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Action prediction in 10-month-old infants at high and low familial risk for Autism Spectrum Disorder

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ABSTRACT

Background: Several studies have reported action prediction difficulties in Autism Spectrum Disorder (ASD). Although action prediction develops in infancy, little is known about prediction abilities in infants at risk for 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 repeatedly presented with actions during which a familiar object (cup/phone) was either brought to a location usually associated with the object (cup-to-mouth/phone-to-ear; usual condition) or to an unusual location (cup-to-ear/phone-to-mouth; unusual condition). We assessed infants' anticipations to the actual target location (i.e., the location where the object was actually brought; the mouth in cup-to-mouth/phone-to-mouth actions; the ear in cup-to-ear/phone-to-ear actions) and the alternative target location (the ear in cup-to-mouth/phone-to-mouth actions; the mouth in cup-to-ear/phone-to-ear actions).

Results: Anticipation frequencies were modulated by object knowledge across all infants: We found more frequent anticipations towards the alternative target location for unusual compared to usual actions. This effect was in particular present for mouth anticipations which were also overall more frequent than ear anticipations. Across usual and unusual actions, infants showed more frequent anticipations towards the actual target location, potentially representing a learning effect elicited by the repeated action presentation. Importantly, there were no differences between the low- and high-risk infants in predictive eye movements.

Conclusion: Whereas our results suggest that familial risk for ASD does not affect action prediction in infancy, future research needs to investigate whether differences are apparent in those high-risk infants who later receive a diagnosis.

1. 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

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diverse deficits associated with ASD (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 (Brock, 2012; 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 differences in action prediction (Boria et al., 2009; Cattaneo et al., 2007; Hudson, Burnett, & Jellema, 2012; Schuwerk, Sodian, & Paulus, 2016; Senju et al., 2010; Vivanti, Trembath, & Dissanayake, 2014; Vivanti et al., 2011; Zalla, Labruyere, & Georgieff, 2006; Zalla, Labruyère, Clément, & Georgieff, 2010). Cattaneo et al. (2007), for instance, found that typically developing 5–9-year-old children show 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 (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), and is thought to reflect the mapping of observed actions onto own motor representations. This mapping is thought 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, Friston, & Frith, 2007; Prinz, 2006). A deficit in the MNS as proposed by several researchers is hypothesized to affect the mapping of observed behavior and may underlie the social deficits associated with ASD (Iacoboni & Dapretto, 2006; Oberman et al., 2005; Théoret et al., 2005; Williams et al., 2001; 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, Gredebäck, & von Hofsten, 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). This study revealed that children with ASD showed predictive eye movements that were similar to typically developing children, suggesting intact action prediction abilities. These findings are in contrast to other eye-tracking studies that do report differences (Schuwerk, Sodian, & Paulus, 2016; Senju et al., 2010; Vivanti et al., 2014; Vivanti et al., 2011). Schuwerk, Sodian, and Paulus (2016), for instance, assessed the influence of statistical learning on predictive gaze behavior in 10-year-old children 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. These findings suggest that prediction difficulties may arise when individuals are presented with more complex tasks where information needs to be integrated. Moreover, studies assessing action prediction tasks that require the interpretation of social cues or inference of mental states also report difficulties in individuals with ASD (Marsh, Pearson, Ropar & Hamilton, 2014; Senju et al., 2010; Vivanti et al., 2014). Vivanti et al. (2014), for instance, showed that predictive eye movements were influenced by the actor's gaze direction cues for control participants but not for individuals with AS. Marsh et al. (2014) investigated action prediction during the observation of rational and irrational actions, and 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. Interestingly, findings by Hudson et al. (2012) suggest that while showing typical performance during action prediction, the strategy that individuals with ASD apply may differ from controls.

Taken together, these studies provide a complex picture of intact and impaired action prediction abilities in individuals with ASD (see also Vivanti et al., 2011). Findings thus far suggest that anticipatory eye movements appear typical in children with ASD in the context of a simple goal directed actions (Falck-Ytter, 2010). However, difficulties arise when individuals are confronted with more complex actions (Schuwerk et al., 2016) and with tasks that require the interpretation of social cues or inference of mental states (Marsh et al., 2014; Senju et al., 2010; Vivanti et al., 2014).

Thus far, our knowledge about action prediction in ASD is limited to school-aged children and older individuals but 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 et al., 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 individuals have an increased risk of receiving a diagnosis themselves (ranging from 10 to 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, Gliga, Bedford, Charman, &

Johnson, 2014). These early differences can help to deepen our understanding of 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 for ASD 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. The infants' anticipations were also 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 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). The usual and unusual actions in our study were presented in separate blocks during which one action type was shown repeatedly. Such a block design allowed for the assessment of potential changes in the infants' predictions elicited by the repeated presentation of the usual or unusual actions. In contrast to Hunnius and Bekkering (2010), the lifting phase of the actions presented in our experiment was occluded, after familiarizing the infants once with the completely visible action. Several previous studies have used occluded stimuli to measure action prediction, showing that infants track movements behind occluders (Paulus et al., 2011; Stapel, Hunnius, Meyer, & Bekkering, 2016). The occlusion of our stimulus videos served two purposes: On the one hand, we aimed to reduce the distraction in the visual scene and thereby increase the infant's attention towards the action's (potential) target location (i.e. the mouth or the ear). If the movement of the lifting phase was visible to the infant, this could capture the infant's attention since movement is highly visually salient. With the occlusion of the lifting phase, however, no movement was visible during this phase, which we thought would lead to an increase of the infant's attention to the two target locations, resulting in a higher frequency of predictions. The second reason for occluding the lifting phase of our stimulus videos was that this enabled us to create a specific moment in time in each stimulus video during which the goal of the action is not yet clear, and thus any looks towards end locations during this time must be based on object knowledge. More specifically, during the occluded lifting phase the goal of the action remains ambiguous, but once the object reappears from behind the occluder, the goal is clear. This allows for an unambiguous selection of the time window of interest where a fixation to the goal area can be considered predictive.

For the usual and unusual actions, we 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. 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 also 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.

2. Methods

2.1. 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). The infants assessed for this study will be followed up at 24 and 36 months of age where an assessment of ASD symptoms and ASD classification will be made. At the time of writing, complete information on the diagnostic outcome of the sample was not yet available to the research team.

Table 1
Sample characteristics.

	N	Age	MSEL-ELC
HR	29 (14♀)	10.18 (.51)	92.66 (13.93)
LR	23 (9♀)	10.13 (.41)	96.57 (13.33)
Total	52 (23♀)	10.15 (.46)	94.38 (13.68)

We verified that the two infant groups were similar in age ($t(50) = -.44$, $p = .67$) and their developmental stage as measured by the Mullen Scales of Early Learning (MSEL) Composite Score ($t(50) = 1.02$, $p = .31$). The gender distribution between the two groups did also not differ significantly ($\chi 2(1, n = 52) = 0.44$, $p = .51$).

2.1.1. 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 healthy 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 by the local ethics committee and all parents gave written informed consent for participation prior to the testing. At the end of the testing day, families received monetary compensation for their participation as well as travel reimbursement and the infant received a small present.

2.2. Procedure

2.2.1. 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.

2.2.2. 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 everyday 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 (i.e. a short video clip showing a moving colorful animation, such as a wiggling rattle or dancing animals) 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. Full completion of the experiment took approximately 8 min. 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.

2.3. 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 Fig. 1) after familiarizing the infants once with the completely visible actions. Although the previous study by Hunnius and Bekkering (2010) used non-occluded videos, there are several recent eye tracking studies assessing action prediction in infants using occluded stimuli (Paulus et al., 2011; Stapel et al., 2016). These studies suggest that infants track presented movements behind occluders and predict the reappearance of observed actors or objects. As mentioned in the introduction, by occluding part of the action, we aimed to reduce the distraction in the

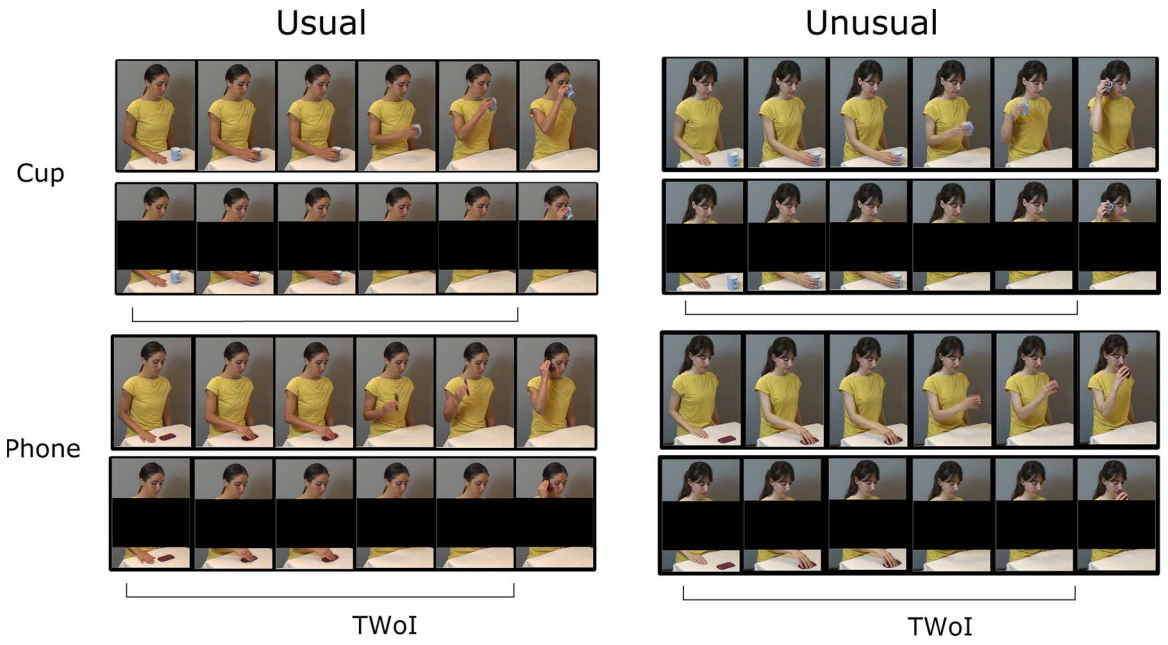

Fig. 1. Stimulus Material.
This figure shows four examples of the experimental stimuli presented during the experiment. At the start of each block a non-occluded video was presented in which a female actor was grasping either a cup (upper examples) or a phone (lower examples) and bringing this object either to a usual location (left examples) or to an unusual location (right examples). Consecutively, the infants were presented with a partly occluded version of the video for another 7 trials. The time window of interest (TWoI) that was analyzed in the main analysis is indicated by the black bracket underneath the video illustrations.

visual scene during the object-lifting phase and increase the infant's attention towards the (potential) target locations of the presented actions. In addition, occluding the movement path also allowed us to select the time window of interest where a fixation to the goal area is clearly predictive.

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). Besides the identity of the two actors, all other aspects of the visual scene in the videos were kept the same. 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 (618 × 395 pixels, about 43.89% of the whole videoimage), but the location shifted to best occlude the movement trajectory for each individual video. The average stimulus video durations ranged from 3.76 s to 4.60 s and did not differ between the two conditions (Usual: $M = 4.08$s, $SD = .22$s; *Unusual:* $M = 4.21$s, $SD = .25$s; $t(14) = -1.09$, $p = .29$). The elapsed time between the disappearance and reoccurrence of the object behind the occluder was variable across the different stimulus videos ($M = 429$ ms, $SD = 75.19$ ms) to ensure that infants could not predict the reappearance based on occlusion duration. Importantly though, the occlusion duration did not differ between the two conditions (Usual: $M = 404$ ms, $SD = 62.11$ ms; *Unusual:* $M = 454$ ms, $SD = 82.64$ ms; $t(14) = -1.37$, $p = .19$).

In each of the four experimental blocks, infants were presented with the same actor-object-location combination repeatedly. Blocks of the two conditions (usual and unusual actions) were presented in alternation. 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 Fig. 1). To mark the beginning of a new condition, blocks were separated with a visual attention-getter stimulus (i.e. a short video clip showing a moving colorful animation, such as a wiggling rattle or dancing animals) that was played in between two blocks. In addition, the two conditions were performed by two different actors for each infant in order to enhance the distinctiveness between usual and unusual action blocks. Thus, 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. The actors were counterbalanced 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.

2.4. Analysis of eye tracking data

For the main 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. An additional

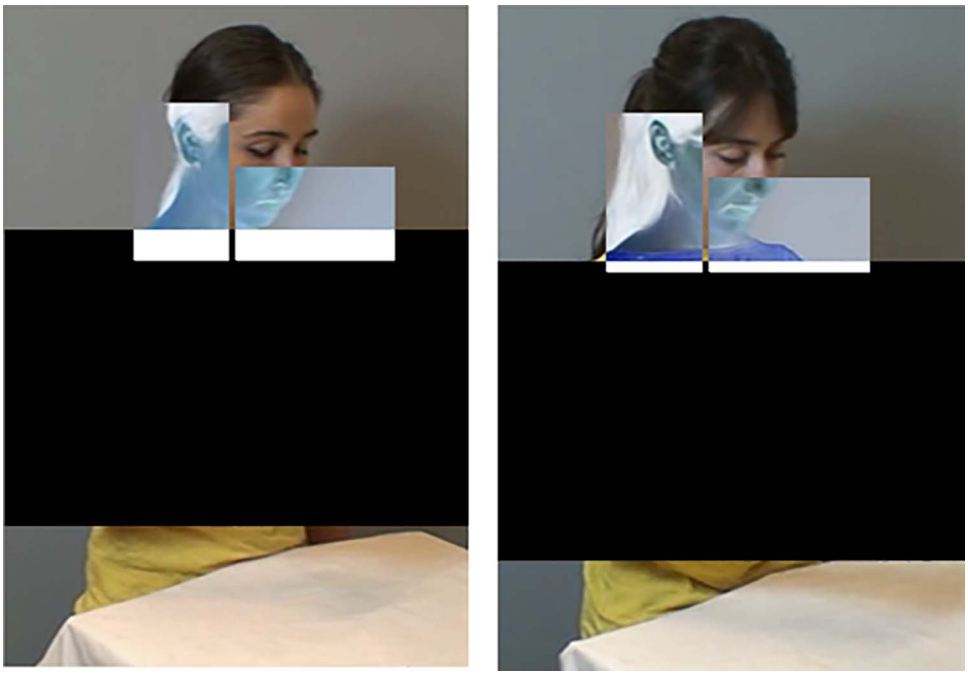

Fig. 2. Areas of Interest.
This figure shows two examples of the Areas of Interest (AoIs) used for the analysis of occluded trials. AoIs were the same size for both mouth and ear area and across the different stimulus videos.

detailed analysis of all four actions (cup-to-mouth, phone-to-ear, cup-to-ear, phone-to-mouth) confirmed the conclusions of the main analysis and can be found in the Supplementary materials (see Supplementary analysis 1). 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. Our block design in principle also allowed for an assessment of potential changes in the infants' predictions elicited by the repeated presentation of the usual or unusual actions. However, an analysis of separate trials for each action block was not possible due to insufficient numbers of valid trials. 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 (210 × 125 pixels, see Fig. 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 S1). For each video, there was one TWoI, which started 200 ms 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 40 ms (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 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).

For all performed parametric tests, we assessed the assumption of normality by means of a Shapiro-Wilk test. In cases where the assumption of normality was not met, additional non-parametric tests were performed to confirm the results obtained from the parametric tests. These instances are reported in the results section where applicable. To detect potential outliers, we assessed the

Table 2
Overview of the experimental conditions and corresponding definitions of actual and alternative target anticipations.

	Action	Actual Target	Alternative Target
Usual	Cup-to-Mouth	Mouth	Ear
	Phone-to-Ear	Ear	Mouth
Unusual	Cup-to-Ear	Ear	Mouth
	Phone-to-Mouth	Mouth	Ear

data based on the interquartile range (IQR, i.e. the range between the 25th (Q1) and the 75th (Q3) percentiles). The IQR was calculated by subtracting the value of Q1 from the value of Q3. Consequently, a lower and an upper boundary were defined based on 1.5 times the IQR (lower boundary: Q1 − 1.5*IQR; upper boundary Q3 + 1.5*IQR) and any value outside these boundaries was considered as an outlier (cf. Ghasemi & Zahediasl, 2012). Our data contained no outliers based on this criterion, except for one outlier in the Mullen ELC data. Specifically, one high-risk participant scored below the lower range of the ELC score. To ensure that the inclusion of this outlier did not influence our results, we ran the main analysis as well as the analysis of group differences in the Mullen ELC scores again, excluding this participant. These additional analyses (see Supplementary analysis 2) did not change any of the findings reported in the main manuscript.

2.4.1. Frequency of anticipatory looks

In a first step, we assessed whether a given trial was valid or not: A trial was considered valid if the infant looked at the screen during the time of the TWoI (i.e. before the object reappeared behind the occluder). Importantly, infants were not required to look at the target AoIs for a trial to be considered valid. A trial during which the infant was not looking at the screen at all (i.e. did not have at least one fixation to the screen) during the TWoI, however, was considered invalid and excluded. Infants were only included in the analysis if they contributed at least four valid trials per condition.

In a second step, we assessed per valid trial whether the infant showed a target anticipation. A fixation was considered a target anticipation if the infant looked at one of the two target AoI (i.e. the mouth or the ear) during the TWoI (i.e. before the object reappeared behind the occluder). A target anticipation was further classified as an *actual* or an *alternative* target anticipation (see also Table 2): If the infant anticipated to the target AoI 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 labeled 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 labeled an *alternative* target anticipation. In a last step, the number of trials that contained (one or more) actual and alternative target anticipations were counted and relative anticipation frequencies were determined per infant. It should be noted that a trial could be counted as containing both an actual and an alternative target anticipation if the infant fixated both the actual and the alternative target location during the time window of interest.

Our main analysis then focused on group and condition differences in the relative anticipation frequencies for actual and alternative targets. Specifically, differences in relative anticipation frequencies were analyzed using a 2 × 2 × 2 repeated measures ANOVA with Anticipation Location (actual target vs. alternative target) and Condition (usual vs. unusual) as within-group factors and Group (HR vs. LR) as between-subject factor.

2.4.2. 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.

2.4.3. 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 Fig. S1) 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 (80 × 100 pixels). The TWoI started 200 ms 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.

Table 3
Overview of the number of trials and total anticipations.

	Valid Trials		Total anticipations (actual + alternative)	
	Usual	Unusual	Usual	Unusual
HR	10.14 (3.10) [4–14]	9.45 (3.14) [4–14]	5.62 (3.09) [0–12]	5.66 (3.89) [0–14]
LR	11.04 (2.72) [4–14]	10.83 (2.53) [5–14]	6.87 (3.27) [0–13]	6.78 (3.93) [2–16]
Total	10.54 (2.95) [4–14]	10.06 (2.94) [4–14]	6.17 (3.20) [0–13]	6.15 (3.91) [0–16]

The number of valid trials that the infants contributed to the Usual and Unusual condition is shown in the middle column. The total number of anticipations – representing the number of actual and alternative target anticipations over all trials- is shown in the right column. The first presented value 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 ($t(51) = 1.21$, $p = .23$) or the total target anticipations ($t(51) = .04$, $p = .91$) between the Usual and Unusual condition across all infants. There were no group differences in the number of valid trials for the Usual condition ($t(50) = 1.10$, $p = .28$) or for the Unusual condition ($t(50) = 1.71$, $p = .09$) and there were also no group differences in the number of total anticipations for the Usual condition ($t(50) = 1.41$, $p = .16$) or for the Unusual condition ($t(50) = 1.03$, $p = .31$). As the Shapiro-Wilk test suggested that the distribution for the number of trials did deviate from normality for both conditions (Usual: $W = 0.901$, $p < 0.001$; Unusual: $W = .924$, $p = .003$), we performed additional non-parametric tests that confirmed the findings. A Mann-Whitney U test showed that there was no difference in the number of trials between the low- and high-risk infants for either condition (Usual: $W = 388.50$, $p = .311$: Unusual: $W = 418.5$, $p = .117$). A Wilcoxon Signed-Rank test further showed that there was no difference in number of trials between the Usual and Unusual condition across infants ($W = 564.0$, $p = .271$). HR = high-risk infants, LR = low-risk infants.

3. Results

3.1. Frequency of anticipatory looks

Table 3 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. Fig. 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 ($F(1,50) = 1.66$, $p = .20$) and no interaction effects involving Group (Group x Condition: $F(1,50) = .12$, $p = .73$; Group × Anticipation Location: $F(1,50) = .58$, $p = .45$; Group × Condition × Anticipation Location: $F(1,50) = .25$, $p = .62$). Frequencies of anticipations thus did not differ significantly between low- and high-risk infants.

The analysis, however, did reveal a significant main effect of Anticipation Location ($F(1,50) = 49.61$, $p < .01$, $\eta_p^2 = .50$). Uncorrected post-hoc paired sample t-tests revealed that for both conditions, infants looked more frequently at the actual target (Usual: $M = .40$, $SD = .22$; Unusual: $M = .37$, $SD = .22$) compared to the alternative target (Usual: $M = .17$, $SD = .15$, $t(51) = 5.92$, $p < .01$; Unusual: $M = .21$, $SD = .16$, $t(51) = 5.19$, $p < .01$). Importantly, our analysis also revealed a marginally significant Interaction effect between Condition and Anticipation Location ($F(1,50) = 3.07$, $p = .09$, $\eta_p^2 = .06$). Uncorrected 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 ($t(51) = -2.18$, $p = .03$). There was no difference between the two conditions in the frequency of looks towards the actual target location ($t(51) = .83$, $p = .41$). As the Shapiro-Wilk test suggested that the assumption of normality was not met for the alternative anticipation frequency for either condition (Usual: $W = .923$, $p = .003$; Unusual: $W = .942$, $p = .014$), we performed additional non-parametric tests that confirmed the findings. A Mann-Whitney U test showed that there was no difference in the alternative anticipation frequency between the low- and high-risk infants for either condition (Usual: $W = 357.00$, $p = .670$: Unusual: $W = 362.5$, $p = .599$). More importantly, a Wilcoxon Signed-Rank test showed a significant difference between the usual and unusual condition for the alternative anticipation frequency ($W = 375.5$, $p = .045$).

3.2. 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

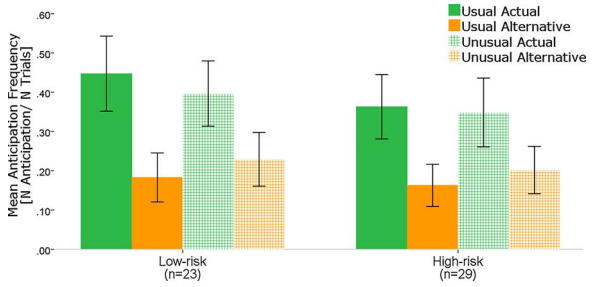

Fig. 3. Mean Anticipation Frequency.
This figure shows the average relative anticipation frequency to the actual and alternative target location for the Usual and Unusual action conditions separated for the low- and high-risk infants. Our results showed no significant effects of group on the anticipation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

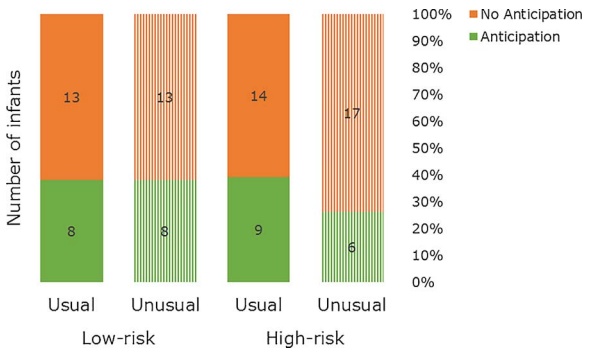

Fig. 4. Anticipations during the first trial.
This figure shows the number of infants that did and did not show an actual target anticipation during the first trial for the two condition and separated for the low- and high-risk infants. Our results showed no group differences in the distribution of anticipating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

measures ANOVA in JASP (Love et al., 2015) using the same factors as in the repeated measures ANOVA. 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 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 S2.

3.3. 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 ($t(42) = .11$, $p = .92$), MSEL ELC score ($t(42) = 1.09$, $p = .28$) or gender distribution ($\chi^2(1,n = 44) = .38$, $p = .54$) for this subset of infants. In addition, the number of valid first trials was not different between the two groups (Usual: $t(42) = .73$, $p = .47$; Unusual: $t(42) = .55$, $p = .59$). As the Shapiro-Wilk test suggested that the distribution of the number of trials did deviate from normality for both conditions (Usual: $W = .599$, $p < .001$; Unusual: $W = .519$, $p < .001$), we performed additional non-parametric tests that confirmed the findings. A Mann-Whitney U test showed that there was no difference in the number of trials between the low- and high-risk infants for either condition (Usual: $W = 267.00$, $p = .474$: Unusual: $W = 258.50$, $p = .593$). A Wilcoxon Signed-Rank test further showed that there was no difference in number of trials between the Usual and Unusual condition across infants ($W = 18.00$, $p = .145$).

Fig. 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 ($\chi^2(1,n = 44) = .01$, $p = .94$) or Unusual condition ($\chi^2(1,n = 44) = .73$, $p = .39$) 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 revealed that the distribution of anticipating and non-anticipating infants was not significantly different between the two conditions ($p = .58$).

4. 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; 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; Senju et al., 2010; Schuwerk et al., 2016; Vivanti et al., 2014; Vivanti et al., 2011), little is known about the early development of action prediction in ASD. The present study assessed whether 10-month-old infants at high risk for ASD show anticipatory eye movements during the observation of goal-directed actions. 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 also did not observe any 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 risk for ASD and low-risk controls.

Our results showed a significant main effect of anticipation location across all participants: Infants overall showed more frequent anticipations towards the actual goal location of the observed action. Additional analyses assessing mouth and ear anticipations separately for the four different actions, suggest that infants tended to look more often at the actual goal location for both actions directed at the mouth and at the ear (see Supplementary analysis 1). As actions were repeatedly presented within each experimental block, this general main effect of anticipation location might reflect a learning effect.

In line with previous work in typically developing infants (Hunnius & Bekkering, 2010), we also found 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 (e.g. looking at the mouth during a cup-to-ear action). Our additional analysis assessing mouth and ear anticipations separately for the four actions (see Supplementary Analysis 1), suggests that this effect was in particular present for the mouth anticipations whereas for ear anticipations the interaction effect was not significant. This finding could potentially be explained by an overall lower frequency of ear anticipations compared to mouth anticipations (see Supplementary analysis 1) which may reduce the sensitivity to find a relatively small interaction effect. Moreover, it might be the case that the association between cup and mouth is particularly strong in young infants whereas the associations between phone and ear might be weaker. Overall our findings are in line with the notion that by 10 months of age, infants have acquired knowledge about 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] Differences between the two studies 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 adapted to 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 et al. (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 they observed no differences in predictive eye movements. These findings are in line with the notion of Hunnius and Bekkering (2010) that object knowledge influences the processing of observed actions. The absence of an effect in the study by Stapel et al. (2010) may also be explained by 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 (η_p^2 = .06), the pattern of anticipation frequencies we observed was similar to Hunnius and Bekkering (2010) and we did observe a difference between the two 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 ASD 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 familial risk (see Supplementary Table S2). 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 (Cruys et al., 2014; Lawson et al., 2014; Pellicano & Burr, 2012; Sinha et al., 2014). In an eye tracking study, Schuwerk et al. (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. Noteworthy, some studies have reported group differences by 10 months, suggesting that atypicalities can already be detected at this young age. For instance, Elsabbagh et al. (2009) found slower attentional disengagement in 9–10-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, Palmer, Jacobi, Childress, & Arndt, 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 autism risk 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 within 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 and 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 3), 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. How these descriptive findings relate to eventual ASD outcome remains to be assessed in future work.

[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 in our experiment, whereas this difference was present for most stimuli in the previous study.

4.1. Limitations and future directions

While multiple recent eye-tracking studies have used partially-occluded video stimuli – similar to ours – to assess action prediction in young infants (Paulus et al., 2011; Stapel et al., 2016), the use of such stimuli, rather than a more naturalistic approach, possibly limits the generalizability of the findings to non-occluded stimuli. Although our results show that basic predictive eye-movements in 10-month-old high-risk infants do not differ from controls, using more complex and natural stimuli might provide additional insights into prediction abilities in young infants at high risk for ASD.

Another limitation of this study is that no information on diagnostic outcome of the cohort is currently available. Therefore, one must be cautious in interpreting our findings with respect to early prediction difficulties in ASD. Future assessments and diagnostic information will be required to investigate this further. Once outcome data is available, high-risk infants can be divided into groups of infants who receive an ASD diagnosis and infants who do not. We will then be able to investigate whether action prediction is typical or atypical in young infants that later receive an ASD diagnosis. Moreover, in addition to considering the effect of ASD diagnosis on action prediction, future studies could also assess how early action prediction is influenced by additional individual characteristics that have been linked to ASD outcome and severity in high-risk infants, such as gender or family affectedness (Ozonoff et al., 2011; Schwichtenberg, Young, Sigman, Hutman & Ozonoff, 2010).

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, but whether prediction difficulties are present in those high-risk infants that later receive an ASD diagnosis remains to be investigated in future work.

Authors contributions

R.B. contributed to all aspects of the experiment: design, data collection and data analyses. E.W. & R.S.H. contributed to the data collection. H.B., J.K.B. & S.H. contributed to the design. All authors (R.B., E.W., R.S.H., H.B., J.K.B. & S.H.) contributed to interpreting the results and to writing the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.rasd.2018.02.004.

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