Social-Cognitive Processing and Familial Risk for Autism Spectrum Disorder

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General Introduction
GENERAL INTRODUCTION

Walking through a forest, looking at the surrounding nature, you cannot help but notice the differences between the trees that you are passing by. Some trees have a majestic crown full of bright colors, while other tree tops are only sparsely filled with leaves. Whereas these observations can tell us a lot about the tree and its health, there is an equally - if not more - important part of the forest that is invisible to the bypassing observer. Underneath the ground, the tree’s roots are well hidden deep in the forest’s soil. Forming an underground network, the roots are of great importance for the tree’s growth, health and survival (Wohlleben, 2016). A full understanding of the forests and its characteristics, thus requires the assessment of its visible as well as its hidden characteristics. Similarly, when we observe other people, their behavior and other visible characteristics can tell us a lot about them. Yet what remains invisible are the neural processes that underlie what we can observe from the outside. The scientific field of cognitive neuroscience aims to integrate our knowledge about the visible and invisible aspects of human cognition. Researchers study the relationship between brain processing and behavioral as well as cognitive phenomena (such as memory or learning), bridging the gap between neuroscience and cognitive psychology (Purves et al., 2008).

The recent advance in technology and research methods to study neural processing in humans has led to a rapidly growing scientific field over the past decades. Not only do these methods allow researchers to study typical behavior and its neural correlates, they also aid the understanding of neurodevelopmental disorders and how the visible behavioral atypicalities relate to differences in brain development and brain functioning. The current thesis lies within the field of developmental cognitive neuroscience and its focus is the assessment of early neural and behavioral characteristics of individuals at high risk for Autism Spectrum Disorder.
AUTISM SPECTRUM DISORDER

Autism Spectrum Disorder (ASD) is a common neurodevelopmental disorder, affecting approximately 1% of the general population (Elsabbagh et al., 2012; Hahler & Elsabbagh, 2014). Individuals with ASD show social and communication deficits such as the inability to relate to others and understand their thoughts or intentions (APA, 2013; Baron-Cohen, 2000; Constantino & Charman, 2016). The disorder is further characterized by stereotyped, repetitive behaviors and restricted interests. Individuals with ASD can also show atypical sensory processing such as over- or under-sensitivity or abnormal responses to sensory stimuli like touch or sound (APA, 2013; Constantino & Charman, 2016). ASD is a very heterogeneous disorder in which symptom severity and manifestation can vary largely between affected individuals. In line with such a heterogeneity, research has shown that ASD characteristics are continuously distributed in the general population and diagnosed individuals represent the severe end of this continuum (Constantino & Charman, 2016). When first described, ASD was believed to be the result of lacking parental affection (Bettelheim, 1967), however it soon became clear that the disorder has a strong biological basis (Attwood, 2008; Ecker, Bookheimer, & Murphy, 2015; Hernandez, Rudie, Green, Bookheimer, & Dapretto, 2015; Hill & Frith, 2003; Rajendran & Mitchell, 2007). Today, ASD is considered a neurodevelopmental disorder characterized by atypical brain development, and abnormal brain anatomy, functioning and connectivity (Bauman & Kemper, 2003; Ecker et al., 2015; Hernandez et al., 2015). Despite the growing amount of research investigating the behavioral as well as neurocognitive characteristics of ASD, its precise causes remain unknown to date. Twin, family and molecular-genetic studies indicate that the etiology of ASD is rather complex, and that ASD is likely caused by multiple common and rare genetic factors that interact with each other and with environmental risk factors (Betancur, 2011; Huguet, Ey, & Bourgeron, 2013; Persico & Merelli, 2015). As ASD is rarely diagnosed before the age of 3 years when deviations in social and communication skills become apparent (Begeer et al., 2013; Daniels & Mandell, 2014), little is known about the early development of the disorder. Researchers, however, do agree that an earlier detection of ASD is beneficial for the individuals and their families (Bölte et al., 2016; Hahler & Elsabbagh, 2014; Zwaigenbaum et al., 2015; Zwaigenbaum, Bryson, & Garon, 2013) and scientists are currently working towards increasing our knowledge about the early development and potential early markers of the disorder.
THE EARLY DEVELOPMENT OF ASD

Previously, early signs of ASD were predominantly assessed based on home videos which were retrospectively analyzed, or based on retrospective parental reports (Costanzo et al., 2015; Zwaigenbaum et al., 2015, Zwaigenbaum et al., 2013). More recently, researchers have started prospective studies on the early development of ASD, following infants at high familial risk for the disorder. Infant siblings of children with ASD have an increased risk of receiving a diagnosis themselves (ranging from 10-20%; see Constantino, Zhang, Frazier, Abbacchi, & Law, 2010; Ozonoff et al., 2011; Szatmari et al., 2016). By following these siblings during their first years of life, researchers are able to directly assess the infants’ characteristics and their development rather than relying on retrospective analyses (Bölte et al., 2013; Elsabbagh & Johnson, 2010; Szatmari et al., 2016). These prospective studies have increased the range of methods that can be applied to study early autism, allowing the direct assessment of neural processing and brain development in addition to behavioral investigations (Bosl, Tierney, Tager-Flusberg, & Nelson, 2011; Elsabbagh et al., 2009; Orekhova, Elsabbagh, Jones, Dawson, & Charman, 2014; Wolff et al., 2012). In most prospective studies, the development of high-risk infants is compared to a group of low-risk controls, typically infant siblings of children without a family history of ASD. By assessing differences between high- and low-risk infants, researchers can establish which aspects of development are affected by the familial risk for ASD. Infant siblings are followed through development until - typically at the age of 24 or 36 months - a preliminary research diagnosis of ASD can be made. This enables the assessment of early atypicalities that manifest specifically in those high-risk infants who later receive an ASD diagnosis (HR-ASD). Contrasting the HR-ASD group with high-risk infants who do not receive a diagnosis (HR-noASD) can inform researchers about additional risk and protective factors within the high-risk group. While some early atypicalities may be specific to the HR-ASD group, other differences may be present across the entire high-risk group as part of the infant broader autism phenotype (BAP). The BAP refers to the phenomenon that some family members of individuals with autism who do not have an ASD diagnosis can show subclinical characteristics of the disorder (Macy et al., 2013; Piven, Palmer, Jacobi, Childress, & Arndt, 1997). Getting a more detailed picture of the infant BAP as well as of early markers specific to HR-ASD infants will aid our understanding of the disorder and its course over development, providing the basis for future early detection and interventions.
THE ZEBRA-PROJECT

The research conducted for this thesis was embedded within the large European research project EU-AIMS (European Autism Interventions - A Multicentre Study for Developing New Medications) in which siblings of children with and without ASD were followed longitudinally at multiple European research sites. In the Netherlands, infants were followed at two sites (at the Radboud University Medical Centre and the Baby Research Center in Nijmegen, and at Utrecht University in Utrecht) within the so-called Zebra-project (Zusjes En BRoertjes van kinderen met Autisme, Sisters And Brothers of Children with Autism). Infant siblings at high- and low-risk for ASD were enrolled in the study at 5 or 10 months of age and assessed again at 14, 24 and 36 months. During each visit, infants and their parents participated in a battery of experimental tasks, developmental assessments and behavioral observations. The study is currently ongoing and the research presented in this thesis focuses on the investigation of behavioral and neural differences between at-risk infants and low-risk controls at the ages of 5 (Chapter 1), 10 (Chapter 2), and 14 months (Chapter 3). This thesis describes several experimental studies investigating different aspects of social cognition: The first chapter of this thesis focuses on the neural processing of social stimuli in high- and low-risk infants. The remainder of this thesis assesses the development of action prediction in infants at low and high risk for ASD as well as the neural mechanisms underlying action prediction in typical individuals (Chapter 4).
SOCIAL INFORMATION PROCESSING IN INFANTS AT RISK FOR ASD

As the social deficits associated with ASD are often considered the core symptoms of the disorder, it was initially hypothesized that social atypicalities had the largest potential to manifest as an early marker (Elsabbagh & Johnson, 2016). Some researchers have argued that deviant social attention may be present in ASD early in life, leading to an altered exposure to social stimuli and prohibiting typical social development (Chevallier, Kohls, Troiani, Brodkin, & Schultz, 2012; Dawson et al., 2004). As preferential orienting towards social stimuli, like faces, can be observed in typically-developing infants from birth onwards (Farroni, Csibra, Simion, & Johnson, 2002; Johnson, Dziurawiec, Ellis, & Morton, 1991; Johnson, 2005; Morton & Johnson, 1991; Valenza, Simion, Cassia, & Umiltà, 1996), fundamental deficits in social attention in ASD were thought to be detectable already in very young infants (Chevallier et al., 2012; Dawson et al., 2004). Contrary to these clear expectations of early social atypicalities, most studies assessing high-risk infants suggest that core behavioral symptoms, such as limited eye contact or lack of social smiling, do not manifest prior to 12 months of age (Elsabbagh & Johnson, 2016a; Jones, Gliga, Bedford, Charman, & Johnson, 2014; Szatmari et al., 2016). With respect to deviant social attention in young infants, results have been mixed with most studies showing typical performance during the first year (Elsabbagh et al., 2014; Gliga, Jones, Bedford, Charman, & Johnson, 2014; Ozonoff et al., 2010). Ozonoff and colleagues (2010), for instance showed that the frequency of gaze to faces was similar between low- and high-risk infants at 6 months of age. Similarly, Elsabbagh and colleagues (2013) reported that spontaneous orienting towards static faces was typical in 7-month-old infants who later received an ASD diagnosis. Another study by Elsabbagh and colleagues (2014) assessed visual scanning of complex as well as simple scenes and reported similar attention to eye regions relative to mouth regions in 7-month-old high- and low-risk infants. On the other hand, there are some recent studies that have reported differences in social attention during the first year of postnatal life (Di Giorgio et al., 2016; Jones et al., 2016; Jones & Klin, 2013). Jones and Klin (2013), for instance, reported that high-risk infants who later received an ASD diagnosis showed a decline in eye fixations from 2 to 6 months of age. Another study by Jones and colleagues (2016) further showed differences in visual habituation to social stimuli at 6 but not 12
months of age comparing high-risk infants who did and did not meet criteria for ASD at 24 months. Recently, Di Giorgio and colleagues reported differences in visual preferences to social stimuli between newborns at high risk and low-risk controls. While these findings give a first indication that social attention might be atypical at birth, replication will be required and further research needs to establish how the findings relate to later ASD outcome. Overall, evidence for atypical social attention during the first year is mixed and results from behavioral prospective studies thus far suggest that social atypicalities become more stable and pronounced only after the infant’s first birthday (Elsabbagh & Johnson, 2016a; Gliga et al., 2014; Jones et al., 2014).

Next to behavioral measures, prospective studies also allow for the assessment of neural processing in infants at high risk for ASD. Importantly, studying brain function prior to the clinical onset of ASD, enables researchers to investigate the underlying neural mechanisms of ASD without being confounded by potential effect of interventions or later compensatory mechanisms. In typically-developing individuals, social information is processed within specific regions of the brain, including the superior temporal sulcus (STS), temporoparietal junction (TPJ), fusiform face area (FFA), orbitofrontal cortex, and the amygdala (Adolphs, 2003). Atypical activation of these social brain regions has been found in adults and children with ASD during the processing of social information (Amaral, Bauman, & Mills Schumann, 2003; Baron-Cohen et al., 2000; Misra, 2014; Pelphrey, Shultz, Hudac, & Vander Wyk, 2011; Zilbovicius et al., 2006). Individuals with ASD for instance show diminished responses to social sounds (Gervais et al., 2004) and faces (Jemel, Mottron, & Dawson, 2006) but also altered processing of biological motion (Kaiser & Pelphrey, 2012). The selectivity of social brain regions for processing social information has been shown to emerge during the first few months of postnatal development (Blasi et al., 2011; Grossmann, Oberecker, Koch, & Friederici, 2010; Lloyd-Fox et al., 2009). Therefore, potential deviations in brain development affecting the selectivity of social brain regions in ASD may be detectable early in life and could precede the onset of behavioral symptoms. Using different neuroimaging techniques, several researchers have indeed shown atypicalities in brain activation elicited by social stimuli during the first year of life in infants at high risk for ASD (Elsabbagh et al., 2009; Elsabbagh et al., 2012; Lloyd-Fox et al., 2013). Elsabbagh and colleagues (2012), for instance, used Electroencephalography (EEG) and found that the neural
processing of eye gaze shifts at 6 to 10 months of age differed in infants who were later diagnosed with ASD compared to both low-risk controls and high-risk infants who did not receive a diagnosis. More recently, a study by Lloyd-Fox and colleagues (2013) using functional near infrared spectroscopy (fNIRS) reported reduced neural sensitivity to social stimuli in 5-month-old high-risk infants in brain regions typically associated with social processing. While low-risk controls showed posterior temporal cortex activation during the observation of complex social dynamic stimuli, high-risk infants did not. These studies have illustrated that atypical brain activation to social stimuli is detectable during the first year of life. Deviations in cortical processing may thus proceed visible behavioral signs and could provide a more sensitive measure of atypical development in high-risk infants compared to the assessment of behavioral characteristics. A critical role for future research lies in the further investigation of these neural atypicalities and their relation to early behavioral differences as well as later ASD outcome. As the earlier neuroimaging results were based on relatively small samples, replication and extension of the previous findings in independent cohorts is an essential first step.

Chapter 1 of the current thesis investigates cortical differences in social processing of 5-month-old high- and low-risk infants using fNIRS. The study is based on the paradigm previously used by Lloyd-Fox and colleagues (2013) in which infants are presented with complex social and non-social dynamic video stimuli. Chapter 1 assesses whether the cortical specialization of posterior temporal cortex to social dynamic stimuli differs between the low- and high-risk infant groups. The remaining chapters of this thesis, focus on an aspect of social cognition that has thus far not been assessed in infants at risk for ASD: the ability to form predictions about actions observed in others.
ACTION PREDICTION IN ASD

Several studies in adults and children with ASD have shown that the social symptoms associated with the disorder include an impairment in the ability to predict others’ actions and intentions (Boria et al., 2009; Hudson, Burnett, & Jellema, 2012; Sparaci, Stefanini, D’Elia, Vicari, & Rizzolatti, 2014; Zalla, Labruyère, Clément, & Georgieff, 2010; but see Falck-Ytter, 2010; Marsh, Pearson, Ropar, & Hamilton, 2014). A common way of studying action prediction is by means of eye tracking. In eye tracking, the participant’s eye gaze position on a screen is estimated, usually based on the eye’s corneal reflection of infrared light that is emitted by the eye tracker. The participants’ eye gaze position is continuously sampled, typically at a rate between 50 and 300 Hz. Based on the recorded eye position data, eye movements and fixations can be extracted and further analyzed. Researchers can then, for instance, assess looking times, scanning patterns, oculomotor tracking or saccade latencies (Gredebäck, Johnson, & von Hofsten, 2010). Eye tracking is also widely-used in developmental research and has been implemented in a variety of studies assessing infant cognition (Aslin, 2012; Gredebäck et al., 2010; Oakes, 2012). While eye tracking has the advantage that infant looking behavior can automatically be captured, there are several challenges researchers have to consider (Aslin, 2012; Gredebäck et al., 2010; Oakes, 2012). For instance, as infants cannot be instructed to sit still during the measurement, researchers are confronted with data loss as well as lower data quality and reduced spatial accuracy compared to adults. These aspects need to be considered during experimental design as well as data analysis. Generally, the rich dataset that is acquired during eye tracking provides a challenge for both adult and infant eye tracking studies, as researchers need to make choices regarding the relevant metrics extracted for analysis (Aslin, 2012).

Using eye tracking, it has been established that typically-developing children and adults predict the course of an observed action by fixating the action target before it is reached (Falck-Ytter, Gredebäck, & von Hofsten, 2006; Flanagan & Johansson, 2003; Gredebäck & Falck-Ytter, 2015; Hunnius & Bekkering, 2010). Hunnius and Bekkering (2010), for instance, showed that infants who observed an actor bringing a cup to the mouth, fixated at the mouth area already before the cup had arrived there. Eye tracking studies assessing action prediction in autism have reported that individuals with ASD can show typical predictive eye
movements for very simple actions, such as a ball being placed into a bucket (Falck-Ytter, 2010). Yet when presented with more complex anticipation tasks in which an action can unfold in multiple ways, individuals with ASD show less frequent predictions and an impaired ability to use frequency information (Schuwerk, Sodian, & Paulus, 2016).

In addition to the empirical findings suggesting atypical action prediction (Boria et al., 2009; Hudson, Burnett, & Jellema, 2012; Sparaci, Stefanini, D’Elia, Vicari, & Rizzolatti, 2014; Zalla, Labruyère, Clément, & Georgieff, 2010), several theories aiming to explain the underlying mechanisms of the disorder propose that prediction difficulties form a core symptom of ASD (Sinha, Kjelgaard, Gandhi, Tsourides, Cardinaux, et al., 2014). An influential account suggests that a deficit in the cortical mirror neuron system (MNS) in ASD could explain the social and communication difficulties of the disorder (Gallese, Rochat, & Berchio, 2013; Iacoboni & Dapretto, 2006; Rizzolatti, Fabbri-Destro, & Cattaneo, 2009; Williams, Whiten, Suddendorf, & Perrett, 2001). The MNS is typically activated during the execution as well as the observation of others’ actions (Cochin, Barthelemy, Roux, & Martineau, 1999; Hari et al., 1998; Kilner, Friston, & Frith, 2007; Muthukumaraswamy & Johnson, 2004). By mapping observed behaviors onto own action representations, the MNS is thought to facilitate the generation of action predictions (Kilner, Friston, & Frith, 2007; Prinz, 2006; Rizzolatti & Sinigaglia, 2016).

A deficit in the MNS in ASD, as proposed by some researchers, would affect this mapping resulting in the impairments to understand and predict others’ actions (Cattaneo et al., 2007; Galles et al., 2013; Iacoboni & Dapretto, 2006; Rizzolatti et al., 2009). Although in line with the reported action prediction difficulties, the MNS deficit account of ASD remains highly debated (Fan, Decety, Yang, Liu, & Cheng, 2010; Oberman et al., 2005; Raymaekers, Wiersema, & Roeyers, 2009; Southgate & Hamilton, 2008). Like other theories focusing on the social symptoms of ASD, the MNS deficit account is unable to explain the range of non-social symptoms that are also characteristic of the disorder. While the social symptoms have for long been the focus of much of the ASD research, it has been argued that future theories of ASD should provide explanations covering the entire range of symptoms including the social and non-social sensory features of ASD (Elsabbagh & Johnson, 2016; Leekam, 2016).
Novel theoretical accounts that aim to provide such a unifying explanation of ASD suggest that ASD may be a disorder of prediction (Brock, 2012; van de Cruys et al., 2014; Gomot & Wicker, 2012; Lawson, Rees, & Friston, 2014; Pellicano & Burr, 2012; Sinha et al., 2014). As argued by Sinha and colleagues (2014), an impairment to form and update predictions about the environment could lead to an experience of a “world wherein events occur unexpectedly and without cause” (Sinha et al., 2014, p.15220). The authors further propose that several of the symptoms associated with ASD could be explained by such a prediction deficit, including the adherence to routine and repetitive behaviors, sensory hypersensitivity and the inability to predict others’ behavior. Researchers have further started to speculate about the underlying cause and neural mechanisms of atypical prediction in ASD (Brock, 2012; van de Cruys et al., 2014; Lawson et al., 2014; Pellicano & Burr, 2012; van Boxtel & Lu, 2013). Pellicano and Burr (2012) proposed that a Bayesian framework could explain the observed deviations in ASD. In such a framework, perception is seen as an inference which requires the integration of incoming sensory information and prior knowledge (priors) about the environment. Pellicano and Burr (2012) argued that individuals with ASD may have attenuated priors leading to a weaker influence of prior expectations on perception. Such “hypo priors” could explain some of the perceptual features of ASD, like, for instance, the superior performance in visual search tasks (Joseph, Keehn, Connolly, Wolfe, & Horowitz, 2009). More recently, predictive coding accounts of ASD have been put forward (van de Cruys et al., 2014; Lawson et al., 2014; van Boxtel & Lu, 2013). These accounts also assume Bayesian inferences, but aim to explain the entire range of associated symptoms and propose underlying neurobiological mechanisms (van Boxtel & Lu, 2013). Two recent predictive processing accounts both suggest that in ASD the precision (i.e. the weight) attributed to the different aspects of the inferential process is distorted (van de Cruys et al., 2014; Lawson et al., 2014). Both researchers argue that such distortions would result in broad atypicalities across multiple domains, such as observed in ASD. Importantly, the authors further explain that the suggested distortions in predictive processing would in particular affect individuals in social situations because social situations are typically complex and characterized by high ambiguity and uncertainty. Under uncertainty, the correct integration and weighing of sensory information and prior knowledge is of particular importance in order to understand the situation and extract the meaningful information from the environment (van de Cruys
et al., 2014; Lawson et al., 2014). The social symptoms of ASD as well as the reported difficulties in action prediction (Boria et al., 2009; Cattaneo et al., 2007; Schuwerk et al., 2016; Zalla, Labruyère, Clément, & Georgieff, 2010) are therefore in line with the novel accounts proposing that atypicalities in predictive processing may underlie ASD.
**ACTION PREDICTION IN INFANTS AT RISK FOR ASD**

Despite the evidence for prediction difficulties in ASD, we know very little about the early development of these processes in infants at high risk for ASD. Yet, difficulties may arise early as the ability to predict the actions of others develops early in life. Using eye tracking, it has been shown that typically-developing infants can display predictive eye movements during action observation from 6 months onwards (Falck-Ytter, Gredebäck, & von Hofsten, 2006; Hunnius & Bekkering, 2010). In line with the notion that predictions are generated based on the motor representations of the observer (Elsner, D'Ausilio, Gredebäck, Falck-Ytter, & Fadiga, 2013; Kilner et al., 2007; Prinz, 2006), action experience has been shown to modulate predictions in infants (Kanakogi & Itakura, 2011; Stapel, Hunnius, Meyer, & Bekkering, 2016). As young infants already show the capacity to predict others’ actions, atypicalities in the development of action prediction in infants at high risk for ASD may also be visible at a young age. A study by Brisson, Warreyn, Serres, Foussier, and Adrien-Louis (2012) provides preliminary evidence for an action anticipation difficulty in high-risk infants. The authors analyzed feeding situations and found that 4- to 6-month-old infants who later received an ASD diagnosis showed a deficit in anticipatory mouth-opening during feeding. How high-risk infants predict goal-directed actions of others, however, has thus far not been assessed. The investigation of action prediction differences between high- and low-risk infants is the topic of **Chapter 2** and **Chapter 3** of this thesis. **Chapter 4** concludes the empirical work of this thesis with an assessment of the neural correlates of behavioral action predictions in a typical population.
OUTLINE OF THE THESIS

The aim of this thesis is to assess differences in the neural and behavioral characteristics of young infants at high risk for ASD and same-aged low-risk controls. In Chapter 1, I use functional near infrared spectroscopy (fNIRS) to assess the cortical processing of social information in 5-month-old high- and low-risk infants. Based on previous findings (Lloyd-Fox et al., 2013), I expect high-risk infants to show reduced neural sensitivity for social stimuli in the posterior temporal cortex. In the following two Chapters of the thesis, I then focus on the assessment of action prediction abilities in 10- and 14-month-old infants at high and low risk for ASD. Although multiple studies have shown action prediction differences in adults and children with ASD (Boria et al., 2009; Cattaneo et al., 2007; Schuwerk et al., 2016; Sinha, Kjelgaard, Gandhi, Tsourides, & Cardinaux, 2014; Zalla et al., 2010), it remains to be assessed whether action prediction difficulties are present in high-risk infants and form a potential early marker of the disorder. In Chapter 2, I assess action prediction abilities in 10-month-old infants at high and low risk for ASD. Using eye tracking, I investigate whether knowledge about objects and their associated actions influenced the frequency of action predictions in high-risk infants in the same way as in typically developing controls. In Chapter 3, I study action prediction abilities in 14-month-old infants at high and low risk for ASD. By means of eye tracking, I assess whether motor experience influenced the accuracy and stability of action predictions in high-risk infants in the same way as in low-risk controls. Finally, Chapter 4 examines the link between neural and behavioral markers of action prediction in a population of typically-developing adults. By simultaneously measuring predictive eye movements using eye tracking, and neural responses using Electroencephalography (EEG), I aim to increase our understanding of the underlying neural mechanisms of behavioral action predictions providing a future basis for further research into action prediction abilities in individuals with ASD. The dissertation is concluded with a general discussion in which I revisit and evaluate the findings from the empirical studies described in Chapter 1 to 4.
Chapter 1

Diminished socially selective neural processing in 5-month-old infants at high familial risk for autism

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ABSTRACT

The social and communicative difficulties that characterize Autism Spectrum Disorder (ASD) are considered the most striking feature of the disorder. Research has reported that individuals with ASD show abnormalities in the brain regions associated with the processing of social information. Importantly, a recent study using functional near-infrared spectroscopy (fNIRS) found the first evidence of atypicalities in the neural processing of social information in four to six-month-old infants at high familial risk for ASD. These findings provide an important step in the search for early markers of ASD and highlight the potential for neuroimaging techniques to detect atypical patterns of neural activity prior to the manifestation of most behavioral symptoms. This study aimed to extend the findings of reduced neural sensitivity to social stimuli in an independent cohort. Twenty-nine 5-month-old infants (13 low-risk infants, 16 high-risk infants) were presented with social and non-social visual stimuli, similar to the previous experiment. Importantly, a non-social dynamic motion control condition was introduced allowing the comparison between social dynamic and non-social, static, as well as dynamic stimuli. We found that while low-risk infants showed activation to social stimuli in the right posterior temporal cortex, this activation was reduced in infants at high risk for ASD. Although the current sample size was relatively small, our results replicate and extend previous work and provide evidence for a social processing difference in infants at risk for autism. Future research will determine whether these differences relate to an eventual ASD diagnosis or may rather reflect the broader autism phenotype.
INTRODUCTION

The social and communication difficulties that characterize Autism Spectrum Disorders (ASD) are considered the most striking feature of the disorder. Many researchers have studied the atypical patterns of behaviour related to understanding others’ minds, goals, and intentions that can be observed in individuals with ASD (Baron-Cohen, 2000; Brent, Rios, Happé, & Charman, 2004; Peterson, Slaughter, Moore, & Wellman, 2016; Yirmiya, Erel, Shaked, & Solomonica-Levi, 1998; Zalla, Labruyère, Clément, & Georgieff, 2010, Boria et al., 2009; Sparaci, Stefanini, D’Elia, Vicari, & Rizzolatti, 2014). In typically developing adults, the processing of social information is associated with specific brain regions including areas in the temporal lobe - in particular the superior temporal sulcus (STS), temporoparietal junction (TPJ), orbitofrontal cortex, fusiform face area (FFA), as well as the amygdala (Adolphs, 2003). Several neuroimaging studies have reported that individuals with ASD show atypical responses to the processing of social information in these social brain regions (Amaral, Bauman, & Mills Schumann, 2003; Baron-Cohen et al., 2000; Misra, 2014; Pelphrey, Shultz, Hudac, & Van der Wyk, 2011; Zilbovicius et al., 2006). Atypicalities include, for example, diminished responses to social sounds (Gervais et al., 2004) and faces (Jemel et al., 2006) as well as altered processing of biological motion (Kaiser & Pelphrey, 2012).

Cortical activation selective for social stimuli begins to develop early in infancy. Functional near-infrared spectroscopy (fNIRS) is one of the neuroimaging techniques best suited to study cortical activation in young infants (Lloyd-Fox, Blasi, & Elwell, 2010; Lloyd-Fox et al., 2014). In fNIRS, measurements of change in absorption of near-infrared light from sensors placed on the infant’s head are used to infer cortical oxy- (HbO₂) and deoxy-hemoglobin (HHb) concentration changes associated with neuronal activation in the underlying tissue (Ferrari, Mottola, & Quaresima, 2004). Using fNIRS, Lloyd-Fox and colleagues (2009) were the first to show that - similar to adults - visual social stimuli elicit activation in the posterior temporal cortex in infants by five months of age. In the following years, other studies followed reporting similar early cortical selectivity to social stimuli, such as vocal sounds or social gaze cues (Blasi et al., 2011; Farroni et al., 2013; Grossmann, Lloyd-Fox, & Johnson, 2013; Grossmann, Oberecker, Koch, & Friederici, 2010; Johnson et al., 2005; Lloyd-Fox, Blasi, Mer-
cure, Elwell, & Johnson, 2011). Given that neural tuning towards social stimuli is already present at such a young age, atypical information processing within social brain regions in ASD may also be visible early in infancy and could serve as a potential early marker of the disorder.

One way to study early social processing deficits in ASD is by means of prospective longitudinal studies. Siblings of children diagnosed with ASD have an increased risk of receiving a diagnosis themselves (ranging from 10-20%, compared to 1% in the general population, Constantino, Zhang, Frazier, Abbacchi, & Law, 2010; Ozonoff et al., 2011). Following those infants during early development hence provides a unique opportunity to directly assess early markers of the disorder that may aid early detection, diagnosis and eventual treatment of ASD (Sven Bölte et al., 2013; Elsabbagh & Johnson, 2010). Using such a prospective study design, Lloyd-Fox and colleagues (2013) investigated the processing of complex dynamic social stimuli in 5-month-old infants at risk for ASD. In their experiment, infants were presented with engaging social videos of a female actor which were compared to a baseline of full-color static non-social images. In addition, infants were also presented with auditory vocal and non-vocal stimuli to assess temporal cortex responses to auditory stimuli. While typically developing infants showed specific activation in posterior temporal regions to the social compared to the non-social stimuli, this activity was reduced in the high-risk infants. Group differences were visible for both modalities, suggesting a generic difference in social information processing in the temporal cortex of infants at risk. In line with these findings, other studies have also reported differences in neural processing during the first year of life in high-risk infants (Bosl, Tierney, Tager-Flusberg, & Nelson, 2011; Elsabbagh et al., 2009; Luyster, Wagner, Vogel-Farley, Tager-Flusberg, & Nelson, 2011; Mc Cleery, Akshoomoff, Dobkins, & Carver, 2009; Wolff et al., 2012). Most behavioral atypicalities, on the other hand, become manifested gradually between the end of the first and the second year of life and are often subtle in nature (Elsabbagh & Johnson, 2010; Jones, Gliga, Bedford, Charman, & Johnson, 2014, but see Di Giorgio et al., 2016). Neuroimaging methods hence play an important role in the detection of early neural markers that precede the onset of behaviorally expressed symptoms.
The recent findings of atypical social processing in infants at risk for ASD by Lloyd-Fox and colleagues (2013) are promising, yet data was collected from a relatively small number of participants (18 high-risk and 16 low-risk infants) and findings thus require replication. The present study aimed to extend the previous findings of reduced neural sensitivity in high-risk infants (Lloyd-Fox et al., 2013) in an independent infant cohort. Functional NIRS data was collected from 5-month-old infants at high and low familial risk for ASD who were presented with social dynamic and non-social static visual stimuli. In addition, our current experimental design was extended to include a dynamic non-social control condition (similar to that used in a previous study of typically-developing infants; Lloyd-Fox et al., 2009). This condition was added to assess group differences in processing of social stimuli controlling for the amount of motion in the stimulus display. Although the spatial resolution of fNIRS is much better than that of electrophysiological measures (Lloyd-Fox et al., 2010), it is difficult to determine the exact anatomical location from which the measured signal originates. Since motion-sensitive areas such as MT/V5 are located close to the posterior STS, differences in motion information between stimuli may result in a potential confound: Increased cortical activity may represent sensitivity to motion (MT/V5) rather than the processing of social aspects of the stimuli (STS) (Lloyd-Fox et al., 2009). The previous study of infants at familial risk for ASD contrasted dynamic social stimuli with static non-social stimuli (Lloyd-Fox et al., 2013) and did not include a motion control condition. Therefore, while we believe from previous research in typically-developing infants (see Lloyd-Fox et al., 2009) that the channels identified by Lloyd-Fox and colleagues (2013) were over the pSTS-TPJ region for the low-risk infants, we do not know whether stimulus motion could have had an impact on the observed response in the high-risk infants. By contrasting social dynamic stimuli with non-social dynamic stimuli, the present study is able to assess differences between high- and low-risk infants in the cortical processing of social information controlling for the amount of motion presented. Based on previous research (Lloyd-Fox et al., 2013), we expected to find diminished neural hemodynamic responses in posterior temporal cortex to social stimuli in the high-risk infants compared to low-risk controls.
METHODS

Table 1. Characteristics of the final sample. Age and the MSEL Early Learning Composite (ELC) standard score are mean values with standard deviations reported in the parentheses. The range for the age and the ELC scores is reported in the square brackets. There were no significant differences between high- and low-risk infants in age ($t(27)=-0.07, p=0.55$), gender distribution ($X^2(1, N=29)=1.88, p=0.17$) or ELC standard scores ($t(27)=1.44, p=0.16$).

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Age</th>
<th>ELC standard score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-risk</td>
<td>13</td>
<td>5.27(0.50)[4.63-6.01]</td>
<td>95.62(09.01)[74-110]</td>
</tr>
<tr>
<td>High-risk</td>
<td>16</td>
<td>5.37(0.58)[4.70-6.51]</td>
<td>90.06(11.22)[61-106]</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>5.34(0.54)[4.63-6.51]</td>
<td>93.55(10.49)[61-110]</td>
</tr>
</tbody>
</table>

Participants

All infants who participated in this experiment were taking part in a longitudinal study on the early development of autism. High-risk (HR) infants were included if they had at least one older sibling with a clinical diagnosis on the autism spectrum. For all children, a clinical report was available to the research team that was used to confirm the diagnosis of the older sibling. Low-risk (LR) infants were included if they had at least one older typically-developing sibling and no family history of autism. In addition, all infants had to be born full-term (>36 weeks) to be included. The study was approved by the local medical ethics committee and written informed consent was given by both parents prior to the start of the experiment. Data are stored in the EUAIMS Data Repository and requests for data should go through the EUAIMS network data access policies. Thirty-five 5-month-old infants (16LR, 19HR) were enrolled and participated in the fNIRS experiment. Six infants (3HR) were later excluded due to poor data quality (3: 2HR), insufficient number of trials (1: LR) or experimenter error (2: 1HR). The final sample used for analysis hence consisted of 29 infants (13LR, 16HR, see Table 1). In addition to the fNIRS experiment described below, the development of each infant was assessed using the Mullen Scales of Early Learning (MSEL, Akshoomoff, 2006; Mullen, 1995). The MSEL is a standardized measure consisting of five scales (visual reception (VR), expressive (EL) and receptive language (RL) and gross (GM) and fine motor (FM)) which combined lead to the Early Learning Composite (ELC) standard score reflecting the overall development of the child. Importantly, we found no differences between the two infant groups for the overall ELC score ($t(27)=1.44, p=0.16$)
as well as for any of the five distinct sub-scales (GM: $t(27)=0.95, p=0.35$; VR: $t(27)=0.96, p=0.35$; FM: $t(27)=1.39, p=0.18$; RL: $t(27)=1.28, p=0.21$; EL: $t(27)=-0.55, p=0.58$), suggesting that our groups matched in their overall as well as domain-specific development.

**Stimulus material**

The stimulus material consisted of social and non-social (dynamic) videos as well as non-social static images. The non-social static baseline images were the same stimulus material as used by Lloyd-Fox and colleagues (2013) and consisted of pictures of transportation devices (such as cars or helicopters). Per baseline block, images were shown randomly for a variable duration of 1-3s per picture. The social dynamic video stimuli were also based on Lloyd-Fox and colleagues (2013) and the current social dynamic condition was the same as the visual-social condition of the previous study. The social videos showed a combination of actions or movements performed by a female actor. These included, for instance, the actor moving her eyes from one side to the other, moving her mouth, or performing hand games such as “peek-a-boo” (Figure 1). To match the amount and distribution of presented motion, the non-social video stimuli were selected from previous studies (Lloyd-Fox et al., 2009), as well as newly created stimulus material. The non-social videos showed a combination of moving toys or objects. These included, for instance, moving machines, spinning toys or toys that contained moving balls (Figure 1). The amount of motion was estimated for each stimulus video by inspecting the frame by frame changes in the sum of the squared differences in the red, green, and blue color channels of all pixels (Meyer, Braukmann, Stapel, Bekkering, & Hunnius, 2015; Schippers, Roebroeck, Renken, Nanetti, & Keysers, 2010). Consecutively, the motion energy was visually compared between the social and non-social stimuli and a selection of non-social videos was made for inclusion in the current study.

**Procedure**

Infants were seated in a baby carrier on their parent’s lap in a sound-proof testing booth in front of a computer screen monitor (24 inch, 16:9, 1920x1080 pixels). After the infants were capped with the NIRS headgear, they were presented with blocks of dynamic video clips in alternation with a non-social static baseline period (Figure 1). Stimulus presentation was realized using Matlab (Mathworks, Inc., Natick, MA; http://mathworks.com, Version 2011b) and
infants’ behavior was monitored online and videotaped for offline coding. If the participant disengaged from the display the experimenter could play an attention getting sound to redirect the infant’s attention back to the screen. The experiment continued until the infant was bored or showed signs of discomfort.

**Figure 1.** Time line of the experimental blocks. Infants were presented with blocks of social and non-social dynamic stimuli interspersed with a static non-social baseline block. The experiment always started with a baseline block but whether the first dynamic block was social or non-social was counterbalanced between participants.

**NIRS data acquisition**

NIRS data was collected using the University College London (UCL) topography system (Everdell et al., 2005) that emits near-infrared light at two wavelengths (780nm and 850nm). A custom-built headgear similar to Lloyd-Fox and colleagues (2013) was used to collect data from 26 channels (10 detector and 10 source optodes) which were placed over the temporal cortex at an inter-optode distance of 2cm (Figure 2). The headgear positioning was based on external anatomical landmarks of the infant’s head and placement was done such that the posterior area of the headgear approximately covered the STS-TPJ area according to previous research, and a NIRS-MRI co-registration map of scalp location to anatomy developed for this age range (Lloyd-Fox et al., 2009; Lloyd-Fox et al., 2013; Lloyd-Fox et al., 2014).

**Data pre-processing**

Infants looking behavior was coded offline to ensure that trials were only included in the analysis if an infant had watched at least 60% of the dynamic stimuli as well as 30% of the pre- and post stimuli static baseline (Lloyd-Fox et al., 2013). Importantly, we verified that there were no differences in looking time for the two conditions and between low- and high-risk infants (see supplementary analyses 1).
Neural processing of social stimuli in 5-month-old infants

The collected NIR attenuation data from each channel were assessed using artifact detection algorithms (Lloyd-Fox et al., 2009, Lloyd-Fox et al., 2013; Lloyd-Fox, Blasi, Everdell, Elwell, & Johnson, 2011) implemented in Matlab (Mathworks, Inc., Natick, MA; http://mathworks.com, Version 2015b). In line with previous work, channels were excluded if the coefficient of variation of the attenuation exceeded 10% or if the normalized power was larger than 35% with respect to the total power (Lloyd-Fox et al., 2013). If an infant showed artifacts on more than half of the channels, the infant was excluded from further analysis. Consecutively, the attenuation data was low-pass filtered at 1.2 Hz and blocks of 22s were extracted for each of the dynamic trials, consisting of the last 4s of the static baseline trial, the dynamic trial (8-10s) and the following static baseline trial (9-12s). Linear trends within each block were removed by subtracting a line defined between the first and last 4s of each block. Next, the data was transformed to HbO\textsubscript{2} and HHb concentration changes using the modified Beer-Lambert law (Delpy et al., 1988) with a differential pathlength factor.
of 5.13 for infants (Duncan et al., 1995). Finally, a trial was rejected within a channel if HbO$_2$ concentrations exceeded 3.5µM during baseline or 8µM during the dynamic stimuli. For a channel to be included in the statistical analysis for a particular infant, at least 3 valid artifact-free trials were required. The number of infants that were included for a particular channel was hence variable.

Data analysis
Data analysis followed closely the analysis steps from previous studies using a similar paradigm (Lloyd-Fox et al., 2009, Lloyd-Fox et al., 2013). Hence, HbO$_2$ and HHb concentration changes during the dynamic video presentation were assessed within a four-second time-window (10-14s). This window was chosen based on other recent fNIRS studies (Lloyd-Fox et al., 2013, Lloyd-Fox et al., 2016; Lloyd-Fox et al., in press) and taking into account that the hemodynamic response takes time to reach its peak after stimulus onset. We chose a slightly narrower time window in comparison to some previous studies (e.g.: Grossmann et al., 2013; Lloyd-Fox et al., 2011) as recent work by Lloyd-Fox and colleagues (2016) has shown that such a narrow window around the peak of the response provides a more robust marker of activation. Using this window, we extracted concentration changes for both HbO$_2$ and HHb. Although HBO$_2$ has a higher signal to noise ratio and responses are often more consistent in infants, it is recommended to report activation changes for both HbO$_2$ and HHb to provide a complete picture of cortical activation changes and to aid comparability between studies (Lloyd-Fox et al., 2010; Tachtsidis & Scholkmann, 2016) and we followed these recommendations in the current paper.

In a first analysis step, the averaged HbO$_2$ and HHb concentration changes for the two dynamic conditions were compared to baseline using one-sample t-tests. To control for multiple comparisons, p-values were corrected for the number of investigated channels using False Discovery Rate (FDR) methods (Benjamini & Yekutieli, 2001). Channels that showed significant activation from baseline were then further investigated. Importantly, activation was considered valid if channels showed an increase in HbO$_2$ and/or a decrease in HHb. Channels for which HbO$_2$ and HHb were significantly increasing or decreasing in unison were not included in the analysis, as the signal was then considered inconsistent with the usually elicited cortical response (see Lloyd-Fox et al., 2013). For the channels that showed significant signal change from baseline, the peak change
within the four second time window was then extracted for the dynamic conditions for further comparisons. In a first step, differences between the social dynamic and non-social dynamic stimuli were assessed within the two infant groups using paired sample t-tests. Then, in a final step, group differences in the responses to the dynamic stimuli were assessed using independent sample t-tests.
RESULTS

On average, infants watched 8.86 social dynamic blocks (range: 3-17), 8.86 non-social dynamic blocks (range: 3-17) and 16.55 static baseline blocks (range 5-35) and there was no difference in the amount of trials watched between the high-risk and low-risk infants (Social: $t(27)=0.57, p=0.58$; Non-Social: $t(27)=0.37, p=0.72$; Baseline: $t(27)=0.91, p=0.37$, for a more detailed report on the infants’ visual attention see supplementary analyses 1). The mean number of infants included in the final analysis per channel was 27 (12LR, 15HR) ranging from 25 (12LR, 13HR) to 28 (12LR, 16HR).

Figure 3. Results of the analysis comparing cortical activation to dynamic social (left) and non-social stimuli (right) with respect to the non-social static baseline. Low-risk infants (upper panels) showed increased HbO$_2$ concentration changes for the social dynamic stimuli in channel 25. High-risk infants (lower panels) showed increased HbO$_2$ concentration changes for the non-social dynamic stimuli in channel 22. Significant group differences were found in channel 25, indicated by the black circle.

Cortical activation to the dynamic stimuli was assessed with respect to the non-social static baseline period$^1$. An overview of the results is shown in Figure 3. For the low-risk infants, the analysis revealed a significant increase of HbO$_2$

$^1$: The results reported as significant have been corrected for multiple comparisons using False Discovery Rate (FDR) methods. An overview of the significant channels based on the uncorrected p-values can be found in the supplementary table 1.
for the social dynamic condition in channel 25 ($t(11)=4.07$, $p=0.048$, corrected using FDR), which was positioned over the right pSTS-TPJ region. No significant activation was found for HbO$_2$ for the non-social dynamic condition ($p>0.46$ for all tests, corrected using FDR). No significant activation was found for HHb for either condition ($p>0.12$ for all tests, corrected using FDR). For the high-risk infants, no channels showed significant HbO$_2$ activation for the social dynamic condition ($p>0.27$ for all tests, corrected using FDR). There was, however, a significant increase in HbO$_2$ concentration changes for the non-social dynamic condition in channel 22 ($t(15)=3.92$, $p=0.04$, corrected using FDR), which was positioned over the same right pSTS-TPJ region. No significant activation was found for HHb for either condition ($p>0.33$ for all tests, corrected using FDR). Channel 22 and 25 were hence selected for further analysis of condition and group differences in HbO$_2$ concentration changes. Figure 4 shows the averaged HbO$_2$ and HHb time courses for the social and non-social dynamic condition in

**Figure 4.** Averaged Hemoglobin concentration changes for the social and non-social dynamic condition for the low-risk (LR, upper panels) and high-risk (HR, lower panels) infants in channel 25 (left) and 22 (right). Time point 0 represents the onset of the dynamic video block and the dotted window shows the time window on which the statistical comparisons are based. Group differences are indicated by the asterisk.
those channels for the low- and high-risk infants. Condition differences were significant for the low-risk infants in channel 25, as the social dynamic stimuli elicited significantly larger HbO$_2$ concentration changes than the non-social dynamic stimuli ($t(11)=2.82, p=0.02$, Cohen’s $d=0.94$). There were no condition differences in channel 22 ($t(10)=1.47, p=0.17$) for the low-risk infants. Importantly, for the high-risk infants, no condition differences were found for either channel (22: $t(14)=-0.67, p=0.51$; 25: $t(15)=0.34, p=0.74$). In a last step, group differences were assessed and independent sample t-tests showed that the HbO$_2$ response to social dynamic stimuli in channel 25 was significantly larger for the low-risk infants compared to the high-risk group ($t(26)=-2.22, p=0.04$, Cohen’s $d=0.87$, Figure 3). There were no group differences for the non-social dynamic stimuli in channel 25 ($t(26)=-0.09, p=0.93$) and no group differences were found for channel 22 for either condition (social: $t(24)=-0.57, p=0.57$, non-social: $t(24)=-0.67, p=0.51$).
DISCUSSION

Previous research found that 5-month-old infants at high risk for developing Autism Spectrum Disorders show reduced neural sensitivity to social stimuli (Lloyd-Fox et al., 2013). In the present study, a similar experimental design was implemented and our results extend the original findings. In line with our hypothesis, low-risk infants showed significant activation over right posterior temporal cortex in response to social stimuli, whereas this response was not significant in the high-risk infants. Importantly, we compared social and non-social dynamic stimuli which were matched on the amount of motion in the stimulus display. This contrast enabled us to assess whether activation of the posterior temporal cortex was modulated by the social aspects rather than representing activation originating from motion-sensitive brain regions. Confirming our hypothesis, low-risk infants’ showed a socially selective cortical response in the right posterior temporal cortex: HbO$_2$ concentration changes were larger in response to the social dynamic than the non-social dynamic condition, suggesting that the reported activation indeed originated from regions involved in the processing of social information (i.e. pSTS-TPJ, see also Lloyd-Fox et al., 2009). In contrast, social dynamic stimuli did not elicit any significant cortical activation in the high-risk infants. Rather, we found significant activation for the non-social condition in the right posterior temporal cortex with respect to baseline for this infant group. Similar activation from baseline to non-social dynamic stimuli has also been reported previously in typically developing infants (Lloyd-Fox et al., 2009) and is likely due to the more engaging nature of the dynamic stimuli compared to the static baseline. Although it is interesting that the activation to the non-social condition in the high-risk infants did survive FDR-correction whereas the social activation did not, it is important to note that we did not find significant differences between the social and non-social stimuli. Rather the time courses of the HbO$_2$ responses shown in Figure 4 were very similar for both conditions. These results suggest that both conditions were processed similarly by the high-risk infants and that the socially selective processing visible in the low-risk infants was diminished in the high-risk group.

Our findings complement previous studies reporting early social processing differences in at-risk infants compared to typically-developing controls (Elsabbagh et al., 2012; Jones et al., 2016; Lloyd-Fox et al., 2013). Moreover, our results
are in line with previous work in older individuals with ASD showing atypical social processing (Gervais et al., 2004; Jemel et al., 2006) and attentional orienting (Klin, Lin, Gorrindo, Ramsay, & Jones, 2009), as well as difficulties in the integration of complex dynamic social information (Shah, Bird, & Cook, 2016). It has been theorized that the atypicalities in social processing observed in ASD may be the result of an early failure to orient towards social information (Johnson, 2014; Jones et al., 2008). Typically-developing infants show an early bias drawing their attention towards socially relevant stimuli, like faces (Farroni, Csibra, Simion, & Johnson, 2002; Johnson, 2005; Morton & Johnson, 1991). The interactive specialization theory postulates that the cortical social brain network emerges through an interaction of those early attentional biases and environmental experiences. Abnormalities in the bias to orient towards socially-relevant stimuli in ASD may hence lead to a cascade, disrupting typical developmental processes (Johnson, 2001; Johnson, 2011). There is an ongoing debate about this hypothesis as several studies have recently reported typical patterns of attention to social stimuli in young infants at risk for ASD (Elsabagh et al., 2013; Jones & Klin, 2013). Jones and Klin (2013), for instance, showed that fixations to the eye-region during the presentation of faces were similar for 2-month-old high-risk infants who later received an ASD diagnosis and low-risk controls. The researchers reported that differences in looking at the eyes of others only emerged later, between 2 and 6 months of age. In contrast, Di Giorgio and colleagues (2016) recently reported differences in attention to social stimuli already in newborns at high risk for ASD. Future studies will need to integrate the different findings and provide more detailed reports of the development of social processing in infants at risk for ASD. Our current findings, showing that by 5 months of age socially selective cortical activation is diminished in high-risk infants, suggest that atypicalities in social processing are present during the first half year of development, but more research will be needed to assess when these deviations first emerge.

One difference between the current study and the previous findings by Lloyd-Fox and colleagues (2013) that needs consideration is the extent of cortical activation we observed. While, previously, broader bilateral temporal cortex activation to social stimuli was found (Lloyd-Fox et al., 2009, Lloyd-Fox et al., 2013), significant activation was limited to right posterior temporal cortex in the current experiment. We argue that this difference can be explained by the
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strict measures that we applied to control for multiple comparisons. Whereas
the previous study reported uncorrected p-values, our results were corrected
using a False Discovery Rate approach which reduced the number of channels
that were considered significant (see supplementary table 1 for a complete
report of all significant corrected and uncorrected p-values). In addition, the
sample size of the current experiment was slightly lower than in the previous
study which may have led to less power for detecting cortical activation.

An observation we would like to further discuss is the apparent initial decrease
of the HbO₂ response for both the social and non-social condition visible in
channel 25 in the high-risk infants (see Figure 4). To assess this response fur-
ther, we performed an additional analysis testing for activation from baseline
using a 3-7 seconds time window surrounding the minimum of the response
visible in Figure 4. This analysis revealed that both dynamic conditions indeed
elicited a significant decrease of HbO₂ in channel 25 for the high-risk infants
(social: t(15)=-2.18, p=0.046; non-social: t(15)=-3.57, p=0.003, uncor-
rected). Importantly, a deactivation was only significant for HbO₂ but not HHb
(social: t(15)=-1.37, p=0.191; non-social: t(15)=1.91, p=0.075, uncorrected).
As the two chromophores were thus not decreasing in unison or mimicking each
other, we would not consider this response to represent an artifact. We would
rather argue that the observed initial deactivation in the high-risk infants may
represent a meaningful characteristic of the hemodynamic response to the
presented social and non-social stimuli. Interestingly, Lloyd-Fox and colleagues
(in press) found a similar pattern of early decreased activation in a group of
high-risk infants that went on to receive an ASD diagnosis in toddlerhood,
supporting the notion that this phenomenon may be a relevant characteristic
of early autism. However, deactivation of HbO₂ is difficult to interpret and it
remains unclear what is driving this response. Therefore, additional research
using larger samples is needed to replicate this finding and further investigate
its underlying physiology and significance.

Following multiple comparison correction, our results showed significant ac-
tivation for the low-risk infants for the social dynamic stimuli in channel 25 only,
and for the high-risk infants for the non-social dynamic stimuli in channel 22
only. Since both channels are located over the pSTS-TPJ region, we may have
expected more similar patterns of results for both channels, rather than a group
and condition specific difference. Therefore, we further investigated whether patterns of activation were indeed disparate for these two channels, or whether the differences in significant results for the two channels may have been influenced by the relatively low sample size and strict multiple correction criteria we applied. In line with this interpretation, one should note that the HBO response to the social condition for the low-risk infants in channel 22 was significant based on an uncorrected p-value as shown in the supplementary table 1. To further assess channel differences, we performed a 2x2 repeated measures ANOVA for each of the infant groups separately, using Channel (Ch22, Ch25) and Condition (social, non-social) as within subject factors (see supplementary analyses 2). For both infant groups, we did not find a main effect of channel or an interaction between channel and group. These results suggest that overall there was no substantial difference in the responses in channel 22 and 25. Crucially though, this additional analysis did show a significant main effect of Condition for the low-risk group only, but not for the high-risk group, in line with the results from the main analysis.

Despite a slightly smaller sample size and more conservative analysis approach, we replicated the expected pattern of increased cortical activation within the pSTS-TPJ region to social stimuli in low-risk controls which was absent in the high-risk group. Our study thus illustrates that fNIRS is a powerful technique which is able to detect atypicalities in brain function during early infancy. While many of the behavioral red-flags of developing ASD - such as lack of response to own name or difficulties in joint attention - start to emerge only around the end of the first or second year of life (Jones et al., 2014; Palomo et al., 2006; Zwaigenbaum et al., 2005), this study and other neuroimaging experiments have shown group differences earlier in development (Lloyd-Fox et al., 2013; Bosl, Tierney, Tager-Flusberg, & Nelson, 2011; Wolff et al., 2012). We currently have no information on whether the infants in our sample will develop typically or receive a diagnosis within the autism spectrum at a later age. Therefore, while our results may be related to early autism, they may also indicate a risk group effect that is unrelated to a later ASD diagnosis. Likewise, some studies have shown that early group differences can be present in high-risk infants (Merin, Young, Ozonoff, & Rogers, 2007) while not being related to a later ASD diagnosis (Young, Merin, Rogers, & Ozonoff, 2009). Whilst others have shown that early neural responses can be associated with a later diagnoses of ASD (Elsabbagh et al. 2012, Lloyd-Fox et al., in press). Whether our current results
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represent an early marker of ASD or rather a characteristic of the risk group can be investigated once outcome data is available for our sample.

At the age of 36 months, a preliminary diagnosis of ASD can be made enabling researchers to classify high-risk participants into groups of infants who do develop ASD (HR-ASD) and those who do not (HR-noASD). Recent findings from a collaborating lab using a similar fNIRS paradigm (Lloyd-Fox et al., 2013) suggest that atypicalities in social processing may indeed be especially pronounced in high-risk infants that receive a diagnosis of ASD at 36 months (Lloyd-Fox, et al., in press). The researchers found that HR-ASD infants showed diminished social brain network activation to visual and auditory social stimuli compared to low-risk controls, providing the first evidence that these neural signatures may have the potential to be an early marker of the disorder. Their results were based on a small sample of 5 HR-ASD infants, so it will be important to establish whether those infants from our sample who go on to receive a diagnosis of ASD at 36 months also show similar patterns of atypicality. Furthermore, in line with previous prospective infant ASD research (Elsabbagh et al., 2013; Kaiser et al., 2010), Lloyd-Fox and colleagues (in press) found that differences between HR-ASD and HRD-noASD infants were not as strong as those with LR infants, suggesting that altered cortical responses to social stimuli may also be present in the broader autism phenotype (BAP). The BAP describes the finding that unaffected family-members of individuals with autism share characteristics of the disorder at a subclinical level (BAP, Macy et al., 2013; Piven, Palmer, Jacobi, Childress, & Arndt, 1997). Getting a clearer picture of the characteristics of the BAP over development as well as of differences that can be predictive of ASD in the high-risk infants will greatly benefit our understanding of the disorder and aid early detection and diagnosis. To enable those more detailed analyses in the future, data from larger samples will be required. Once the infants from the current study reach the age of 36 months, data can be pooled with other samples - such as the sample from Lloyd-Fox and colleagues (in press) – to create a large dataset for further analysis.

Taken together our findings provide compelling evidence for an early social processing difference in 5-month-old infants at risk for ASD. Future research will determine whether these differences relate to an eventual diagnosis or may rather reflect the broader autism phenotype.
SUPPLEMENTARY MATERIAL

A) Supplementary Table 1. Results from the one-sample t-tests assessing significant changes in HbO₂ and HHb with respect to baseline in the low-risk and high-risk infant group. Channels reaching significance based on uncorrected p-values are shown for each of the contrasts together with their FDR-corrected values which were used to determine significance in the main manuscript. Ch represent the channel number and p_FDR represents the corrected p-value using False Discovery Rate correction. FDR corrected values were calculated using the mafdr function implemented in Matlab and corrections were done for the number of channels (n=26) used within each contrast. Significant channels after correction are highlighted bold.

<table>
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<th>HbO2</th>
<th>t</th>
<th>p</th>
<th>p_FDR</th>
<th>HHB</th>
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### Low-risk infants

#### Non-Social Dynamic vs. Baseline

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<td>Ch 12</td>
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### High-risk infants

#### Non-Social Dynamic vs. Baseline

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<td>Ch 13</td>
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<td>0.370</td>
<td>Ch 21</td>
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<tr>
<td>Ch 22</td>
<td>3.92</td>
<td>0.002</td>
<td><strong>0.040</strong></td>
<td>Ch 24</td>
<td>2.51</td>
<td>0.024</td>
<td>0.296</td>
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</table>
B) Supplementary analyses

1. Analysis of group and conditional differences in stimulus attention

To ensure that our results were not confounded by group or condition differences in stimulus attention, we performed additional analyses on the video coding data. More specifically, we determined the percentage of looking for all of the social, non-social, and baseline blocks and compared the average looking time between blocks and between the high- and low-risk infant groups. An overview of the average looking time per block can be found in the table below (Table BT1). Importantly, infants’ average looking time for the social and non-social stimuli exceeded 80% which is comparable to previous studies (Shimada & Hiraki, 2006) and suggests that infants were generally very attentive in the current study.

<table>
<thead>
<tr>
<th>Block Type</th>
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<th>High-risk</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Social</td>
<td>86.84 (10.34)</td>
<td>81.12 (10.42)</td>
<td>83.68 (10.60)</td>
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<tr>
<td>Non-social</td>
<td>81.87 (11.51)</td>
<td>82.19 (12.85)</td>
<td>82.05 (12.05)</td>
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<tr>
<td>Baseline</td>
<td>70.67 (11.62)</td>
<td>69.79 (13.78)</td>
<td>70.18 (12.64)</td>
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</table>

To assess condition differences, group differences and interaction effects, we performed a 3x2 repeated measures ANOVA with Block (social vs. non-social vs. baseline) as within subject factor and Group (high-risk vs. low-risk) as between subject factor. There was no main effect of Group ($F(1,27)=0.31, p=0.58$) and no interaction between Group and Block ($F(2,54)=1.25, p=0.30$). We found a significant main effect of Block ($F(2,54)=27.08, p<0.01$). Paired-sample t-tests showed that there was no difference between looking time during social and non-social blocks ($t(28)=0.81, p=0.43$) but that infants’ looking time was lower for the baseline blocks compared to the social ($t(28)=5.65, p<0.01$) and non-social ($t(28)=7.56, p<0.01$) blocks. Given the nature of the stimuli (dynamic videos vs. static baseline images) a difference between looking time between the two dynamic conditions and the baseline is expected. More importantly, the absence of a difference between the two dynamic conditions and between the two groups suggest that attentional effects are unlikely to have influenced the current results.
2. *Analysis of possible differences between channel 22 and 25*

To investigate whether responses in channel 22 and 25 were different between conditions for the low- and high-risk infants, we performed a 2x2 repeated measures ANOVA for each of the infant groups using Channel (Ch22, Ch25) and Condition (social, non-social) as within subject factors. For the low-risk group, we found a main effect of Condition ($F_{(1,10)}=10.46, \ p<0.01$), indicating that HBO$_2$ responses for the social condition were overall larger than responses in the non-social condition. The main effect of Channel ($F_{(1,10)}=2.31, \ p=0.16$) and the interaction effect were not significant ($F_{(1,10)}=0.50, \ p=0.50$) for the low-risk infants. For the high-risk group, there was no significant main effect of Condition ($F_{(1,14)}=1.27, \ p=0.28$) or Channel ($F_{(1,14)}=2.56, \ p=0.13$), and the interaction effect did not reach significance either ($F_{(1,14)}=2.53, \ p=0.13$). These results suggest that for both infants the two channels did not differ significantly from each other. Moreover, the finding that a main effect of Condition is present for the low-risk group but not for the high-risk group is in line with the results reported in the main manuscript.
Chapter 2

Action prediction in 10-month-old infants at high and low familial risk for autism spectrum disorder

Ricarda Braukmann
Emma Ward
Roy S. Hessels
Harold Bekkering
Jan K. Buitelaar
Sabine Hunnius

Based on: Action prediction in 10-month-old infants at high and low familial risk for autism spectrum disorder (submitted).
ABSTRACT

Background: Several studies have reported action prediction difficulties in individuals with Autism Spectrum Disorder (ASD). Although action prediction develops in infancy and plays an important role in social interactions, little is known about early prediction abilities and potential atypicalities in ASD.

Methods: Using eye tracking, we measured action anticipations in 52 10-month-old infants at high and low familial risk for ASD. Infants were presented with actions during which a familiar object was either brought to a location usually associated with the object or to an unusual location.

Results: We investigated infants’ anticipations to the actual target location (i.e. the location where the object was actually being brought to) and the alternative target location for both usual and unusual actions. Across the low- and high-risk infant groups, anticipation frequencies were modulated by object knowledge and the actions associated with them. In particular, participants tended to look more frequently to the alternative target location when presented with unusual compared to usual actions. Importantly, we did not find any differences between the low- and high-risk infants in predictive eye movements.

Conclusion: We found that object knowledge modulated action predictions in the low- and high-risk infants, and there were no significant differences between the two infant groups. Whereas our results suggest that familial risk for ASD does not affect action prediction at 10 months of age, future research needs to investigate whether prediction atypicalities might manifest in the subgroup of high-risk infants who later receive an ASD diagnosis.
INTRODUCTION

Autism Spectrum Disorder is defined by deficits in social interaction and communication as well as stereotyped behavior and restricted interests (APA, 2013). Recently, various researchers have proposed that prediction difficulties may underlie multiple of the diverse deficits associated with ASD (van de Cruys et al., 2014; Gomot & Wicker, 2012; Lawson, Rees, & Friston, 2014; Pellicano & Burr, 2012; Sinha et al., 2014). Several of these accounts aim to explain the ASD symptoms from a Bayesian perspective and suggest that the inferential processes that integrate prior information and incoming sensory evidence may be affected in individuals with ASD (Brook, 2012; van de Cruys et al., 2014; Lawson et al., 2014; Pellicano & Burr, 2012). It is argued that atypical predictive processing could explain altered perception and sensory experiences in ASD (e.g., Pellicano & Burr, 2012), but may also result in the associated social and communication deficits by affecting the individual’s ability to predict others’ actions and intentions (Sinha et al., 2014). In line with these theoretical propositions, several empirical studies have reported that individuals with ASD show difficulties in action prediction (Boria et al., 2009; Cattaneo et al., 2007; Schuurk et al., 2016; Zalla et al., 2010; Zalla, Labruyere, & Georgieff, 2006). Cattaneo and colleagues (2007), for instance, found that typically developing 5 to 9-year-old children showed anticipatory muscle activation when performing and observing action sequences. Children with ASD, on the other hand, lacked this anticipatory activation, both during action execution and action observation. Although these results were, at the time, interpreted in the light of a proposed deficit in the human mirror neuron system (MNS, Iacoboni & Dapretto, 2006; Oberman et al., 2005; Rizzolatti, Fabbri-destro, & Cattaneo, 2009; Rizzolatti & Fabbri-Destro, 2010; Southgate & Hamilton, 2008; Théoret et al., 2005; Williams, Whiten, Suddendorf, & Perrett, 2001), these findings are also in accordance with recent theories suggesting a general prediction deficit in ASD (Van de Cruys et al., 2014; Gomot & Wicker, 2012; Lawson et al., 2014; Pellicano & Burr, 2012; Sinha et al., 2014). In typically-developing individuals, the MNS is activated during action execution and observation (e.g., Cochin, Barthelemy, Roux, & Martineau, 1999; Hari et al., 1998), which is thought to reflect the mapping of observed actions onto own motor representations. This mapping is proposed to play a crucial role in the generation of action predictions based on the observer’s own motor plans (Elsner, D’Ausilio, Gredebäck, Falck-Ytter, &
Fadiga, 2013; Kilner et al., 2007; Prinz, 2006). A deficit in the MNS as proposed by several researchers is hypothesized to influence the mapping of observed behavior and may affect aspects of social cognition in ASD (Théoret et al., 2005; Williams et al., 2001; Iacoboni & Dapretto, 2006; Oberman et al., 2005; but see Fan, Decety, Yang, Liu, & Cheng, 2010; Southgate & Hamilton, 2008), including the reported difficulties in action prediction (Boria et al., 2009; Fabbri-Destro, Cattaneo, Boria, & Rizzolatti, 2009; Zalla et al., 2010).

Multiple studies assessing action prediction differences in individuals with ASD have made use of eye tracking to study anticipatory eye movements during action observation. Previous research has established that typically-developing individuals predict ongoing goal-directed actions, as they fixate the target area of an observed action before it is reached (Elsner, Falck-Ytter, & Gredebäck, 2012; Falck-Ytter et al., 2006; Flanagan & Johansson, 2003; Hunnius & Bekkering, 2010). Falck-Ytter (2010) used eye tracking to assess whether five-year-old children with ASD showed anticipatory eye movements when observing an actor performing a series of simple actions (i.e. moving balls into a bucket). Contrary to previous findings suggesting prediction difficulties (Cattaneo et al., 2007; Zalla et al., 2010), this study reported that children with ASD showed predictive eye movements that were similar to typically-developing children. Recent work by Schuwerk, Sodian and Paulus (2016), on the other hand, suggests that 10-year-old children and adults with ASD do differ from controls in more complex action prediction tasks. They assessed the influence of statistical learning on predictive gaze behavior and found that the overall frequency of predictions was lower in individuals with ASD. The repetition of the stimulus lead to accurate predictions in controls but had a weaker effect on the ASD group. Marsh, Pearson, Ropar, and Hamilton (2014) investigated action prediction during the observation of rational and irrational actions, and also reported that individuals with ASD looked less at the action target and had fewer trials during which they showed anticipations. However, when participants with ASD did anticipate, their goal anticipations were similar to controls in this study. Although more research is needed to further assess action prediction difficulties in individuals with ASD and integrate the different results, the findings thus far suggest that anticipatory eye movements can be typical in ASD in the context of a simple goal directed action (Falck-Ytter, 2010), but that individuals show less anticipations when viewing actions which can unfold in several ways (Marsh et al., 2014;
Schuwerk et al., 2016) and that prediction differences may especially arise when frequency information about the observed action needs to be integrated (Schuwerk et al., 2016).

Thus far, our knowledge about action prediction in ASD is limited to school-aged children and older individuals, and little is known about its early development. Yet, the ability to form and update predictions about others’ actions develops already early in infancy (Falck-Ytter et al., 2006; Hunnius & Bekkering, 2014; Meyer, Bekkering, Haartsen, Stapel, & Hunnius, 2015; Stapel, Hunnius, van Elk, & Bekkering, 2010). Multiple studies have reported that infants as young as six months show anticipatory eye movements towards observed action goals (Falck-Ytter, Gredebäck, & von Hofsten, 2006; Hunnius & Bekkering, 2010), similar to adults (Flanagan & Johansson, 2003). Further, toddlers’ precision in predicting the timing of an observed action has been linked to the ability to act jointly with a partner (Meyer et al., 2015), stressing the importance of action prediction abilities for other social skills. Despite its early development and important role in social interactions, our knowledge about early action prediction in young children and infants with ASD to date is limited.

The early characteristics of ASD can be studied by following infants who have an older diagnosed sibling (Bölte et al., 2013; Zwaigenbaum et al., 2007) as these children have an increased risk of receiving a diagnosis themselves (ranging from 10-20%; see: Constantino, Zhang, Frazier, Abbacchi, & Law, 2010; Ozonoff et al., 2011). From past cohort studies, we know that these infants at high risk can already show behavioral abnormalities during their first two years of life (Jones et al., 2014). These early differences can help to deepen our understanding the development of ASD, and to provide possibilities for earlier detection which is expected to be beneficial for individuals with ASD and their families (Zwaigenbaum et al., 2007). The first evidence for a potential early marker related to action anticipation difficulties comes from a study by Brisson, Warreyn, Serres, Foussier, and Adrien-Louis (2012), who used retrospective video recordings to analyze feeding situations. The authors reported that those infants who later received an ASD diagnosis showed fewer mouth-opening anticipations during feeding between 4 and 6 months of age. How young infants at high risk observe and predict goal-directed actions of others, however, has to our knowledge not yet been investigated.
The current study examined predictions about others’ actions in a cohort of 10-month-old high- and low-risk infants. More specifically, we used eye tracking to assess infants’ anticipatory eye movements during the observation of usual and unusual goal-directed actions performed on everyday objects. The present study was based on research by Hunnius and Bekkering (2010), who found that infants as young as six months old showed predictive eye movements during action observation. Moreover, the infants’ anticipations were already modulated by their object knowledge at this young age. In their study, infants performed anticipatory eye movements to the target location of an action more frequently when they were presented with an object that was usually associated with that target location (e.g. bringing a cup to the mouth) rather than when they observed an unusual action (e.g. bringing a hair brush to the mouth). The aim of the current study was to assess whether infants at high risk for ASD show differences compared to low-risk controls in anticipatory eye movements during the observation of usual and unusual goal-directed actions as used by Hunnius and Bekkering (2010). Infants were presented with an actor picking up either a phone or a cup and bringing the object to either the ear or the mouth. This resulted in two experimental conditions: an action ending at a location usually associated with the object (i.e. the phone to the ear, or the cup to the mouth) or at an unusual location (i.e. the phone to the mouth, or the cup to the ear). We then assessed the frequency of anticipations to the actual target location (i.e. where the object was actually being brought to) and the alternative target location for the usual and unusual condition. Based on the previous findings by Hunnius and Bekkering (2010), we expected typically-developing infants to show more frequent anticipations towards the actual target location in the usual compared to the unusual condition (i.e. looking more frequently at the mouth during a cup-to-mouth action vs. a phone-to-mouth action, and looking more frequently at the ear during a phone-to-ear action vs. a cup-to-ear action). Reversely, we expected low-risk infants to show more frequent anticipations towards the alternative target location in the unusual compared to the usual condition (i.e. looking more frequently at the mouth during a cup-to-ear action vs. a phone-to-ear action, and looking more frequently at the ear during a phone-to-mouth action vs. a cup-to-mouth action). We compared action predictions from 10-month-old infants at high familial risk for ASD with low-risk age-matched control participants. Given the previous findings of prediction difficulties in ASD (Boria et al., 2009; Cattaneo et al., 2007; Schuwerk et al.,
2016; Sinha et al., 2014; Zalla et al., 2010), our study aimed to assess whether atypicalities in action prediction manifest early in the development of infants at increased risk for ASD.
METHODS

Participants
All infants in the current sample participated in a longitudinal multi-centre study on the early development of autism. Infants were tested at one of two sites (S1, S2). Procedures for both sites were identical unless noted. Families were invited to participate in a set of experiments at several time points during the first three years of the infants’ life after birth. The current eye tracking experiment was one task during the visit at 10 months of age. In total, 61 participants - 36 high-risk infants (HR; S1:24, S2:12) and 25 low-risk infants (LR; S1:18, S2:7) - participated in the current experiment. Nine infants (7HR, 2LR) had to be excluded from data analysis due to lack of sufficient valid trials (n=7, 6HR) or technical problems with the eye tracking equipment (n=2, 1HR), leading to a final sample of 52 participants (29HR (S1:18, S2:11); 23LR (S1:17, S2T:6), see Table 1).

Table 1. Sample characteristics. We verified that the two infant groups were similar in age (t(50)=-0.44, p=0.67) and their developmental stage as measured by the Mullen Scales of Early Learning (MSEL) Composite Score (t(50)=1.02, p=0.31). The gender distribution between the two groups did also not differ significantly (χ²(1, n=52)=0.44, p=0.51).

<table>
<thead>
<tr>
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<th>N</th>
<th>Age</th>
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<tbody>
<tr>
<td>HR</td>
<td>29 (14♀)</td>
<td>10.18 (0.51)</td>
<td>92.66 (13.93)</td>
</tr>
<tr>
<td>LR</td>
<td>23 (9♀)</td>
<td>10.13 (0.41)</td>
<td>96.57 (13.33)</td>
</tr>
<tr>
<td>Total</td>
<td>52 (23♀)</td>
<td>10.15 (0.46)</td>
<td>94.38 (13.68)</td>
</tr>
</tbody>
</table>

Inclusion criteria
High-risk infants were included in the study if they had at least one full older sibling with a clinical diagnosis on the autism spectrum. The diagnosis of the older child was confirmed with a clinical report made available to the research team. Low-risk infants had at least one older typically-developing sibling and no family history of autism. All included infants were born full term (>36 weeks) and were spoken to in Dutch at home by at least one parent. Infants with visual or hearing impairments or a history of epilepsy were not eligible for inclusion in the study. In addition, infants could not participate in the control group, if parents reported concerns about their child’s development. The study was approved
Procedure

Assessment of general development and motor abilities
In addition to the eye tracking experiment (see below), the infant’s development was assessed using the Mullen Scales of Early Learning (MSEL, Akshoomoff, 2006; Mullen, 1995) which is a standardized measure that can be administered with children up to 6 years of age. The MSEL consists of five scales on which scores can be computed separately (visual reception, expressive and receptive language and gross and fine motor skills). From these sub-scores, the Early Learning Composite Score (ELC) was computed as an index of the overall development of the child.

Eye tracking
Infants were invited to the lab (S1) or visited at home by the research team (S2) and participated in a set of eye tracking assessments, including the reported experiment. Infants were seated in an infant chair or on the parent’s lab in front of a Tobii eye-tracker (S1: Tobii T120, S2: Tobii TX300, Tobii Technology, Danderyd, Sweden, see Supplementary Table 1 for more details) at a distance of approximately 65 cm. Calibration and stimulus presentation was realized using Matlab (Mathworks, Inc., Natick, MA; http://mathworks.com), Psychtoolbox (Brainard, 1997; Kleiner et al., 2007; Pelli, 1997), the Tobii SDK 3.0 toolbox (Tobii Technology, Danderyd, Sweden) and Talk2Tobii (Deligianni, Senju, Gergely, & Csibra, 2011). A five-point calibration, with one point in each of the four screen corners and one point in the middle of the screen, was used to calibrate the eye-tracker (cf. Hessels, Andersson, Hooge, Nyström, & Kemner, 2015). Inwards turning spirals were used together with sounds to draw the infant’s attention to the calibration points. If four or more points were calibrated successfully, the experiment was started. Otherwise, the calibration procedure was repeated for the missing calibration points. During the experiment, the infant was presented with video stimuli of a female actor manipulating an every-
day object in either a usual or an unusual way. The infant was monitored by the experimenter and attention-getting sounds were played if the infant disengaged from the screen. In case the infant continued to disengage, a visual attention getter could be played in between the stimulus presentation. The experiment ended once the infant had completed all 32 trials or terminated prematurely in case s/he showed signs of discomfort. For two participants, the experiment was terminated and then administered again fully at a later point during the testing day. To ensure that the total number of trials included in the analysis was the same for these participants as for the other infants, blocks that were already presented during the first demonstration were excluded from the second run and only novel trials were included in the final analysis. The infant’s behavior was video recorded throughout the session to allow for online monitoring.

**Stimulus material**

Presented stimulus videos were based on the material used by two previous studies with a very similar paradigm (Hunnius & Bekkering, 2010; Stapel et al., 2010) in which participants viewed an actor performing usual and unusual actions with everyday objects. In contrast to these previous studies, the current study used partly occluded stimuli (see Figure 1) after familiarizing the infants once with the completely visible actions. By occluding part of the action, we aimed to reduce the distraction in the visual scene during the object lifting phase and increase the infant’s attention towards the target locations of the presented actions.

The experiment consisted of four blocks containing eight videos each. Each video had a duration of approximately four seconds and showed a female actor picking up either a cup or a phone and bringing the object to either a location usually associated with the object (i.e. the cup to the mouth, or the phone to the ear) or to an unusual location (i.e. the phone to the mouth, or the cup to the ear).

For the different stimulus videos, two female actors were recorded performing both actions (usual and unusual) on the two objects (cup and phone). Two different exemplars of each object were used leading to 16 different videos in the final stimulus set. Of each video, an occluded version was created. Using Virtual Dub 1.9.11 (http://virtualdub.org/), a black bar was placed over each video, covering the movement trajectory of the hand. The occluder was of same size
for all videos (618x395 pixels, about 43.89% of the whole videoimage), but the location was shifted to best occlude the movement trajectory for each individual video. The average stimulus video durations ranged from 3.76s to 4.60s and did not differ between the two conditions (Usual: $M=4.08s$, $SD=0.22s$; Unusual: $M=4.21s$, $SD=0.25s$; $t(14)=-1.09$, $p=0.29$). The elapsed time between the disappearance and reoccurrence of the object behind the occluder was variable across the different stimulus videos ($M=429ms$, $SD=75.19ms$) to ensure that infants could not predict the reappearance based on occlusion duration. Importantly though, the average occlusion duration did not differ between the two conditions (Usual: $M=404ms$, $SD=62.11ms$; Unusual: $M=454ms$, $SD=82.64ms$; $t(14)=-1.37$, $p=0.19$).

**Figure 1.** Stimulus Material. This figure shows two examples of the experimental stimuli presented during the experiment. At the start of each block a non-occluded video was presented in which a female actor was grasping either a cup (left example) or a phone (right example) and bringing this object either to a usual location (left example) or to an unusual location (right example). Consecutively, the infants were presented with a partly occluded version of the video for another 7 trials.

In each of the four experimental blocks, infants were presented with the same actor-object-location combination repeatedly. The first trial of each block was a full, non-occluded, presentation of the action, after which the infants were presented with seven trials where the action was partly occluded (see Figure 1). Each infant was presented with both conditions and both objects, but saw one actor performing the usual actions and the other actor performing the unusual actions. Blocks of the two conditions (usual and unusual actions) were presented in alternation. The actors were counterbalanced between participants as to which performed the usual actions and which the unusual actions, and the condition, object, and actor combination that was presented first was also counterbalanced.
**Analysis of Eye tracking data**

For the analysis of the eye tracking data, the cup-to-mouth actions and phone-to-ear actions were collapsed into a usual action condition and the cup-to-ear and phone-to-mouth actions were collapsed into an unusual action condition. The main analysis focused on comparing low- and high-risk infants’ anticipation frequencies in the occluded trials for these two conditions. Anticipations during the first trials were analyzed separately, as described below. To analyze the eye movement data, we used analogous procedures to previous studies (Hunnius & Bekkering, 2010; Stapel et al., 2010).

In a first step, Areas of Interest (AoIs) and Time Windows of Interest (TWoIs) were defined for each of the stimulus videos separately. There were two AoIs in each video: the mouth AoI and the ear AoI. The AoIs were defined as equal-sized rectangular-shaped areas around the ear and mouth and had the same size across all stimulus videos (210x125 pixels, see Figure 2). Given that the eye tracker’s accuracy for infants is generally lower than its optimal value (e.g. Hessels et al., 2015), the size of the AOIs was a multiple of the optimal accuracy value reported by the manufacturer (see supplementary Table 1). For each video, there was one TWoI, which started 200ms after the beginning of the video and ended when the hand and object reappeared behind the upper part of the occluder.

To extract the infants’ fixations from the raw gaze data, a custom-made software tool (GSA, Donders Institute, Nijmegen, The Netherlands) was used. Successive gaze points were marked as a fixation if they remained within a radius of 30 pixels for at least 40ms (cf. Meyer et al., 2015). From the extracted fixation data, we calculated anticipation frequencies using Matlab 2015b (Mathworks, Inc., Natick, MA; http://mathworks.com) as described below. Although previous studies suggest that standard event-detection algorithms are susceptible to data quality differences that may exist between individuals with and without ASD (Hessels, Niehorster, Kemner, & Hooge, 2016; Shic, Chawarska, & Scassellati, 2008), we chose to follow the same fixation detection procedures as used in previous studies (Hunnius & Bekkering, 2010; Meyer et al., 2015; Stapel et al., 2010) for the following reasons: Most importantly, while measures as fixation durations and number of fixations have been shown to be influenced by data quality, the designation of a given fixation as within an AoI should
not be affected. As we use the fixations only to establish whether or not an infant looked at a certain AoI, and not as a measure of how often, or how long they looked, we would not expect potential differences in general data quality between the two groups to affect our results. Moreover, we were interested in group differences of the within-subject modulation by condition which also should not be influenced by group differences in data quality. Lastly, as we aimed to replicate the previous findings (Hunnius & Bekkering, 2010; Stapel et al., 2010), we considered it essential to stay as close as possible to the original data analysis strategy in order to be able to compare the current results with the previous findings. Statistical analyses were performed using IBM SPSS Statistics for Windows (Version 23.0. Armonk, NY: IBM Corp) and JASP (Version 0.8.0.1, Love et al., 2015).

**Figure 2. Areas of Interest.**

This figure shows an example of the AoIs used for the analysis of occluded trials for the Usual (left) and Unusual (right) condition. AoIs were the same size for both mouth and ear area and across the different stimulus videos.

**Frequency of anticipatory looks**

All fixations to the target AoIs during the TWoI were considered to be an anticipation towards this target location. A trial during which the infant was not looking at the screen (i.e. did not have at least one fixation to the screen) during
the TWoI was considered invalid, and infants were only included in the analysis if they contributed at least four valid trials per condition. For each infant, it was assessed per trial whether s/he showed an anticipatory fixation (i.e. a fixation during the TWoI) towards one or both of the two AoIs. If the infant anticipated to the target location where the object was also actually brought (i.e. looking at the mouth during cup-to-mouth and phone-to-mouth actions and looking to the ear during cup-to-ear and phone-to-ear actions), this was considered an actual target anticipation. If the infant showed a predictive fixation towards the other location (i.e. looking at the ear during cup-to-mouth and phone-to-mouth actions and looking to the mouth during cup-to-ear and phone-to-ear actions), this was considered an alternative target anticipation. A trial could contain both actual and alternative target anticipations. Differences between the two groups and conditions were analyzed using a 2x2x2 repeated measures ANOVA with Anticipation Location (actual target vs. alternative target) and Condition (usual vs. unusual) as within-group factor and Group (HR vs. LR) as between-subject factor.

Bayesian analysis of the frequency of anticipatory looks
To estimate the strength of the evidence associated with our results, we conducted Bayesian repeated measures analyses using JASP (Love et al., 2015) using the same factors as in the repeated measures ANOVA reported above.

Analysis of the first trial
In order to assess whether high- and low-risk infants differed in their spontaneous anticipation to the actual target location without prior familiarization, we analyzed anticipation frequency in the first, unoccluded, trials separately. As the first trials did not contain an occluder, AoIs were adjusted to fit around the mouth and ear area (see Supplementary Figure 1) to be more comparable with previous studies (Hunnius & Bekkering, 2010; Stapel et al., 2010). Importantly, the AOI size was identical for the mouth and ear AoIs and the same across all stimulus videos (80x100 pixels). The TWoI started 200ms after stimulus onset and ended when the object entered the target AoI. Infants watched a total maximum of two first trials per condition and were included in the analysis if they had at least one valid trial per condition. A trial was considered invalid if the infant did not look at the screen during the TWoI. For each of the two conditions, infants were then classified as anticipating if they fixated at the actual target
AoI during the TWoI in one or both of the first trials of the specific condition. To investigate group differences, a chi-square analysis was performed for the two conditions separately. Condition differences were assessed across the groups by means of a McNemar’s test.
RESULTS

Frequency of anticipatory looks
Table 2 gives an overview of the number of trials infants contributed for each condition as well as the number of total anticipations infants made during the experiment. Figure 3 shows the averaged frequency of anticipations to the actual and alternative targets per condition, separated by risk group. The repeated measures ANOVA revealed no significant main effect of Group ($F(1,50)=1.66, p=0.20$) and no interaction effects involving Group (Group x Condition: $F(1,50)=0.12, p=0.73$; Group x Anticipation Location: $F(1,50)=0.58, p=0.45$; Group x Condition x Anticipation Location: $F(1,50)=0.25, p=0.62$). Frequencies of anticipations thus did not differ significantly between low- and high-risk infants.

Figure 3. Mean Anticipation Frequency. This figure shows the average relative anticipation frequency to the actual and alternative target location for the Usual and Unusual action conditions separated for the low- and high-risk infants. Our results showed no significant effects of group on the anticipation frequency. Error bars indicate +/- 2 SE.

The analysis, however, did reveal a significant main effect of Anticipation Location ($F(1,50)=49.61, p<0.01, \eta_p^2 =0.50$). Post-hoc paired sample t-tests revealed that for both conditions, infants looked more frequently at the actual target (Usual: $M=0.40, SD=0.22$; Unusual: $M=0.37, SD=0.22$) compared to the alternative target (Usual: $M=0.17, SD=0.15, t(51)=5.92, p<0.01$; Unusual: $M=0.21, SD=0.16, t(51)=5.19, p<0.01$). Importantly, our analysis also revealed
a marginally significant Interaction effect between Condition and Anticipation Location ($F(1,50)=3.07, p=0.09, \eta^2_p =0.06$). Post-hoc paired sample t-test revealed that infants looked significantly more frequently at the alternative target for the Unusual compared to the Usual condition ($t(51)=-2.18, p=0.03$). There was no difference between the two conditions in the frequency of looks towards the actual target location ($t(51)=0.83, p=0.41$).

Bayesian analysis of the frequency of anticipatory looks
To assess the strength of the evidence for the null hypothesis (i.e., no group differences), we conducted a Bayesian repeated measures ANOVA in JASP (Love et al., 2015) using the same factors as in the repeated measures ANOVA reported above. Our goal was to assess whether the null model, without familial risk for ASD as a factor, would explain the observed data better than a model with familial risk. Hence the main and interaction effects of Condition and Anticipation Location were included in the null model which was then evaluated against the model including the main effect and interaction effects of familial risk. The Bayes factor in favor of the null hypothesis ($BF_{01}$) was 2.15 for the model including only the main effect of familial risk and ranged from 7.71 to 113.47 for the other models including the main and interaction effects. This suggests that the null model explained the data two times better than the model including the main effect and at least eight times better than the models including the main effect of familial risk and one or multiple interaction effects involving familial risk. A full overview of the results of this analysis can be found in Supplementary Table 2.

Analysis of the first trials
Eight infants had to be excluded from the first trial analysis (6HR, 2LR) due to insufficient valid trials for one or both conditions, leaving a final sample of 44 infants (23HR, 21LR). There were no differences in age ($t(42)=0.11, p=0.92$), MSEL ELC score ($t(42)=1.09, p=0.28$) or gender distribution ($\chi^2(1,n=44)=0.38, p=0.54$) for this subset of infants. In addition, the number of valid first trials was also not different between the two groups (Usual: $t(42)=0.73, p=0.47$; Unusual: $t(42)=0.55, p=0.59$). Figure 4 illustrates the number of infants that did and did not show a prediction in the first trial, separated by group and condition. There were no significant group differences for the Usual ($\chi^2(1,n=44)=0.01, p=0.94$) or Unusual condition ($\chi^2(1,n=44)=0.73, p=0.39$) in the number of infants that
did show one or more actual anticipations during the first trial. To investigate condition differences, we combined the scores of the two groups and assessed condition differences using a McNemar’s test. This analysis revealed that the distribution of anticipating and non-anticipating infants was not significantly different between the two conditions ($p=0.58$).

**Figure 4.** Anticipations during the first trial. This figure shows the number of infants that did and did not show an actual target anticipation during the first trial for the two condition and separated for the low- and high-risk infants. Our results showed no group differences in the distribution of anticipating and non-anticipating infants.

**Table 2.** Overview of the number of trials and total anticipations. The number of valid trials that the infants contributed to the Usual and Unusual condition is shown in the two left columns. The total number of anticipations - representing the number of actual and alternative target anticipations over all trials- is shown in the two right columns. The first presented value per column is the mean value averaged across participants, followed by the standard deviation and the range. There was no difference in the number of valid trials ($t(51)=1.21$, $p=0.23$) or the total target anticipations ($t(51)=0.04$, $p=0.91$) between the Usual and Unusual condition across all infants. In addition, there were no group differences in the number of valid trials for the Usual condition ($t(50)=1.10$, $p=0.28$) or for the Unusual condition ($t(50)=1.71$, $p=0.09$) and there were also no group differences in the number of total anticipations for the Usual condition ($t(50)=1.41$, $p=0.16$) or for the Unusual condition($t(50)=1.03$, $p=0.31$). HR= high-risk infants, LR=low-risk infants.

<table>
<thead>
<tr>
<th></th>
<th>Valid Trials</th>
<th>Total anticipations (actual + alternative)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Usual</td>
<td>Unusual</td>
</tr>
<tr>
<td></td>
<td>HR</td>
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</tr>
<tr>
<td></td>
<td>LR</td>
<td>11.04 (2.72) (4-14)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>10.54 (2.95) (4-14)</td>
</tr>
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</table>
**DISCUSSION**

Recent theoretical accounts as well as empirical studies suggest that individuals with ASD show difficulties in generating predictions about observed actions (Cattaneo et al., 2007; van de Cruys et al., 2014; Pellicano & Burr, 2012; Sinha et al., 2014; Zalla et al., 2010). While several eye tracking studies have assessed how children and older individuals with ASD process and predict others’ actions (Falck-Ytter, 2010; Marsh et al., 2014; Schuwerk et al., 2016), little is known about the early development of action prediction in ASD. The present study assessed whether 10-month-old infants at high familial risk for ASD show anticipatory eye movements during the observation of goal-directed actions and how these anticipations were modulated by object knowledge. Infants were presented with familiar objects that were either brought to a location usually associated with the object or to an unusual location. We did not find any significant effects of familial risk in our main analysis of anticipation frequencies during the repeated presentation of the stimulus videos. Moreover, we did not observe a significant difference in the number of low- and high-risk infants that showed actual target anticipations during the first trial of each of the presented blocks. These findings suggest that anticipations during initial as well as repeated presentations of object-directed actions did not differ between infants at high familial risk for ASD and low-risk controls.

In line with previous work in typically developing infants (Hunnius & Bekkering, 2010), we did see a marginally significant interaction effect between the two conditions and the two types of anticipation: Infants showed more anticipations towards the alternative target location when they were presented with the unusual actions compared to the usual actions. Participants thus were more likely to look at the location where nothing happened if this location was associated with the presented object (i.e. looking at the mouth during cup-to-ear actions and looking at the ear during phone-to-mouth actions). This finding is in line with the notion that by 10 months of age, infants have acquired knowledge about the presented objects and the associated actions, which allows them to make predictions during action observation (Hunnius & Bekkering, 2010). It should be noted, however, that the differences we observed between the usual and unusual condition were less pronounced compared to the previous
findings reported by Hunnius and Bekkering (2010)\(^1\). The differences between the current and the previous study may be explained by the adaptation of our study design. Hunnius and Bekkering (2010) used a between-subjects design where infants were either presented with usual or unusual actions. Our design, on the other hand, was a within-subjects design and the alternating presentation of usual and unusual objects within our experiment may have reduced the infants’ reliance on their prior object knowledge in making predictions. Previously, Stapel and colleagues (2010) used a similar within-subject design and showed that cortical activation of the motor system differed for the usual and unusual conditions, even though the researchers observed no differences in predictive eye movements. The neuroimaging findings by Stapel and colleagues (2010) are in line with the notion of Hunnius and Bekkering (2010) that object knowledge influences the processing of observed actions. The absence of a behavioral effect in the study by Stapel and colleagues (2010) may be explained by the within-subject design and the small sample size (n=11) which could have reduced their sensitivity to detect a small effect. Crucially, although the size of the interaction effect was indeed small in the current study using a within-subject design (\(\eta_p^2 = 0.06\)), the pattern of anticipation frequencies we observed was similar to Hunnius and Bekkering (2010) and we did observe a difference between the usual and unusual conditions for the alternative target predictions in the expected direction.

Our results suggest that object knowledge influenced action predictions across all infants and that there were no differences in the anticipation frequencies between the low- and high-risk infants. To assess the evidence for the null hypothesis that predictions were the same for low- and high-risk infants, we additionally performed Bayesian analyses. The results showed that the null model (no effect of familial risk) explained the data better than any model including ASD risk as a factor. In particular, there was “moderate” to “strong” evidence (Wetzels, van Ravenzwaaij, & Wagenmakers, 2015) for the null model over those alternative models that included the different interaction effects of

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\(^1\): We only observed conditional differences for the alternative goal anticipations but not for the actual goal anticipations, unlike the previous study. In addition, the number of infants that showed actual goal predictions during the first trial was not different between the two conditions in our experiment, whereas this difference was present for most stimuli in the previous study.
familial risk (see supplementary Table 2). These findings support our interpretation that the pattern of anticipations was similar for all participants and that object knowledge influenced action predictions in a similar way for the low- and high-risk infants.

In older children and adults with ASD, prediction difficulties have been reported in multiple studies (Boria et al., 2009; Cattaneo et al., 2007; Schuwerk et al., 2016; Zalla et al., 2010) and atypical predictive processing is a proposed underlying mechanism of the disorder (van de Cruys et al., 2014; Lawson et al., 2014; Pellicano & Burr, 2012; Sinha et al., 2014). In an eye tracking study, Schuwerk and colleagues (2016) reported that individuals with ASD showed lower anticipation frequencies as well as a diminished sensitivity to repeated stimulus presentation. In the current experiment, we found no group differences in action prediction during the first trial or during the repeated presentations, suggesting that the frequency of stimulus presentation did not affect the low- and high-risk infants differentially. Our findings further showed that predictions in both groups were similarly affected by prior object knowledge at 10 months of age, suggesting that familial risk for ASD does not influence action prediction at this age. Noteworthy, some studies have reported group differences between high and low-risk infants by 10 months, suggesting that atypicalities can already be detected at this young age. For instance, Elsabbagh and collagues (2009) found slower attentional disengagement in 9- to 10-month-old high-risk infants relative to controls, suggesting that atypical visual orienting is part of the infant broader autism phenotype (Macy et al., 2013; Piven et al., 1997). In a follow-up study, they further showed that atypicalities in the development of attentional disengagement between 7 and 14 months were related to a later ASD diagnosis (Elsabbagh et al., 2013). In our study, we found that action prediction did not differ between the low- and high-risk infants suggesting that mere familial risk for ASD is not associated with prediction difficulties at 10 months of age. We currently have no information, however, whether and which high-risk infants from our cohort will receive an ASD diagnosis in the future. Although prediction was typical on average in the high-risk group, it could be the case that those high-risk infants that later receive a diagnosis on the autism spectrum (approximately 20% of our sample, cf. Ozonoff et al., 2011) do show atypicalities in their action prediction compared to typically-developing controls and unaffected high-risk siblings. On the other hand, it is also possible that prediction difficulties may
emerge only at a later point in development. To disentangle these two options, diagnostic outcome of our sample will be required. Interestingly, although there were no group differences in the total number of anticipations that infants made (see table 2), there were five infants in our sample that did not show any anticipatory eye movements to either of the target locations during one or both of the experimental conditions, and four of those five participants were high-risk infants. These descriptive findings suggest that complete absence of anticipations was more frequent in the high-risk group even though this did not influence the overall results. Diagnostic outcome data will be required to assess this further and investigate whether the failure to predict in these individual infants might be related to later ASD outcome.

In summary, the current study revealed that both low- and high-risk infants showed anticipatory eye movements during action observation and that object knowledge modulated action predictions across all infants. Our findings suggest that the mere familial risk for ASD does not influence action prediction at 10 months of age. Whether prediction difficulties are present in those high-risk infants that later receive an ASD diagnosis, however, remains to be investigated in future work.
SUPPLEMENTARY MATERIALS

**Supplementary Table 1.** Hard and software specification of the eye tracker and programmes used for stimulus presentations at the two sites. S1 = infants tested at site 1, S2 = infants tested at site 2.

<table>
<thead>
<tr>
<th></th>
<th>S1</th>
<th>S2</th>
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<tbody>
<tr>
<td>Tobii Eyetracker</td>
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**Supplementary Figure 1.** This figure shows an example of the AoIs used for the analysis of the first trial for the Usual (left) and Unusual (right) condition. AoIs were the same size for both mouth and ear area and across the different stimulus videos. Note that for these two example videos, only fixations to the actual goal AoI (i.e. the mouth) would be analyzed.

¹: Optimal accuracy stated by the manufacturer. In both eye tracker set-ups, 1 degree approximately corresponds to 43 pixels.
**Supplementary Table 2.** Results of the Bayesian repeated measures analysis of the anticipation frequencies. Con=Condition, Ant=Anticipation Location.

Bayesian Repeated Measures ANOVA

| Models                                      | P(M)  | P(M|data) | BF_M | BF_{01} | error % |
|---------------------------------------------|-------|----------|------|---------|---------|
| Null model (incl. Con, Ant, Con ▪ Ant, subject) | 0.167 | 0.578    | 6.857| 1.000   |         |
| Group                                       | 0.167 | 0.269    | 1.843| 2.147   | 4.907   |
| Group ▪ Group ▪ Con                         | 0.167 | 0.056    | 0.294| 10.415  | 4.632   |
| Group ▪ Group ▪ Ant                         | 0.167 | 0.075    | 0.406| 7.707   | 5.230   |
| Group ▪ Group ▪ Con ▪ Group ▪ Ant           | 0.167 | 0.017    | 0.085| 34.712  | 5.760   |
| Group ▪ Group ▪ Con ▪ Group ▪ Con ▪ Ant     | 0.167 | 0.005    | 0.026| 113.446 | 6.775   |

*Note.* All models include Con, Ant, Con ▪ Ant, subject.

| Effects                           | P(incl) | P(incl|data) | BF_{Inclusion} |
|-----------------------------------|---------|---------|----------------|
| Group                             | 0.833   | 0.422   | 0.146          |
| Con ▪ Group                       | 0.500   | 0.077   | 0.084          |
| Ant ▪ Group                       | 0.500   | 0.097   | 0.107          |
| Con ▪ Ant ▪ Group                 | 0.167   | 0.005   | 0.026          |
Action prediction in 10-month-old infants
Chapter 3

Motor experience modulates action prediction in infants at low and high familial risk for autism spectrum disorder

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Emma Ward
Nicolette M. Munsters
Harold Bekkering
Jan K. Buitelaar
Sabine Hunnius

Based on: Motor experience modulates action prediction in infants at low and high familial risk for autism spectrum disorder (submitted).
ABSTRACT

Autism Spectrum Disorder (ASD) has been associated with difficulties in predicting others’ actions. As action prediction develops in infancy, difficulties may be visible in infants at risk prior to ASD diagnosis. We analyzed eye tracking data from 29 14-month-olds at high and low familial risk. Infants were presented with partially occluded actions (i.e. Crawling, Walking or Object movement) which differed in how motorically familiar they were to the participants. We showed that prediction accuracy and stability were modulated by action experience for all infants, suggesting that low- and high-risk infants recruit their own motor representations during the prediction of observed actions. Crucially, there were no group differences, suggesting that familial risk for ASD did not influence prediction abilities at 14 months.
INTRODUCTION

Autism spectrum disorder (ASD) is a developmental disorder defined by deficits in social interaction and communication as well as repetitive, stereotyped behaviors (APA, 2013). Empirical findings have shown that the social deficits associated with ASD include difficulties in predicting the outcome of an observed action (Boria et al., 2009; Hudson, Burnett, & Jellema, 2012; Sparaci, Stefanini, D’Elia, Vicari, & Rizzolatti, 2014; Zalla, Labruyère, Clément, & Georgieff, 2010; but see Falck-Ytter, 2010; Marsh, Pearson, Ropar, & Hamilton, 2014). Zalla and colleagues (2010), for instance, reported that children and adolescents with ASD performed worse in predicting the outcomes of incomplete movie sequences displaying familiar and non-familiar actions compared to both individuals with intellectual disabilities and a typically-developing control group. Boria and colleagues (2009) examined more specifically which aspects of action prediction were impaired in children with ASD, contrasting the understanding of what the other person is doing and why she is doing it (action intention). Their results showed that while individuals with ASD were unimpaired in reporting what the actor was doing (i.e. whether she was “touching” or “grasping” the object), they performed worse than controls in predicting the actor’s intentions (i.e. whether she was grasping the object to “use” it or to “place” it). The researchers further showed that difficulties were in particular present when the action intentions had to be inferred from the motor information conveyed by the actor’s handshape rather than from contextual cues. These findings are in line with theoretical accounts suggesting that individuals with ASD may show atypicalities in the cortical motor system involved in action processing (Rizzolatti, Fabbri-Destro, & Cattaneo, 2009; Rizzolatti & Fabbri-Destro, 2010). The human motor system shows overlapping activity during action execution and observation in typically developing individuals (Cochin, Barthelemy, Roux, & Martineau, 1999; Hari, 2006; Lepage & Théoret, 2006; Kilner, Vargas, Duval, Blakemore, & Sirigu, 2004; Southgate, Johnson, Osborne, & Csibra, 2009). It has been argued that this activity reflects the mapping of observed behavior onto own motor representations, which is hypothesized to facilitate the formation of predictions about others’ actions (Kilner et al., 2007; Prinz, 2006; Rizzolatti & Sinigaglia, 2016). Some researchers have proposed that a deficit in this mapping of observed behavior in the neural mirror system could be an underlying mechanism explaining the observed social symptoms in ASD (Oberman et al., 2005; Rizzolatti &
Fabbri-Destro, 2010). Although such a “mirror neuron deficit” account of ASD is highly debated (Southgate & Hamilton, 2008), some studies do show support for atypical motor activity during action observation in individuals with ASD (Cattaneo et al., 2007; Oberman et al., 2005; Oberman, Ramachandran, & Pineda, 2008; Théoret et al., 2005). Cattaneo and colleagues (2007), for instance, reported that typically-developing children showed anticipatory activation of facial muscles when they were grasping a piece of food to then bring it to their mouth for eating. Crucially, similar anticipatory activation was found during an action observation condition in the typically-developing children. Children with ASD, however, failed to show anticipatory activation during both the performance and observation of those actions. The authors argued that their results may reflect difficulties in integrating different motor acts into a larger action in individuals with ASD. This integration is suggested to be necessary for forming predictions about the end goal of the action.

In line with the empirical findings of atypical action prediction (Boria et al., 2009; Cattaneo et al., 2007; Fabbri-Destro et al., 2009; Zalla et al., 2010), recent theoretical accounts have proposed that impairments in predictive abilities may be at the core of multiple of the deficits associated with ASD (Brock, 2012; van de Cruys et al., 2014; Gomot & Wicker, 2012; Lawson, Rees, & Friston, 2014; Pellicano & Burr, 2012; Sinha et al., 2014). Several researchers have theorized that ASD symptoms could be explained within a Bayesian framework where the process of integrating prior expectations and sensory input is distorted in ASD (Brock, 2012; van de Cruys et al., 2014; Lawson et al., 2014; Pellicano & Burr, 2012). Although it remains debated which particular aspect of the inferential process would be affected (Brock, 2012; van de Cruys et al., 2014; Lawson et al., 2014; Pellicano & Burr, 2012), these accounts all propose that a distortion in the inferential process could lead to both sensory abnormalities, as well as social deficits including atypical action prediction (van de Cruys et al., 2014; Sinha et al., 2014).

Despite the evidence for prediction difficulties in ASD, we know very little about the early development of these processes. The ability to generate predictions about others’ actions develops early in infancy: Several studies using eye tracking have reported that typically-developing infants as young as six months show anticipatory fixations towards the goal of an observed action before that goal
Motor experience modulates action prediction in 14-month-old infants

is reached by the actor (Ambrosini et al., 2013; Falck-Ytter, Gredebäck, & von Hofsten, 2006; Hunnius, & Bekkering, 2014; Hunnius & Bekkering, 2010). This ability to predict others’ actions has also been linked to the infant’s own motor skills (Ambrosini et al., 2013; Kanakogi & Itakura, 2011; Stapel et al., 2016), which is in accordance with the notion that own motor representations are used to form predictions about observed actions (Kilner, Friston, & Frith, 2007; Prinz, 2006; Rizzolatti & Sinigaglia, 2016). As children learn to predict others’ actions early in life, potential atypicalities in action anticipation in ASD may already be visible early in development during infancy and toddlerhood. Currently, ASD is rarely diagnosed before the age of three (Begeer et al., 2013; Daniels & Mandell, 2014), yet an earlier diagnosis and potential treatment is thought to be beneficial for the individuals and their families (Bölte et al., 2016; Hahler & Elsabbagh, 2014). If action prediction difficulties are present during infancy or toddlerhood in individuals with ASD, those deviations could serve as an early marker of the disorder. As far as we are aware, however, action prediction has only been assessed in children and older individuals with ASD. A recent study by Falck-Ytter (2010), for instance, used eye tracking to assess whether 5-year-old children with ASD use predictive eye movements when watching a simple goal-directed action (i.e. an actor moving balls into a bucket). Interestingly, children with ASD performed similarly to typically-developing controls, which is in contrast to previous studies showing prediction differences in children and adolescents with ASD (Cattaneo et al., 2007; Zalla et al., 2010). One reason for these different findings may be that the actions Falck-Ytter (2010) presented were very simple and highly familiar to the children. Previous research has reported action prediction difficulties in children in particular for more complex actions that rely on the integration of different action steps (Cattaneo et al., 2007) or the extraction of motor information for prediction (Boria et al., 2009). Crucially, we have little knowledge about the development of action prediction in infants for neither simple nor complex tasks. Younger individuals with ASD may show differences in action prediction and/or its development may be delayed in ASD, despite the typical performance at a later age (Falck-Ytter, 2010). Assessing action prediction abilities in infants or toddlers at risk for ASD is therefore vital to better understand the nature and development of potential action prediction difficulties.

Atypicalities in infants at high risk for ASD might be particularly visible in action prediction tasks that rely on the recruitment of own motor experience.
First, in infants, motor experience has been shown to play an important role for action prediction (Kanakogi & Itakura, 2011; Stapel et al., 2016). Second, prediction difficulties in ASD have been suggested to be especially pronounced in tasks that require inferences based on motor information (Boria et al., 2009, but see also Cattaneo et al., 2007; Fabbri-Destro et al., 2009). Research on motor development in ASD suggests that many individuals with ASD also show motor problems (Cossu et al., 2012; Gowen & Hamilton, 2013; Silas, Levy, & Holmes, 2012) and some consider motor dysfunctions another core deficit of ASD (Bo, Lee, Colbert, & Shen, 2016). Interestingly, several studies suggest that motor difficulties include atypicalities in motor planning and anticipation of own actions (Fabbri-Destro et al., 2009; Forti et al., 2011; Martineau, Schmitz, Assaiante, Blanc, & Barthélémy, 2004; Schmitz, Martineau, Barthélémy, & Assaiante, 2003).

The current study investigated the influence of motor experience on action prediction in 14-month-old infants at high familial risk for ASD by virtue of having an older diagnosed sibling. Specifically, high-risk infants and typically-developing same-aged controls were presented with partially occluded actions (i.e. Crawling, Walking or Object movement) which differed in how motorically familiar they were to the participant. Using eye tracking, we assessed how accurate infants were in predicting the timing of the observed action (prediction accuracy) and how stable their predictions were across different trials (prediction stability). Both of these measures have been used in a previous studies by Stapel, Hunnius, Meyer, & Bekkering (2016) which investigates the role of motor experience on action prediction in typically-developing infants and toddlers. The researchers demonstrated that action experience modulated both the accuracy and stability of predictions made by 14-month-old infants: Predictions were more accurate and stable for actions that the infants had more experience with (i.e. Crawling actions) compared to less familiar actions (i.e. Walking actions) and object movements. These findings suggest that typically-developing infants recruit own motor representations when predicting observed actions (see also Kanakogi & Itakura, 2011). Using the same paradigm as Stapel and colleagues (2016), the current study investigated whether 14-month-old high-risk infants show atypicalities in prediction accuracy and stability compared to low-risk controls. Moreover, we also assessed whether action experience modulates predictions in a different manner for both infant groups.
METHODS

Participants
In total, 29 14-month-old infants (14HR, 15LR, see table 1) were included in the final analysis of this study. An additional 27 infants participated (19HR, 8LR), but had to be excluded as they did not reach the required number of valid trials for each condition. All infants were taking part in a longitudinal multi-centre study on the early development of autism, in which families were invited to participate in a set of experiments at four to five time points during the first three years of the infant’s postnatal life. The current eye tracking task was one of multiple tasks the infants completed during the visit at 14 months of age. The current sample was tested at two research institutes (S1: n1=18, 10HR, 8LR; S2: n2=11, 4HR, 7LR). Procedures for the two sites were the same unless noted. An overview of the demographic information of the infants included in this study can be found in table 1.

Table 1. Overview of infants who participated in this study. There was no significant difference between the two groups in Age (t(27)=-0.23, p=0.82), ELC (t(27)=1.18, p=0.25), distribution of gender (X²(1, N=29)=0.28, p=0.60), GM (t(27)=0.45, p=0.65), or FM (t(27)=0.35,p=0.73). ELC: Mullen Scales of Early Learning Early Learning Composite Score; GM: Mullen Scales of Early Learning Gross Motor T-Score; FM: Mullen Scales of Early Learning Fine Motor T-Score; HR= high-risk infants; LR=low-risk infants.

<table>
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<tr>
<th></th>
<th>N</th>
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<th>MSEL ELC</th>
<th>MSEL GM</th>
<th>MSEL FM</th>
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<td>LR</td>
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<td>93.73 (19.21)</td>
<td>40.93 (15.30)</td>
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<td>Total</td>
<td>29</td>
<td>14.35 (0.56)</td>
<td>89.41 (17.22)</td>
<td>38.28 (13.36)</td>
<td>52.83 (9.83)</td>
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</tbody>
</table>

Participants were recruited throughout the country and an infant was considered as high-risk if he/she had at least one older sibling with a clinical diagnosis on the Autism Spectrum. Previous studies have shown that siblings of children with ASD are at increased risk of receiving a diagnosis themselves (ranging from 10-20%; Constantino, Zhang, Frazier, Abbacchi, & Law, 2010; Ozonoff et al., 2011). Infants from the control group needed to have at least one older typically-developing sibling (minimum three years of age) and no family history of autism to be eligible for participation. All included infants were born full term (>36 weeks) and were spoken to in Dutch at home by at least one parent.
Infants with a history of epilepsy, or visual or hearing impairments were not included in the study. In addition, infants were not included in the control group if parents reported concerns about their child’s development. Before the testing, all parents gave written informed consent for participation and the study was approved by the local ethics committee.

Procedure

Assessment of general development and motor abilities
Participants came to the lab (S1) or were visited at home by the researchers (S2). During the visit, the infants participated in the eye tracking experiment (see below) and in addition, their development was assessed using the Mullen Scales of early learning (MSEL, Akshoomoff, 2006; Mullen, 1995). The MSEL is a standardized measure consisting of five scales (Visual Reception, Expressive Language, Receptive Language, Gross Motor Skills (GM), and Fine Motor Skills (FM)). For each scale, a T-score can be extracted with a mean of 50 and standard deviation of 10. In addition, the early learning composite (ELC) score is often reported as an indication of the overall development of the child. The ELC score can be calculated from the total of scores on all scales and has a mean of 100 and standard deviation of 15. In addition to the MSEL assessment, we also established whether the infants were able to crawl and walk respectively by evaluating the parent report on the infant’s gross motor abilities administered as part of the Vineland Adaptive Behavior Scale II (Sparrow, 2011).

Eye tracking
Infants sat on the parents lap or in an infant seat while their eye movements were recorded using a Tobii Eyetracker (S1: Tobii T120, S2: Tobii TX300, Tobii Technology, Danderyd, Sweden, see supplementary materials for additional information). Stimulus size (in pixel) and stimulus presentation was the same for both sites and all infants. Calibration and stimuli were presented using Matlab (Mathworks, Inc., Natick, MA; http://mathworks.com) and Psychtoolbox 3.0.11 (Brainard, 1997; Kleiner et al., 2007; Pelli, 1997) using the Tobii SDK 3.0 toolbox (Tobii Technology, Danderyd, Sweden) and Talk2Tobii toolbox (Deligianni, Senju, Gergely, & Csibra, 2011). A five-point calibration, with one point in the middle of the screen and four points in each of the screen corners was used to calibrate the eye tracker (cf. Hessels, Andersson, Hooge, Nyström,
Motor experience modulates action prediction in 14-month-old infants (Kemner, 2015). Calibration images were inwards turning spirals which were presented together with sounds to draw the infant’s attention to the calibration points. The experiment was started, if at least four points were successfully calibrated.

During the experiment the infants were presented with video stimuli of another infant walking or crawling, or of an object moving across the screen at constant velocity. All movement paths were partly occluded (see Figure 1). During stimulus presentation, the infant was monitored by the experimenter who could play attention getting sounds if the infant disengaged from the screen. A visual attention getter could be played in between the stimulus presentation, if the infant continued to disengage from the screen. The experiment was terminated once the infant had completed all trials or in case the infant showed signs of discomfort. The infant’s behavior was video recorded throughout the session to allow for online monitoring and offline coding.

Figure 1. Example of stimulus videos. The infants observed either an infant that was crawling (left), or walking (middle) along the screen, or an object moving at constant velocity (right).

Stimulus material
The stimuli consisted of videos which were taken from a previous experiment by Stapel, Hunnius, Meyer, & Bekkering (2016) who used a comparable paradigm to study action prediction and the influence of motor experience in a group of typically-developing infants, toddlers and adults (see also Meyer, Bekkering, Haartsen, Stapel, & Hunnius (2015)). The stimulus videos (see Figure 1, for an example) showed an infant or an object moving from one side of the screen to the other. The infant actor was either walking or crawling across the screen, and the object moved across the screen with constant velocity. Part of the scene was covered by a black occluder (290x396 pixels) which was located 30 pixels from the edge of the display where the movement ended. Stimulus duration varied
between 3-4 seconds (Crawling: \( M=3.9, SD=0.37 \); Walking \( M=3.25, SD=0.20 \); Object \( M=3.50, SD=0.46 \)) and was slightly shorter for the Walking compared to the Crawling videos due to the naturalistic nature of the video recordings.

To ensure that predictions could not be based on fixed timing after stimulus onset, occlusion durations were varied, by selecting videos of actors who differed in movement velocity. Importantly, the average occlusion duration was 0.52s and this average did not differ between the three conditions (Crawling: \( M=0.55, SD=0.18 \); Walking: \( M=0.54, SD=0.15 \); Object: \( M=0.46, SD=0.15 \)). The time from stimulus onset to full occlusion was fixed for all videos at 55 frames (0.22s). The object stimulus videos were created based on the videos recorded with the infant, using Adobe Premiere (Adobe, San Jose, USA). The moving object had a constant velocity and the range of velocity and occlusion duration was matched to the infant movement videos.

In each condition (crawling, walking or object), there were four actors and two starting locations (left or right), leading to a total of 24 stimulus videos. In the experiment, infants were presented with two repetitions of 24 trials each in which all videos were shown in pseudorandom order. Randomization was achieved using MIX (http://www.mrc-cbu.cam.ac.uk/people/maarten-vancasteren/mixandmatch/, Van Casteren & Davis, 2007) and it was ensured that there were at least two other videos in between a repetition of the same infant actor, and that the same condition was presented maximally two times in a row.

**Analysis of Eye tracking data**
The eye tracking data analysis was based on Stapel and colleagues (2016) as well as Meyer and colleagues (2015) who used the same action prediction task. In a first step, a rectangular area of interest (AoI, 84x434 pixels for all videos) was defined surrounding the area on the screen where the actor or object reappeared from behind the occluder. Given that, for infants, the eye tracker’s accuracy is usually lower than its optimal value (e.g. Hessels et al., 2015), we ensured that our AoI size was a multiple of the optimal accuracy (see supplementary Table 1). The raw fixation data was then read into a custom-made software tool (GSA, Donders Institute, Nijmegen, The Netherlands) which was used to extract the infant’s fixations to the AoI. Successive gaze points were considered a fixation if they stayed within a radius of 30 pixels for at least 40ms
Motor experience modulates action prediction in 14-month-old infants (cf. Meyer et al., 2015). Although some studies have suggested that alternative algorithms for fixation detection may be less susceptible to data quality differences that may exist between individuals with and without ASD (Hessels, Niehorster, Kemner, & Hooge, 2016; Shic, Chawarska, & Scassellati, 2008), we decided to follow the procedures previously used (Meyer et al., 2015; Stapel et al., 2016) for several reasons. First, as we aimed to replicate the previous studies (Meyer et al., 2015; Stapel et al., 2016), we considered it essential to stay as close as possible to the original way of data analysis in order to be able to compare the current results with the previous findings. Second, we were in particular interested in group differences of the within-subject modulation by action experience, which should not be affected by group differences in overall data quality. Last, data quality has been shown to mainly influence the number and duration of fixations rather than affecting the mere classification of a fixation to an AoI. As our analysis did not focus on the number or duration of the detected fixations, we would not expect differences in overall data quality between the two groups to have affected our results. Fixations that occurred in the first 200ms of the video were excluded from the analysis, to control for effects caused by previous stimulus presentation. Due to the naturalistic nature of the videos, the time the actor or object was still visible after reappearance was not consistent for all videos, and on average shorter for the Walking condition (510ms, compared to 1150ms for the Crawling and 835ms for the Object condition). To control for this difference, we excluded fixations that occurred later than 510ms after reappearance for the Crawling and Object condition, ensuring that the average duration of stimulus presentation after reappearance was the same for all conditions. Based on the extracted fixations, each trial was evaluated and a trial was considered valid if the infant made at least one fixation to the goal AoI during the (adjusted) stimulus period (cf. Stapel et al., 2016). If the infant did not fixate at the goal AoI, the trial was considered invalid and not included in the analysis. Infants had to contribute at least two valid trials per condition to be included in the analysis. To control for differences in valid trials between the three conditions, a random selection of trials was used per participant so that there were no differences in the amount of trials between conditions ($F(2,56)=0.91, p=0.41$).

Timing accuracy and stability were determined for each infant and condition as follows: Timing accuracy was calculated per trial by measuring the absolute
distance between the onset of the fixation closest in time and the reappearance of the actor/object behind the occluder. The lower the values, the closer in time the infant’s fixation to the object or agent’s reappearance. For each participant, an average accuracy score was then calculated per condition for later comparison. Timing stability was determined by calculating the standard deviation of the derived accuracy scores per participant and per condition. Again, the lower the values, the more stable the infant’s prediction about the object or agent’s reappearance.

Statistical analyses were performed on the average accuracy and stability scores using IBM SPSS 23 (statistical package of the social sciences). For both measures, a Mixed 2x3 ANOVA was performed with group (high-risk vs. low-risk) as between subject factor and condition (Walking vs. Crawling vs. Object) as within subject factor. In addition, to estimate the strength of the evidence associated with our findings, we performed Bayesian repeated measures analyses using JASP (Version 0.8.0.1, Love et al., 2015). Bayesian analyses were conducted for both the anticipation accuracy and stability, and using the same factors as in the repeated measures ANOVA.
RESULTS

Based on the parental report about the infant’s gross motor abilities, we verified that the participants from our current sample were indeed more proficient in crawling compared to walking. In line with our expectation, 93% of the infants (HR: 100%, LR: 87%) were able to crawl, whereas only 28% of the infants (HR: 21%, LR: 33%) were able to walk at 14 month of age. Importantly, the distribution of the number of infants that were able crawl or walk did not differ between the low- and high-risk group (Crawling: $p=0.48$, two-tailed, Fisher’s Exact Test; Walking: $p=0.68$, two-tailed, Fisher’s Exact Test).

Prediction accuracy

On average, infants contributed 4.33 trials to each of the three experimental conditions. Figure 2 shows the average prediction accuracy in seconds for the three conditions, separated into high and low-risk infant groups. The repeated measures ANOVA revealed no main effect of Group ($F(1,27)=0.28$, $p=0.60$) or interaction effect between Group and Condition ($F(2,54)=0.19$, $p=0.83$) suggesting that there was no difference in the prediction accuracy between the two groups. There was, however, a significant main effect of Condition ($F(2,54)=14.30$, $p<0.01$, $\eta^2_p=0.35$). Separate t-tests revealed that prediction accuracy significantly differed between all three conditions (Crawling vs. Object: $t(28)=-5.54$, $p<0.01$; Crawling vs. Walking: $t(28)=-2.48$, $p=0.02$; Walking vs. Object: $t(28)=-2.89$, $p=0.01$). Infants showed lowest values (i.e. more accurate predictions) for the Crawling condition, intermediate values for the Walking condition and highest values for the Object conditions.

Bayesian analysis of Prediction Accuracy

To further assess the strength of the evidence for the null hypothesis (no group difference), we conducted a Bayesian repeated measures ANOVA in JASP (Love et al., 2015) using the same factors as described above. We aimed to assess whether a model without the factor Group (null model) would explain the data better than alternative models including Group as a factor. Hence the effects of Condition was included in the null model which was then evaluated against the model including the main effect and interaction effects of Group and Condition. The Bayes factor in favor of the null hypothesis ($BF_{01}$) was 3.00 for the model including the main effect of Group and 15.92 for the model including the main
effect of Group and interaction effect of Group and Condition. This suggests that the null model explained the data 3 times better than the model including a main effect of Group and approximately 16 times better than the full model including a main effect and interaction effect of Group and Condition. A full overview of the results of this analysis can be found in Supplementary Table 2.

Prediction stability

The average prediction stability in seconds for the three conditions is shown in Figure 3, separated for the high and low-risk group. The repeated measures ANOVA revealed no main effect of group \( (F(1,27)=2.47, p=0.13) \) and no interaction effect between group and condition \( (F(2,54)=0.05, p=0.96) \). There was hence no evidence for differences in the prediction stability between the two infant groups. The ANOVA did reveal a significant main effect of Condition \( (F(2,54)=10.32, p<0.01 \ \eta_p=0.28) \). Separate t-tests showed that prediction stability significantly differed between the Crawling and Walking condition \( (t(28)=-3.04, p=0.01) \) and between the Crawling and Object condition \( (t(28)=-5.60, p<0.01) \), but there was no significant difference in stability between the Walking and Object condition \( (t(28)=-1.14, p=0.26) \).
Bayesian analysis Prediction Stability
We again used JASP (Love et al., 2015) to conduct a Bayesian repeated measures ANOVA to assess whether a null model without the factor Group would explain the data better than the alternative models to which the main effect of Group and its interaction effect with Condition have been added. The Bayes factor in favor of the null hypothesis ($BF_{01}$) was 1.77 for the model including the main effect of Group and 9.77 for the model including the main effect of Group and the interaction effect of Group and Condition. This suggests that the null model explained the data approximately twice as well as the model including an main effect of Group and 10 times better than the full model including the main and interaction effect. A full overview of the results of this analysis can be found in Supplementary Table 3.

![Figure 3. Average Prediction stability in seconds for the three experimental conditions separated for the low- and high-risk infants. Error bars indicate +/- 2 SE.](image)
DISCUSSION

Previous research suggests that individuals with ASD have difficulties in predicting others’ actions (Cattaneo et al., 2007; Fabbri-Destro et al., 2009; Gomot & Wicker, 2012; Sinha et al., 2014; Zalla et al., 2006). The current study investigated whether prediction difficulties are already present early in the development of infants at high familial risk for ASD. We compared a group of 14-month-old high-risk infants with age-matched typically-developing controls and assessed how accurate the participants were in predicting the timing of an observed action (prediction accuracy) as well as how stable the infants’ predictions were across different trials (prediction stability). Infants were presented with partially occluded actions that varied in how much motor experience the infants had with them. Using this paradigm, we did not find any significant effects of familial ASD risk on prediction accuracy or stability. Rather, we found that action experience modulated predictions in the entire sample: Infants’ predictions of the timing of reappearance were more accurate and stable for motorically more familiar crawling actions compared to walking actions or object motion. Infants fixations to the location of reappearance behind the occluder were thus closer in time to the actual reappearance for the crawling actions with which the infants had more experience compared to the walking actions and object motion. Over time, infants predictions were also less variable for the crawling compared to the walking actions. These findings replicate previous work in typically-developing infants: Stapel and colleagues (2016), who developed the current paradigm, showed the exact same pattern of results in typically-developing 14-month-old infants. Furthermore, our results and the findings by Stapel and colleagues (2016) complement previous research showing that prediction abilities in infants are closely linked to their motor skills (Ambrosini et al., 2013; Kanakogi & Itakura, 2011). Our findings further support the notion that predictions of others’ actions are formed based on own motor representations of the observed action (Kilner et al., 2007; Prinz, 2006; Rizzolatti & Sinigaglia, 2016).

To assess the strength of the evidence for the null hypothesis that familial risk for ASD did not influence the action predictions, we performed Bayesian analyses. These analyses revealed that the null model (excluding all effects of familial risk) explained the data 10 (prediction stability) to 16 (prediction
accuracy) times better than the model including the main and interaction effect of ASD risk. This can be seen as “strong” evidence for the null model over the full model (Jarosz & Wiley, 2014; Wetzels, van Ravenzwaaij, & Wagenmakers, 2015) and supports our interpretation that the modulation by action experience was similar for the two infant groups.

Previous research in older children and adults has demonstrated prediction difficulties in ASD (Boria et al., 2009; Cattaneo et al., 2007; Zalla et al., 2010, 2006) and more recently atypical predictive processing has even been suggested to underlie the diverse deficits of the disorder (van de Cruys et al., 2014; Lawson et al., 2014; Pellicano & Burr, 2012). However, there are also some studies that have not found evidence for a prediction deficit. An eye tracking study by Falck-Ytter (2010) for instance, showed similar action prediction abilities for children with and without ASD. A study by Marsh and colleagues (2014) investigated predictive gaze behavior during the observation of rational and irrational actions. Their results also suggest intact predictions in ASD if participants were able to maintain their attention. To unify the different findings more research will be needed to better understand which aspects of action prediction are intact or impaired and under which circumstances. The current study aimed to increase our knowledge of the early development of action prediction in ASD and our findings suggest that the mere familial risk for ASD does not influence prediction abilities at 14 months of age. However, we currently do not have the diagnostic outcome information from our sample and therefore, we need to be careful in interpreting the present findings with respect to a potential early prediction deficit in ASD. While we did not find group differences at 14 months, those infants who receive an ASD diagnosis later in development may still differ in their action prediction abilities from low-risk controls and from those high-risk infants who continue to develop typically. The rate of ASD diagnoses from previous cohort studies has been estimated at around 10-20% (Constantino et al., 2010; Ozonoff et al., 2011) and therefore the majority of our high-risk infants will not receive a clinical diagnosis on the autism spectrum. Together with the relatively small sample size of the current study, it is possible that we were simply unable to detect potential differences that do exist between the small number of high-risk infants that do develop ASD and the other infants from the current study. In addition, our study required infants to fixate at the end location of the action goal, thereby excluding infants that did not predict
that end location. As more high- than low-risk infants needed to be excluded based on insufficient number of trials, it is possible that the nature of our study and analysis methods (i.e. requiring the infants to fixate at the goal location) hindered the detection of group differences. The fact that high-risk infants provided less useable data than low-risk controls may be a meaningful characteristic of the sample which can further be assessed in relation to ASD outcome at a later stage. Crucially, despite the small sample size, we were nevertheless able to replicate the results of Stapel and colleagues (2016) and showed that experience influenced predictions in all participants and independent of familial risk status.

While we did not find any group differences, previous cohort studies have shown that early differences in social processing are detectable in similarly small samples (Elsabbagh et al., 2009; Lloyd-Fox et al., 2013). Elsabbagh and colleagues (2009), for instance found group differences in the neural responses to direct and averted gaze of 10-month-old low- and high-risk infants. A study by Lloyd-Fox and colleagues (2013) reported diminished neural processing of social information in high-risk infants already at 5 months of age. Interestingly, the relation between early differences and later outcome varies. While some early differences can be predictive of later ASD (Elsabbagh et al., 2013; Jones & Klin, 2013), in other cases established group differences between high- and low-risk infants were not associated with an eventual diagnosis (Merin et al., 2007; Young et al., 2009). Conversely, the absence of an effect of familial risk for ASD in the current study does not necessarily imply that there is no difference in action prediction in the subgroup of infants at risk who do continue to develop ASD. We are therefore following the infants over development as an essential next step in understanding how our results relate to diagnostic outcome. Once the infants have reached the age of 36 months, we will be able to assess whether early prediction abilities are distinct for those infants that meet diagnostic criteria for ASD compared to those who do not.

In summary, our study aimed to assess action prediction in high-risk infants compared to low-risk controls and we found no group differences in prediction accuracy or stability. Crucially, action experience influenced predictions for all infants, suggesting that both low- and high-risk infants recruit own motor representations during the prediction of observed actions. Our results show that
familial risk for ASD did not influence action predictions at 14 months of age. Yet, whether prediction difficulties are outcome-related and therefore visible only in the subgroup of infants that continue to receive an ASD diagnosis remains to be investigated in an upcoming follow-up study.
SUPPLEMENTARY MATERIALS

**Supplementary Table 1.** Hard and software specification of the eye tracker and programmes used for stimulus presentations at the two sites. S2= infants tested at site 1, S2= infants tested at site 2.

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**Supplementary Table 2.** Results of the Bayesian repeated measures analysis of the prediction accuracy Bayesian Repeated Measures ANOVA

| Models                                | P(M) | P(M|data) | BFₘ | BFₒᵣ | error % |
|---------------------------------------|------|----------|-----|-------|---------|
| Null model (incl. Condition, subject) | 0.333| 0.716    | 5.042| 1.000 |         |
| Group                                 | 0.333| 0.239    | 0.628| 2.995 | 6.281   |
| Group + Group * Condition             | 0.333| 0.045    | 0.094| 15.917| 3.640   |

*Note. All models include Condition, subject.*

| Effects                                | P(incl) | P(incl|data) | BFᵣᵢnclusion |
|----------------------------------------|---------|---------|--------------|
| Group                                  | 0.667   | 0.284   | 0.198        |
| Condition * Group                      | 0.333   | 0.045   | 0.094        |

¹: Optimal accuracy reported by the manufacturer. For both set-ups, 1 degree corresponds roughly to 43 pixels.
**Supplementary Table 3.** Results of the Bayesian repeated measures analysis of the prediction stability Bayesian Repeated Measures ANOVA

| Models                                      | P(M) | P(M|data) | BF<sub>M</sub> | BF<sub>01</sub> | error % |
|---------------------------------------------|------|----------|----------------|----------------|---------|
| Null model (incl. Condition, subject)      | 0.333| 0.600    | 2.999          | 1.000          |         |
| Group                                       | 0.333| 0.339    | 1.024          | 1.772          | 2.403   |
| Group + Group * Condition                   | 0.333| 0.061    | 0.131          | 9.766          | 3.028   |

*Note.* All models include Condition, subject.

| Effects                      | P(incl) | P(incl|data) | BF Inclusion |
|------------------------------|---------|---------|--------------|
| Group                        | 0.667   | 0.400   | 0.333        |
| Condition * Group            | 0.333   | 0.061   | 0.131        |
Chapter 4

Predictability of action sub-steps modulates motor system activation during the observation of goal-directed actions

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ABSTRACT

Action perception and execution are linked in the human motor system, and researchers have proposed that this action-observation matching system underlies our ability to predict observed behavior. If the motor system is indeed involved in the generation of action predictions, activation should be modulated by the degree of predictability of an observed action. This study used EEG and eye tracking to investigate whether and how predictability of an observed action modulates motor system activation as well as behavioral predictions in the form of anticipatory eye movements. Participants were presented with object-directed actions (e.g., making a cup of tea) consisting of three action steps which increased in their predictability. While the goal of the first step was ambiguous (e.g., when making tea, one can first grab the teabag or the cup), the goals of the following steps became predictable over the course of the action. Motor system activation was assessed by measuring attenuation of sensorimotor mu- and beta-oscillations. We found that mu- and beta-power were attenuated during observation, indicating general activation of the motor system. Importantly, predictive motor system activation, indexed by beta-band attenuation, increased for each action step, showing strongest activation prior to the final (i.e. most predictable) step. Sensorimotor activity was related to participants’ predictive eye movements which also showed a modulation by action step. Our results demonstrate that motor system activity and behavioral predictions become stronger for more predictable action steps. The functional roles of sensorimotor oscillations in predicting other’s actions are discussed.
INTRODUCTION

It is well established that actions and their observations are tightly linked in the human motor system. Activation of the motor system can be observed not only during action execution but also during action observation (Cochin et al., 1999; Riitta Hari, 2006; Lepage & Théoret, 2006). Researchers have proposed that this action-observation matching system facilitates our ability to predict observed behavior (Kilner, Friston, & Frith, 2007; Palmer, Bunday, Davare, & Kilner, 2016; Prinz, 2006; Schubotz, 2007). It is argued that the outcome of an observed action can be inferred and predicted through a mapping of observed actions onto own motor representations (Rizzolatti & Sinigaglia, 2016). In line with a predictive function of the motor system, studies have shown that the knowledge of an upcoming action elicits motor system activation already prior to the action onset (Kilner, Vargas, Duval, Blakemore, & Sirigu, 2004; Southgate, Johnson, Osborne, & Csibra, 2009). Additional support for a matching between observed actions and own motor representations comes from studies using eye tracking. Flanagan and Johansson (2003) measured participants eye movements during the performance and observation of a block stacking task. They discovered that participants preceded goal-directed hand movements with their gaze in a highly similar manner during both the action execution and action observation condition. Anticipatory eye movements during action observation have since been reported in multiple studies (Elsner et al., 2012; Falck-Ytter et al., 2006; Gredebäck & Falck-Ytter, 2015; Hunnius & Bekkering, 2010) and it is argued that these behavioral predictions are generated due to the activation of the corresponding action plans in the observers’ motor system (Flanagan & Johansson, 2003). Elsner, D’Ausilio, Gredebäck, Falck-Ytter and Fadiga, (2013) recently used transcranial magnetic stimulation (TMS) to directly test this hypothesis. They showed that stimulation of the motor cortex slowed predictive eye movements during an action observation task, providing evidence that the motor system is indeed involved in the generation of anticipatory eye gaze.

Many studies have made use of EEG and MEG recordings assessing attenuation of central oscillatory power in the mu- and beta-frequency range as a marker of motor system activation (McFarland et al., 2000; Muthukumaraswamy & Johnson, 2004; Perry et al., 2010; Pfurtscheller, 1981; Denis, Rowe, Williams, & Milne, 2016; Koelewijn, van Schie, Bekkering, Oostenveld, & Jensen, 2008;
McFarland et al., 2000; Meyer, Braukmann, Stapel, Bekkering, & Hunnius, 2015). In agreement with a predictive function of the motor system (Kilner et al., 2007), studies have shown that sensorimotor oscillations are modulated during the observation of erroneous or unexpected actions (Koelewijn et al., 2008; Meyer et al., 2015; Stapel et al., 2010). Stapel and colleagues (2010) found, for instance, that 12-month-old infants demonstrated greater mu-attenuation when observing unusual actions upon everyday objects (such as bringing a cup to the ear rather than to the mouth) compared to actions usually associated with these objects. The researchers argued that observing actions which deviate from the initially expected trajectory requires the generation of additional predictions which is consecutively reflected in enhanced activation of the motor system (Kilner et al., 2007; Stapel et al., 2010). Similarly, in adults, observing erroneous rather than correct actions has also been shown to elicit increased motor system activation, in particular in the beta-frequency range (Koelewijn et al., 2008, Meyer et al., 2015). Interestingly, several other studies have recently also suggested a relationship between beta-oscillations and predictive processing (Palmer, Zapparoli, & Kilner, 2016; Tan, Wade, & Brown, 2016; van Pelt et al., 2016). Tzagarakis, Ince, Leuthold, and Pellizzer (2010), for example, showed that beta-band desynchronization during motor preparation was modulated by the uncertainty of movement direction in an instructed delay-reaching task. More specifically, beta-power was found to be lower when the target location was more predictable. Similarly, Tan and colleagues (2016) modulated the uncertainty of the forward model parameters in a visuomotor adaptation task and showed that post-movement beta synchronization was modulated by this uncertainty. Taken together, these studies suggest that sensorimotor beta-oscillations may be reflective of the motor systems’ predictive processing and in particular related to the precision of predictions (Palmer et al., 2016).

Altogether, there is strong empirical support for the notion that the motor system is involved in the generation of predictions about observed actions (Elsner et al., 2013; Kilner et al., 2007, 2004; Southgate et al., 2009). To date, however, most studies investigating action prediction made use of simple one-step goal-directed actions, like moving a ball into a bucket (Falck-Ytter et al., 2006) or bringing a cup to the mouth (Hunnius & Bekkering, 2010). Actions we encounter during everyday life, on the other hand, consist of multiple
action steps that depend on each other and need to be executed in a particular sequence in order to achieve an overall action goal. For example, to make a cup of tea, one first grabs a teabag, then puts it in a cup and in the last step, fills the cup with hot water. In such a multi-step action, the distinct action steps depend on each other and while the first step is often ambiguous (one can first grab the teabag or the cup), the later steps become more predictable over the course of the action (once the tea bag has been put into the cup the only missing step in making tea is pouring hot water into the cup). Although it has been established that the motor system shows predictive activation during the observation of simple one-step actions (Kilner et al., 2004; Southgate et al., 2009), it remains unknown whether and in which way activity is also modulated by the predictability of distinct action steps within a multi-step action sequence. A first indication that the predictability of an action step influences action prediction comes from a recent study by Poljac, Dahlslätt and Bekkering (2014). In their action observation paradigm, participants’ eye movements were registered, while they watched object-directed actions consisting of three distinct action steps which increased in predictability (such as making a cup of tea). The researchers showed that over the course of the different action steps, predictive eye movements towards the goal of the next action step became more frequent and rapid. These findings were interpreted as evidence that the action steps are not processed in isolation, but that the semantic information from the distinct action steps is accumulated, facilitating the generation of predictions about the later steps of the observed action. Since their study focused on behavioral measures of predictions only, the role of the motor system in the integration of semantic information in multi-step actions remains to be investigated.

The present study examined neural markers of action prediction during the observation of multi-step actions. We tested the hypothesis that predictive motor system activation is modulated by the predictability of the distinct steps in multi-step actions, reflecting the integration of information as the action unfolds. In a combined EEG and eye tracking study, we measured motor system activation along with predictive eye movements while participants were observing different object-directed multi-step actions (similar to Poljac et al., 2014). For each action, the goal of the first step was ambiguous whereas the later steps became more predictable over the course of the action. Motor system activation was assessed by examining attenuation of central mu- and beta-frequency
power. Based on the predictive role of the motor system (Kilner et al., 2007, 2004; Southgate et al., 2009), we expected to find a step-wise increase of motor system activation, indexed by attenuation of sensorimotor oscillations - in particular in the beta-frequency range-, mirroring the increased predictability of the distinct action steps. Following Poljac et al. (2014), we hypothesized a similar modulation of predictive eye movements. Moreover, we expected a relationship between the neural and behavioral measures of action prediction, reflecting the tight link between the motor system and predictive eye movements that has previously been established (Elsner et al., 2013).
METHODS

Participants
In total, 31 participants (age: $M=23.32$, $SD=3.06$; 21 female) took part in the study. From this set, 28 were included in the EEG data analysis (age: $M=23.04$, $SD=3.09$; 19 female) and 22 participants were included in the eye tracking data analysis (age: $M=23.17$, $SD=3.08$; 14 female). Nineteen participants (age: $M=22.78$, $SD=3.10$; 12 female) contributed data to both the EEG and eye tracking datasets and were included in the correlation analysis of the two measures. Participants were all typically-developing adults, who signed informed consent and received course credits or monetary compensation for their participation. All but one participants were right handed, and all participants had normal or corrected to normal vision and hearing.

For the EEG analysis, two participants were excluded due to technical problems and one participant was excluded due insufficient number of artifact-free trials. The relatively large number of participants excluded from the eye tracking analysis was due to equipment problems ($n=5$) or an insufficient amount of valid trials for each of the three conditions ($n=4$). For one participant, behavioral data to confirm proper attention to the stimulus display (see below) was not collected due to technical problems.

Stimulus material
For the purpose of the study, video recordings were created of a female actor sitting at a table performing a three-step action using everyday objects (see Figure 1). Each video lasted for approximately 15 seconds and started with the actor sitting in a neutral position with her hands placed on the table. During each video, there were three objects situated on the table, one at both sides of the actor and one in the middle in front of the actor. After approximately 2 seconds, the actor started moving her hand towards the first object (Step1). She then picked up the first object and brought it towards the second object (Step2) where usually a short action was performed. Then the actor continued to the last object (Step3) to finalize the overall action. An example of such an action is given in Figure 1.
Figure 1. Stimulus Examples. Two example stimulus are displayed in part A. The actor in the upper example first grasps the spoon (Step1), brings it to the sugar pot (Step2), scoops sugar, and finally brings the spoon to the coffee cup (Step3). In the lower example, the actor first gets the cheese slicer (Step1), brings it to the cheese (Step3), slices off a piece of cheese, and finally brings the piece to her mouth (Step3). The corresponding Areas of Interest (AoIs) of the three goal locations from the two example stimulus videos can be seen in part B.

The actions were chosen such that the initial action step was ambiguous, whereas the last step followed deterministically from the two previous steps of the sequence. Start, middle, and end locations of the action steps were counter-balanced so that participants were unable to predict the next step solely based on the object’s location. Also, the actor’s eyes and a large part of her face were covered by the brim of a black hat to ensure that participants were unable to predict the upcoming action step based on the actor’s gaze. Actions were similar to the ones used by Poljac and collagues (2014), but new material was recorded to enlarge the stimulus set so that sufficient trials could be presented required for the EEG analysis. In line with the original stimulus set, about half (13) of our final 28 videos ended at the mouth or face of the actor and the other half (15) ended at an object on the table. In addition to these experimental videos, eight catch videos were recorded in which the last action step did not lead to the conclusion of the overall action goal (see Supplementary Figure 1 for an example). After the presentation of a catch video, and after 16 pseudo-randomly selected experimental videos, participants were asked to indicate whether the observed action was performed correctly. Participant’s answers were analyzed to ensure that they were paying attention to the stimulus presentation.
Stimulus presentation
Stimulus presentations and communication with the EEG and eye tracking systems was realized using Presentation® software (Version 18.1.06.09.15, Neurobehavioral Systems Inc., Albany, CA, USA).

All participants saw each video (catch and experimental) twice during the experiment, resulting in a total of 72 trials which were presented in a pseudo-random order. There were four blocks during which 18 trials were presented on a 24-inch monitor located in a shielded experimental room. Each trial started with the presentation of a baseline period in which a fixation cross was displayed for 1250ms on average (+/- 250ms). Then an experimental video or a catch video was presented. After each catch trial and after 16 pseudo-randomly selected experimental trials, participants were asked to indicate whether the observed action was performed correctly. Responses were recorded using a button box. The entire experiment lasted for about half an hour and after each of the four blocks participants were able to take a short break and continue the experiment whenever they were ready.

EEG recordings
EEG was recorded using 64 Ag/AgCl active electrodes placed in actiCaps (Brain Products, Munich, Germany) and arranged according to the 10-20 system. Fifty-nine electrodes were used for scalp recordings, four electrodes recorded vertical and horizontal EOG and one electrode was placed on the left mastoid for potential additional reference. Data was collected using BrainVisionRecorder (Brain Products, Munich, Germany) with the right mastoid as online reference and a sampling rate of 1000Hz. Impedances were kept below 10kOhm and data was monitored throughout the session by the experimenter.

Eye tracking recordings
Eye movements were recorded using an SMI RED500 stand-alone eye tracker and the iView X™ SDK 3.0 software (SensoMotoric Instruments GmbH, Teltow, Germany) with a sampling rate of 250Hz. The eye tracker was calibrated using a 9-point calibration at the start of the experiment. Eye and head position were monitored throughout task by the experimenter.
Data analysis

EEG data analysis

EEG data was analyzed using MATLAB (2013b, The MathWorks Inc., Natick, MA, 2000) and Fieldtrip (Oostenveld, Fries, Maris, & Schoffelen, 2011), an open source toolbox for EEG data analysis.

Data segmentation

Data were read into Fieldtrip and segments were created for the three action steps per video and for the baseline period. Action step segments had a duration of 1200ms but a variable onset depending on the particular stimulus video. The timing of the segments was defined for each stimulus video separately and was based on the same segmentation as used in the eye tracking analysis (see Analysis of eye movement data section). The moment when the actor’s hand first entered the Area of Interest surrounding the goal object of that action step represented the end of the EEG action step segment. The beginning of the EEG action step segment was consecutively determined as 1200ms prior to the end point. Baseline period segments had a duration of 1000ms and were locked to fixation cross onset.

Preprocessing and artifact rejection using ICA

In a first step of cleaning and preprocessing the data, extremely noisy or flat channels as well as trials containing excessive artifacts were removed from the data by visual inspection. For 18 participants, no channels were rejected. For the remaining 10 participants, on average two channels were rejected (ranging from one to four, see Supplementary Table 1) but this never included channel Cz. In a following step, Independent Component Analysis (ICA) was performed in order to extract artifacts caused by eye movements and eye blinks. First, ICA components were correlated with the data from the bipolar EOG channels. Consecutively, the spatial distribution of each component was inspected visually and in a last step the time course was visually assessed and components were manually rejected. For all but one participants, at least two components were rejected that correlated highly with the EOG data and showed a specific spatial distribution and time course associated with ocular artifacts. For the remaining participant, only one component could be identified. In addition, for five participants an additional component was rejected which either also matched the criteria for ocular artifacts (n=1) or clearly reflected the heart rate throughout
the experiment (n=4). After determination and removal of the ICA components, the data was reconstructed and further analyzed. Previously excluded channels were interpolated using a nearest neighbor approach and finally, the data was re-referenced to the average of all electrodes. In a last step, each segment was visually screened and segments containing remaining artifacts were excluded manually from further analysis.

Trials for the fixation and the three action steps were then separated for analysis of spectral power. For one participant the amount of artifact-free baseline period segments was extremely low (n=17) and this participant was hence excluded from further analysis. On average the included participants contributed 49.21 (SD=3.73) trials to Step1, 48.57 (SD=4.09) trials to Step2, 49.43 (SD=4.01) trials to Step3, and 43.25 (SD=5.88) trials to the baseline period.

Calculation of spectral power
To calculate the spectral power of the signal, Fast Fourier Transform was applied to the segments using a multitaper frequency transformation. In order to control for individual differences, the resulting power values of the three action steps were normalized for each individual participant using the power values from the baseline period segments. This was achieved by dividing the power from each action step by the power of the baseline period and taking the log of this ratio. This is a common way of normalizing frequency power data (see, for example, Cuevas, Cannon, Yoo, & Fox, 2014; Meyer, Braukmann, Stapel, Bekkering, & Hunnius, 2015).

Based on previous literature, our measure for the activation of the motor system activation during action observation was the attenuation of the central mu- and beta-frequency power. EEG power was extracted from Cz and mu- and beta-band ranges were set from 8-12Hz (mu) and 15-25Hz (beta) (see: McFarland et al., 2000; Meyer et al., 2015; Perry et al., 2010; Pfurtscheller, 1981; Pineda, 2005; Denis et al., 2016). All analyses were performed on the log transformed normalized power values. To investigate whether the power in the mu- and beta-frequency range was attenuated during action observation with respect to baseline period, one-sample t-tests were conducted for each of the action steps and frequency ranges. To test for power differences between the three action steps a repeated measures ANOVA with Step as a within-subject factor was conducted for each of the two frequency ranges.
Analysis of eye movement data

Determination of Areas of Interest (AoI)
Raw eye movement data was read into BeGaze™ 3.0 analysis software (Senso-Motoric Instruments GmbH, Teltow, Germany) where fixations were extracted based on the standard filter settings (minimum fixation duration of 50ms and peak velocity threshold of 40°/s). For each experimental video three rectangular-shaped Areas of Interest (AoIs) were defined around each of the goal objects of the three action steps. AoI size varied per video ($M=30698.07$ pixels, $SD=24404.31$), but the average AoI size did not differ between the three action steps ($F(2,81)=1.431, p=0.245$). Finally, fixation data for the three AoIs over all experimental trials were extracted and further processed using MATLAB (2012b, The MathWorks Inc., Natick, MA, 2000).

Determining Time Windows of Interest (TWoI)
For each experimental video, a predictive (pTWoI) and reactive Time Window of Interest (rTWoI) was determined for each action step. The moment when the actor’s hand started to move towards the goal object was the start of the pTWoI. Conversely, the moment when the actor’s hand first entered the goal AoI was used as the end of the pTWoI and the beginning of the rTWoI, respectively. Finally, the end of the rTWoI was selected such that the reactive and predictive window were of equal length.

Due to the fact that natural stimuli were used, TWoI size ($M=1306.65$ ms, $SD=331.26$) differed between stimulus videos ($F(2,81)=18.96, p<0.001$) and window size was on average smaller for Step1 compared to Step2 ($t(27)=-6.72, p<0.001$) and Steps3 ($t(27)=-5.35, p<0.001$), but equal for Step2 and Step3 ($t(27)=0.41, p=0.685$). To control for differences in TWoI length, we used relative measures for our eye tracking analysis where possible: For the Looking Time a percentage was used, and for the Count Ratio the number of fixations during the pTWoI were divided by the number of fixations during the pTWoI and rTWoI combined (see below). The segments used in the EEG analysis were always equally long (see EEG analysis) leaving no bias for a particular action step.

Classification of predictive and reactive eye tracking trials
In MATLAB, for each action step, trials were classified as either being predictive
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(a goal fixation was made during the pTWoI), reactive (no predictive fixation was made, but a goal fixation was made during the rTWoI) or invalid (no goal fixation was made during either TWoI). Participants with less than 15 valid trials per action step were excluded from the analysis. This concerned four individuals from the initial 31 participants who took part in the experiment. On average, the included participants contributed 29.91 ($SD=9.42$) trials to Step1, 37.77 ($SD=9.01$) trials to Step2, and 27.14 ($SD=7.55$) trials to Step3.

Calculation of the dependent eye tracking measures
Similar to Poljac and colleagues (2014), three eye tracking measures of interest were calculated separately for each action step: Predictive Looking Time, Predictive Gaze Onset, and Predictive Count Ratio. Predictive Looking Time was calculated by extracting the duration of fixations to the AoI during the pTWoI. Looking times were then standardized as a percentage with respect to the length of the respective pTWoI and then averaged over trials for each participant. Predictive Gaze Onset was defined as the onset of the first fixation to the AoI relative to the end point of the pTWoI. A larger value hence reflected an earlier onset of the predictive fixation. Finally, the Predictive Count Ratio was determined by dividing the number of predictive trials by the total number of trials for each action step. To assess differences in predictive eye movements for the three action steps, we performed a Repeated measures ANOVA for each of the three dependent measures with Step as a within-subject factor.

Correlation of EEG and eye tracking measures
To assess the relationship of predictive cortical motor system activation and predictive eye movements, we performed a correlation analysis. For this purpose, difference scores were derived for all dependent measures reflecting the difference in prediction between two neighboring action steps. More specifically, we subtracted Step1 from Step2, and Step2 from Step3. A more negative difference score in the EEG measure thus reflected less power- and hence more motor system activation- for the later compared to earlier action steps. For the eye tracking data, conversely, a larger difference score would reflect enhanced prediction in later compared to earlier action steps. Correlations between EEG-power in the mu- and beta-band with the three eye tracking measures were calculated separately for each of the two action step contrasts.
RESULTS

Attention to stimulus presentation
Participants answered the questions presented after catch and selected experimental trials correctly 94.48% of the time ($SD=0.05$), with no differences in performance for catch compared to experimental trials ($t(29)=0.09$, $p=0.929$). This confirmed that participants were paying attention during stimulus presentation.

EEG results
To assess whether the power in the mu- and beta-frequency range was attenuated during action observation, we conducted a one-sample t-test for each of the action steps and frequency bands separately. As expected, the averaged log ratio of the power was negative in all cases and significantly different from zero for all action steps for the mu-frequency range and the second and third action step for the beta-frequency range ($ts(27)<-3.29$, $ps<0.004$ for all five contrasts, see Supplementary Table 2 for an overview of the exact test statistics and effect sizes). In addition, the first action step for the beta-frequency range reached marginal significance ($t(27)=-2.02$, $p=0.054$). These results showed that for both the mu- and the beta-frequency range, power was attenuated during the action observation periods compared to the baseline period. Next, we assessed differences between the three action steps using a repeated measures ANOVA. No effect of Step was found for the mu-frequency band ($F(2,54)=1.92$, $p=0.156$, see supplementary Figure 2 for a visualization of mu-power over the three action steps). For the beta-frequency band, on the other hand, there was a significant main effect of Step ($F(2,54)=19.54$, $p<0.001$, $\eta^2_p=0.42$). Figure 2A shows the averaged relative beta-power for the three Steps at Cz, and Figure 2B illustrates the topographic distribution of the effect. To further investigate the main effect, we conducted paired-sample t-tests which showed that relative power was larger for Step1 compared to Step2 ($M=0.10$, $SD=0.16$, $t(27)=3.33$, $p=0.003$, $d=0.61$) and Step3 ($M=0.17$, $SD=0.16$, $t(27)=5.71$, $p<0.001$, $d=1.00$), and larger for Step2 compared to Step3 ($M=0.07$, $SD=0.11$, $t(27)=3.30$, $p<0.003$, $d=0.49$). A decrease of beta-power is seen as a reflection of increased motor system activity (Perry & Bentin, 2009; Pfurtscheller, 1981; Pineda, 2005) and these results hence suggest that participants showed increased motor system activation for the later compared to the earlier action steps.
Figure 2. A) The bar graph showing the relative beta-frequency power over central electrode Cz for the three action steps. Error bars indicate +/- 2 SE; significant differences (p<0.05) between the action steps are marked by the asterisk B) Topographic plot of the difference in beta-power for neighboring action steps. Blue colors indicate less power for later compared to earlier action steps.

**Beta-frequency power during catch trials**

In our design, predictability always increased gradually during video presentation: the first step was least predictable and the last step was most predictable. It could be argued that our findings of stronger beta-attenuation for later action steps described above are therefore not due to an increase in predictability of the action steps, but due to stimulus duration, reflecting a build-up of motor system activation during the observation of a complex action. To assess this potential alternative explanation, we performed an additional analysis of beta-frequency attenuation during the catch trials. In the catch trials, participants were also presented with a complex multi-step actions similar to the actions used in the main experiment (see Supplementary Figure 1 for an example). Yet for the catch trials, the presented action steps never lead to the conclusion of the overall action goal, and predictability hence did not increase over the course of the action. If our results were due to a build-up of motor system activity during the continuous observation of complex human actions, rather than due to predictability of the different action steps, a build-up of beta-attenuation should also be visible during the catch trials as well.
Catch trial were analyzed following the same procedures as used in the main analysis and a full description of the analysis can be found in the supplementary materials. Importantly, a repeated measures ANOVA on the relative beta-frequency power showed no effect of Step on beta-power attenuation during the catch trials ($F(2,54)=2.15, p=0.127$, see supplementary Figure 1B for a visualization). There was thus no gradual decrease of beta-power during the observation of the catch trials which suggests that an effect of stimulus duration cannot explain the main findings of stronger beta-attenuation for later, more predictable action steps.

Eye tracking results
An overview of the eye tracking results can be found in Figure 3. A significant main effect of Step was found for all three dependent measures (Predictive Looking Time: $F(2,42)=21.45, p<0.001, \eta_p=0.51$; Predictive Gaze Onset: $F(2,42)=58.21, p<0.001, \eta_p=0.74$; Predictive Count Ratio: $F(2,42)=15.89, p<0.001, \eta_p=0.43$). To further assess differences between the three Steps, paired-sample t-tests were performed. Results were similar for all three measures, showing a difference between Step1 and Step2 (Predictive Looking Time: $t(21)=-5.90, p<0.001, d=-1.20$; Predictive Gaze Onset: $t(21)=-10.82, p<0.001, d=-2.83$; Predictive Count Ratio: $t(21)=-4.92, p<0.001, d=-0.97$) as well as Step1 and Step3 (Predictive Looking Time: $t(21)=-5.24, p<0.001, d=-1.11$; Predictive Gaze Onset: $t(21)=-8.30, p<0.001, d=-2.27$; Predictive Count Ratio: $t(21)=-3.79, p=0.001, d=-0.82$). There was, however, no difference between Step2 and Step3 (Predictive Looking Time: $t(21)=0.51, p=0.616$; Predictive Gaze Onset: $t(21)=1.51, p=0.147$; Predictive Count Ratio: $t(21)=1.38, p=0.182$). This indicates that participants showed more, as well as, longer and faster predictions during the last two action steps compared to the first one.

Correlation analysis
To examine the relationship between predictive motor system activity and predictive eye movements, we derived the difference score between consecutive action steps for each of our dependent measures and assessed whether eye tracking and EEG measures were correlated with each other. An overview of all correlation coefficients, test-statistics and correlation plots can be found in Supplementary Table 3 and Supplementary Figure 3 and 4. Differential beta-
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Figure 3. Eye tracking results. Error bars +/- 2SE; significant differences (p<0.05) between the action steps are marked by the asterisk.
power was significantly correlated with Predictive Gaze Onset when contrasting Step 1 and Step 2 ($r=-0.54$, $p=0.018$, see Figure 4, upper left panel). Stronger behavioral predictions, indicated by an earlier gaze onset for the later step, were related to a larger decrease in beta-power from Step 1 to Step 2. Similarly, we also found a marginally significant correlation between Predictive Looking Time and beta-power for the difference score contrasting Step 2 and Step 3 ($r=-0.41$, $p=0.083$, see Figure 4 upper right panel). Again, stronger behavioral predictions, indicated by a longer looking times for the later step, were correlated with a larger decrease in beta-power from Step 2 to Step 3.

Figure 4. Scatter plot of the (marginally) significant correlations between the EEG and Eye tracking data derived from subtracting Step 1 from Step 2 (upper panel) and Step 2 from Step 3 (lower panel). The left plots shows the relationship between beta-power and Gaze Onset (upper), and beta-power and Looking Time (lower). The right plots shows the relationship between mu-power and Gaze Onset (upper), and mu-power and Looking Time (lower). Scatter plots of the remaining investigated correlations can be found in supplementary material Figure 3 and 4.
We also found significant correlations between the same two eye tracking measures and mu-power, although in opposing directions. For the contrast comparing Step 2 and Step 3, mu power - like beta-power - was negatively correlated with Predictive Looking Time ($r=-0.46$, $p=0.046$, see Figure 4 lower left panel). For the contrast comparing Step1 and Step2, however, we found a positive relationship between mu-power and Predictive Gaze Onset $r=0.46$, $p=0.048$, see Figure 4 lower right panel): An earlier gaze onset for the later step was related to a smaller decrease in mu-power from Step1 to Step2.
DISCUSSION

Previous research on the role of the motor system in action prediction has primarily focused on simple one-step goal-directed actions (Elsner et al., 2013; Kilner et al., 2004; Koelewijn et al., 2008). The present study investigated the role of the motor system in the integration and prediction of distinct action steps within a multi-step action sequence. Using EEG and eye tracking, we assessed participants’ motor system activation and predictive eye movements during an action observation task. We found significant attenuation of both mu- and beta-power during action observation compared to baseline. This is in line with previous research, linking attenuation in these frequency ranges to motor system activation (Brinkman, Stolk, Dijkerman, de Lange, & Toni, 2014; McFarland et al., 2000; Meyer et al., 2015; Perry et al., 2010; Pfurtscheller, 1981; Pineda, 2005; Denis et al., 2016). More importantly, and confirming our hypothesis, we found an increase in motor system activity depending on the action step predictability: Central sensorimotor beta-power decreased over the course of the action showing the least attenuation prior to the first (least predictable) action step and most attenuation prior to the last (most predictable) action step. Importantly, no such decrease in beta-power was evident for the catch trials, supporting the interpretation that the observed activity was related to the action’s predictability rather than simply being the result of build-up of activation elicited by the observation of a complex multi-step action. Furthermore, our results showed that participants’ anticipatory eye movements were also modulated by predictability, and we found a relationship between neural and behavioral measures: Participants who showed a larger attenuation of beta-power for later compared to earlier action steps, also showed a larger increase in duration and onset of behavioral anticipations. Although this relationship was not significant for all of the eye tracking measures we assessed and needs to be interpreted with caution, a link between neural and behavioral markers of prediction is in accordance with previous findings showing that the motor system is involved in the generation of predictive eye movements (Elsner et al., 2013). Notably, we also found correlations between the eye tracking measures and mu-attenuation. However, as will be discussed below in more detail, the pattern of the relationship was inconsistent across action steps (see supplementary Figure 3 and 4) and one needs to be cautious in interpreting these findings because of the absence of a main effect of Step for the mu-frequency band in our main analysis.
Predictability modulates motor system activation during action observation

Predictions in the motor system: the role of sensorimotor oscillations
The results of current study suggest that motor system activation, reflected by attenuation of beta-power, increased based on the predictability of observed action steps. These findings are in line with the suggested role of the motor system in the generation of action predictions (Elsner et al., 2013; Kilner et al., 2007; Prinz, 2006; Schubotz, 2007). Moreover, our findings complement recent fMRI research suggesting that brain regions involved in action perception (i.e. premotor, parietal and occipitotemporal areas, often referred to as the action observation network (AON), see Cross et al., 2012; Cross, Kraemer, Hamilton, Kelley, & Grafton, 2009; Gazzola & Keysers, 2009; Schubotz, 2007) are modulated by action predictability (Plata Bello, Modroño, Marcano, & González-Mora, 2015; Wurm, Hrkać, Morikawa, & Schubotz, 2014). Wurm and colleagues (2014), for instance, assessed the processing of multi-step actions which were either characterized by the presence of an overarching action goal or not (see also Hrkać, Wurm, & Schubotz, 2014). Using this paradigm, the researchers investigated, among other things, the effect of goal predictability on brain activation. In the goal-coherent action observation condition, the overall action goal became more predictable towards the end of the action sequence. Wurm and colleagues (2014) showed that activity in several regions of the AON – in particular in the inferior frontal gyrus and occipitotemporal cortex - decreased as a function of goal predictability. These findings support the notion that the action observation network is modulated by action predictability (see also Plata Bello et al., 2015).

Our study focused on activation of the neural motor system during action observation and aimed to assess whether and in what way neural oscillations associated with the processing of own and other’s actions are also modulated by action predictability. Using EEG, we found that attenuation of central sensorimotor beta-power became stronger over the course of the observed actions when the distinct action steps became more predictable. Importantly, beta-attenuation did not differ over time during the observation of catch trials, which displayed similar complex multi-step actions but without the increase in predictability towards the last action step. These results suggest that the observed activity during the experimental trials cannot simply be explained by the continued presentation of a complex action sequence. Rather, the findings suggest a modulation of motor system activity by action predictability and an involvement of beta-oscillations in the predictive processing of the motor system.
As naturalistic stimuli of everyday action sequences were used, the presented action steps differed in movement complexity. The action sequence usually started with the actor reaching towards one of the objects, followed by a manipulation of the object, and finally continuing with another reaching action. Performed movements during the middle action step were hence more complex, whereas the first and last action step consisted of simple reaching actions. Differences in movement complexity, however, cannot explain our current results. If movement complexity modulated motor system activation, one would expect to find a peak of activation at Step2 and no differences between Step1 and Step3, rather than an increase of activity over the course of the action. Our study yielded a significant difference between Step1 and Step3 for the eye tracking measures as well as an overall decrease of beta-band attenuation between all action steps. This supports our interpretation that the current findings reflect a modulation by predictability, rather than reflecting other stimulus features such as movement complexity.

Interestingly, we found a modulation by predictability for the beta-frequency range, but no effect was present for mu-oscillations. Although both oscillations are typically associated with motor system activation (McFarland et al., 2000; Muthukumaraswamy & Johnson, 2004; Perry et al., 2010; Pfurtscheller, 1981), research on mental simulation of goal-directed actions has also suggested that mu- and beta-oscillations serve distinct functions (Brinkman et al., 2014). In addition, neuroimaging studies have reported differences in the origin of the two sensorimotor rhythms (Ritter, Moosmann, & Villringer, 2009; Salmelin & Hari, 1994). Two studies that investigated the processing of errors in the motor system have shown that beta-power is modulated by observing erroneous compared to correct actions (Koelewijn et al., 2008; Meyer et al., 2015). One of those studies also assessed mu-power and showed no modulation by action correctness in adults (Meyer et al., 2015). This specificity of beta-power being modulated by erroneous - or unpredicted- events is in agreement with our current findings, suggesting that oscillations in the beta-frequency range may be associated with predictive processing in the motor system. Moreover, a recent study by Tzagarakis and colleagues (2010) has linked beta-oscillations to response uncertainty, showing that beta-power prior to the execution of a movement was lowest when the movement direction was most predictable. Similarly, Tan and colleagues (2016) also showed a relationship between post-movement
beta-power and model uncertainty which was modulated during a visuomotor adaptation task. Using MEG, van Pelt and colleagues (2016) studied beta- and gamma-oscillatory in an action observation paradigm in which the probability of kinematic aspects and action outcomes were manipulated, leading to different probabilities for the different kinematic-outcome combinations. The researchers found an increase in beta-band power in the temporoparietal junction along with the kinematics-outcome predictability. Although their study did not focus on motor system activation and utilized a different paradigm, the link between beta-oscillations and predictability is in keeping with our findings.

Sensorimotor mu-oscillations, on the other hand, were attenuated during action observation but did not show a modulation by predictability in the current study. These findings suggest that mu-oscillations may reflect a general non-specific mechanism of motor system activation. This interpretation is in agreement with findings by Meyer and colleagues (2011) who investigated motor system activation in toddlers during the observation of a joint action partner. The authors reported that activity in the beta-frequency range was related specifically to the timing of the other person’s action, while power in the mu-frequency range was persistent throughout the whole observation window that was investigated.

Motor system activity and predictive eye movements

In this study, we examined three measures of predictive eye movements which all showed significant differences between the first and the last two action steps. These findings are consistent with work by Poljac and colleagues (2014), showing that during the observation of an unfolding action sequence, stronger behavioral predictions can be observed for later, more predictable, action steps. Slight differences between the results of the two studies are likely to be caused by differences in stimulus material as well as the resulting time windows and AoIs used in the analysis.

1: In their action observation paradigm, Poljac and colleagues (2014) found an identical pattern of results (i.e. significant differences between the first and the last two action steps) for the Predictive Looking Time measure only. For Predictive Gaze Onset and Predictive Count Ratio, on the other hand, they reported a difference between the third and the first two action steps, whereas the present study found a difference between the first and last two steps for all three measures.
The current study extended the previous findings by examining the neural underpinnings of predictions during action observation as well as the relationship between neural and behavioral markers of predictions. Recent work showed that the motor system is directly involved in the generation of predictive eye movements (Elsner et al., 2013). In accordance with this, we found a significant correlation between the two measures of prediction: Participants who showed a stronger beta-attenuation from the first to the second action step, also showed a greater increase in Predictive Gaze Onset. Although only marginally significant, we found a similar relationship between beta-attenuation from the second to the third action step and Predictive Looking Time. While these findings support the tight link between motor system activation and behavioral action predictions, it needs to be noted that the remaining correlations between beta-power and predictive eye movements did not reach significance and the findings thus need to be interpreted with caution. Interestingly, while mu-power was not modulated by predictability, we did find significant correlations between eye tracking measures and mu-attenuation. However, while the relationship between beta-power and predictive eye movements was consistent (with more motor activity being related to stronger predictions, see also Supplementary Figure 3), the relationship between mu-power and predictive eye movements was inconsistent: A stronger mu-attenuation was associated with weaker predictions for Step1 compared to Step 2, whereas it was associated with stronger predictions for Step2 compared to Step3. Given that we found no main effect of action step in our main analysis, the results of the correlational analysis are difficult to interpret. The rational of this analysis was to see whether increased neural activity for later action steps was related to increased behavioral predictions. However, for the mu-frequency, we did not find any increased activity for later action steps as the main effect of Step was not significant. This absence of a main effect may have resulted in the observed inconsistent relationship between mu-power and behavioral predictions. Overall, we showed that both mu- and beta-power were related to behavioral predictions, but that only for beta-power there was a consistent relationship with stronger attenuation being related to stronger predictions. These findings are in line with our interpretation of the main analysis, suggesting that beta-oscillations in particular are related to predictions in the motor system.
As participants were performing eye movements during the task, one could argue that the relationship between the EEG and eye tracking measures reflects a mere artifact of eye movements in the EEG data rather than reflecting a true connection between two distinct measures of action prediction. However, we consider this explanation to be unlikely for multiple reasons: First, ICA was applied to detect and remove components from the EEG data that reflected overt eye movements. Several studies suggest that ICA is a powerful method to correct for eye-artifacts in the EEG data due to the distinct temporal and spatial activation pattern of the eye movement components (Jung et al., 1998; Plöchl, Ossandón, & König, 2012). Second, eye movement artifacts in the EEG data have been shown to affect mostly higher frequency ranges such as gamma rather than the lower frequency ranges we investigated (Reva & Aftanas, 2004; Yuval-Greenberg, Tomer, Keren, Nelken, & Deouell, 2008). Third, the topographic plots in Figure 2b show that the difference between the action steps was densely localized around central electrodes making a contamination by eye movement artifacts unlikely. Finally, additional analyses assessing beta-attenuation during the catch trials suggested that beta-power was not different for the distinct action steps in the catch trials. During the catch trials, one would expect similar eye movements as the catch trials also contained complex goal-directed multi-step actions. If the reported beta-attenuation was merely reflecting eye movement artifacts, we would thus expect a similar pattern of activation during the catch trials. We therefore would argue that our main results are not confounded by eye movement artifacts and that correlation analysis reflects a true link between neural motor system activation and predictive eye movements (see also Elsner et al., 2013).

In summary, the present study demonstrated that attenuation of beta-power, reflecting activity in the motor system, and behavioral predictions become stronger for more predictable sub-steps within a multi-step action. Our findings are in accordance with recent empirical work suggesting distinct functional roles for the sensorimotor mu- and beta-rhythms (Brinkman et al., 2014; Meyer, Hunnius, van Elk, van Ede, & Bekkering, 2011) and linking beta-oscillations to predictions in the motor system (Palmer et al., 2016).
SUPPLEMENTARY MATERIALS

Supplementary Figure 1. A. Catch trial example. The first part of the actions displayed in the catch trials consisted of typical actions steps, whereas in the last part the overall action goal is not concluded. In this example, the actor first grabs the spoon, brings it to the sugar pot to scoop sugar, but then fails to bring the spoon to the cup in the last part of the action. B. Beta-power during catch trials. The bar graph shows the relative beta-frequency power over central electrode Cz for the three action steps during the catch trials.

Supplementary Analysis of the Catch trials

To assess the effect of video duration on beta-attenuation, we performed an additional analysis of the catch trials. The catch trials also contained a continuous presentation of a complex action but without an increase in action step predictability (see Supplementary Figure 1A for an example). Catch trials were analyzed following the same procedures as used in the main analysis. For each catch trial, three segments of 1200ms were extracted from the data and further processed as described above. As the catch videos did not consist of three distinct action steps themselves, the segmentation was based on the average timing of the three action steps from the main analysis (Step1: 1.89-3.09; Step2: 5.33-6.53; Step3: 12.78-13.98). For three of the catch stimulus videos, the duration of the video exceeded the duration of the last step window and therefore the onset of the last segment was adjusted for these catch videos.
to fit the overall video duration, resulting in an average onset of the third step window at 12.28s post-stimulus onset [$SD=0.78$, range: 10.92-12.78]. Baseline trials were extracted from the fixation trials preceding the catch trials and had a duration of 1000ms similar to the main analysis. On average the included participants contributed 14.75 ($SD=1.48$, range: 10-16) trials to Step1, 14.68 ($SD=1.54$, range: 11-16) trials to Step2, 14.93 ($SD=1.15$, range: 12-16) trials to Step3, and 14.04 ($SD=1.55$, range: 10-16) trials to the baseline period. As reported in the main manuscript, we performed a repeated measures ANOVA on the relative beta-frequency power using Step as a between subject variable and this analysis showed no effect of Step on beta-power attenuation ($F(2, 54)=2.15$, $p=0.127$).

Supplementary Figures 2 to 4

**Supplementary Figure 2.** *A* Topographic plot of the difference in mu-power for neighboring action steps. *B* Bar graph displaying averaged relative mu-frequency power during the three action steps at electrode Cz.
Supplementary Figure 3. Scatter plots of all correlations between beta-frequency power and the three eye tracking measures. A. Contrasting Step 1 and Step 2. B. Contrasting Step 2 and Step 3. Significant correlations are marked by asterisk and marginally significant correlations by cross.
Supplementary Figure 4. Scatter plots of all correlations between mu-frequency power and the three eye tracking measures. A. Contrasting Step 1 and Step 2. B. Contrasting Step 2 and Step 3. Significant correlations are marked by the asterisk.
**Supplementary Tables**

**Supplementary Table 1. Overview of interpolated channels**

<table>
<thead>
<tr>
<th>Participant</th>
<th>Interpolated Channels</th>
</tr>
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<tbody>
<tr>
<td>07</td>
<td>T7, T8</td>
</tr>
<tr>
<td>11</td>
<td>T7, T8, TP7, TP8</td>
</tr>
<tr>
<td>13</td>
<td>AF8</td>
</tr>
<tr>
<td>15</td>
<td>F4, T8</td>
</tr>
<tr>
<td>16</td>
<td>AF7, AF8, T7, TP7</td>
</tr>
<tr>
<td>17</td>
<td>CPz</td>
</tr>
<tr>
<td>19</td>
<td>FT8</td>
</tr>
<tr>
<td>21</td>
<td>CPz, CP4</td>
</tr>
<tr>
<td>23</td>
<td>F2, CPz, CP4</td>
</tr>
<tr>
<td>30</td>
<td>POz</td>
</tr>
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</table>

**Supplementary Table 2. Overview of the test statistics and effect sizes of the one-sample t-tests assessing mu- and beta-power differences from zero.**

<table>
<thead>
<tr>
<th></th>
<th>t-value</th>
<th>p-value</th>
<th>Effect size d</th>
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<tr>
<td>Mu Step 1</td>
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<td>0.64</td>
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<tr>
<td>Mu Step 2</td>
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</tr>
<tr>
<td>Beta Step 2</td>
<td>-6.86</td>
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<td>1.30</td>
</tr>
<tr>
<td>Beta Step 3</td>
<td>-8.76</td>
<td>&lt;0.001</td>
<td>1.66</td>
</tr>
</tbody>
</table>

**Supplementary Table 3. Correlation coefficients and test statistics for the correlational analysis between the EEG and eye tracking measures. A. Contrasting Step 1 and Step 2. B. Contrasting Step 2 and Step 3. S1=Step1, S2=Step2, S3=Step3 CR=Count Ratio, LT=Looking Time, GO=Gaze Onset.**

**A**

<table>
<thead>
<tr>
<th></th>
<th>CR (S1vs.S2)</th>
<th>LT (S1vs.S2)</th>
<th>GO (S1vs.S2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta (S1vs.S2)</td>
<td>r=-0.11, p=0.646</td>
<td>r=-0.32, p=0.188</td>
<td>r=-0.54, p=0.018</td>
</tr>
<tr>
<td>Mu (S1vs.S2)</td>
<td>r=-0.08, p=0.760</td>
<td>r=0.16, p=0.502</td>
<td>r=0.46, p=0.046</td>
</tr>
</tbody>
</table>

**B**

<table>
<thead>
<tr>
<th></th>
<th>CR (S2vs.S3)</th>
<th>LT (S2vs.S3)</th>
<th>GO (S2vs.S3)</th>
</tr>
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<tbody>
<tr>
<td>Beta (S2vs.S3)</td>
<td>r=0.13, p=0.587</td>
<td>r=-0.41, p=0.083</td>
<td>r=-0.34, p=0.157</td>
</tr>
<tr>
<td>Mu (S2vs.S3)</td>
<td>r=-0.30, p=0.209</td>
<td>r=-0.46, p=0.048</td>
<td>r=-0.11, p=0.665</td>
</tr>
</tbody>
</table>
General Discussion
GENERAL DISCUSSION

The aim of this thesis was to investigate differences in neural and behavioral characteristics of social cognition between young infants at high familial risk for ASD and typically-developing controls. In Chapter 1, I studied the neural processing of complex dynamic social stimuli in 5-month-old high- and low-risk infants. Using functional near infrared spectroscopy (fNIRS), I found that while typically-developing infants showed enhanced posterior temporal cortex activation for social stimuli, this activation was diminished in the high-risk group. In the following two chapters of this thesis, I investigated action prediction abilities in 10- (Chapter 2) and 14-month-old infants (Chapter 3). While we did observe differences in neural processing of social stimuli between the high- and low-risk infants in Chapter 1, the behavioral assessments of action prediction using eye tracking did not reveal any group differences. Rather, anticipatory eye movements were found to be similarly modulated for all infants by object knowledge (Chapter 2) and motor experience (Chapter 3). Finally, Chapter 4 consisted of an investigation of the neural processes associated with action prediction in typical adults. By co-registering electroencephalography (EEG) and eye tracking, I showed that neural oscillations in the beta-frequency range were modulated by the predictability of the distinct action steps and correlated with predictive eye movements. These insights provide a basis for further investigations of neural processes related to action prediction in ASD. In the following, I will discuss the findings of this thesis in detail and outline their implications as well as directions for future research.
SOCIAL INFORMATION PROCESSING IN INFANTS AT HIGH RISK FOR ASD

The results of Chapter 1 are in line with previous neuroimaging studies that have reported atypical cortical processing of social information in high-risk infants during the first half year of postnatal development (Elsabbagh et al., 2012; Lloyd-Fox et al., 2013). The findings extend the work of Lloyd-Fox and colleagues (2013) by showing that posterior temporal cortex activity in low-risk infants was specifically elicited by social dynamic stimuli, whereas activation in the same region was similar for the social and non-social condition in the high-risk infants. The cortical selectivity for social stimuli in posterior temporal cortex that is present in typically-developing infants within the first few months of development (Blasi et al., 2011; Lloyd-Fox et al., 2009), is thus diminished infants at high risk for ASD. Atypical cortical sensitivity to social stimuli may therefore form part of the infant broader autism phenotype (BAP) at this age. The BAP describes a set of sub-clinical features that can be found in non-autistic relatives of individuals with ASD (Pisula & Ziegart-Sadowska, 2015; Piven et al., 1997). BAP characteristics are related to the core deficits that characterize ASD and include social difficulties, language delays and deficits, as well personality characteristics such as behavioral rigidity (Hurley, Losh, Parlier, Reznick, & Piven, 2007; Ozonoff et al., 2014). The results of Chapter 1 suggest that atypical social processing is visible in infant siblings of children with ASD already at 5 months of age. However, currently, high-risk infants that later receive an ASD diagnosis were included in this group comparison and it remains to be assessed if differences between unaffected high-risk siblings and low-risk infants persist once outcome information is available. Future work will need to establish whether the early differences in cortical activation to social stimuli reflect a state characteristic (i.e. characterizing in particular those high-risk infants who later receive an ASD diagnosis) or a trait characteristic (i.e. characterizing all high-risk infants irrespective of diagnostic outcome) of ASD. With respect to this question, preliminary findings by Lloyd-Fox and colleagues (in press) suggest that in particular those high-risk infants who receive a diagnosis later show reduced socially-selective activation at 5 months compared to low-risk controls. High-risk infants that continue to develop typically, on the other hand, show an intermediate response. Once information about the outcome of our sample is available, we will be able to investigate this further. In addition to assessing
the relationship between early social processing and later ASD outcome, an important task for future research will lie in unraveling the deviations in the development of cortical specialization that lead to the observed differences in Chapter 1.

**Social-specific vs. domain-general deficits**

From birth onwards, typically-developing infants show attentional biases towards social stimuli, like faces (Farroni, Csibra, Simion, & Johnson, 2002; Johnson, Dziurawiec, Ellis, & Morton, 1991; Morton & Johnson, 1991; Valenza, Simion, Cassia, & Umiltà, 1996). These initial biases are thought to play an important role in the process of cortical specialization during the early stages of postnatal brain development (Johnson, 2001; Johnson, 2005). It has been suggested that a deviation in this early bias towards social stimuli may be present in ASD and could result in atypical social development including the observed atypical cortical specialization reported in Chapter 1 (Chevallier, Kohls, Troiani, Brodkin, & Schultz, 2012; Dawson et al., 2004). Prospective studies assessing social attention in infants at high risk for ASD have, however, shown limited support for these theories. Rather, most studies suggest that social attention is typical at 6 months of postnatal development and only begins to deviate towards the end of the first year (Elsabbagh & Johnson, 2016; Elsabbagh et al., 2014; Gliga, Jones, Bedford, Charman, & Johnson, 2014; Jones, Gliga, Bedford, Charman, & Johnson, 2014; Di Giorgio et al., 2016; Elsabbagh et al., 2010). Few studies have shown earlier differences (Jones et al., 2016; Jones & Klin, 2013), although recently Di Giorgio and colleagues (2016) reported atypicalities in preferential looking comparing newborns at low and high risk for ASD. As this was the first study to assess social attention in newborns, more research will be needed to replicate the findings and, importantly, assess how differences relate to an eventual ASD diagnosis. Overall, prospective studies thus far do not show strong support for generic atypicalities in social attention during the first six months of development (Elsabbagh & Johnson, 2016; Gliga et al., 2014; Jones et al., 2014). Rather, early deviations in the social domain seem to be subtle and more pronounced social deficits emerge only after 12 months of age (Elsabbagh & Johnson, 2016; Gliga et al., 2014; Jones et al., 2014). Importantly, research has shown that the early development of high-risk infants is also characterized by differences from low-risk controls in a variety of non-social domains (Elsabbagh & Johnson, 2010, Elsabbagh & Johnson, 2016; Jones et al., 2014). Reported deviations include
for instance, differences in temperament (Clifford et al., 2013; Garon et al., 2009), atypical disengagement of visual attention (Elsabbagh et al., 2013), and atypical motor coordination and movement patterns (Bhat, Landa, & Galloway, 2011). Given this broad range of differences, Elsabbagh and Johnson (2016) argued that domain-general accounts of ASD may provide a better explanation of the early signs associated with ASD than accounts focusing solely on the social deficits (see also Gliga et al., 2014). Domain-general accounts propose that widespread atypicalities in (brain) development, including but not limited to social brain regions, are present in ASD. Deviations in social brain activity such as observed in Chapter 1 may then be caused by generic problems with information processing rather than localized deficits in social brain regions (Elsabbagh & Johnson, 2016). In line with this notion, neuroimaging studies of high-risk infants have shown that early differences are not limited to atypicalities in social brain regions, but include alterations in overall cortical connectivity and brain structure (Bosl et al., 2011; Keehn, Wagner, Tager-Flusberg, & Nelson, 2013; Orekhova et al., 2014).

A domain-general account of ASD that has recently been proposed by several researchers is a deficit in prediction or predictive processing (Brock, 2012; van de Cruys et al., 2014; Gomot & Wicker, 2012; Lawson, Rees, & Friston, 2014; Pellicano & Burr, 2012; Sinha et al., 2014). It is argued that problems in forming and updating predictions about the environment could explain multiple of the ASD symptoms (Sinha et al., 2014). Several recent models propose that individuals with ASD might show a disturbance in the integration of prior knowledge about the environment and incoming sensory information (van de Cruys et al., 2014; Lawson et al., 2014; Pellicano & Burr, 2012). While these theoretical accounts first focused on providing an explanation of the sensory symptoms of the disorder (Pellicano & Burr, 2012), such as superior performance in visual search tasks (Joseph et al., 2009), more recent proposals suggest that predictive processing could also explain the social aspects of the disorder. Social situations are seen as complex and highly ambiguous which require individuals to rely more strongly on prior information and specific contextual cues (van de Cruys et al., 2014; Lawson et al., 2014). Thus far, empirical studies assessing whether atypical predictive processing could explain social difficulties in ASD are sparse. A recent study by Chambon and colleagues (2017), to my knowledge, was the first to directly assess this question. The researchers investigated
participant’s reliance on sensory and prior information during an action prediction tasks. Individuals were asked to infer the intentions of an actor who was manipulating non-meaningful objects either in a social or non-social context. It was shown that social priors had a greater influence on performance in typical adults compared to adults with ASD. The study by Chambon and colleagues (2017) thereby provides first evidence that differences in action prediction could be explained by deviations in the integration of social priors. However, more research will be needed to assess this further and also establish whether atypical predictive processing may be present in infants at high risk for ASD.
ACTION PREDICTION IN INFANTS AT HIGH RISK FOR ASD

While older individuals with ASD can show difficulties in predicting the actions of others (Boria et al., 2009; Cattaneo et al., 2007; Chambon et al., 2017; Schuwerek, Sodian, & Paulus, 2016; Zalla, Labruyère, Clément, & Georgieff, 2010), action prediction abilities have thus far rarely been assessed in high-risk infants. Yet, the ability to predict others’ actions has been shown to develop early in infancy (Falck-Ytter et al., 2006; Hunnius & Bekkering, 2010) and deviations may then also emerge early in atypical development. Chapter 2 and Chapter 3 of this thesis therefore aimed to investigate action prediction abilities in young high-risk infants. Specifically, the infants’ eye movements were recorded during the observation of actions performed by others. Both studies showed that there were no differences in predictive eye movements between the low- and high-risk infants. More importantly, action prediction was shown to be modulated by similar factors across all participants: In Chapter 2, I replicated previous findings showing that object knowledge influenced infants’ target predictions (Hunnius & Bekkering, 2010). Participants looked more frequently at an alternative target location - where nothing was happening- for unusual compared to usual actions performed on everyday objects. Importantly, this modulation was present across the low- and high-risk group, suggesting that prior knowledge influenced predictions irrespective of familial risk.

The results of Chapter 3 illustrated the importance of motor experience on action prediction, replicating previous work in typically-developing infants (Hunnius & Bekkering, 2014; Kanakogi & Itakura, 2011; Meyer, Bekkering, Haartsen, Stapel, & Hunnius, 2015; Stapel, Hunnius, Meyer, & Bekkering, 2016). Specifically, I showed that infants were more accurate and less variable in predicting the timing of actions that they were motorically more familiar with. Patterns of anticipations were again strikingly similar for the low- and high-risk infants, suggesting that motor experience influenced predictions irrespective of familial risk. The close link between action prediction abilities and motor proficiency in young infants has been interpreted as evidence that observed behavior is mapped onto own action representations (Kanakogi & Itakura, 2011; Prinz, 2006; Stapel et al., 2016). While it has been suggested that this mapping might be distorted in individuals with ASD (Cattaneo et al., 2007; Gallese et al., 2013; Iacoboni &
Dapretto, 2006; Rizzolatti et al., 2009), our results suggest that infants at high risk do recruit their own motor system during the observation of others’ actions at 14 months of age. Taken together, the findings of Chapter 2 and 3 suggest that observational and active action experience influenced action predictions not only in typically-developing infants (Hunnius & Bekkering, 2014), but also in infants at high familial risk for ASD to the same degree.

In the assessment of early prediction abilities, the two eye tracking studies reported in this thesis complement each other by assessing different aspects of action prediction. In Chapter 2, it was assessed how frequently infants predicted the target of an observed action, whereas in Chapter 3, the accuracy and stability of the predictions were measured. Combined, the results suggest that none of these aspects of action prediction were atypical in infants at high familial risk for ASD. In Chapter 3, infants were required to fixate at the end point of the observed action in order to be included in the analysis. As our exploration focused on group differences in fixation accuracy and stability, this requirement was necessary to perform the analysis. However, the exclusion rate of Chapter 3 was relatively high and one might argue that group differences in other parameters of action prediction could have been present in the larger sample. Although we did not assess this in Chapter 3, the results of Chapter 2 showed that performance remained similar for the low- and high-risk infants, even if a larger sample was assessed and fixations to the action goal were not required. In this study, I assessed how frequently infants fixated at the target goal before the action was completed without requiring the infant to perform those predictions. Overall, we thus found no differences in the number of action predictions that were made (Chapter 2), nor in the accuracy and stability of the performed predictions (Chapter 3).

Although the findings from this thesis thus strongly suggest that action prediction is not impaired in infants at high familial risk, one needs to be careful in interpreting the present results with respect to a proposed prediction deficit in ASD (van de Cruys et al., 2014; Lawson et al., 2014; Sinha et al., 2014). At this point in time, we do not know whether the sub-group of high-risk infants who receives an ASD diagnosis later may show deviations from low-risk controls and high-risk infants who continue to develop typically. While, according to our results, action prediction difficulties do not reflect a risk or trait marker of ASD,
they may reflect a state marker of the disorder (i.e. characterizing specifically those high-risk infants who later receive an ASD diagnosis). With this in mind, it is interesting that in both Chapter 2 and Chapter 3, more high-risk infants than low-risk infants needed to be excluded from the analysis. Moreover, and despite the absence of a group difference, more high- than low-risk infants failed to anticipate completely during the study reported in Chapter 2. These characteristics may turn out to be meaningful, but need to be further assessed once outcome information for the high-risk infants is available.
BEHAVIORAL VS. NEURAL ATYPICALITIES IN INFANTS AT HIGH RISK

Overall, the results from the first three chapters of this thesis suggest that infants at high risk for ASD do show atypicalities in brain activation elicited by social stimuli at 5 months of age but do not show behavioral differences during the observation and prediction of others’ actions at 10 or 14 months of age. Interestingly, the analysis of the looking behavior during the fNIRS experiment in Chapter 1 suggested that visual attention to the social and non-social stimuli was similar for the low- and high-risk infants at 5 months. While the two groups were thus not distinguishable on basis of behavior, differences were visible in neural activity. Deviations in neural activity may therefore provide a more sensitive measure of early atypical development. In line with this notion, other studies also suggest that abnormalities in brain measures can be present in young infants at high risk for ASD in the absence of behavioral differences (Elsabbagh et al., 2012; Orekhova et al., 2014). Elsabbagh and colleagues (2012), for instance, reported atypical event-related potentials during the observation of eye gaze in 6 to 10-month-old high-risk infants. Importantly, no differences in visual scanning of the scene were present in the sample, suggesting that the observed neural differences could not be explained by behavioral atypicalities. In a more recent study, Orekhova and colleagues (2014) found that patterns of alpha hyper-connectivity characterized 14-month-old high-risk infants who later received an ASD diagnosis compared to other high-risk infants and low-risk controls. Again, no behavioral group differences during EEG data collection were present in the study. While Chapter 2 and 3 of this thesis did not reveal differences in behavioral measures of action prediction, it thus remains possible that actions were processed differently in the high-risk infants’ brain. Although preliminary results from an unpublished study in our lab did not reveal differences in cortical motor system activation during action prediction between low- and high-risk infants at 14 months of age, future research should investigate this further.

1: In an ongoing follow-up EEG study of Chapter 2, I aimed to assess differences in neural processing between low- and high-risk infants during the observation of usual and unusual actions at 14 months of age. The study was based on a paradigm developed by Stapel and colleagues (2010) who reported that typically-developing 12-month-olds showed enhanced motor system activation (reflected by stronger attenuation of central sensorimotor mu-power) during the observation of unusual compared to usual actions. Using the same paradigm, I thus far gathered valid EEG data from 14 high-risk and 8 low-risk infants. Preliminary analyses of this sample did not reveal significant differences.
The cortical mirror neuron system (MNS) has been suggested to play an important role in the generation of action predictions. The MNS is activated during the observation of own and others’ actions and this overlap is suggested to reflect the mapping of observed behavior onto own action representations (Kilner et al., 2007; Prinz, 2006; Rizzolatti & Sinigaglia, 2016). A proposed deficit in this system in ASD could then result in observed difficulties to understand and predict others’ actions (Cattaneo et al., 2007; Gallese et al., 2013; Iacoboni & Dapretto, 2006; Rizzolatti et al., 2009). Many studies assessing MNS activation in individuals with ASD have used EEG and focused on the investigation of attenuation of mu-oscillations over sensorimotor areas (Martineau, Cochin, Magne, & Barthelemy, 2008; Oberman et al., 2005; Oberman et al., 2013; Oberman et al., 2008; Raymaekers et al., 2009). Mu-attenuation is seen to reflect motor system activation (McFarland et al., 2000; Muthukumaraswamy & Johnson, 2004; Perry et al., 2010; Pfurtscheller, 1981) and can be observed during action execution as well as observation in typically-developing individuals (Denis, Rowe, Williams, & Milne, 2016; Koelewijn, van Schie, Bekkering, Oostenveld, & Jensen, 2008; McFarland et al., 2000; Meyer, Braukmann, Stapel, Bekkering, & Hunnius, 2015) Thus far, EEG studies assessing MNS difficulties in individuals with ASD have shown mixed results (Martineau et al., 2008; Oberman et al., 2005; Oberman et al., 2013; Oberman et al., 2008; Raymaekers et al., 2009). While some studies reported reduced mu-attenuation during action observation (Oberman et al., 2005; Oberman et al., 2008), other studies have shown no differences (Raymaekers et al., 2009; Ruyschaert, Warreyn, Wiersema, Oostra, & Roeyers, 2014). Given the mixed empirical evidence, the MNS account of ASD remains highly debated (Fan et al., 2010; Southgate & Hamilton, 2008). Yet, most studies assessing oscillatory power during action observation in ASD have focused on contrasting observation and execution conditions of simple goal-directed actions (Oberman et al., 2005; Ruyschaert et al., 2014). It remains to be assessed whether more subtle differences are present in the processing of complex actions. Moreover, given the behavioral findings of action prediction difficulties in ASD (Boria et al., 2009; Cattaneo et al., 2007; Schuwerk et al., 2016; Sinha et al., 2014; Zalla et al., 2010), neural atypicalities in the process-
ing of others’ actions may be present specifically in aspects related to action prediction. The last chapter of this thesis presents a paradigm, which could be applied in future studies assessing neural and behavioral action prediction difficulties in individuals with ASD. More specifically, in Chapter 4, I investigated the neural correlates of action prediction during the observation of complex multi-step actions in typical adults.
NEURAL CORRELATES OF ACTION PREDICTION

In Chapter 4 of this thesis, I investigated motor system activation in typical adults during the observation of complex actions consisting of multiple steps, which differed in their predictability. In particular, I focused on the assessment of attenuation of mu- and beta-oscillations over central sensorimotor sites, which have been previously associated with motor system activation (Hari, 2006; Honaga et al., 2010; Meyer, Braukmann, Stapel, Bekkering, & Hunnius, 2015; Stapel et al., 2010; Tzagarakis, Ince, Leuthold, & Pellizzer, 2010; Van Elk, Van Schie, Hunnius, Vesper, & Bekkering, 2008). The results suggested that beta-oscillations were modulated by action predictability and correlated with predictive eye movements. Oscillations in the mu-frequency range, on the other hand, were not modulated by action predictability. The findings complement previous work showing motor system involvement during action prediction (Elsner et al., 2013) and suggest a specific role for beta-oscillations in the predictive function of the motor system. The findings from Chapter 4 can be applied in future work assessing the neural correlates of action prediction in ASD. While previous studies on MNS functioning in ASD have often focused on mu-oscillations and relatively simple actions (Oberman et al., 2013; Perkins, Stokes, McGillivray, & Bittar, 2010; Raymaekers et al., 2009), future studies could assess more complex actions and report on both mu- and beta-oscillations given their suggested distinct functions in action processing and the potential role for beta-oscillations specifically related to action prediction.
DIRECTIONS FOR FUTURE RESEARCH

The Zebra-project is an ongoing study in which the infants from the cohort tested for Chapter 1 to 3 of this thesis are invited back to the lab at 24 and 36 months of age. The crucial next step during these follow-up visits will be to assess who of the high-risk infants receives a diagnosis on the autism spectrum. Therefore, the Zebra research team will assess the children’s development and ASD outcome at these later time points. One of the most important outcome measures is the Autism Diagnostic Observation Schedule (ADOS; Lord, Luyster, Gotham, & Guthrie, 2012; Lord et al., 1989, Lord et al., 2000) which is a semi-structured assessment of social and communicative behavior used to establish a (preliminary) diagnosis of ASD. Based on the diagnostic outcome, the gathered data from the current thesis can be re-analyzed and a comparison can be made between high-risk infants who receive an ASD diagnosis (HR-ASD), high-risk infants who do not (HR-noASD) and low-risk controls. These future analyses will provide crucial insights into the early differences in social processing and action prediction associated with later ASD.

While the neuroimaging results reported in Chapter 1 suggest that atypical cortical processing is present in the entire high-risk group, it will be interesting to assess whether social-selective activation further distinguishes the HR-ASD and the HR-noASD group. Preliminary findings from a collaborating lab suggests that differences from low-risk controls are especially pronounced in the HR-ASD group while the HR-noASD group displays an intermediate pattern of activation (Lloyd-Fox et al., in press). Since the paradigm used in Chapter 1 was similar to the paradigm by Lloyd-Fox and colleagues (in press, but see also Lloyd-Fox et al., 2013), the data from both studies can be combined resulting in a larger sample that can be further investigated. As approximately 20% of the infants followed for this thesis will receive an ASD diagnosis (Constantino, Zhang, Frazier, Abbacchi, & Law, 2010; Ozonoff et al., 2011), larger samples like that will be necessary to increase the statistical power of the dataset and enable the application of more complex analyses.

Using the acquired data from Chapter 2 and Chapter 3, future assessments of differences between low-risk, HR-ASD and HR-noASD infants will also provide interesting insights into the early development of action prediction within
these three sub-groups. Thus far, the eye tracking results from Chapter 2 and Chapter 3 suggested that action prediction abilities did not differ between the high and low-risk infants. It may, however, be the case that anticipations are impaired specifically in those high-risk infants who do continue to receive an ASD diagnosis. On the other hand, action prediction may also not distinguish the HR-ASD and HR-noASD group. Once diagnostic information from our cohort is available, we will be able to assess this further and establish whether action prediction differences manifest as an early marker of ASD or not.

The growing amount of research on the development of infant siblings of children with ASD provides an increasingly more detailed picture of infant BAP as well as early markers associated with later ASD diagnosis. Knowledge about early ASD characteristics provides the basis for timely detection and interventions starting in infancy and early toddlerhood when symptoms are first detectable (Constantino & Charman, 2016; Dawson, 2008). While there is consensus that early identification and intervention will be beneficial for affected individuals and their families (Charman, 2014; Webb et al., 2017; Zwaigenbaum, 2015), their implementation proves to be highly complex. Recently, Camarata (2014) and Charman (2014) outlined the challenges of ASD identification at 24 months as well as the mixed results of current interventions for infants and toddlers with ASD. One of the major challenges for early ASD detection is the substantial heterogeneity associated with the disorder. While infants who are severely affected and show symptoms across the full autism spectrum (i.e. social atypicalities, as well as repetitive behaviors and sensory atypicalities) can potentially be relatively easily detected, more subtle cases and infants that deviate only on a sub-set of ASD characteristics are difficult to identify. Importantly, some of the early deviations are not specific for ASD but can signal other developmental atypicalities. For instance, common early signs in infants and toddlers with ASD are speech and communication problems such as delayed language (Camarata, 2014). Despite the difficulties in establishing a reliable distinction between early ASD and other disorders, targeting social-communication skills in infants and toddlers that display deviations early in development is thought to improve later outcome (Webb et al., 2017). With respect to early interventions, both Camarata (2014) and Charman (2014) stressed the need for randomized control trials (RCTs) to reliably assess the effectiveness of the intervention programs. Charman (2014) reviewed the to date published RCTs
of early interventions targeting social communication and language skills in infants and toddlers with ASD. Although some early interventions have shown improvements in language and communication features (Dawson et al., 2010; Kasari, Freeman, & Paparella, 2006) as well as behaviors that were specifically targeted during the intervention – such as joint attention (Kasari et al., 2006) or parent-child synchrony (Green et al., 2010) -, no intervention effects on ASD severity were found in those studies that did include a measure of ASD outcome (Dawson et al., 2010; Jonathan Green et al., 2010). While current results from early intervention studies thus provide mixed findings for their effectiveness, interventions are further being developed and evaluated (Green et al., 2017; Wass, Porayska-Pomsta, & Johnson, 2011). As the broad assessment of infants at high familial risk for ASD continues, our understanding about the mechanisms behind ASD and its early developmental advances which will provide novel insights for new avenues of ASD research and interventions.
CONCLUSION

This thesis investigated different aspects of social cognition in young infants at high risk for ASD and typically-developing controls. I showed that atypical cortical processing of social information can be observed in 5-month-old high-risk infants (Chapter 1). While differences in neural processing thus seem to be part of the infant broader autism phenotype, I did not observe behavioral differences in action prediction at 10 or 14 months of age. Rather action prediction appeared to be similarly modulated by object knowledge (Chapter 2) and action experience (Chapter 3) in low- and high-risk infants. The last chapter of this thesis showed that cortical sensorimotor beta-oscillations were related to predictive eye movements elicited during action observation in typical adults. These results provide a basis for further investigations of the neural underpinnings of action prediction in ASD. Importantly, as the Zebra-project continues, the infants assessed in Chapter 1 to 3 of this thesis will be invited back to the lab at 24 and 36 months, and diagnostic outcome information will be gathered. Follow-up studies will be then able to investigate the relation between the aspects of early social cognition described in this thesis and later ASD outcome.
Appendix

References
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Appendix


ENGLISH SUMMARY

This thesis is embedded into the field of developmental cognitive neuroscience. It focuses on the scientific assessment of early behavioral and neural characteristics of infants at high familial risk for developing Autism Spectrum Disorder and low-risk typically-developing controls. Autism Spectrum Disorder (ASD) is a common neurodevelopmental disorder, affecting approximately 1% of the general population. Individuals with ASD show social communication deficits, stereotyped behaviors and restricted interests, as well as atypical sensory processing. As ASD is typically not diagnosed before the age of 3, little is known about its early development. Researchers, however, do agree that an earlier identification of ASD and timely intervention could benefit the individuals and their families.

The research described in this thesis was part of the Zebra-project (Zusjes En BRoertjes van kinderen met Autisme, Sisters And Brothers of Children with Autism) which aimed to assess the early development of infant siblings of children with ASD and low-risk controls. Siblings of children with ASD have an increased risk (ranging from 10-20%) of receiving a diagnosis themselves. For this project, infants and their parents were assessed at five time points during the first three years of the infants’ life. During each visit, experimental tasks, developmental observations and behavioral assessments were administered. This thesis describes several experimental studies investigating different aspects of social cognition at the ages of 5 (Chapter 1), 10 (Chapter 2), and 14 months (Chapter 3). The first chapter of this thesis focused on the neural processing of social stimuli in high- and low-risk infants. The remainder of this thesis assessed the development of action prediction in infants at low and high risk for ASD (Chapter 2 and 3) as well as the neural mechanisms underlying action prediction in typical individuals (Chapter 4).

The social and communication difficulties that characterize ASD are considered the most striking feature of the disorder and previous findings suggested that atypical processing of social information may be present already at 5 months of age in infants at high risk. To replicate and extend these findings, in Chapter 1, I used functional near infrared spectroscopy (fNIRS) to assess the neural processing of social and non-social dynamic stimuli in 5-month-old high- and
low-risk infants. The results showed that social dynamic stimuli elicited activation in the right posterior temporal cortex in the low-risk infants, but that this activation was reduced in infants at high risk for ASD. My results thus replicated and extended previous research providing evidence for early deviations in socially selective processing in infants at high risk for ASD.

Chapter 2 to 4 of this thesis focused on action prediction, assessing potential early atypicalities in ASD. Several previous studies have reported action prediction difficulties in children and adults with ASD. Although action prediction develops early in infancy and plays an important role in social interactions, few studies to date have assessed early prediction abilities and potential atypicalities in infants at high risk. The experimental study of Chapter 2 assessed the influence of object knowledge on action prediction in 10-month-old high- and low-risk infants. Using eye tracking, I measured the infants’ action anticipations during the presentation of different actions performed on familiar everyday objects. Importantly, actions displayed an object being brought either to a location usually associated with this object or to an unusual location. I investigated infants’ anticipations to the actual target location (i.e. the location where the object was actually being brought to) and the alternative target location for both usual and unusual actions. For all infants, anticipation frequencies were modulated by object knowledge and the actions associated with them: Participants tended to look more frequently to the alternative target location when presented with unusual compared to usual actions. Importantly, I did not find any differences between the low- and high-risk infants in predictive eye movements. These results suggested that familial risk for ASD did not affect action prediction at 10 months of age.

In the second eye tracking study of this thesis, described in Chapter 3, the influence of motor experience on action prediction was investigated in 14-month-old high- and low-risk infants. Previous research in typically-developing infants has shown that prediction accuracy and stability are influenced by the infants’ own motor skills. The aim of Chapter 3 was to assess whether action experience modulates predictions in the same way in high-risk infants. Participants were presented with partially occluded actions (i.e. Crawling, Walking or Object movement) which differed in how motorically familiar they were to the infants. I assessed how accurate and stable infants were in predicting the reappearance of the actor or
object behind the occluder. I found that prediction accuracy and stability were modulated by action experience for all infants, suggesting that both low- and high-risk infants recruited their own motor representations during the prediction of observed actions. Crucially, there were no group differences, suggesting that familial risk for ASD did not influence prediction abilities at 14 months.

The last chapter of this thesis, Chapter 4, consisted of a combined electroencephalography (EEG) and eye tracking study assessing the neural processes associated with action prediction in typical adults. In particular, I focused on activation of the neural motor system which has been proposed to play an important role in the generation of predictions about observed actions. I investigated whether motor system activation was modulated by the degree of predictability of an observed action. Typical adult participants were presented with object-directed actions (e.g., making a cup of tea) consisting of three action steps which increased in their predictability. While the goal of the first step was ambiguous (e.g., when making tea, one can first grab the teabag or the cup), the goals of the following steps became predictable over the course of the action. Motor system activation was assessed by measuring attenuation of sensorimotor mu- and beta-oscillations. I showed that mu- and beta-oscillations were attenuated during action observation, indicating general activation of the motor system. Importantly, predictive motor system activation, indexed by beta-attenuation, increased for each action step, showing strongest activation prior to the final (i.e. most predictable) step. Moreover, sensorimotor activity was related to participants’ predictive eye-movements which also showed a modulation by action step. The findings from Chapter 4 demonstrated that motor system activity and behavioral predictions were modulated by the predictability of action sub-steps. In particular, beta-oscillations seem to play a role in the predictive function of the motor system.

In summary, the results of my thesis suggest that infants at high risk for ASD show differences in the neural processing of social stimuli at 5 months of age. The behavioral assessment of action prediction conducted at 10 and 14 months of age, on the other hand, did not reveal group differences between low- and high-risk infants. Rather, anticipatory eye movements were found to be similarly modulated for all infants by object knowledge and motor experience. Taken together, my findings suggest that deviations in neural activity may provide
a more sensitive measure of early atypical development in high-risk infants compared to behavioral assessments. While the two eye-tracking studies of this thesis did not reveal differences in behavioral measures of action prediction, it remains possible that actions were processed differently in the high-risk infants’ brain. Additional research needs to be conducted to investigate this further. The last chapter of this thesis presents an assessment of the neural processes associated with action prediction in typical adults which could sever as basis for future studies in individuals with ASD.

An important role for future research will be to determine how the results from the first three chapters of this thesis relate to an eventual ASD diagnosis of the participants. The Zebra project is an ongoing study in which the infants are invited back to the lab at 24 and 36 months where the research team will assess ASD symptoms. Based on the outcome of this preliminary diagnosis, the gathered data from the current thesis can be re-analyzed and a comparison can be made between high-risk infants who receive a diagnosis (HR-ASD), high-risk infants who do not (HR-noASD) and low-risk controls. These future analyses will provide crucial insights into the early differences in social processing and action prediction associated with later ASD. While the neuroimaging results reported in Chapter 1 suggested that atypical cortical processing is present in the entire high-risk group, it will be interesting to assess whether social-selective activation further distinguishes the HR-ASD and the HR-noASD group. Using the acquired data from Chapter 2 and Chapter 3, future assessments of differences between low-risk, HR-ASD and HR-noASD infants will provide important insights into the early development of action prediction within these three sub-groups. Thus far, the eye tracking results from this thesis suggested that action prediction abilities did not differ between the high- and low-risk infants. It may, however, be the case that anticipations are impaired specifically in those high-risk infants who do continue to receive an ASD diagnosis. On the other hand, action prediction may also not distinguish the HR-ASD and HR-noASD group. Once diagnostic information from the cohort is available, this can further be assessed, and it can be established whether action prediction differences manifest as an early marker of ASD or not. Overall, future research following infants at high familial risk for ASD will advance our understanding of the mechanisms behind the disorder and its early development, providing novel insights for new avenues of ASD research and early interventions.
**NEDERLANDSE SAMENVATTING**

Dit proefschrift beschrijft een aantal wetenschappelijke studies op het gebied van de klinische ontwikkelingspsychologie en de cognitieve neurowetenschappen. Het doel van dit proefschrift was het onderzoeken van vroege gedrags- en hersenkenmerken van baby’s met een verhoogd risico voor het ontwikkelen van een Autisme Spectrum Stoornis. Autisme Spectrum Stoornis (ASS) is een pervasieve ontwikkelingsstoornis, die bij ongeveer 1% van de bevolking voorkomt. ASS wordt gekenmerkt door sociale interactie- en communicatieproblemen, repetitief gedrag en beperkte interesses, alsook door afwijkingen in het verwerken van zintuiglijke prikkels. Doordat ASS meestal niet voor het derde levensjaar wordt gediagnosticeerd is er tot nu toe vrij weinig bekend over de vroege ontwikkeling van de stoornis. Onderzoekers zijn het er wel over eens dat een vroege diagnose en tijdige behandeling positieve invloed kunnen hebben op de ontwikkeling van kinderen met ASS en hun families.

Het onderzoek dat in dit proefschrift staat beschreven maakt deel uit van het Zebra-project (Zusjes En BRoertjes van kinderen met Autisme). Het Zebra-project richt zich op het bestuderen van de vroege ontwikkeling van broertjes en zusjes van kinderen met en zonder ASS. Baby broertjes en zusjes van kinderen met ASS hebben zelf een verhoogd risico (tussen de 10-20%) om op latere leeftijd ook een diagnose binnen het autisme spectrum te krijgen. Binnen het Zebra-project worden de broertjes en zusjes en hun ouders op vijf meetmomenten gedurende de eerste drie levensjaren bestudeerd. Bij elk bezoek worden er verschillende experimentele taken afgenomen en wordt de ontwikkeling en het gedrag van de kinderen in kaart gebracht. Dit proefschrift is gebaseerd op de eerste drie meetmomenten en beschrijft meerdere wetenschappelijke studies waarin op 5 (Hoofdstuk 1), 10 (Hoofdstuk 2) en 14 maanden (Hoofdstuk 3) verschillende aspecten van de sociale cognitie werden onderzocht. In het eerste experimentele hoofdstuk werden de neurale processen die betrokken zijn bij het verwerken van sociale stimuli in baby’s met hoog en laag risico op ASS onderzocht. De rest van dit proefschrift beschrijft onderzoek naar het voorspellen van handelingen. In Hoofdstuk 2 en 3 heb ik onderzocht hoe kinderen met een hoog of laag risico voor het ontwikkelen van ASS naar handelingen van andere mensen kijken. Met name was ik geïnteresseerd hoe en of zij voorspellingen maken over de voortgang van de bekeken handelingen.
Appendix

In het laatste hoofdstuk van dit proefschrift heb ik tot slot gekeken naar de neurale processen die bij het voorspellen van handelingen betrokken zijn in volwassenen zonder ASS (Hoofdstuk 4).

Problemen op het gebied van sociale interactie en communicatie worden vaak als de meest opvallende symptomen van ASS gezien. Recent hersenonderzoek heeft aangetoond dat afwijkende verwerking van sociale informatie al aanwezig is bij 5 maanden oude baby’s met verhoogd risico op ASS. Om deze bevindingen te repliceren en uit te breiden heb ik in Hoofdstuk 1 gebruikt gemaakt van functional near-infrared spectroscopy (fNIRS), een methode om de activiteit van hersengebieden bij jonge kinderen in kaart te brengen. Met behulp van fNIRS heb ik de neurale verwerking van sociale en niet-sociale dynamische stimuli in 5 maanden oude baby’s met hoog en laag risico op ASS onderzocht. De resultaten lieten zien dat het kijken naar sociale stimuli bij baby’s met een laag risico leidde tot een toename in hersenactiviteit in het achterste gedeelte van de rechter temporale kwab, een gebied dat deel uit maakt van het netwerk dat sociale stimuli verwerkt. Bij de baby’s met verhoogd risico voor ASS was deze hersenactiviteit verminderd. Mijn bevindingen uit Hoofdstuk 1 repliceren eerder onderzoek en tonen aan dat er al vroeg verschillen te zien zijn in de neurale verwerking van sociale informatie in baby’s met een verhoogd risico op ASS.

In hoofdstuk 2 tot en met 4 van dit proefschrift heb ik het voorspellen van handelingen onderzocht. Verschillende wetenschappelijk studies hebben aangetoond dat kinderen en volwassenen met ASS problemen hebben om handelingen en intenties van andere mensen correct te voorspellen. De vaardigheid om handelingen te voorspellen ontwikkelt zich al vroeg in de babytijd en speelt een belangrijke rol bij sociale interacties. Hierdoor zouden problemen met het voorspellen van handelingen ook al vroeg zichtbaar kunnen zijn bij kinderen die op latere leeftijd een ASS diagnose krijgen. Echter was hier tot nu toe nog maar weinig onderzoek naar gedaan en was het onbekend of kinderen met verhoogd risico op ASS problemen vertonen op het gebied van handelingsvoorspellingen. In de experimentele studie van Hoofdstuk 2 heb ik gebruik gemaakt van eye tracking om te bestuderen hoe 10 maanden oude kinderen met hoog en laag risico voor ASS handelingen van andere mensen voorspellen. Ik heb onderzocht in hoeverre kennis over een voorwerp dat in een handeling gebruikt wordt de voorspellingen van de kinderen ging beïnvloeden. De kinderen keken tijdens het onderzoek naar verschillende handelingen met een bekend voorwerp (een kopje
of een telefoon). Het voorwerp werd of op een gewone manier gebruikt (d.w.z. het kopje werd naar de mond gebracht; de telefoon werd naar het oor gebracht) of op een ongewone manier (d.w.z. het kopje werd naar het oor gebracht; de telefoon werd naar de mond gebracht). Eerder onderzoek in kinderen zonder verhoogd risico op ASS heeft aangetoond dat de kennis van voorwerpen al vanaf 6 maanden invloed heeft op de voorspellingen die gemaakt worden. Kinderen verwachten bijvoorbeeld eerder dat een kopje naar de mond wordt gebracht en zijn verrast wanneer een kopje naar het oor worden gebracht. In de studie van Hoofdstuk 2 heb ik gekeken of 10 maanden oude kinderen de juiste eindlocatie van de handelingen konden voorspellen en of zij even goed waren in het voorspellen van de gewone en ongewone handelingen. De resultaten van dit onderzoek lieten zien dat voorspellingen voor alle kinderen beïnvloed werden door de kennis van het voorwerp: Als het voorwerp naar een ongewone eindlocatie werd gebracht keken kinderen vaker naar de andere eindlocatie (d.w.z. zij keken naar het oor terwijl een telefoon naar de mond werd gebracht en naar de mond terwijl een kopje naar het oor werd gebracht). De belangrijkste bevinding van dit onderzoek was dat er geen verschillen waren in de voorspellingen tussen kinderen met hoog en laag risico op ASS. Bij 10 maanden oude kinderen heeft een verhoogd risico om ASS te ontwikkelen dus geen effect op de vaardigheid om handelingen van anderen te voorspellen.

In de tweede eye tracking studie van dit proefschrift, beschreven in Hoofdstuk 3, heb ik gekeken naar de invloed van motorische ervaring op het voorspellen van handelingen in 14 maanden oude kinderen. Eerder onderzoek in kinderen zonder verhoogd risico op ASS heeft aangetoond dat de nauwkeurigheid en stabilitéit van handelingsvoorspellingen beïnvloed worden door de motorische vaardigheid van de kinderen. Het doel van Hoofdstuk 3 was om te bestuderen of de invloed van motorische ervaring op de vaardigheid om handelingen te voorspellen verschilt tussen kinderen met hoog of laag risico op ASS. De kinderen keken naar video’s van handelingen waar zij zelf meer of minder motorische ervaring mee hadden. Video’s toonden of een kruipende baby (een handeling waar de kinderen zelf ook al motorische ervaring mee hadden), een lopende baby (een handeling waar de meeste kinderen nog geen of maar weinig motorische ervaring mee hadden), of van een bewegend voorwerp (een handeling waar kinderen helemaal geen motorische ervaring mee hadden). De handelingen waren gedeeltelijk verborgen achter een occluder en er werd
gemeten hoe precies en stabiel de kinderen waren in het voorspellen van de verschijning van de (lopende of kruipende) baby of het bewegende voorwerp achter deze occluder. De resultaten van dit experiment toonden aan dat de nauwkeurigheid en stabiliteit van de voorspellingen in alle kinderen beïnvloed werden door hun motorische ervaring. De kinderen -die zelf bijna allemaal al goed konden kruipen maar vaak nog minder goed waren in het lopen- waren namelijk nauwkeuriger en stabiler in het voorspellen van de kruipende dan van lopende baby. Het voorwerp, waar geen motorische ervaring voor mogelijk is, werd het minst goed voorspelt. Zowel kinderen met een laag alsook met een hoog risico op ASS gebruikten dus hun eigen motorische representaties in het voorspellen van handelingen. De belangrijkste bevinding van dit onderzoek was dat er wederom geen verschil werd gevonden tussen kinderen met laag of hoog risico voor ASS. Ook bij 14 maanden oude kinderen heeft een verhoogd risico om ASS te ontwikkelen dus geen effect op de vaardigheid om handelingen van anderen te voorspellen.

Het laatste hoofdstuk van dit proefschrift, Hoofdstuk 4, beschrijft een gecombineerde Elektro-encefalografie (EEG) en eye tracking studie waarin de neurale processen van het voorspellen van handelingen in volwassenen zonder ASS werden bestudeerd. Deze studie richtte zich met name op het neurale motorische systeem dat volgens recent onderzoek een belangrijke rol speelt bij het voorspellen van handelingen. Ik heb onderzocht of het motorische systeem beïnvloed wordt door de mate van voorspelbaarheid van een gepresenteerde handeling. Volwassen proefpersonen keken naar alledaagse handelingen (bijvoorbeeld het maken van een kopje thee) bestaande uit drie handelingsstappen die in voorspelbaarheid toenamen. Terwijl de proefpersoon het doel van de eerste stap van de handeling nog niet kon weten, werden de volgende handelingsstappen steeds beter te voorspellen (bijvoorbeeld: als men een kopje thee wil maken kan men eerst het zakje pakken of eerst het kopje, maar nadat het zakje gepakt is en in het kopje is gelegd mist er alleen nog heet water). Activiteit van het motorische system werd geanalyseerd door naar de sterkte van de mu- en beta-oscillaties in het EEG-sinaal te kijken. Eerder onderzoek heeft laten zien dat de sterkte van deze oscillaties over motorische gebieden van de hersenen afneemt als deze gebieden worden geactiveerd. Ook in het onderzoek van Hoofdstuk 4 nam de sterkte van de mu- en beta-oscillaties af tijdens het kijken naar de handelingen wat suggereert dat het motorische systeem
In dit proefschrift heb ik gekeken naar verschillende aspecten van de sociale cognitie in kinderen met een hoog risico voor het ontwikkelen van ASS en controles. De resultaten van het hersenonderzoek in Hoofdstuk 1 toonden aan dat 5 maanden oude baby’s met een verhoogd risico voor ASS sociale informatie anders verwerken dan kinderen met een laag risico. De gedragsstudies naar het voorspellen van handelingen van anderen lieten echter geen verschil zien tussen 10 en 14 maanden oude kinderen met hoog of laag risico voor ASS. In plaats daarvan werden de voorspellingen van alle kinderen beïnvloed door hun kennis over de voorwerpen die in de handelingen gebruikt werden en door hun motorische ervaring. Mijn bevindingen suggereren dat verschillen in neurale activiteit wellicht een gevoeligere maat zijn voor een afwijkende ontwikkeling in kinderen met een verhoogd risico voor ASS dan gedragsmetingen. Terwijl in de twee eye tracking studies geen verschillen tussen de twee groepen kinderen werden gevonden is het mogelijk dat er verschillen bestaan in de neurale verwerking van de bekeken handelingen. Aanvullend onderzoek zal nodig zijn om dit verder te bestuderen. In het laatste hoofdstuk van dit proefschrift staat een experimentele taak beschreven waarmee wellicht in vervolgonderzoek de neurale verwerking van handelingen en de invloed van voorspelbaarheid op het motorische systeem in ASS zou kunnen worden onderzocht.

Een belangrijke rol voor toekomstig onderzoek is het relateren van de bevindingen van de eerste drie hoofdstukken van dit proefschrift aan een latere ASS
diagnose van de proefpersonen. Het Zebra-project is een langlopende studie waarbij de broertjes en zusjes voor verder onderzoek worden uitgenodigd wanneer ze 24 en 36 maanden oud worden. Tijdens deze meetmomenten zal het onderzoeksteam onder meer de ASS symptomen van de kinderen in kaart brengen. Gebaseerd op de uitkomsten van deze voorlopige diagnose kan de data van dit proefschrift opnieuw worden geanalyseerd. Er kan gekeken worden of er verschillen zijn in de afgenomen experimentele taken tussen kinderen met een verhoogd risico op ASS en een latere diagnose, kinderen met een verhoogd risico op ASS maar zonder latere diagnose en controle kinderen met een laag risico. Deze vervolganalyses zullen belangrijke inzichten geven in hoe de verwerking van sociale stimuli en de voorspelling van handelingen in de vroege kindertijd gerelateerd zijn aan een latere ASS diagnose. Terwijl de bevindingen van Hoofdstuk 1 suggereren dat de neurale verwerking van sociale informatie afwijkend is in de gehele groep kinderen met een verhoogd risico zouden er verdere verschillen kunnen bestaan tussen de subgroepen. Het is belangrijk om te bestuderen of de kinderen die later wel een diagnose krijgen onderscheiden kunnen worden van de kinderen die later geen diagnose krijgen op basis van de gemeten hersenactiviteit. Met de verzamelde data van Hoofdstuk 2 en Hoofdstuk 3 zal verder onderzoek gedaan kunnen worden naar verschillen in het voorspellen van handelingen van anderen tussen de drie subgroepen van kinderen (d.w.z kinderen met laag risico, kinderen met verhoogd risico en latere diagnose, en kinderen met verhoogd risico maar zonder latere diagnose). Tot dusver laten de eye tracking resultaten geen verschillen zien in het voorspellen van handelingen tussen kinderen met hoog en laag risico. Het zou echter zo kunnen zijn dat voorspellingen wel afwijkend zijn maar dan alleen in de groep kinderen die later een ASS diagnose krijgt. Aan de andere kant zou er ook geen verschil kunnen bestaan tussen kinderen met of zonder latere diagnose. Zodra informatie over de ASS diagnoses van dit cohort beschikbaar is zal dit verder onderzocht kunnen worden en kan worden vastgesteld of een afwijkende voorspelling van handelingen een vroege marker van ASS vormt of niet. In zijn geheel, zal het toekomstige onderzoek naar de vroege kenmerken van kinderen met een verhoogd risico op ASS onze kennis over de onderliggende mechanismen van de stoornis en zijn ontwikkeling vooruitbrengen waaruit nieuwe inzichten voor vervolgonderzoek en vroege behandelingen zullen voortkomen.
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**CURRICULUM VITAE**

Ricarda Braukmann was born on the 12th of December 1987 in Coesfeld, Germany. In 2007, Ricarda moved to the Netherlands where she started her Bachelor in Psychology at the University of Twente in Enschede. Having received her Bachelor’s degree (cum laude) in 2010, she was admitted to the Cognitive Neuroscience Research Master at the Radboud University in Nijmegen. After graduating from the Master’s program (cum laude), Ricarda started her PhD research at the Radboud University Medical Centre in August 2012. As a PhD candidate, Ricarda worked within the European project EU-AIMS where she studied the early development of infants at high and low familial risk for autism spectrum disorder. Ricarda was part of the of the Department of Cognitive Neuroscience (Radboud UMC) working within the *Neuropsychiatric and Developmental Disorders* group of Prof. Jan Buitelaar at the Donders Centre for Medical Neuroscience, and part of the Faculty of Social Sciences (Radboud University) working within the *BabyBRAIN* group of Dr. Sabine Hunnius and with Prof. Harold Bekkering at the Donders Centre for Cognition. Next to her PhD research, Ricarda received funding from the VSBfonds supporting a four months research project with Dr. Sarah Lloyd-Fox at the *Centre for Brain and Cognitive Development* in London, United Kingdom. During her last year as a PhD candidate, Ricarda furthermore did a four months part-time internship at the policy and communication department of DANS (Data Archiving and Networked Services) in The Hague. DANS is a national data and knowledge center for sustainable access to research data which promotes data sharing and open access. Ricarda will continue to work for DANS as a program leader for the social sciences after finishing her PhD.
**PUBLICATION LIST**


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