Latest findings in autism research

How do they support the importance of early diagnosis and immediate intervention?

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Summary

It is increasingly recognised that early intervention starting during the first three years of life is critical for improving the outcome of children affected with autism spectrum disorders (ASD). Accurately diagnosing autism at a very early age remains however challenging. Here, we review recent research studies that support the hypothesis that a deficit in motivational aspect in orienting to people, and maintaining interactions with them, explains how autistic symptoms and cognitive difficulties develop progressively during early childhood. The social motivation theory represents a unified framework to help us diagnose children with autism earlier on, and to understand the mechanisms by which early intensive intervention helps in restoring their developmental trajectories. After reviewing the current state of eye-tracking and neuroimaging research in the field, we present the research strategies that we are currently deploying within the NCCR Synapsy (supported by the Swiss National Science Foundation) to advance our understanding of these critical issues.

Key words: autism; early diagnosis; eye-tracking; neuroimaging; social motivation

Introduction

Autism is a heterogeneous disorder characterised by a triad of symptoms including impairments in social interactions, delayed development of spoken language and repetitive patterns of behaviour. To satisfactorily account for the observed clinical heterogeneity, the term of autism spectrum disorders (ASD) is most commonly used, regrouping clinical manifestations that vary in severity along the continuum of autistic traits. Recent epidemiological records estimate the global prevalence of ASD to range from 1 on 68 [1] to 1 on 160 individuals [2].

Diagnostic criteria specify that autistic symptoms have to emerge prior to the age of three years [3]. However, autism is most often diagnosed later, at an age when therapeutic interventions are less effective [4]. In 2002, the median age of diagnosis of ASD in the United States was 5.7 years [5]; it decreased to 4.4 years in 2010 [1]. In Europe and Switzerland, such epidemiological data are less systematically recorded and the age at first diagnosis is difficult to estimate. One Swiss study however reported an average age at diagnosis of 6.1 years old at the Lausanne University Child and Adolescent Psychiatry Service, over the period from 1996 to 1999 [6]. This study showed that actively informing private practitioners can substantially lower the average age at first diagnosis, but unfortunately the benefit did not persist after the end of the programme. Although there is a definite trend for an earlier age of diagnosis in every country, a significant amount of children are still diagnosed after the period during which behavioural therapies have shown to be the most successful. The first three years of life may indeed represent a “window of opportunity”, when therapeutic interventions yield the most optimal long-term outcome [7–9]. In Switzerland, a few early intervention centres are being opened, and their efficacy is currently examined by the invalidity insurance. In Geneva, the “Centre d’Intervention Précoce en Autisme” (CIPA) opened in 2010, led by Hilary Wood. The CIPA offers intensive intervention following the Early Start Denver Model (ESDM [8]) for children, younger than 30 months. In sum, as such centres are being opened, clinicians are increasingly facing the challenging task to identify children with ASD as young as possible, to give these children the best chances for their future.

One of the main challenges of early diagnosis is that the current diagnostic criteria are not fully appropriate for children younger than 36 months [10, 11]. A large number of studies thus examined what are the most reliable “red flags” that put children at risk to develop typical autistic symptoms as they grow (see table 1 for a summary of these signs). The “red flags” were mostly observed in videotapes of the child recorded before diagnosis or through retrospective interviews of the parents [12, 13]. An alternative to identify the early signs of autism is to follow prospectively cohorts of children at high risk of developing autism. With a 19% risk to develop autism [14], siblings of an affected child represent the most studied population. Nowadays, structured questionnaires have been developed to screen for autism [15, 16]. The classical autism diagnostic tools such as the Autism Diagnostic Interview (ADI, [17]) and the Autism Diagnostic Observation Schedule (ADOS [18]) have also been adapted to offer specific algorithms and modules for toddlers aged 12 to 30 months [19, 20]. However, diagnosing autism at an early age remains difficult; novel techniques informed by the latest research, such as eye-tracking, may thus offer promises to help clinicians identify children at risk.
In addition to the fact that the behavioural symptoms may not fully emerge before the age of three years, another challenge of early diagnosis is the substantial heterogeneity of the disorder. The facets of heterogeneity in autism are multiple and complicate the development of interventions that are specifically suited to the needs of each individual child. First, clinical and cognitive manifestations vary largely, from individuals who are severely socially impaired and minimally verbal, to those presenting subtle difficulties in social interaction that are often accompanied by high-levels of intellectual social interest that are often accompanied by high-levels of intellectual achievements [21]. Second, the genetic heterogeneity is tremendous, with more than 100 different genetic defects identified to date [22]. Finally, the absence of strong and consistent neuroanatomical differences between patients with ASD and controls [23] suggests that the neurological mechanisms may substantially differ from one individual with autism to another (see also [24]). Despite this high variability, there are however common traits that support the rationale for regrouping ASD into a single entity.

Table 1  Early development between 12 and 24 months of age: which signs should alert for ASD? The “red flags” summarised here were identified from previous reviews [112–114]. The presence of several of these signs should motivate a specialised consultation with a psychologist or psychiatrist trained to diagnosed autism in young children as soon as possible, to avoid delaying the introduction of appropriate therapeutic interventions.

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The social motivation hypothesis of ASD

Over the last decade, behavioural, eye-tracking, and biological studies have converged to propose the hypothesis that disrupted social motivation plays a central role in autism (reviewed in [30]). The “social motivation theory of autism” posits that a lack of interest for social stimuli deprives the child of adequate experiences during the critical period spanning the first years of life, limiting opportunities for social and cognitive learning. Concomitantly, reduced exposure to relevant stimuli impairs the development of cerebral regions specialised in social information processing (the “social brain” [32]). This developmental cascade is schematically illustrated in figure 1.

Social orienting is a concept that has been largely examined in normative developmental psychology. From the first day of life, newborns preferentially orient to social stimuli, with innate attention to faces [33, 34]. As reviewed by Klin [35], looking at people, and specifically into the eyes, is one of the most important factor to sustain social adaptation throughout the life span (see also [36, 37]). From the first clinical descriptions, it was noted that children with autism don’t look into the eyes [38], a finding which is known to distinguish autism from mental retardation already by one year of age [39]. These alterations in social contact can be observed very early, as demonstrated by retrospective analysis of home-recorded videotapes: one-year-old children later diagnosed with autism already interact significantly less with others, and display fewer joint attention behaviours. The reduced social interest persists into adolescence and adulthood, as behavioural studies have reported that social interactions are less rewarding for adolescents with autism.
than for their typically developing peers [41]. As detailed in the next section, eye-tracking technology provides a unique opportunity to quantify social orienting, thus validating part of the social motivation hypothesis.

The social motivation hypothesis suggests that reduced attention to social cues subsequently impairs the experience-driven development of the cerebral regions responsible for social information processing by a cascading effect. The social brain [32, 42] comprises a network of widespread brain regions, each of which processes a different component of social information (see fig. 2). The superior temporal cortex is thought to be responsible for the processing of biological motion and for understanding the other’s intentions through the direction of their gaze [43, 44]. The fusiform gyrus and the amygdala process face and emotion [45, 46]. The inferior frontal and the inferior parietal lobule are part of the mirror neurons, responsible for action perception and imitation behaviours [47]. The temporo-
Figure 3

Example of eye-tracking set-up, with an adult participant performing an eye-tracking task. The video is displayed on a standard screen, and the participant doesn’t need to wear any specific or invasive material. The infra-red sensors that capture the reflection of the light on the cornea are located in the small device below the screen, allowing to precisely locate the focus of the participant’s gaze.

Eye-tracking refers to the technology that captures the location of the gaze focus, based on the reflection of infra-red light on the cornea and the pupil. The direction of the gaze can be recorded using portable glasses, measuring what the individual is looking at in everyday situations. However, most research studies to date have preferred the eye-tracking setting where the participant looks at pictures or movies displayed on a screen, for a more convenient and standardised approach (see fig. 3). Since Klin and colleagues proposed that eye-tracking provides an unequalled opportunity “to see the world through the eyes of an individual with autism” in their seminal paper [52], the technique has received an ever increasing interest in the field of autism. In this first study, the authors recorded the focus of the gaze of one high-functioning adult male with autism, and one IQ-, gender- and age-matched neurotypical adult while they watched clips of complex social interactions from the movie “Who’s Afraid of Virginia Woolf?”. They reported that the adult with autism focused less in the eyes of the actors, and was attempting to extract information on facial expression from the mouth rather than the eyes. Also, he relied less on nonverbal social cues to identify a target, and was thus less efficient at following the focus of the conversation. For the first time, this study allowed observers to reliably quantify phenomena that were previously observed only in clinical settings. The collected eye gaze data supported the idea that individuals with autism do not fully understand all the complexities of social interactions, because they are not looking at the correct cues. Thus, lot of questions arose from this first case report: is the visual preference similarly altered in all individuals with ASD? What are the mechanisms driving this atypical visual exploration? Do faces attract less the visual interest of individual with ASD? Is that context-dependent? Is there a more general alteration in interest for biological motion in autism? And finally and more crucially, is the lack of interest for salient social cues already apparent in very young children even before they even show the full expression of autistic symptoms? Over the last decade, a variety of eye-tracking studies have attempted to answer these questions; a selection of these studies is presented below.

Is the atypical gaze pattern when looking at social interactions similarly altered in all individuals with ASD?

Several studies using video clips of naturalistic social interactions have replicated the first case report’s findings by Klin and colleagues, suggesting that abnormal patterns of visual exploration within social scenes is a consistent hallmark of autism. First, the same authors confirmed their preliminary observations in a larger sample of 15 individuals with ASD and 15 controls [53]. They found that all individuals with autism spent less time looking at the eyes, but fixated more the mouth, the body, or objects in the scenes. Time spent looking at the mouth and objects were strong predictors of the degree of social competence, confirming that eye-tracking measurements represent an appropriate way to understand how individuals with autism extract meaning in complex social situations. Other studies with different video clips also consistently reported that children and adults with ASD look less at faces than neurotypical individuals in video clips that reproduce typical everyday situations. Nakano and colleagues observed that both children and adults with ASD look less at faces and saccade less between two speakers. They also observed that individuals with autism are easily distracted by objects moving in the background, such as curtains or floating letters added to the video [54]. Shic and collaborators showed that this pattern of looking less at faces and focusing more on the background of the scenes was already observed in 20-month-old toddlers with a diagnosis of autism [55]. All this evidence robustly points to the fact that, in a naturalistic scene where two or more persons interact together, individuals with ASD of all ages find the faces less salient than neurotypical individuals.

Because faces convey important information about emotional status and intentions of the person, not looking at faces may result in difficulties in understanding social interactions. The hypothesis that children with autism will not capture all the important information needed to understand the content of the social scene was further tested by Falk-Yititer and colleagues [56, 57]. In 2012 [56], they examined
whether children with autism were responding to joint attention behaviours by following the direction of another person’s gaze shift or pointing gesture. In typically developing toddlers, the ability to accurately follow referential gaze cues is established around one-year-old [58], and supposedly facilitates learning processes by helping the child to associate between the targeted object and the label used by the adult to name this object. Using an eye-tracking paradigm where an actress was looking specifically at one object among others, Flack-Ytter et al. observed that children with autism performed less correct gaze shifts in response to the gaze cues [56]. Importantly, the amount of correct gaze shifts correlated with adaptive communication scores measured with the Vineland, corroborating the hypothesis that not attending to the referential cues has a deleterious impact on the broader aspects of social and communication learning in children with ASD. In a more recent study, the same group used semi-naturalistic interactions between two children and a toy, with a video clip where the younger child asks the other one for a toy, using nonverbal gestures [57]. They observed that five-year-old children with autism failed to follow the non-verbal gesture and orient to the face of the child holding the toy, as typically developing (TD) children were doing. They speculate that looking away from the child who requested the toy, to fixate on the face of the child who is able to give the toy, is probably highly adaptive, as this child holding the toy is the only one who determines the next event in the interaction (i.e., whether he will give it or not). This study demonstrates that children with ASD diverge very early on from TD in how they read someone else’s intentions and anticipate the next step in the social interactions.

How do children with ASD look at the face of someone speaking to them?

The studies summarised above all contributed important knowledge about how individuals with ASD attend to social interactions that they are witnessing, but left unaddressed the question of what happens when they are part of the interaction. To examine this important question using eye-tracking, a few groups of researchers started using video clips in which the actor is speaking at and looking at the camera, simulating a direct interaction with the child. For instance, Jones and colleagues [59] recorded a video in which an actress looks at the camera, engaging the child to play games such as pat-a-cake or peek-a-boo. They showed that preferential looking to the eyes was already affected in two year old toddlers with autism, who looked more at the mouth. In contrast, typically developing toddlers, and toddlers with developmental delay but not autism showed preferential fixation to eyes. The authors discuss how the eyes are critical to extract information on the facial expression, and how decreased looking at the eyes already at very young age will affect the development of the child’s expertise about social cues and have further cascading effects on his socialisation. Another study by Chawarska and colleagues [60] also examined whether looking at the faces and eyes was dependent on the context of the scene in 13 to 25 month old toddlers. They presented the child with a video clip of an actress trying to engage the child with direct speech and visual contact, or making a sandwich without specifically looking at the child. They observed that, when the actress was not specifically looking at the child and speaking to him, there was no difference in how children with ASD explored the scene compared to controls. However, when the adult was trying to engage the child using directed speech and eye contact (“dyadic bid”), the toddlers with autism looked significantly less at her face than TD children. This study importantly conveyed the information that children with autism are not more attracted by objects than by people in a situation that doesn’t specifically elicit interaction, but that they fail to attend to engaging social stimuli in the same way neurotypical children do.

Is the visual exploration of static faces altered in ASD?

To further understand why individuals with ASD are consistently less attracted by faces engaged in social interactions, numerous eye-tracking studies used pictures of faces to quantify how these individuals explore them visually. Conversely to the dynamic video clips, which have high ecological validity, the use of static stimuli allows a more rigorous exploration of specific experimental paradigms. For instance, orienting to faces (or social orienting more generally) can be quantified using preferential looking when stimuli of different natures are presented simultaneously on the same screen [61, 62]. Sasson and colleagues [61] used a paradigm where 24 images were presented simultaneously on the screen; part of these images was social stimuli (i.e., pictures of faces or entire people) and part of them were objects. They measured whether children with ASD explored visually the set of images differently than the typically developing children. Children with ASD explored fewer images and had a longer fixation time per image compared to TD, which characterises a more detail-oriented exploration. Further, images such as vehicles or electronic equipment captured better the attention of ASD children than other objects such as plants, clothing or furniture. This result is not surprising, given that vehicles and electronic equipment are classically known to be of high interest in everyday situations for children with ASD. No difference in the patterns of exploration of social stimuli was however observed between children with ASD and TD in this study. In a subsequent study, the same authors limited to two the number of pictures presented simultaneously on the screen, with only one face and one object [62]. Based on the many consistent results using video clips of films, we could have expected that the children with ASD would be less drawn to look at faces when presented alone with an object on the same screen. However, they indeed had a similar visual attention to faces than TD, unless the competing object was part of their circumscribed interests. This similar attention to faces’ pictures also corroborated previous observations by McPartland and colleagues [62], who showed that children with ASD look as much at faces’ pictures than TD children, even though they have more difficulties to recognise them. There is however some evidence that individuals with autism are less attracted by faces displaying emotion than neurotypical individuals [64].
In sum, it thus appears that static faces, except when conveying emotion, are on average similarly salient for children with ASD and neurotypical children, but that the divergence in visual exploration emerge more prominently when using dynamic, naturalistic, stimuli. As a side note to this topic, it is of clinical relevance that children with ASD have relatively normal exploration of pictures of people and faces, given that the picture exchange communication systems (PECS) is often proposed to aid communication in children with autism [65]. Indeed, Gillespie-Smith et al. [66] observed that children with ASD attended the faces in the PECS cartoon similarly to neurotypical children. They concluded that children with autism paid attention to the pictures for a sufficient amount of time to encode the relevant information and use the PECS appropriately.

Does the type of motion influence the visual interest of individuals with ASD?

The repeated finding of mostly preserved visual exploration of faces’ pictures in children and adults with ASD largely contradicts the initial findings from Klin and colleagues [52, 53] that individuals with ASD look less at faces and persons. One of the hypotheses for the discrepancy is the static vs dynamic nature of the stimuli. A study by Von Hofsten and colleagues [67] provides interesting observations about how children with ASD pay attention to different types of dynamic motion. They compared how children with ASD and controls were looking at four different motions in video clips: a rolling ball with acceleration and deceleration, a rolling ball which was occluded from sight for part of its trajectory, a turn-taking conversation between two women and two objects alternatively moving and making sounds like in a turn-taking conversation. They showed that the two groups of children did not differ in the smooth pursuit of an object on the screen, nor in their ability to predict the timing of the reappearance of the temporarily occluded moving object. The children with ASD also looked at the turn-taking object motion in a similar way than TD. However, children with ASD showed a significantly different pattern of visual fixation in the conversation paradigm: they fixated less on the speakers and their gaze did not predict turns in conversation. This study elegantly demonstrates that it is exclusively the social nature of the dynamic stimuli that fails to capture the attention of children with ASD.

A variety of different paradigms have corroborated these results, pointing to a lack of orienting to social or biological motion in children and adults with ASD. For instance, a few studies have used point-light paradigms, where biological motion is reduced to its sparsest information. Only a few light points placed on the joints of a person or an animal walking or running in the dark [68] is usually sufficient to recognise the biological motion from the very first moments of life. Indeed, Bardí et al. [69] have shown that a typical two-day-old newborn already orients preferentially to biological motion using such a point-light paradigm. Conversely, it has been shown that children with ASD aged eight to ten years fail to orient preferentially to point-light human walkers [70]. In addition to be less drawn by the point-light human walkers compared to scrambled motion, children with autism also have more trouble recognising that the point-lights represent a person walking [71]. More recently, Klin and colleagues added some complexity to the point-light paradigm, using real actors performing children’s games (such as pat-a-cake) to create the point-light movies retaining the vocalisations of the actors [72]. On one side of the screen, the movie featuring the actor was presented together with the audio soundtrack, while on the other side of the screen the same movie was presented backward (disrupting the biological motion) and without its corresponding sound. Compared to typically developing children and children with developmental delay, two-year-old children with ASD failed to show a preference for the biological motion, exploring randomly both sides of the screen. Interestingly however, children with ASD oriented dramatically to audio-visual synchrony when it occurred: when the actor was clapping his hands in the clip, children with ASD looked systematically at the hands. More sophisticated analyses of the audiovisual synchrony in the entire recording showed that synchrony between the audio soundtrack and the visual motion explained 90% of the variance in the visual interest of children with autism. This high preference for audiovisual synchrony may explain why children with ASD look more at the mouth than at the eyes of someone speaking: in the absence of a preferential interest for the eyes, the synchronisation between lip motion and speech sound may be an important attractor to these children.

It thus seems that the difficulties to understand social cues in children with autism is rooted in a failure to orient to biological motion, even when modeled in its simplest form with point-lights. Paying attention to biological motion is in turn very important for all levels of social perception, in that we use the direction of other’s gaze, their facial expression, their hand and body gestures to interpret their intentions [73].

At which age do we see the first alterations in visual exploration in children with autism?

We have shown above how the different eye-tracking studies progressively dissected different levels of social cognition to quantify how individuals with autism fail to orient to social cues and to biological motion. These studies have shown that young children with ASD (starting from two years old for most of them) are on average equally affected as adults with ASD, supporting the theory that the lack of social and biological orienting is one of the primary deficits in autism (as stated in the social motivation hypothesis summarised in fig. 1). One of the crucial questions that emerges from this observation is whether lack of social orienting is already identified in children before they show the full behavioural symptoms of autism, so that eye-tracking could be used to guide very early diagnosis of autism.

To address this question, Pierce and colleagues [74] examined very young children with autism, from the age of 14 months old. At this age, the ADOS-2 [20] provides standardised scores delimiting a “range of concerns” for autism, and the children were prospectively followed until they reached the age of two years, when the diagnosis was...
confirmed. They designed an elegant paradigm to quantify social orienting, where the video of children moving (biological motion) was shown on one side of the screen, while geometric motion (screen-saver like motion) was displayed on the other side of the screen (see also the task displayed on the screen of figure 3 for our own adaptation of their paradigm). With this one-minute video, they observed that, as a group, the children with ASD spent significantly less time looking at biological motion (and more looking at dynamic geometric patterns). There was an important variance in the social orienting of children with ASD, but all toddlers who spent more than two-thirds of the time looking at geometric patterns belonged to the ASD group. These results suggest that a short and simple eye-tracking test to measure social orienting can have a remarkable specificity in screening very young children at risk for autism.

Other studies have examined the hypothesis that children at high risk to develop autism would already show altered gaze patterns in their first year of life. For instance Merin and colleagues [75] tested 31 younger siblings of children with autism, when they were 6 months old. They used a live eye-tracking paradigm using a close-circuit TV video system where the mother was first interacting with her child for 60 seconds, then presenting a still face for 60 more seconds, and finally interacting again. No difference between the at-risk and the comparison groups was observed in how the children reacted to the effect of the still face, both groups showed reduced smiling, gaze aversion and increased fussing or crying. In subsequent analyses, the authors used hierarchical cluster analyses to identify a group of children who looked less at the eyes of their mother compared to her mouth. Interestingly, 10 out of 11 of the children who looked less at the eyes belonged to the at-risk group, suggesting that part of the abnormal gaze pattern may start very early in autism. Unfortunately, the authors did not publish any information about diagnostic outcome after the age of 2 years, so that it remained difficult to disentangle whether this abnormal looking pattern corresponded to an endophenotype, i.e. a phenotype clustering in affected families along with genetic traits [76], or whether abnormal eye looking could predict the future onset of autism in these infants.

To overcome this limitation, a few research groups have recently started to publish studies in which they follow prospectively the sibling of a child with autism from the first months of life until the age at which diagnosis can reliably be made. Chawarska and colleagues [77] examined gaze patterns in six-month-old high risk infants, using the same paradigm that was detailed above, where an actress was either engaging the child in a dyadic bid by speaking to him, or making a sandwich without paying particular attention to the child. They observed that the infants later diagnosed with ASD looked less at the social scene than the controls; and when they looked at the scene, they spent less time looking at the actress’ face. These data provided the first confirmation that gaze patterns, as recorded with eye-tracking, can substantially differ long time before a reliable clinical diagnosis of ASD can be made. Two other groups used similar prospective designs to identify early signs of autism. Elsabaggh and colleagues [78, 79] performed two relatively standardised experiments in a cohort of children at risk for autism, for which eye-gaze data was recorded at 7 and 14 months old, and formal ASD diagnosis established at 2 and 3 years old. In their first paper [78], they examined how infants reorient their attention to peripheral, nonsocial stimuli. Their results showed that reduced flexibility in the control of visual attention starts to emerge only after 14 months old, which indeed suggests that the visual processing of non-social stimuli is not primarily affected in very young children that will develop autism later on. In their second paper [79], they used a variety of paradigms where an actress was directly looking at the child, moving in a relatively standardised way her eyes, her mouth, or her hands in front of the face. They found few differences between groups and concluded that scanning of faces do not begin as strikingly different in individuals who later on develop autism compared to typically developing toddlers. However, they observed that a trend for excessive mouth looking in the mouth moving condition predicted the later emergence of autistic symptoms. As discussed above for eye-tracking results in older children and adults with ASD, it may be the case that the visual exploration of highly standardised stimuli may not be as altered as more naturalistic stimuli, explaining why Elsabaggh and colleagues found less robust difference in infants who developed autism later on compared to the significant findings by Chawarska et al. Indeed, the most recent study by Jones and Klin [80] demonstrated that preferential looking into the eyes was already robustly altered from the age of 2 months of age in infants who will develop autism later on. With the overall goal to create growth charts of infant social engagement, they collected an impressive amount of data in 110 children. Each of them received an eye-tracking assessment for 10 times between the age of 2 and 24 months. Using video scene of naturalistic caregiver interaction, they showed that the infants who were later diagnosed with ASD showed a decline in preferential interest for eyes from 2 to 6 months of age, which was not observed in the infants who did not develop ASD. This outstanding observation represents the earliest known time at which trajectories of children with ASD start to diverge from typical development. The authors also argue that the initial preservation of the eye-looking mechanisms before the age of 2 months offers potentially promising avenues for looking for treatment, as it may be easier to build on a primarily intact social orientation compared to a congenital absence of it.

In sum, the most recent studies using naturalistic stimuli [77, 80] provide robust support for a first observation of decreased social orienting between 2 and 6 months old in individuals who will later on be diagnosed with autism. The simplicity of such eye-tracking assessment will probably soon make it possible to broadly screen infants at risk (e.g., siblings or infants with developmental delay), to offer extremely precocious identification of children who should receive intensive intervention. Finally, the conceptualisation that social orienting is the primary deficit in ASD, that cascades into more complex alterations as the child grows, may also prove the utility of eye-tracking measurement as a biomarker to predict or quantify outcome.
**Neuroimaging studies**

Brain imaging can be used to measure differences in brain anatomy, using structural MRI, or to examine cerebral activity, using functional MRI (fMRI) or electro-encephalography (EEG). Given that the acquisition of good quality MRI data requires the individual to remain still in the scanner for an extended period of time (ranging from 10 to 90 minutes), initial studies examined mainly high-functioning adolescents or adults with ASD. More recently however, Nordahl and colleagues [81] have proposed to scan young children during natural sleep without sedation, to better understand when the neurodevelopmental trajectories start to diverge in individuals with autism. They reported great success in these acquisitions from the age of 6 months [82] to 4.5 years old [81]. A summary of the main findings of anatomical differences and alterations in cerebral activity observed in individuals with autism is described below.

Structural brain imaging studies examining cerebral morphology in individuals with autism have provided somehow inconsistent results to date. One of the most replicated findings is the cerebral enlargement observed in children aged 2–4 years old [83, 84]. The study examining the largest sample to date (n = 586) [85] indeed observed that cerebral volumes are increased up to the age of 7, but decreased thereafter in older children, adolescents and adults with autism. This observation has led to the hypothesis of early brain overgrowth followed by arrest in development in autism [85]. Scanning high-risk infants during natural sleep from the age of six months, a recent prospective study reported that cerebral enlargement and increased cephalo-spinal quantity can already be observed in six-month-old infants who will later develop autism [82]. This observation of enlarged brain in infants provides robust evidence that the trajectories of neurodevelopment also deviate very early in children with autism, corroborating the eye-tracking results that the pattern of visual interest starts to differ between 2 and 6 months of age. However, heterogeneity in the clinical characteristics of individuals with autism may be an important factor affecting cerebral morphometric differences. Indeed, thanks to a larger sample than previous studies, Nordahl and colleagues have recently demonstrated that enlarged brain volume is not characteristic of all young children with autism, rather it is specific for those who present regressive autism onset [86]. Further, inconsistent findings across studies that include older children and adults with ASD may be driven by inter-individual heterogeneity. Indeed, whereas Courchesne et al. [85] reported reduced cerebral volume from the age of 7 years, Ecker and colleagues observed a mixture of increased and decreased gray matter volumes in a different sample of adults with ASD [87]. Similarly, meta-analyses summarising the observations of large number of studies indeed do not point to a consistent and robust pattern of anatomical changes associated with autism [23, 88, 89]. As suggested by Lenroot and Yeung [24], future studies will have to include large sample size and subdivide individuals with autism into subgroups to examine whether specific anatomical differences can be observed in more homogeneous samples to provide a more coherent account for changes in the structure of the brain in autism.

In contrast to the relatively inconsistent findings across all structural brain imaging studies to date, functional studies have brought us a much more coherent picture of altered activity in several brain regions specialised in social information processing (see [90] for a review). Some studies have used original paradigms to measure cerebral activity in tasks involving social meaning. For instance, Klin adapted the Social Attribution Task developed decades ago [91]: he showed to high functioning individuals with autism a video cartoon depicting simple animated geometric patterns such as triangles and circles moving as if they were displaying interactions [92]. He then asked all participants to describe these cartoons, and observed very different types of narratives for individuals with autism compared to neurotypical controls. Control participants generally attributed social meaning and personified the shapes, e.g., describing how the cartoon seemed to them like “kids playing together”. Conversely, individuals with autism described the cartoon mostly with technical terms, precisely referring to the shape of the triangles, and describing the motion with physical rules, e.g., stating that the shapes were “attracted by a magnetic field”. Despite the fact that the cartoon doesn’t display any explicit social content, this paradigm has been shown to activate all social brain regions in typically developing individuals [93], such as medial prefrontal regions, the temporal superior gyrus, the amygdala, and even the region of the fusiform gyrus that is known to be specialised in face processing. In individuals with autism however, those social brain regions were significantly less activated when watching the cartoon as compared to the controls [94], providing a biological correlate for the between-groups differences in information processing. Further studies using more explicit social stimuli also showed differences in the social brain networks in autism. For instance, reduced activation in the fusiform gyrus was observed in adolescents with autism who performed a facial discrimination task [95]. In the group of individuals with autism, activation of the fusiform gyrus was strongly correlated with time spent fixating the eyes, further strengthening the idea that not attending to the eyes will shape the cerebral circuits differently over time. Pelphrey et al. [96] examined cerebral activity in a paradigm of joint attention: they observed that the superior temporal sulcus of individuals with autism is not sensitive to the intentions conveyed by the gaze shift of another person. This failure to respond to gaze shift seems to occur very early in infants who will develop autism, as Elsabbagh et al. [97] demonstrated using EEG: cerebral response to eye-gaze shift at 6 to 10 months of age distinguished children who will develop ASD later from those who don’t. The authors confirmed using eye-tracking that this difference in brain activity was not driven by differences in the visual scanning during the task, thus confirming that cerebral response to similar social stimuli start to deviate very early on in autism.

All together, the studies summarised above provide compelling evidence that activation of the brain areas specialised in social information processing is altered very early on in autism. Attending differently to social cues from the very first months of life is very likely to shape differently the cerebral circuitry of individuals with autism over time (see e.g. [98]). Contrasting with the large body of literature...
pointing to social orienting as an initial and stable deficit in the pathogenesis of ASD, there is however a dearth of knowledge about the neural correlates responsible for reduced social orienting. One hypothesis is that the cerebral network responsible for allocating attentional resources to different kinds of relevant stimuli, the salience network, is altered in autism [99, 100]. The salience network involves mainly the anterior insula and the dorsal anterior cingulate cortex, and is tightly connected with subcortical affect and reward processing systems. From a meta-analysis of 24 functional neuroimaging studies [101], hypoactivation of the right anterior insula has been consistently evidenced in studies using different social cognition paradigms in autism, providing a common mechanism for less attention to social stimuli. An alternative explanation could be that the lack of social orienting is driven by more deep alterations in the reward system in individuals with autism. Indeed, a recent study observed decreased functional connectivity between the numerous nodes of the dopaminergic reward pathways and the voice-selective cortex, providing a mechanism by which the sound of the voice may be less enjoyable to children with autism [102]. Similarly, a recent optogenetic study in rodents [103] suggested that the initiation and maintenance of social interactions could indeed rely on a single pathway of the reward system, connecting the ventral tegmental area (VTA) and the nucleus accumbens. These recent studies all provide support for the hypothesis that lack of social orienting in individuals with autism may be rooted very deeply in the brain, so that social interactions are inherently less rewarding for individuals with autism compared to neurotypical individuals.

**Impacting the developmental cascade: opportunities for therapeutic interventions?**

If the lack of salience of social stimuli is encoded so deeply in the brain of individuals with autism, one of the most important challenges that future studies have to address is to understand whether social orienting and the rewarding aspect of social interactions can be modulated in affected individuals. One pharmacological approach that seems to have important potential for this purpose targets the oxytocin system [104]. Oxytocin, sometimes named the “hormone of love”, has been shown to play a key role in infant-mother attachment and in adult pair-bonding (see [105] for a review). Given its crucial role in attachment, oxytocin has received an increased interest in many psychiatric conditions [106]. Oxytocin also seems to play a crucial role in processing simple biological motion: administered to healthy adults, oxytocin improves their sensitivity to identify biological motion in a point light paradigm [107], and modulate their electrical brain activity while watching these stimuli [108]. In autism, administration of oxytocin has been shown to promote eye contact, and normalise cerebral activity in tasks of face processing [109] or paradigms of social vs non-social judgement [110]. These highly promising results demonstrate that the saliency of social stimuli can be increased by modulating the oxytocin system. Unfortunately, oxytocin penetrates poorly the blood brain barrier, and effects following the most commonly used intranasal application last only for about an hour. Research is thus being conducted to find alternative approaches to target the oxytocin system [104], with great potential to provide highly specific drugs that treat autistic disorders by increasing the saliency of social cues.

In the meantime, the most satisfying approach showing long-term beneficial outcome in autism is early intensive, non-pharmacological, intervention [4, 9]. It is currently unknown whether such approaches have an effect on social interest, but it is commonly accepted that early intensive interventions provides optimal experiences in a period of maximal plasticity to partially restore neurodevelopmental trajectories [4]. Indeed, a promising initial study by Dawson and colleagues showed that EEG patterns in response to face processing normalise after two years of intervention following the Early Start Denver Model, compared to children who receive unspecific treatment [111]. This important study suggests that early social intervention can help shaping the neural circuits responsible for social processing in children with autism. Unfortunately, the cross-sectional design of this study did not allow to measure potential developmental changes within each individual. Further, the authors did not collect any eye-tracking measures, so that we don’t know whether the observed cerebral normalisation was associated with normalised, or partly restored, social orienting. The latter point is of particular importance, given that if we had a way to reorient the child to socially relevant stimuli, then we could expect this child to gain every day exposure and experience that will generalise outside the treatment setting and continue shaping his maturing brain on the long term, even after the end of the intensive intervention programme. To the best of our knowledge, no study to date addressed this important question, measuring longitudinal changes in cerebral structure, activity and connectivity, alterations in social orienting and visual preference for faces, or the relationship between these variables in toddlers with autism receiving early intensive intervention. To address this gap in the field, we built a unique research project, which we briefly present in the next section.

**The autism project in the NCCR Synapsy**

The National Center of Competence in Research (NCCR) “SYNAPSY – The Synaptic Bases of Mental Diseases” (http://www.nccr-synapsy.ch) is an initiative funded by the Swiss National Science Foundation. The NCCR Synapsy started in 2010 and aims at enhancing collaborations between researchers around different psychiatric diseases. Groups of fundamental and clinical researchers located in Geneva, Lausanne and Basel started working together to further our understanding of the neural pathways that lead to early psychosis (K. Do, P. Conus, S. Clarke, M. Murray, O. Blanke), 22q11 deletion syndrome (S. Eliez, C. Michel, S. Antonarakis, P. Caroni, D. Muller, A. Carleton), developmental stress (F. Ansermet, D. Schechter, S. Clarke, M. Murray, C. Sandi, A. Dayer, J. Gräff, A. Holtmaat, C. Lüscher, A. Lüthi), mood disorders (J.-M. Aubry, M. Preisig, P. Marquet, A. Dayer, P. Magistretti, A. Volterra, P. Bezzi) and autism...
The goal of the project is to constitute a longitudinal cohort of young children with autism that receive varying degree of therapeutic interventions (i.e., treatments typically available in the community, or intensive intervention following the Early Start Denver Model). The toddlers with autism are recruited as soon as they receive their first diagnosis, in average around two years old. The same data are collected on a matched group of typically developing toddlers. As illustrated in fig. 4, all children are repeatedly examined at six months intervals using an extensive protocol that combines standardised behavioural and neuro-developmental assessments, high-density EEG, eye-tracking and epigenetics. We also expect to include structural and functional MRI in our protocol, for the older children who are able to stay still in the scanner. The broad scope of the project is to assess the trajectories of social, cognitive and cerebral development of children with ASD in their first years following diagnosis, and to understand how interventions can alter these trajectories. In particular, we aim at establishing developmental trajectories of the visual preference in children with ASD and typically developing children, to guide the development of tools for early diagnosis of autism. For that purpose, we designed our own social orienting paradigm where biological motion is presented on one side of the screen and geometric motion on the other side (see fig. 3, and fig. 5). As further detailed in fig. 5, we also designed a variety of eye-tracking paradigms to examine increased level of complexity in social information processing, including following attention, predicting intentions and actions of others, theory of mind, or attribution of prosocial intentions to shape. We specifically want to examine how early intensive intervention affect quantitatively

spectrum disorders (M. Schaefer, S. Eliez, C. Michel, T. Rihs, P. Scheiffele, P. Caroni, R. Schneggenger, C. Bellone, D. Muller). One unique aspect of the NCCR Synapsy is the emphasis on translational research, bringing researchers with highly different backgrounds together, where clinical cohorts and basic neuroscience work together to test specific hypotheses about the neural circuits involved in a disease. The clinical cohort of children with autism started recently within this fruitful environment.

Figure 4
Detailed protocol of the clinical autism project in the NCCR Synapsy. Young children are examined directly after they receive a diagnosis, usually around two years old. The figure describes the battery of assessments that is performed. Repeated assessments are performed every 6 months, with small variations from the initial examinations, to precisely quantify the progresses made by each child.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>+6 months</th>
<th>+1 year</th>
<th>+18 months</th>
<th>+2 years</th>
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<tr>
<td>All children receive some degree of therapeutic interventions</td>
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<td>Standardized clinical assessment of the child:</td>
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<td>- autistic symptoms and social communication (ADI, ADOS, ESCS)</td>
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<td>- developmental, cognitive level and language skills (PEP, WPPSI)</td>
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<td>- adaptive functioning (Vineland)</td>
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<td>- other symptoms (CECL, sensory profile)</td>
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<td>- home video</td>
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<td>Assessment of the family and environment:</td>
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<td>- cognitive level and socio-economic level of the parents</td>
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<td>- parental stress</td>
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<td>Detailed examination of medical history</td>
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<td>Eye-tracking</td>
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<td>High-resolution EEG</td>
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<td>Epigenetics</td>
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<tr>
<td>Measure of the intensity and type of therapeutic intervention</td>
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Figure 5
Illustration of the eye-tracking and EEG paradigms collected to quantify trajectories of social cognition and social brain development. We designed our own paradigms to measure different levels of complexity in social cognition, from social orienting, to simple social scenes to quantify following of joint attention, understanding of intentions and representation of other’s beliefs. In addition, we also use a paradigm developed by K. Hamlin [112] to measure the attributions of good and bad intentions. Finally, we record EEG during most of these eye-tracking tasks, to understand the neural basis of biological motion and social interactions processing.
Different levels of translational research in the NCCR autism project. For each of the aspects examined in the clinical cohorts, basic neuroscientists involved in the NCCR Synapsy are using corresponding read-outs in rodent models. Fundamental experiments are being conducted to assess social learning, social reward, and to precisely dissect the neural pathways involved in social cognition, and altered in mouse models of autism. Experimental manipulations of these pathways are also examined, with the ultimate goal develop of pharmacological compounds and to understand the effects of social enrichment in the cerebral circuitry of social information processing.

**Development of social cognition**
- Eye-tracking
- Evoked potentials
- Paradigms of social learning
- Assessment of social reward system

**Cortical structure, connectivity and maturation**
- Resting state EEG
- MRI & fMRI
- Synaptic morphology and function
- Alterations of cortico-cerebellar loops, rewards circuits and amygdalo-striatal network

**Behavioral & pharmacological interventions**
- Behavioral intervention therapy
- Preclinical pharmacological interventions and early social environment enrichment
- Set the stage for pharmacological interventions

**Epigenetics**
- DNA methylation

The mouse models are specifically used to identify precisely the neural circuitry involved in autistic symptoms, and use experimental paradigms to manipulate them. A description of how the projects using mice relate to the questions examined in the human project is provided in fig. 6. Specifically, behavioural assays to quantify social cognition and the affected neural pathways in rodent models are being developed (P. Caroni, C. Bellone, P. Scheiffele). Read-outs for delineating the neural correlates of a critical period for social enrichment in rodent models, as a paradigm that corresponds to early intensive intervention, will be explored (P. Caroni). Analysis of synaptic morphology and plasticity will be examined in mouse models of human neureligin-3 and Fmr1 mutation (D. Muller, P. Scheiffele). Finally, rodent models will be used to test the postulate that reward circuit is primordial for social orienting, dissecting neuronal circuits underlying social reward and social learning (C. Bellone, P. Caroni, R. Schneggenburger). On the long term, we further envision that the experience acquired by quantifying the effect of behavioural therapies in children with ASD will serve to prepare the tools for future clinical drug trials. Some candidate compounds that could be assessed in such trials are currently being assessed in the preclinical rodent studies (P. Scheiffele). In sum, the NCCR Synapsy is a unique opportunity to create a pole of excellent international neuroscience research in autism in Switzerland, with a deep exploration of the alterations in specific neural circuits but also examining questions that are highly relevant for the
do these children evolve during the first years following diagnosis?

References


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