 2005), empathie (Iacoboni & Dapretto, 2006) en taal (Rizzolatti & Arbib, 1998). Vaak zijn deze vaardigheden beperkt bij personen met ASS. Dit leidt dan ook tot de veronderstelling dat een dysfunctionele werking van spiegelneuronen aan de basis van deze beperkingen van ASS zouden liggen (Gallese, Gernsbacher, Heyes, Hickok, & Iacoboni, 2011).

Spiegelneuronen zijn actief tijdens het observeren en het uitvoeren van acties. Deze automatische match tussen de observatie en de uitvoering van acties is identiek aan het onderliggende proces werkzaam tijdens imitatie (Marshall & Meltzoff, 2011). Imitatie speelt een belangrijke rol in het verwerven van sociaal-communicatief gedrag (Ogawa & Inui, 2012; Rogers, Young, Cook, Giolzette, & Ozonoff, 2010). Meer bepaald vervult het een manier van communiceren en van het aanleren van sociale kennis en gedrag (Cochin, Barthelemey, Roux, & Martineau, 2001). Bij ASS echter is imitatie vaak beperkt en wordt vaak verondersteld dat dit andere sociaal-communicatieve beperkingen kan veroorzaken (Williams, Whiten, & Singh, 2004). De meerderheid van de studies vonden evidentie voor moeilijkheden met het imiteren van bijvoorbeeld gebaren (vb. Roeyers, Van Oost, & Bothuyne, 1998), onbekende acties met voorwerpen (vb. Smith & Bryson, 1994) of de stijl waarmee de acties worden uitgevoerd (vb. Hobson & Lee, 1999) bij individuen met ASS. Omwille van de heterogeniteit van ASS-symptomen (Levy, Mandell, & Schults, 2009) en het gebrek aan een eenduidige definitie van imitatie (Sevlever & Gillis, 2010), is er echter nog steeds geen duidelijkheid omtrent de ontwikkeling van imitatie bij ASS.

DOEL VAN HET DOCTORAATSONDERZOEK

Hoewel imitatie en de onderliggende neuronale processen van ASS reeds grondig werden onderzocht, ontbreekt uniformiteit in de resultaten (vb. Jones, 2007; Paulus, Hunnius, Vissers, & Bekkering, 2011). Onderzoek werd recent beïnvloed door de ontdekking van spiegelneuronen, actief zowel tijdens de observatie als tijdens de uitvoering van acties (Di Pellegrino et al., 1992; Rizzolatti et al., 1996). Williams, Whiten, Suddendorf, en Perrett (2001) waren enkele van de eerste onderzoekers die suggereerden dat imitatieproblemen, vaak geobserveerd bij personen met ASS, het gevolg kunnen zijn van beperkingen in het matchen van observatie en uitvoering van acties, veroorzaakt door een verminderde werking van spiegelneuronen. Nadien onderzochten heel wat onderzoekers de relatie tussen imitatie en spiegelneuronen bij ASS (vb. Hamilton, Brindley, & Frith, 2007; Oberman et al., 2005; Raymaekers, Wiersema, & Roeyers, 2009). De diverse resultaten in de literatuur rond imitatie en de werking van spiegelneuronen in ASS vragen om verder onderzoek van deze concepten

en het verband tussen beide. Het voornaamste doel van dit doctoraatsonderzoek was dan ook het onderzoeken van imitatie en de werking van neurale spiegelsystemen bij typisch ontwikkelende kinderen, kinderen met ASS en jongere siblings van kinderen met ASS.

Het eerste doel van dit onderzoeksproject was inzicht krijgen in de spiegelneuronen respons bij typisch ontwikkelende kinderen en de ontwikkeling van een aangepast paradigma om neurale spiegelprocessen te onderzoeken bij jonge kinderen. Om dit te onderzoeken, werd spiegelneuronen activiteit gemeten aan de hand van centrale mu suppressie tijdens het observeren en imiteren van doelgerichte acties en tijdens het observeren van handbewegingen die ofwel live ofwel op televisie werden aangeboden. Op basis van de conclusies uit deze studie, onderzochten we aanvullend de mu suppressie respons bij typische ontwikkelende kinderen tussen 18 en 30 maanden oud tijdens de observatie en imitatie van live doelgerichte acties en tijdens de observatie van live handbewegingen.

Ten tweede onderzochten we spiegelneuronen activiteit bij kinderen met een risico op het ontwikkelen van ASS (d.i., jongere siblings van kinderen met ASS) en kinderen gediagnosticeerd met ASS om meer inzicht te krijgen in de spiegelneuronen werking in ASS en in het BAF. Meer specifiek onderzochten we spiegelneuronen activiteit bij jongere siblings tussen 18 en 36 maanden oud en jonge kinderen met ASS tussen 24 en 48 maanden oud aan de hand van centrale mu suppressie tijdens observatie- en imitatietaken.

Het laatste doel van dit doctoraatsproject was het onderzoeken van gebaren en object imitatievaardigheden bij siblings van kinderen met ASS, vergeleken met kinderen met ASS en typisch ontwikkelende kinderen, allen tussen de 48 en 69 maanden oud.

VOORNAAMSTE ONDERZOEKSRESULTATEN

Voordat we de belangrijkste onderzoeksresultaten van dit doctoraatsproject samenvatten, geven we graag een toelichting over de gebruikte methodiek voor het meten van activiteit van spiegelneuronen. Een vaak gehanteerde en kindvriendelijke methode is het meten van hersenactiviteit tijdens elektro-encefalografische opnames

(EEG). Meer specifiek werd de onderdrukking van de mu golf, ook wel mu suppressie genaamd, als indicatie voor activiteit van spiegelneuronen binnen onze studies gebruikt (Muthukumaraswamy, Johnson, & McNair, 2004). Mu golf activiteit werd meestal gemeten over centrale elektroden en in één studie (bij de typische ontwikkelende kinderen tussen 18 en 30 maanden oud) werd ook de activiteit over de frontale en pariëtale elektroden gemeten.

Een eerste onderzoek, gericht op het nagaan van de bruikbaarheid van een paradigma om de werking van neurale spiegelneuronen systemen te onderzoeken bij jonge kinderen, toonde aan dat het gebruik van live acties ecologisch valieder was dan acties gepresenteerd op televisie. Kinderen tussen 18 en 36 maanden oud toonden immers significante mu suppressie tijdens het observeren van live acties, maar niet tijdens het observeren van acties gemodelleerd op televisie. Daarenboven bleek de mu suppressie ook sterker te zijn tijdens imitatie in de live setting in vergelijking met de televisie setting. Deze resultaten suggereren een differentiële invloed van live en televisie acties op de werking van spiegelneuronen. Dit zorgt ervoor dat het paradigma dat werd toegepast in de volgende studies van dit doctoraatsproject enkel gebruik maakte van live demonstraties.

Verder bleek bij kinderen tussen 18 en 30 maanden oud reeds een werkzaam actie observatie-uitvoering matching proces aanwezig te zijn. Specifiek vertoonde deze groep kinderen significante centrale mu suppressie tijdens zowel het observeren als het uitvoeren van doelgerichte acties. Daarnaast observeerden we eveneens activiteit in de spiegelneuronen tijdens het observeren van handbewegingen. Naast centrale mu golf activiteit, werd ook pariëtale mu suppressie gevonden tijdens de observatie- en imitatietaken. Enkel tijdens actie-observatietaken was mu suppressie even sterk over centrale, frontale en pariëtale elektroden. Verder werd er nagegaan of sociaal-communicatieve vaardigheden, zoals imitatie, taal en leeftijd, gerelateerd zijn aan centrale mu suppressie. Mu suppressie was niet significant gerelateerd aan taal en leeftijd. De kwaliteit van imiteren was positief gerelateerd aan mu suppressie tijdens observatie van handbewegingen en tijdens het uitvoeren van doelgerichte acties.

Naast neurale spiegelneuronen activiteit bij typisch ontwikkelende kinderen, onderzochten we ook de werking van spiegelneuronen gemeten aan de hand van mu suppressie bij siblings en bij kinderen met ASS. Beide studies toonden aan dat kinderen

met een verhoogd risico op het ontwikkelen van ASS, met name de sibings, en jonge kinderen gediagnosticeerd met ASS, niet significant verschillen van typisch ontwikkelende kinderen wat betreft centrale mu suppressie. Hieruit blijkt dat beperkte of verminderde werking van spiegelneuronen als kenmerk van ASS en het breder autisme fenotype niet werd bevestigd in dit doctoraatsonderzoek. Kwaliteit van imitatie was slechts beperkt geassocieerd met centrale mu suppressie tijdens imitatie bij de siblings. Bij de groep met ASS werden geen significante verbanden gevonden tussen centrale mu suppressie en andere kindkenmerken, met name imitatie, leeftijd en ernst van de symptomen.

Nader onderzoek van de imitatievaardigheden bij siblings, kinderen met ASS en typisch ontwikkelende kinderen toonde aan dat ASS niet wordt gekenmerkt door een algemeen imitatietekort. Meer bepaald scoorden de kinderen met ASS zwakker op gebarenimitatie, maar niet op objectimitatie in vergelijking met typisch ontwikkelende kinderen en siblings. Daarenboven vertoonden de siblings imitatievaardigheden gelijklopend aan de imitatieprestaties van de typisch ontwikkelende kinderen. De ernst van de ASS-symptomen was enkel gecorreleerd met objectimitatie bij de siblings. De prestaties inzake gebaren- en objectimitatie waren onderling gerelateerd bij de kinderen met ASS en de typisch ontwikkelende kinderen, maar niet bij de siblinggroep. Hieruit kan worden gesuggereerd dat procedurele imitatie anders lijkt te verlopen bij ASS en bij het BAF.

Samenvattend kunnen we stellen dat onze resultaten de hypothese van een verminderde werking van spiegelneuronen als kenmerkend neurologische eigenschap van ASS en het breder autisme fenotype niet bevestigen. Veel vragen blijven echter onbeantwoord en verder onderzoek is nodig om meer duidelijkheid te scheppen. Daarbij kunnen de bevindingen van dit onderzoeksproject in acht worden genomen.

PRAKTISCHE IMPLICATIES

Dit onderzoeksproject bevestigt dat jonge kinderen met ASS zwakker scoren op gebarenimitatie, maar niet op objectimitatie. Bijgevolg onderschrijven deze bevindingen het belang en de effectiviteit van imitatie als onderdeel van interventietechnieken voor

ASS. Daarenboven was imitatie van gebaren en objecten significant aan elkaar gerelateerd bij de kinderen met ASS en de typisch ontwikkelende kinderen die werden onderzocht binnen onze studies. Dit suggereert dat interventies die focussen op 1 vorm van imitatie (d.i., gebaren of objecten) tijdens therapie de ontwikkeling van de andere imitatievorm positief kunnen beïnvloeden. Vermits imitatie een belangrijke rol speelt in sociaal-communicatieve ontwikkeling en omdat de effectiviteit van imitatie als deel van interventietechnieken voor ASS werd aangetoond, zou dit een belangrijke focus moeten zijn in de aanpak en behandeling van ASS om zo een algemene positieve uitkomst te beogen.

In tegenstelling tot het belang van imitatie als deel van de interventie bij kinderen met ASS, vond dit doctoraatsonderzoek geen evidentie voor beperkte werking van spiegelneuronen bij ASS als onderdeel van de diagnostiek en de behandeling van deze stoornis. Bijgevolg blijkt de bruikbaarheid van interventies, zoals neurofeedbacktraining (= het creëren van zelfregulatie door trial en error aan de hand van visuele feedback; Holtmann et al., 2011) of het corrigeren van chemisch onevenwicht (vb. het stimuleren van de vrijlating van neuromodulators gerelateerd aan spiegelneuronen activiteit; Ramachandran & Oberman, 2006), geen ondersteuning te vinden in dit onderzoeksproject. Hoewel vaak wordt gesuggereerd dat louter gedragsmatige interventies dienen te worden aangevuld met neurologische behandelingen, vinden weinig studies duidelijke eenduidige resultaten betreffende de rol van spiegelneuronen bij ASS. Vermits dit doctoraatsproject geen evidentie vond voor verminderde werking van spiegelneuronen bij kinderen met ASS of met een verhoogd risico op het ontwikkelen van ASS (d.i., siblings), lijkt het ineffectief om (preventief) dit matchingproces aan te pakken of te verbeteren tijdens interventietechnieken. Verder werd het idee om spiegelneuronen activiteit als diagnostische focus of als biomarker voor ASS of het BAF te aanvaarden, niet ondersteund binnen dit onderzoeksproject.

CONCLUSIE

Ondanks het groeiend aantal studies rond de werking van spiegelneuronen en hun rol bij de ontwikkeling van imitatie bij ASS, blijven de resultaten onduidelijk en

tegenstrijdig. Het doel van dit doctoraatsproject was dan ook deze kennis te verruimen en dit onderzoeksgebied uit te breiden. De hypothese van vertraagde of verminderde werking van neurale spiegelneuronen bij kinderen met ASS en bij hun jongere broertjes/zusjes werd door onze resultaten verworpen. Deze resultaten ontkrachten het idee van verminderde werking van spiegelneuronen als neurologisch kenmerkende factor van ASS en het BAF. Als laatste ondersteunen onze resultaten niet het idee van een algemeen imitatietekort bij ASS. Samenvattend werd binnen dit doctoraatsproject geen evidentie gevonden voor het idee van een verminderde werking van spiegelneuronen als verklarend model voor imitatie en andere sociaal-communicatieve symptomen kenmerkend voor ASS en het BAF.

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