Social visual engagement in infants and toddlers with autism: Early developmental transitions and a model of pathogenesis

Ami Klin, *Emory University*
Sarah Shultz, *Emory University*
Warren Jones, *Emory University*

Journal Title: Neuroscience and Biobehavioral Reviews
Volume: Volume 50
Publisher: Elsevier | 2015-03-01, Pages 189-203
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1016/j.neubiorev.2014.10.006
Permanent URL: https://pid.emory.edu/ark:/25593/tr36g

Final published version: http://dx.doi.org/10.1016/j.neubiorev.2014.10.006

HHS Public Access

Author manuscript

Neurosci Biobehav Rev. Author manuscript; available in PMC 2016 March 01.

Published in final edited form as:

Neurosci Biobehav Rev. 2015 March ; 0: 189–203. doi:10.1016/j.neubiorev.2014.10.006.

Social visual engagement in infants and toddlers with autism: Early developmental transitions and a model of pathogenesis

Ami Klin[*], **Sarah Shultz**, and **Warren Jones**

Marcus Autism Center, Children's Healthcare of Atlanta & Emory University School of Medicine, 1920 Briarcliff Rd NE, Atlanta, GA 30329, United States

Abstract

Efforts to determine and understand the causes of autism are currently hampered by a large disconnect between recent molecular genetics findings that are associated with the condition and the core behavioral symptoms that define the condition. In this perspective piece, we propose a systems biology framework to bridge that gap between genes and symptoms. The framework focuses on basic mechanisms of socialization that are highly-conserved in evolution and are early-emerging in development. By conceiving of these basic mechanisms of socialization as quantitative endophenotypes, we hope to connect genes and behavior in autism through integrative studies of neurodevelopmental, behavioral, and epigenetic changes. These changes both lead to and are led by the accomplishment of specific social adaptive tasks in a typical infant's life. However, based on recent research that indicates that infants later diagnosed with autism fail to accomplish at least some of these tasks, we suggest that a narrow developmental period, spanning critical transitions from reflexive, subcortically-controlled visual behavior to interactional, cortically-controlled *and social* visual behavior be prioritized for future study. Mapping epigenetic, neural, and behavioral changes that both drive and are driven by these early transitions may shed a bright light on the pathogenesis of autism.

Keywords

Autism; Autism spectrum disorders; Social visual engagement; Biological motion; Eye fixation; Infancy; Prodromal; Pathogenesis; Visual imprinting; Epigenetics

1. Introduction: From an array of lights and sounds to reciprocal social interaction, to hypotheses on autism pathogenesis

Were we to trace the evolutionary steps of a species whose brain specialization is shaped primarily by sociality – as is the case with primates (Ghazanfar and Santos, 2004) – the first hurdle would be to solve a pressing problem of initial conditions. Human neonates enter the world in a state of utter fragility. They are immersed in a complex array of lights and sounds and changing sensations. They will only survive and thrive through the intervention of another being—a caregiver. Given these initial conditions, what mechanisms would increase

[*]Corresponding author. +1 404 785 5762. ami.klin@emory.edu (A. Klin).

the odds of that neonate surviving? How, in that sea of sensations, would a newborn's perceptually-guided actions be successfully canalized toward the critical interactions with another being (the being who offers the greatest probability of helping that infant to continue)? Ample research already indicates that reciprocal social interaction becomes the platform for future development of social and communicative competence (Klin et al., 2003). This process, in turn, results in fast-paced and iterative social brain specialization (Johnson, 2001). But apart from cataloging certain physical features of the stimulus, we still know very little as to how that distal figure, the caregiver, initially attracts and then further maintains the attention of an infant. We know that interaction typically succeeds, beginning in the first hours and days of life, and that it then becomes an almost inexhaustible source of reinforcement in later weeks and months, leading the infant through what will be its period of greatest post-natal change in structure and function of brain and body. But beyond those descriptions of typical success, we know very little about the biological mechanisms that make those steps possible.

In no other human condition is this initial problem more pressing than in the case of autism. A highly prevalent neurode-velopmental condition of genetic origins, autism is characterized primarily by early-onset, lifelong, and potentially devastating disabilities in social and communicative function (Volkmar et al., 2004). Hundreds of genetic variants associated with autism have been identified, but none has yet accounted for any more than a very small percentage of cases (Geschwind, 2011). Linking specific genetic findings to causal mechanisms has been particularly challenging because autism is defined behaviorally, by a cluster of symptoms – impairments in social communication and restricted patterns of interests and behavior (APA, DSM-5, 2013) – which only become visible, and can therefore serve as the basis for conventional clinical diagnosis, toward the end of the second year of life (Chawarska et al., 2008). These symptoms are likely the complex and heterogeneous outcomes or end results of genetically-based disruptions of the child's ongoing development, and therefore cannot be mapped directly onto genetic processes (Meyer-Lindenberg and Weinberger, 2006; Jones and Klin, 2009). To leverage genetic discoveries in pursuit of causes of autism, we need successful systems biology approaches, leading the field from gene to protein to cellular function to neuronal circuitry to behavior impacted in pathogenesis (State and Sestan, 2012). Clearly, tractable model systems are crucial to the study of autism, but creation of appropriate systems has, to date, been modest (Crawley, 2007). Once a system is developed, one of the greatest challenges in this field is the development of behavioral assays that hold adaptive relevance (as evolutionary adaptations) to the human syndrome (or to a mediating phenotype; Gould and Gottesman, 2006) and to the species used as a model system (Lederhendler and Schulkin, 2000; Moy et al, 2006). Here, we argue that the gene-symptom gap may be narrowed through a focus on adaptive skills that (1) are known to be more proximal to genetic expression (and thus relatively less complicated by later learning and compensatory mechanisms); (2) are central to the expression of the syndrome (and thus relate directly to the development of social interaction and communication); and (3) are equally relevant to species-specific adaptive survival in model systems that may be used to elucidate molecular mechanisms (Insel and Fernald, 2004; Jones and Klin, 2009). Such adaptive skills are likely to be the solutions to our initial

problem: how infant and caregiver action become successfully connected to form adaptive, reinforcement-driven interaction.

In this perspective piece, we describe two important means to this social-adaptive end. As such, each is a basic mechanism of social engagement. Each one is also a mechanism of social *visual* engagement: preferential attention to biological motion (the movements of vertebrate animals), and preferential attention to others' eyes. Both are evident in the earliest expression of behavior in human infants and in infants of several other species whose survival depends upon the care of an adult conspecific. Of course, neither of these is the only such means to social engagement (in visual or non-visual domains), but new research indicates that both of these mechanisms are compromised in infants and toddlers with autism. They are not a cause of autism, rather they are signs of the unfolding of autism: evidence of the derailment of typical development that almost assuredly adds to the ongoing disruption of subsequent social and communicative growth. However promising these findings may be, they are currently descriptive in nature. Insights into their biology await lessons from model systems that can generate hypotheses about gene–brain–behavior relationships. Nature is parsimonious in its solutions (Thompson, 1942). Whether we see the same functions appear via evolutionary conservation (a solution inherited from a common ancestor), or via convergent evolution (a solution evolving independently in different species as a result of similar selective pressures), there may be lessons to learn from well-studied model systems.

This issue of *Neuroscience & Biobehavioral Reviews* is celebrating the work of Gabriel Horn. Over several decades of advances in research of visual imprinting in the domestic chick (Horn, 2004), Horn and colleagues painstakingly documented a cascade of genetic, epigenetic, cellular, and brain transformations resulting from this event—the preferential movement toward and subsequent recognition of the mother hen. Interestingly, young chicks and human infants share a very similar adaptive task (the same that formed the basis of our initial value problem): both species need to detect and orient to the caregiver, and both species need to learn the characteristics of the caregiver, as the caregiver becomes the anchor of infants' experiences and guarantees their survival. In the chick, visual recognition of the natural parent happens within hours from hatching (Horn, 2004). In the human infant, visual recognition of mother is well established by three months of age (Mash et al., 2013). Many studies have drawn parallels between chicks and human infants in the evolution of social orienting (e.g., Hoffman and Ratner, 1973; Horn and Johnson, 1989; Rosa Salva et al, 2011). This model system suggests a hypothesis to explain a new finding from our laboratory (Jones and Klin, 2013) by focusing on a narrow set of early neurodevelopmental transitions that infants later diagnosed with autism fail to make. Elucidating the biological bases of these transitions could shed a bright light on the pathogenesis of autism.

2. Social orienting in autism spectrum disorders (ASD), Part 1: Perception of biological motion

For human infants, engagement with the caregiver is the initial task upon which survival depends. Given their fragility at birth, success in this task is of immediate survival value and is of fundamental evolutionary significance. A central skill facilitating this adaptive task is

preferential attention to biological motion—a form of perceptual “life detector” (Troje and Westhoff, 2006). Biological motion refers to the movement of vertebrate species; in humans, it corresponds to actions that range from gait and bodily gestures, to facial expressions and change in gaze direction. Special sensitivity and preferential orientation to forms of biological motion are widely present across species – from humans (Johansson, 1973; Fox and McDaniel, 1982) to monkeys (Oram and Perrett, 1996) to cats (Blake, 1993) to birds (Omori and Watanabe, 1996) – and are developmentally very early-emerging. Signs can be found in newly-hatched chicks (Vallortigara et al, 2005) and in human infants as young as 2 days of age (Simion et al., 2008). These abilities are believed to be critical for filial attachment and for detection of predators in many species (Johnson, 2006). In addition, in humans, this ability has been postulated to be the forerunner of the capacity for attributing intentions to others, a cardinal social cognitive skill (Frith and Frith, 1999).

The evidence pointing to the key role of biological motion perception in social brain networks is impressive:

- The neural underpinnings of biological motion perception are overlapping with brain regions involved in perception of basic social signals such as facial expression and gaze direction (Pelphrey et al., 2005).
- Biological motion perceived through other sensory modalities – as when listening to sounds of human action (Bidet-Caulet et al., 2005) – evokes activity in the same areas of the brain that are typically responsive to visual presentations.
- Perception of biological motion remains intact in a variety of forms, from degraded presentations, through varying states of occlusion, and in cases when information-bearing components are reduced to their most minimal (Thompson et al., 2005; Neri et al., 1998).
- Perception of biological motion can be preserved even when other types of motion perception are impaired, as in individuals with Williams syndrome (Jordan et al., 2002) (a condition noted for severe visuo-spatial deficits) and in patients suffering from circumscribed brain lesions (Jokish et al, 2005).

Collectively, this evidence describes a mechanism that is evolutionarily well-conserved; emerges early in development; is highly robust in signal-detection (withstanding degradation on signaling and receiving sides); and is redundantly represented via multiple sensory modalities, at least later in life. Each of these aspects suggests ready benefits for adaptive interaction with other living beings: following the movements of a conspecific, looking at others to entreat or avoid interaction, learning by imitation, or directing preferential attention to cues that build on biological motion (such as facial expression and gaze direction).

Strikingly, reductions in many of those same behaviors are hallmark symptoms in children with autism spectrum disorders (ASD): deficits in social interaction, diminished eye contact and reduced looking at others, problems with imitation, deficits in recognizing facial expressions, difficulties following another's gaze, and limited ability to attribute intentions to others (Volkmar et al., 2004). Given the importance of perception of biological motion to social adaptive behavior, our group began to examine this construct in young children with

autism. Using a classic method to measure visual perception of biological motion in which the movement of people, or other vertebrates, are rendered as point-light animations (Johansson, 1973), we presented 2-year-olds with ASD and typically developing (TD) and non-autistic but developmentally delayed (DD) controls a series of such animations consisting of caregivers' approaches enacting children's games, such as “peek-a-boo” or “pat-a-cake,” which were created with live actors and motion capture technology (Klin et al., 2009). These animations included simultaneous audio recording. The experimental task was a preferential looking paradigm (Fig. 1A) a point-light animation was presented on one half of a computer screen together with the audio soundtrack of the actor's vocalizations. On the other half of the screen, the same animation was presented, but that point-light figure was inverted in orientation (shown upside down) and played in reverse order. Only the one (forward) audio soundtrack was presented. Inverted presentation disrupts perception of biological motion in young children (Pavlova and Sokolov, 2000) and is processed by different neural circuits in infants as young as eight months of age (Reid et al., 2006). Evidence for recognition and preferential attention to biological motion was measured by the child's viewing patterns: increased looking toward the upright figure indicated preferential attention to biological motion (Simion et al., 2008) and the perceptual matching of human voice with a mental template of human action (Klin et al, 2003). The children's visual scanning was measured with eye-tracking equipment (Fig. 1B–D).

The results of the experiment (Fig. 1E–G) were surprising on several counts. First, two-year-olds with ASD showed no preferential attention for human biological motion; instead, their levels of looking at point-light displays of biological motion were equivalent to those expected by chance (Fig. 1E). In contrast, both control groups (even those with significant cognitive delays) preferentially attended the upright displays of human biological motion (Fig. 1F and G). Second, a serendipitous observation in an earlier study (Klin and Jones, 2008) had revealed that one of our point-light animations contained a confound. While the others presented only moving point-lights with accompanying human voice, one animation – “pat-a-cake” – included a different sound: the sound of clapping is heard at the same time that two point-lights, representing the actor's hands, collide. The collision of point-lights and the resulting clapping sound created a *causal* physical contingency: rather than merely co-occurring (as with speech sounds and movements in the other animations), the movements of the point-light hands in this case actually *caused* a noise to occur. During the clapping, the causal physical contingency only exists on the upright side: the single audio track plays normally (forward), matching the upright movements, but the action of the inverted figure, playing in reverse, does not move in time to the clapping sounds. In contrast to the chance levels of preferential looking exhibited in all of the other animations (Fig. 1E), the toddlers with ASD displayed a strong and significant preference for the upright figure during the “pat-a-cake” animation (Fig. 2A). These results suggested that the toddlers with ASD had failed to recognize and preferentially orient toward point-light displays of social action but were acutely sensitive to the presence of physical contingencies in the same stimuli. We pursued this observation by quantifying the amounts of audiovisual synchrony contained in all of our animations in order to examine whether more subtle synchronies might have played a role in the children's viewing of animations. The results of these new analyses supported this hypothesis (Fig. 3A–D). When viewing point-light animations of human

action, 90% of the variance in their preferential attention of 2-year-olds with ASD could be directly accounted for by the presence of non-social, physical contingencies (i.e., the audiovisual synchronies) (Fig. 3A); these synchronies, however, were unrelated to the visual scanning of the control groups (Fig. 3B and C). Finally, these findings were then replicated and extended in a subsequent experiment with a new cohort of toddlers with ASD (Fig. 3D).

In summary, toddlers with ASD fail to recognize and preferentially orient toward point-light displays of biological motion and, are instead, highly sensitive to the presence of non-social, physical contingencies. Thus toddlers with ASD fail to demonstrate a skill that is already present in very young human infants (Fox and McDaniel, 1982) and even newborns (Simion et al., 2008), and is highly conserved across various species, including newly hatched chicks (Regolin et al., 2000). This finding implicates the disruption of an evolutionarily highly conserved and developmentally early-emerging social adaptive skill in the pathogenesis of autism, suggesting early onset, possibly in the newborn period. While the literature on biological motion perception in autism or its impact on social visual engagement is not entirely consistent (e.g., Falk-Ytter et al., 2013; Shic et al., 2014), the persistence of biological motion perception abnormalities in the later lives of individuals with ASD has been shown extensively via behavioral, electrophysiologic and functional MRI studies (Castelli et al., 2002; Blake et al., 2003; Redcay, 2008; Pelphrey and Carter, 2008; Kaiser et al., 2010; Kröger et al, 2014).

3. Social orienting in autism spectrum disorders (ASD), Part 2: Looking at others'eyes

Human infants are drawn to the eyes of others from the first days and weeks of life. Four-day-old newborns distinguish between a face looking toward them and a face looking away (Farroni et al., 2002), and by three months, human infants look more at a person's eyes than at other parts of the face, and more at a person's face than elsewhere on the body (Haith et al, 1977). Similar findings have been reported in non-human primates via both experimental and ethological observations (Emery, 2000): mother–infant chimpanzees (*Pan troglodytes*) engage in mutual gaze frequently, and social engagement appears to be interchangeable across the tactile and visual modalities. As the dyads display more mutual gaze, the caregiver cradles the infant less (Bard et al., 2005). Infant chimpanzees prefer to look at a face with eyes open than closed (Myowa-Yamakoshi et al., 2003), and mutual gaze is commonly observed in mother-infant dyads in their natural ecosystem (de Waal, 2003). In both humans and non-human primates, the importance of mutual gaze for early social development (Brooks and Meltzoff, 2002; Emery, 2000) and for social adaptation throughout the lifespan (Kampe et al., 2001; Emery, 2000) underscores the critical role it plays in socialization. In fact, even the external morphology of the eye itself may reflect evolutionary selection for adaptive social function: among all primates, humans have the largest ratio of exposed sclera in the eye outline, apparently as an adaptation to enhance the gaze signal (Kobayashi and Kohshima, 1997). Furthermore, although eye looking appears to be primarily the province of primates (Emery, 2000), other mammals, such as dogs, orient to faces (Miklóski et al., 2003), and preferential attention to face-like stimuli has been shown

in both human newborns and newly-hatched chicks using identical stimuli (Rosa Salva et al., 2011).

Like biological motion perception, the evidence pointing to the key role of eye fixation in human social brain networks is extensive:

- Cortical processing of faces in infants as young as four months is enhanced when faces are gazing at the infant, rather than away (Farroni et al, 2004), and face recognition in these infants is in fact modulated by direct gaze (Farroni et al, 2007).
- Eye gaze modulates emotional arousal via amygdala activation to emotion expressions in faces (Sato et al., 2010).
- Eye gaze modulates brain activity associated with social reward brain circuitry, increasing activation in the ventral striatum with eye contact, and decreasing activation when eye gaze is directed away (Kampe et al, 2001).
- Direct eye gaze rapidly and specifically enhances spontaneous mimicry of other people's actions – an action that enhances affiliation and social engagement – via shared activation of the medial prefrontal cortex, superior temporal sulcus and inferior frontal gyrus (Wang et al, 2011).

In ASD, deficits in eye gaze are a defining feature of the condition (APA, DSM-5, 2013) and a key item in standardized diagnostic tests (Lord et al., 2000). These deficits have been extensively demonstrated in behavioral studies (e.g., Klin et al., 2002a,b); in electrophysiologic reports (e.g., Elsabbagh et al., 2009, 2012) including intracranial recordings (Rutishauser et al., 2013); and also in functional MRI studies (e.g., Dalton et al., 2005; Kliemann et al., 2012; Davies et al., 2011).

The conserved nature, early onset, and critical role of eye fixation in socialization prompted our group to examine preferential looking to the eyes of approaching adults in infants and toddlers with autism spectrum disorders (ASD). In an early study (Jones et al., 2008), we presented 2-year-old children with videos showing an actress looking directly into the camera, playing the role of caregiver, and engaging the viewer in typical infant-directed interaction games such as “pat-a-cake” and “peek-a-boo” while the children's visual fixation patterns were measured by eye tracking. There were three groups: toddlers with ASD (ASD), typically developing (TD) controls, and non-autistic but developmentally delayed (DD) controls. Children with ASD exhibited significantly less eye fixation relative to the two other groups: median eye fixation was in fact less than half that of TD and DD children. Two additional observations added importance to this finding. First, eye fixation in the toddlers with ASD was significantly correlated with their level of social disability (as measured via standardized clinical instruments), thus imbuing this behavioral assay with clinical validity. Second, toddlers with ASD also displayed significantly increased mouth-fixation relative to controls. In light of our findings in studies of preferential orientation to biological motion – in which visual behavior of toddlers with ASD appeared to be guided primarily by audiovisual synchrony rather than the social nature of the point-light animations – we raised the hypothesis that their mouth-fixation resulted from their engaging with the video stimuli as a composite of physical characteristics, without social meaning,

given that the mouth is the locus of greatest audiovisual synchrony in speaking faces (i.e., speech sounds and lip movements covary; Klin et al, 2009).

Once again, toddlers with ASD failed to display a pattern of preferential attention that is otherwise both early-emerging and evolutionarily-conserved. Together with our findings in studies of biological motion, our results supported the contention made in the very first description of autism by Kanner (1943), who characterized “autistic disturbances of affective contact” as “congenital”. Our group and others have long advocated the notion that disruptions of typical, extremely early-emerging mechanisms of adaptive social action give rise to the social disability known as autism (Klin, 1989, 1991; Klin et al., 2003; Dawson et al., 1998, 2004; Schultz, 2005). However, this hypothesis is still only supported by indirect evidence since knowledge of the first two years of life of children with autism is quite limited (though growing), primarily because children with autism are diagnosed between two and three years of life at the earliest (Chawarska et al., 2008). Although several studies show atypical neural processing of social stimuli in infants at risk for autism, or in infants who were subsequently diagnosed with autism (e.g., Luyster et al, 2011; Elsabbagh et al., 2012; Lloyd-Fox et al, 2013), direct observation and quantification of the early developmental progression of autism is still scant (Rogers, 2009), including possible disruptions in early-emerging social adaptive behaviors. This gap in clinical and research knowledge is a critical one. The first two years of a baby's life encompass the most substantial and rapid period of neural and behavioral growth in postnatal human development (Johnson, 2001). For a condition as strongly heritable as autism (Gupta and State, 2007), and for one in which multifactorial genetic etiologies are likely to begin their impact on development from birth if not before (Volkmar and Pauls, 2003), a thorough mapping – of social behavior, brain changes, and gene processes – in the first two years of life is a critical step for understanding the pathogenesis of the condition and constraining gene–brain–behavior hypotheses (DiCicco-Bloom et al., 2006).

There are several alternatives to a congenital model of autism pathogenesis. And by “congenital”, we mean to characterize a behaviorally defined condition for which there are specific, visible and measurable behavioral abnormalities by the time of birth or soon thereafter; not the obvious observation that because of the genetic nature of its etiology, the biological vulnerabilities leading to the behavioral syndrome are, naturally, already present at birth. In this sense, one non-congenital model originates from the observation that a large proportion of children with autism appear to undergo regression in development during the second year of life, particularly in the language domain, *after* a period of entirely typical development (Lord et al., 2004; Parr et al., 2011; Barger et al., 2013). It is not yet clear whether a true regression of previously acquired language skills occurs, or, alternatively, whether a failure of early vocalizations to progress into full-blown communicative vocalizations is a better explanation for this phenomenon (as initial vocalizations might fade away in the absence of a child's intent to communicate; Klin et al., 2008). Also, the phenomenon of regression, to date studied primarily via *retrospective* studies, has not been documented in *prospective* studies (Rogers, 2009). A second competing non-congenital model of pathogenesis focuses on hallmark deficits in older children with autism, particularly joint attention (i.e., sharing a focus of attention with another person; e.g., Mundy

and Neal, 2000) and Theory of Mind (i.e., attributing mental states to others; e.g., Frith and Frith, 1999), skills that emerge in the second to fourth year of life in typically developing children. Here, too it is unclear whether these deficits are the culmination, rather than the genesis, of social dysfunction in these children (Klin et al., 2003).

This state of affairs was the impetus for our most recent study of eye fixation in ASD (Jones and Klin, 2013). Infants who were later diagnosed with ASD and typically developing infants (TD) were shown pre-recorded video scenes of actresses playing the role of caregivers while engaging their children in infancy games. Like in our previous study (Jones et al, 2008), the children's visual scanning was measured by eye tracking. Data were collected monthly, from two to six months of age, and then every three months until the age of 18 months, with a final data point at 24 months (10 time points overall). Ascertainment of diagnostic status and its stability happened at 24 and 36 months, respectively. Eye-fixation data for the typical children delineated “growth charts” of social visual engagement (Fig. 4A) against which we compared the data for the infants later diagnosed with autism (Fig. 4B). TD children, from two to six months, looked more at the eyes than at any other region of the screen (mouth, body, objects); eye fixation increased steadily during this period and remained rather stable until the age of 24 months.

Given our hypothesis that children with ASD have a congenital deficit impairing their ability to preferentially orient to others' eyes, our expectation was that their levels of eye fixation would be reduced relative to those of typically developing infants from the earliest time of data collection (Fig. 4C). Our results falsified this hypothesis (Fig. 4D and E): eye fixation began at a level similar to TD children but then declined steadily from the two-month starting point, arriving at a level that was approximately half that of TD children by the 24-month endpoint. This decline in eye fixation was already underway within the first 6 months. Two additional observations added significance to this finding. First, the decline in eye fixation within the first six months alone was strongly and significantly associated with diagnostic outcome at the age of 36 months. Thus developmental differences in level of preferential attention to the eyes of other people was a strong marker of later diagnosis 1 and 1/2 years before the children could be diagnosed conventionally and 2 and 1/2 years before they would be diagnosed stably (Jones and Klin, 2013). Second, in the children with ASD, the degree of decline in eye looking was a strong predictor of level of social disability at outcome (as measured with standardized clinical instruments): children whose levels of eye looking declined most rapidly were also most socially disabled in later life.

These findings represent the earliest indicators of atypical social development in children with ASD, with mean decline in eye fixation evident already between two and six months of age. However, our results also showed, despite that decline, that eye looking appears to be present within the normative range in those early months, contrary to our expectation of a reduction in preferential attention the eyes that we expected would be present from birth. Instead, at two months of age, the cross-sectional data suggested eye fixation values of approximately 60% to 30% in ASD to TD looking—meaning infants later diagnosed with ASD looked more at the eyes than TD infants, not less. This difference was reduced, but still present, when the data were analyzed longitudinally (Fig. 4E), with a difference of approximately 50% to 40% (the change in estimates of eye fixation from cross-sectional to

longitudinal analyses reflects the fact that longitudinal estimates weigh the two-month data in relation to all subsequent estimates). Given the developmental nature of this phenomenon, the longitudinal data are likely the more accurate estimate. As shown in Fig. 4E, where *F* value functions for pointwise comparisons of fixation data are given, eye fixation is briefly significantly different at that early age.

It is important to highlight the fact that, consistent with other studies (Chawarska et al., 2013; Elsabbagh et al, 2012), cross-sectional comparisons of eye fixation between infants later diagnosed with autism relative to controls may not show differences at some points in development (for example, at the age of 6 months); what our data emphasize is that it is the developmental trajectory that is already different, even at that early age. If replicated in larger studies, this set of findings would place several constraints on theories of autism pathogenesis: The developmental process leading to autistic symptomatology is present as early as the two-to six-month period of life; but, for this assay at least, eye-fixation is not reduced at the beginning of this period, suggesting that this process did not begin at birth. What critical developmental transitions – in behavior, brain and gene expression – occur around that time that could account for this profile?

4. Endophenotypes and model systems

To answer this question, there is a need to situate our findings in systems biology research. Addressing two issues may be critical.

First, how do we bridge the gap between genes and symptoms in complex neurodevelopmental or neuropsychiatric disorders? The concept of “endophenotypes” has emerged as one answer to this continuing frustration of how to connect molecular, cellular, brain, behavior, and ecological factors in the study of complex psychiatric disorders, in which myriad genes and genetic processes overlap across a wide variety of psychiatric entities (Gottesman and Gould, 2003).

Second, are there model systems that can inspire and inform this project? Here, avian studies of visual imprinting provide an intriguing and insightful answer (Horn and McCabe, 1984). In this system, as in the human developmental period of interest, there is a discrete transition from congenital predisposition to new learning processes. Highlighting this transition is not a recapitulation of the conceptual dichotomy between nature and nurture, which has been sufficiently discredited by close analysis (Oyama, 2000); rather it is a strategy for research focused on manageable transitions within a process that is admittedly more continuous in nature.

4.1. Endophenotypes

“Endophenotypes” (Gould and Gottesman, 2006) are quantifiable components of a neurodevelopmental or psychiatric condition, but not the symptoms themselves; they capture a core feature of the condition that is in between gene and disease entity. They measure the instantiation of pathogenesis that leads to, but precedes, condition-specific symptomatology (Meyer-Lindenberg and Weinberger, 2006). Endophenotypes could potentially be behavioral, brain system (structural or functional), metabolic/hormonal, or even molecular

measurements provided the given measure parallels, quantitatively, the disease process that precedes the advent of the symptoms used to define the condition. Endophenotypes are thought to be of great importance in understanding complex neurodevelopmental and psychiatric disorders because they – more so than symptoms – are likely to mark the derailment of normative functions, which in turn would get us closer to causal mechanisms and pathogenesis (Dawson et al, 2002). It is in this context that we judge the value of our assays of social adaptive behavior: Can they be used as workable endophenotypes?

Our biological motion findings highlighted the importance of foundational social adaptive mechanisms in symptomatology and pathogenesis of autism. But there is a gap in evidence, as the first two years of life remain unstudied in children with autism using this paradigm. Our eye fixation assay, however, might take us further already: it quantifies a developmental decline in normative adaptive function. That decline is proximal, developmental, pre-symptomatic, highly quantitative, and is ultimately predictive not only of categorical (diagnostic) outcome, but of individual, dimensional levels of symptom severity. And yet, while the connection with symptomatic aspects of autism appears strong, the question remains as to how our assay could help us constrain hypotheses and guide genetics and brain research.

In principle, our data provide evidence for the notion that clinical outcome is shaped not only by initial genetic vulnerabilities, but also by the atypical experiences that arise as a consequence of those vulnerabilities (Jones and Klin, 2009). Given the dependence of developmental changes in brain and epigenesis on individual experience (Insel and Fernald, 2004), our assays of eye fixation decline could provide the quantitative measure for a process that parallels the emergence of autism—quantitatively in terms of both *timing of disruption* (i.e., developmental timelines or “when”) and *dosage of disruption* of (i.e., degrees or “how much”). Timing and dosage of disruptions should be measured as deviations from normative benchmarks or “growth charts” as obtained in typically developing infants and toddlers, from birth through the emergence of symptoms defining the condition. If so, the next generation of studies could co-register, prospectively and longitudinally, over the prodromal period of autism, behavioral endophenotypes as quantitative benchmarks (or regressors) for biological measures, also quantitative and longitudinal, of brain and epigenesis. The candidate endophenotype of decline in eye fixation defines a narrow window of developmental transitions that may hold promise for this gene–brain–behavior research enterprise. One model system can inform this enterprise: visual imprinting in the domestic chick.

4.2. From behavioral endophenotype to brain and genes: Lessons from an avian model system

While it is clear that any comparisons across species needs cautionary warnings, most of all when comparing species as phylogenetically distant as chicks (a precocial bird; Rose, 2000) and humans (powered by neoteny; Gould, 1977), the presumption is that the genetic and epigenetic, neural and hormonal processes subserving social adaptive behaviors are likely to show evolutionary conservation (Insel and Fernald, 2004). An emblematic cross-species example is the expression of the oxytocin receptor gene (*OXTR*) in brains of rodents and

primates: *OXTR* plays a role in recognition memory of conspecifics (Skuse et al., 2014), an important adaptive task in both species. Distal mechanisms of rodent recognition and bonding are primarily facilitated by olfaction (Sullivan and Wilson, 2003), and mapping of *Oxtr* in the mouse brain reveals selective expression in olfactory networks (Gould and Zingg, 2003). In contrast, distal mechanisms for primate recognition and bonding are primarily facilitated by vision and audition—and mapping of *OXTR* in primate brain reveals selective expression in areas associated with modulation of visual attention, with processing of auditory and multimodal sensory stimuli, and with control of orienting responses to visual stimuli (Freeman et al, 2014). Thus, the common adaptive task of individual identification and distal bonding shares a common biochemical basis in these different species, and that common basis is differentially distributed in the brain according to species-typical relevance: olfactory for rodent and visual and auditory for primate.

When then comparing chicks and primates, both are born with predispositional biases to certain distal forms of environmental stimuli—biological motion and “face-like” stimuli in chicks (Regolin et al., 2000; Rosa Salva et al., 2011); biological motion, “face-like” stimuli and eye/gaze in humans (Simion et al., 2008; Rosa Salva et al, 2011; Farroni et al., 2002). Since Konard Lorenz's (1935) description of the process of visual imprinting in birds, that process has become an important paradigm for studying the neural mechanisms of socially-mediated learning and memory (Horn, 1998; Insel and Fernald, 2004). Filial imprinting is the process through which a young animal forms a preference for, and attachment to, its parent, and is most obvious in precocial birds (which imprint upon their parents and then follow them around). Precocial birds start walking soon after hatching, looking for their parents for their safety; thus, the process of imprinting is vital to early survival. In the domestic chick, visual imprinting happens as the newly-hatched chick moves toward its mother hen, learns her visual characteristics, and bonds to her.

In a series of studies of visual imprinting in the chick, Horn and McCabe (1984) mapped two separable phases of the imprinting process: predispositional perception and learned memory. That these two phases can be dissociated has been shown via manipulations such as drug administration and lesions that affect each of the phases differentially (e.g., Davies et al, 1985). In the first phase of visual imprinting, filial behavior is evidenced through preferential orientation toward a distal stimulus without prior exposure to that stimulus. The predisposition to orient toward or approach stimuli resembling a conspecific is independent of experience, is bound by a sensitive period, and, as noted above, appears to depend on the configural properties of the stimulus (Johnson et al, 1989; Johnson, 2006). This initial predisposition is then shaped by subsequent experience. How this evolutionarily essential predisposition is encoded in the brain is not yet known (Insel and Fernald, 2004). In contrast, the second phase of visual imprinting – establishment of filial bonding via learning of the parent's characteristics – has been extensively studied (Horn, 2004). Employing autoradiographic, biochemical, lesion, and electrophysiological techniques to study visual imprinting in newly-hatched chicks, Horn and Bate-son, as well as others, have established that a localized region of the chick forebrain, the intermediate and medial part of the mesopallium (IMM, formerly called IMHV, an association area of the telencephalon), is

crucially involved in visual imprinting (Horn, 2004). The evidence for behavior–brain causal links is impressive:

- Bilateral ablation of this area prevents imprinting and abolishes retention of imprinting-acquired preferences (McCabe et al., 1981).
- Morphology of synapses in the IMM is modified by imprinting (Horn et al., 1985): there is increase in synaptic density and elevated glutamate binding (reviewed in Rose, 2000), and synaptic density is associated with the strength of the learned behavior (Horn et al., 1985).
- Imprinting simultaneously initiates two brain processes involving the control of cell proliferation—one related to maturation of a species-specific functional system for tracking individuals of the same species, and one related to remembering the characteristics of the actual parent (Komissarova and Anokhin, 2008).

This is, therefore, a model of how extensive reorganization of a neural network occurs as a result of a learning process. This model system has also substantially advanced our understanding of the impact of experience upon gene expression:

- Total RNA synthesis is up-regulated in the IMM during the acquisition phase of imprinting (Horn et al., 1979), suggesting that gene expression in the IMM is involved in the process of imprinting.
- Neural cell adhesion molecules expressed in the IMM in the chick brain play a time-dependent role in the learning process of imprinting (Solomonia et al, 1998).
- Up-regulated genes involved in filial imprinting – such as c-Fos, NMDA (*N*-methyl-$_D$-aspartate), γ-aminobutyric acid (GABA), and neural cell adhesion molecule (NCAM) - show a significant correlation between degree of protein synchesis and strength of imprinting (reviewed in Yamaguchi et al., 2008).
- Strikingly, NMDA receptor antagonists substantially extend the sensitive period for imprinting (Parsons and Rogers, 2000).

Collectively, several lessons may be drawn from this genetically modulated period of plasticity, during which specific brain regions acquire information essential for survival of the individual. First, early social experience and learning are themselves sufficient to trigger major, time-sensitive, and time-locked neural and epigenetic re-organization processes, and these hold a direct causal and quantitative association with the adaptive task in question. Second, and more speculatively, the avian model system may inform research on gene–brain–behavior studies of humans by providing precedents, if not specific hypotheses, for the inter-relationships between early social experiences, neural re-organization and epigenetics. In what ways are these lessons relevant to advancing systems biology autism research?

While notable advances in the genetics of autism have been made in recent years, the field as a whole remains at an impasse: we now have several hundred genes implicated in autism, but only the most general of theories as to how to tie those findings to the clinical phenotype (Geschwind, 2011; Casci, 2011; State and Sestan, 2012; Scherer and Dawson, 2011; Sanders

et al, 2012; Iossifov et al., 2012; Gillman et al., 2011; Green et al, 2008; Gupta and State, 2007; Buxbaum et al., 2012). The current state of the science was summarized in a recent editorial in *Nature*: "finding the genetic variations is one mission; understanding what they mean is another" (2012. 491:S4–S6).

One common theme is emerging from the multiplicity of recent genetic advances. Several identified autism susceptibility genes encode neuronal cell-adhesion molecules that are critical in anchoring, stabilizing and maintaining synapses (Betancur et al., 2009, for a review); both common and rare genetic variants have been implicated (Wang et al., 2009; Bucan et al, 2009; Glessner et al., 2009; Südhof, 2008; Morrow et al., 2008). Intriguingly, this theme emerged also from the chick model system, in which cell-adhesion molecules play a key role in the establishment of visual imprinting (Solomonia et al, 1998; Parsons and Rogers, 2000; Yamaguchi et al., 2008). This convergence raises hypotheses concerning related gene–brain relationships. The brain processes information by transmitting signals at synapses, which connect neurons into vast networks of communication cells. Alongside the genetic focus on synaptic function, an increasing number of anatomical and functional neuroimaging studies have highlighted atypical cortical connectivity in individuals with ASD (Just et al., 2012; Kana et al., 2009; Keown et al., 2013; Supekar et al, 2013), including in infants and toddlers (Wolff et al, 2012; Keehn et al., 2013). While the confluence of molecular and neuroimaging findings have prompted some to suggest that ASD may represent a generalized 'neuronal disconnection syndrome' (Wang et al, 2009), the profile of varied neuropsychological strengths and weaknesses in ASD has more specific implications for our expectations of which networks should be most disrupted (e.g., so-called social brain networks; Johnson et al., 2005; Grossmann and Johnson, 2007) and which should be relatively spared; more specific hypotheses and greater consideration of early development is warranted (McPartland et al., 2011).

In the chick model, visual imprinting is localized to the IMM, where specific and correlated synaptic proliferation and organization takes place, and where equally specific and correlated genetic and epigenetic changes occur. The specificity of gene–brain relationships in the chick system is, of course, still missing in models of autism, but the analogy may be more than heuristic. Clearly, the avian forebrain lacks the layered structure of mammalian cortex; yet, the relation of the forebrain to subcortical structures is similar, following a basic higher vertebrate brain design (Nauta and Karten, 1970). The IMM functional homologue in primates is thought to be the cingulate cortex (Panksepp, 1998). In humans, the cingulate cortex is implicated in the processing of social visual information and reward-related factors guiding action and decision-making (Apps et al., 2013). Following this analogy, the pursuit of a hypothesis focused on the role of cell-adhesion proteins in pathogenesis of autism (e.g., Südhof, 2008) might be constrained in terms defined by the model system: The relationship should be time-dependent (early infancy), time-locked (to the acquisition of discrete social visual adaptations), focused on a more constrained brain system (social brain network), and quantitatively related (expression of cell-adhesion molecules should hold a strength association with anomalies in connectivity and tractography among key social brain nodes). Studies of early white matter development and visual orienting are underway (Elison et al., 2013; Wolff et al., 2012). New studies combining analyses of gene, brain, and behavior,

under the same conceptual framework, will help to further elucidate the earliest mechanisms of both typical social engagement and the disruption thereof in ASD.

At present, the weak link in this system biology approach in human development is our understanding of the role, if any, of epigenetics. In other animals, behaviorally distinct phenotypes can be created, via epigenetic modification, from the same initial genotype (Herb et al., 2012). One such modification, DNA methylation, is dynamic in cells such as neurons, and can make them responsive to different environmental stimuli, including exposure to social stimuli (Lockett et al, 2012). Several mechanisms linking the social environment in early life to the epigenetic programming of behavior have already been identified (Szyf et al, 2008). Thus in brain cells, epigenetic information reflects the developmental history, neuronal activity, and environmental exposures of those cells. A recent study found distinct differences in DNA-methylation patterns between monkeys reared by, and those separated from, their mothers; these differences were long-lasting (Provencal et al., 2012). Several other programs of research are examining epigenetic changes as a result of social experiences, in humans and in non-human primates, and not only in early life but through the lifespan (Champagne, 2010). Environmental influences on epigenetic mechanisms are now being tentatively connected to disease susceptibility (Jirtle and Skinner, 2007). Alterations in epigenetic pathways can directly result in neurodevelopmental disorders such as Rett syndrome (Amir et al., 1999; Schanen, 2006). In a much more complex condition such as autism, however, the various layers of genetic and epigenetic information have not as yet been integrated with precise measurements of behavior, and certainly not during the early periods of post-natal life (LaSalle, 2013). Although still in its incipience, developmental transcriptome projects (which localize – to brain region, and quantify – by developmental period, the expression of candidate transcripts [Kang et al, 2011]) may help constrain the focus of attention to genes whose expression in key nodes of the social brain network vary in syntony with major, discrete behavioral and brain transitions in the prodromal period of autism. The chick model system of visual imprinting, with its more established behavior–brain–epigenetics causal inter-relationships, looms large as a model to be emulated.

Clearly, epigenetics – the functionally relevant changes in gene expression that do not involve a change in the nucleotide sequence – is not the only mechanism through which genes and the environment work together to influence human social behavior. A more complete understanding of any human behavior should consider each type of gene-environment interplay, but studies of gene–environment correlations, gene–environment interactions, and heritability-environment interactions are also lacking in autism research (Meek et al, 2013). We focused our discussion on epigenetics here because of the immediate connection with the avian model system. But the principle epigenetic research exemplifies is the need to consider the potential multiplier effect, and the iterative process involved, of gene–environment longitudinal effects in which initial vulnerabilities may lead to breakdown in behavioral adaptations, which in turn compound and amplify genetic, brain and behavioral disruptions eventually leading to the expression of behaviorally defined developmental disabilities such as autism (Meek et al, 2013). How to define a road map through this complexity is the topic of this perspective piece.

5. A model of autism pathogenesis

While the challenges for fully integrated gene–brain–behavior studies are still formidable, the very concept of such studies was unimaginable even a few years ago. In the interim, however, the most straightforward lesson from the avian model system is that the emergence of foundational mechanisms of social adaptation may follow two dissociable phases. The first phase corresponds to early-emerging predispositions to orient to certain stimuli in the immediate ecosystem of the organism—stimuli that have survival value; and the second phase corresponds to subsequent learning processes by which the organism ascertains the characteristics of its natural parent and is motivated, and endogenously reinforced, to bond to it. It is specifically in this context that our findings on developmental changes in eye fixation in infants later diagnosed with autism might serve more readily as a useful autism endophenotype.

Results from our studies of eye looking in infants and toddlers with ASD pinpoint, for the first time, in a quantitative fashion, the timing and magnitude of a specific developmental disruption: although looking at others' eyes is already in decline within the first 6 months of life in infants later diagnosed with autism, the average magnitude of eye looking (despite declining) is within the normative range. These results are consistent with several recent studies documenting abnormalities in neural processing of social information at cross-sectional time points later in the first year of life in infants at risk for, or later diagnosed with, ASD (Dinstein et al, 2011; Elsabbagh et al., 2012; Lloyd-Fox et al, 2013; Luyster et al, 2011). Since systems that subserve orienting responses are overlapping with systems necessary for active attention and processing (e.g., Fries et al., 2001; Gregorious et al., 2009; Lakatos et al., 2008; Varela et al., 2001; Siegel et al, 2008), the abnormalities in neural processing are well predicted by the earlier disruptions in social orienting.

Our results also specify which aspects of orienting and processing, in a developmental sense, appear to be intact and which are not. This insight bears directly on an influential theory of early neural development of adaptive reactions in humans proposed by Johnson and colleagues (Johnson, 1990; Johnson and Karmiloff-Smith, 2004). Drawing from Horn and McCabe's (1984) model of visual imprinting in the domestic chick, this theory states that early adaptive reactions map onto two distinct mechanisms, which in turn map onto the neuronal maturation of two, distinct neural networks. The first was termed *experience-expectant* (more proximal to genetic determination) whilst the second was termed *experience-dependent* (covering the iterative learning and experiences that result from engagement with the adaptive task toward which the organism was first reflexively oriented).

Evidence for Johnson and colleagues' model comes from a series of social orienting studies, maturational schedules of human brain, and the emerging field of developmental social neuroscience. Behaviorally, longitudinal studies demonstrate that preferential orientation to faces, seen in the first days and weeks of life, gradually declines in typical development around four and six weeks after birth before re-emerging at two months or so (Johnson et al., 1991). This pattern of decline mirrors that of other reflexive behaviors in neonates such as orienting to auditory sounds and imitating others (Dodwell, 1983; Field et al., 1980, 1986;

Maratos, 1982; Vinter, 1986). In this model, the initial orientation to face-like stimuli (as in Rosa Salva et al., 2011) is considered a reflexive, genetically determined, experience-expectant mechanism. What takes over is described as the learned, environmentally determined, experience-dependent mechanism. In support of this contention, several studies have shown that two- to three-month old infants respond to faces differently than newborns, requiring more veridical stimuli, with moving internal features (not only face-like, schematic faces), to elicit preferential attention (Johnson et al., 1991, 1992; Turati et al., 2005). More strikingly, the second month of life marks the first clear sense that infants are actively engaged with faces in an interactive and socially meaningful way (Wolff, 1987; Trevarthen, 1979; Lamb et al., 1987; Tronick et al., 1978; Bertin and Striano, 2006), and interactive signals (like contingent smiling) are at that point responded to by infant and caregiver as forms of social communication (Messinger and Fogel, 2007).

Connecting these behavioral findings to a model of brain specialization subserving these transitions, Johnson and colleagues' model poses a shift from subcortical to cortical control as the neural substrate underlying the change in face processing at approximately two months of age (Johnson and Morton, 1991; Johnson, 2001; Johnson, 2005). The thesis is that initial predispositions are served by subcortical structures, which decline due to inhibition by developing cortical circuits (as visual preferences come under cortical control) (Barrera and Maurer, 1981; Maurer and Barrera, 1981; Johnson et al., 1991; de Haan et al., 2002). To date, support for this hypothesis comes only from indirect evidence, and primarily on the basis of known maturational and specialization schedules of the visual pathways:

- While the subcortical visual pathway is functional at birth, the primary visual cortex remains relatively immature (Atkinson, 2000; Martin et al, 1999; Johnson, 1990; Morita et al, 2000), and has little initial influence over visually guided behavior (Csibra et al., 2000).
- Myelination of the subcortical visual pathways begins by about two months prenatally and is complete by about three months after birth, whereas myelination of cortical visual pathways begins at the time of birth and does not finish until about four months (Yakovlev and Lecours, 1967).
- While subcortical structures such as the LGN have completed the majority of their developmental change before birth (Hitchcock and Hickey, 1980; Garey and de Courten, 1983; Khan et al, 1994), primary visual cortical areas undergo an increase in synaptogenesis in the first months of life (Huttenlocher et al., 1982).
- Retinocortical pathways become fully functioning approximately two months after birth (Atkinson, 2000; Braddick et al., 1986).
- ERP and PET studies show evidence of cortical specialization for face processing at two to three months of age (Halit et al., 2003; Tzourio-Mazoyer et al., 2002); and
- There are differences in EEG oscillatory activity in familiar vs. unfamiliar face processing demonstrating three-month-olds' ability to visually recognize their mothers (Mash et al., 2013).

Although the transition hypothesized between subcortical and cortical networks has not been tested directly in brain–behavior studies of human infants, evidence for the subcortical route for social information processing, including faces, includes many functional neuroimaging studies of adults (Johnson, 2005). This route implicates the superior colliculus, pulvinar and amygdala, and corresponds to processing that is fast, involves low-spatial frequency stimuli, and precedes more detailed processing by the ventral visual pathway. The subcortical route to face processing is also activated in the absence of conscious awareness, as when face stimuli are presented via binocular rivalry experimental paradigms (Pasley et al., 2004). This evidence is consistent with several decades of studies in model systems, including newly hatched chicks (Johnson and Horn, 1988; Vallortigara et al., 2005; Rosa Salva et al., 2011), which shows that rapid detection of biologically relevant stimuli is mediated by subcortical visual pathways from birth (Sewards and Sewards, 2002; Vuilleumier, 2005).

Against the backdrop of Johnson and colleagues' model, our eye fixation data for typically developing (TD) infants map very closely onto this existing evidence and onto the developmental timeline for the emergence of experience-dependent cortical control of preferential attention to faces. We hypothesize that our TD data at two months are in a behavioral “valley” between the initial reflexive orientation and the emerging, cortically-mediated, experience-dependent preferential eye fixation. The two-month time point is situated between one downward curve, corresponding to the waning of subcortically-controlled, reflexive eye fixation, and one upward curve, corresponding to the incipient cortically-controlled, reward-based, interactive eye fixation (which is observed in the TD increase in fixation on eyes from two to nine months) (Fig. 4A, above). In contrast, the data for infants later diagnosed with ASD suggest a reflexive orientation that appears to persist beyond its developmentally appropriate time window, meaning that reflexive, subcortically-mediated orientation is not inhibited by the emergence of experience-dependent eye fixation. The absence of a cortically-controlled, experience-dependent eye fixation curve is suggested by the steady, continuing decline in their eye fixation beginning at two months (Fig. 4B, above). As shown in Fig. 4E (above), total fixation time on eyes for the ASD group at two months is somewhat higher relative to the TD group because, at that transition point from subcortical-to-cortical control of eye fixation, uninhibited reflexive eye fixation results in higher absolute percentages of eye fixation than those observed in the TD group. In contrast, the TD data at two months represent decaying reflexive eye fixation and the beginning of increasing interactive, experience-dependent eye fixation.

We do not suggest an outright failure of cortical control of all preferential visual attention in children with ASD; to the contrary, our data suggest a developmentally delayed, but still present, co-opting of those mechanisms by attention to other features of the environment (such as physical movement and audiovisual synchrony; Klin et al, 2009; Shultz et al, 2011). The lack of decline in early body fixation (seen in Fig. 4G), increase in mouth fixation (Fig. 4F), and increase in object fixation (Fig. 4H) observed in their data are all consistent with the lack of emergence of experience-dependent, adaptive attention to the eyes of others. Elsewhere we also posed this process as the onset of some of the hallmark characteristics of children with autism later in life, whereby the blockage of the normative social adaptive trajectories biases the child to forms of learning that are not grounded in social interaction: preponderance of learning about the physical (rather than social) environment (Klin, 2000;

Klin et al., 2002a,b, 2003; Klin and Jones, 2006); echolalia and rote speech over contextualized communication (Tager-Flusberg et al., 2005); hyperlexia over conceptual reading (Grigorenko et al., 2003); memorization of facts and information over episodic and personal information (O'Shea et al, 2005; Klin et al., 2007) among other “autistic” styles of learning well-noted in later-life clinical expression (Klin et al., 2003). Emblematic of the way social and non-social object perception is associated with brain specialization in ASD is a series of functional neuroimaging studies of adolescents and adults with ASD: when viewing faces, subjects with ASD display hypoactivation of the right fusiform gyrus (the now well-known “fusiform face area”; Kanwisher and Yovel, 2006) relative to controls; tellingly, they also display hyperactivation of the right inferior temporal gyri, the pattern found in controls when viewing inanimate objects (Schultz et al., 2000). In one particularly intriguing case (Grelotti et al., 2005), an adolescent with ASD displayed activation of his amygdala and fusiform gyrus in perceptual discriminations involving cartoon characters but not human faces, suggesting that structures typically subserving face processing were “co-opted” by a non-social area of circumscribed interest (he had a prodigious ability to identify “Digimon” characters).

We do not suggest that autism is caused by abnormalities in eye fixation (Jones and Klin, 2013). Rather, this assay is likely a reflection of more generalized failures in social adaption at the level of brain and epigenesis, causing and being caused by the iterative processes tying gene, brain and behavior. In this light, the model of autism pathogenesis derived from our longitudinal data on eye fixation specifies that the normative transition from subcortically-to cortically-controlled social visual behavior – as reward-based, interactional eye fixation takes over and co-opts initial reflexive eye fixation – fails to occur. If so, the focus of future research should be this critical transition occurring between about four and twelve weeks of life. In brain studies, next steps should include identification of developmental changes in brain systems associated with decline in automatic, reflex-like behaviors, and with the emergence of voluntary social adaptive action. Candidate targets for studies of subcortical and cortical brain networks guiding visual behavior are available (e.g., Johnson, 2005; Itier and Batty, 2009) for connectivity and tractography studies in human infants. Stringent hypotheses should specify the differential maturational schedules and inter-connectivity of these networks relative to behavioral assays of social visual engagement. These hypotheses should be quantitative, in terms of timing and magnitude of brain-behavior relationships.

6. Concluding remarks

To leverage genetic discoveries in pursuit of causes and mechanisms of pathogenesis in research of autism spectrum disorders (ASD) there is a need for an integrative systems biology approach via a mutually constraining set of hypotheses in gene–brain–behavior studies. Like in other complex neurodevelopmental and psychiatric conditions, the concept of “endophenotypes” should be called upon to aid in multilevel research, from molecular to cell, to brain, to ecosystems. The work of Gabriel Horn and colleagues on visual imprinting in the domestic chick provides a model in which temporally-locked and localized neurodevelopmental reorganization and molecular changes occur as results from discrete actions that signify the accomplishment of foundational adaptive tasks—the chick orients to,

and then learns the characteristics of its natural parent, engaging in behaviors that promote the chick's survival.

In this perspective piece, we described two parallel mechanisms of social adaptive behavior in human infants, which are evolutionarily highly-conserved and developmentally early-emerging, and which promote reciprocal engagement between infant and caregiver, an iterative processes that sets in motion brain specialization and the acquisition of key human functions such as social cognition and communication.

These two adaptive mechanisms – preferential orientation to biological motion, and preferential attention to the eyes of others – are considerably disrupted in infants and toddlers with ASD. Eye fixation, in particular, has been shown to quantify developmental decline in normative adaptive action from the first months of life, but not from birth. This decline is proximal to genetic determination (thus unconfounded by subsequent learning); it is pre-symptomatic (as symptoms of ASD are visible and can only be reliably diagnosed by 2 to 3 years); and it predicts not only categorical diagnostic outcome but also individual levels of symptom severity. These findings delineate a narrow set of early neurodevelopmental transitions – between two and six months of life – which infants later diagnosed with ASD fail to make. Elucidating the biological bases of these transitions will involve the mapping of brain reorganization and epigenetic changes that accompany these transitions in typically-developing babies, and determining which are altered in infants later diagnosed with ASD.

This project emulates the accomplishments of the chick model system by regressing, upon the behavioral endophenotype, quantitative measurements of brain morphology, connectivity and tractography associated with the transition from reflexive/subcortically-controlled, to interactional/cortically-controlled visually guided behavior. While similar measurements of gene expression are still impeded by conceptual and methodological challenges, they likely hold the key to understanding how the multiplicity of genetic findings in ASD research are causally linked to the pathogenesis of the condition.

Acknowledgments

This work was supported by grants from the Simons Foundation and the National Institute of Mental Health (R01 MH083727; P50-MH100029). Additional support was provided by the Marcus Foundation, the J.B. Whitehead Foundation, and the Georgia Research Alliance. We wish to thank the families and children for their time and participation in our various studies reviewed here. We also wish to thank Jessica Jones, Andrea Trubanova, Jeremy Borjon, Jenn Moriuchi, Kate Rice, Jessie Northrup, Laura Edwards, Jennings Xu, Anna Krasno, Casey Zampella, Kelley Knoch, David Lin, Katelin Carr, and Amanda Blank for their assistance in data collection and analysis; Peter Lewis, Jose Paredes, Philip Gorrindo, and Marilyn Ackermann for assistance in designing and building lab hardware and software; Gordon Ramsay and Courtney McCracken for discussions of data analysis and statistics; Irene Zilber, Amy Margolis, Daniela Blum, Martha Dye, Deanna Simeone, Amanda Smith, and Kerry O'Loughlin for project supervision, coordination, and data collection; Tammy Babitz for administrative support; and Kasia Chawarska, Celine Saulnier, Suzanne Macari, Rhea Paul, Amy Carney, Tina Goldsmith, Amanda Steiner, Grace Gengoux, Diane Goudreau, Erin Loring, James McGrath, and Abha Gupta for their contributions to the clinical characterization of the samples; and Chris Gunter for editing the manuscript.

References

American Psychiatric Association (APA). Diagnostic and Statistical Manual of Mental Disorders (DSM-5). fifth. American Psychiatric Association; Washington, DC: 2013.

Amir RE, et al. Rett syndrome is caused by mutations in X-linked MECP2, encoding methy-CpG-binding protein 2. Nat Genet. 1999; 23:185–188. [PubMed: 10508514]

Apps MA, et al. The role of the midcingulate cortex in monitoring others' decisions. Front Neurosci. 2013; 7:251. [PubMed: 24391534]

Atkinson, J. The Developing Visual Brain. Oxford University Press; Oxford: 2000.

Bard KA, et al. Group differences in the mutual gaze of chimpanzees (*Pan troglodytes*). Dev Psychol. 2005; 41(4):616–624. [PubMed: 16060808]

Barger BD, et al. Prevalence and onset of regression within autism spectrum disorders: a meta-analytic review. J Autism Dev Disord. 2013; 43(4):817–828. [PubMed: 22855372]

Barrera ME, Maurer D. The perception of facial expressions by the three-month-old. Child Dev. 1981; 52(1):203–206. [PubMed: 7238144]

Bertin E, Striano T. The still-face response in newborn, 1.5-and 3-month-old infants. Infant Behav Dev. 2006; 29(2):294–297. [PubMed: 17138285]

Betancur C, et al. The emerging role of synaptic cell-adhesion pathways in the pathogenesis of autism spectrum disorders. Trends Neurosci. 2009; 32(7):402–412. [PubMed: 19541375]

Bidet-Caulet A, et al. Listening to a walking human activates the temporal biological motion area. NeuroImage. 2005; 28(1):132–139. [PubMed: 16027008]

Blake R. Cats perceive biological motion. Psychol Sci. 1993; 4(1):54–57.

Blake R, et al. Visual recognition of biological motion is impaired in children with autism. Psychol Sci. 2003; 14(2):151–157. [PubMed: 12661677]

Braddick OJ, et al. Orientation-specific cortical responses develop in early infancy. Nature. 1986; 320:617–619. [PubMed: 3702994]

Brooks R, Meltzoff AN. The importance of eyes: how infants interpret adult looking behavior. Dev Psychol. 2002; 38(6):958–966. [PubMed: 12428707]

Bucan M, et al. Genome-wide analyses of exonic copy number variants in a family-based study point to novel autism susceptibility genes. PLoS Genet. 2009; 5(6):e1000536. [PubMed: 19557195]

Buxbaum JD, et al. The autism sequencing consortium: large-scale,high-throughput sequencing in autism spectrum disorders. Neuron. 2012; 76(6):1052–1056. [PubMed: 23259942]

Casci T. Disease genetics: converging models for autism. Nat Rev Genet. 2011; 12:456–457. [PubMed: 21681205]

Castelli F, et al. Autism, Asperger syndrome and brain mechanisms for the attribution of mental states to animated shapes. Brain. 2002; 125(Pt 8):1839–1849. [PubMed: 12135974]

Champagne FA. Epigenetic influence of social experiences across the lifespan. Dev Psychobiol. 2010; 52(4):299–311. [PubMed: 20175106]

Chawarska, K., et al. Autism Spectrum Disorders in Infants and Toddlers. Chawarska, K.; Klin, A.; Volkmar, F., editors. The Guilford Press; New York, NY: 2008.

Chawarska K, Macari S, Shic F. Decreased spontaneous attention to social scenes in 6-month-old infants later diagnosed with autism spectrum disorders. Biol Psychiatry. 2013; 74(3):195–203. [PubMed: 23313640]

Crawley JN. Mouse behavioral assays relevant to the symptoms of autism. Brain Pathol. 2007; 17(4): 448–459. [PubMed: 17919130]

Csibra G, et al. Cortical development and saccade planning: the ontogeny of the spike potential. NeuroReport. 2000; 11(5):1069–1073. [PubMed: 10790884]

Dalton KM, et al. Gaze fixation and the neural circuitry of face processing in autism. Nat Neurosci. 2005; 8(4):519–526. [PubMed: 15750588]

Davies DC, et al. Noradrenaline and learning: effects of the noradrenergic neurotoxin DSP4 on imprinting in the domestic chick. Behav Neurosci. 1985; 99:652–660. [PubMed: 3843732]

Davies MS, et al. Neural bases of gaze and emotion processing in children with autism spectrum disorders. Brain Behav. 2011; 1(1):1–11. [PubMed: 22398976]

Dawson G, et al. Children with autism fail to orient to naturally occurring social stimuli. J Autism Dev Disord. 1998; 28(6):479–485. [PubMed: 9932234]

Dawson G, et al. Defining the broader phenotype of autism: genetic, brain, and behavioral perspectives. Dev Psychopathol. 2002; 14:581–611. [PubMed: 12349875]

Dawson G, et al. Early social attention impairments in autism: social orienting, joint attention, and attention to distress. Dev Psychol. 2004; 40(2):271–283. [PubMed: 14979766]

de Haan M, et al. Specialization of neural mechanisms underlying face recognition in human infants. J Cogn Neurosci. 2002; 14(2):199–209. [PubMed: 11970786]

de Waal FX. Darwin's legacy and the study of primate visual communication. Ann N Y Acad Sci. 2003; 1000:7–31. [PubMed: 14766618]

DiCicco-Bloom E, et al. The developmental neurobiology of autism spectrum disorder. J Neurosci. 2006; 26(26):6897–6906. [PubMed: 16807320]

Dinstein I, et al. Disrupted neural synchronization in toddlers with autism. Neuron. 2011; 70(6):1218–1225. [PubMed: 21689606]

Dodwell, PC. Spatial sense of the human infant. In: Hein, A.; Jeannerod, M., editors. Spatially Oriented Behavior. Springer-Verlag; New York, NY: 1983.

Elison JT, et al. White matter microstructure and atypical visual orienting in 7-month-olds at risk for autism. Am J Psychiatry. 2013; 170(8):899–908. [PubMed: 23511344]

Elsabbagh M, et al. Neural correlates of eye gaze processing in the infant broader autism phenotype. Biol Psychiatry. 2009; 65(1):31–38. [PubMed: 19064038]

Elsabbagh M, et al. Infant neural sensitivity to dynamic eye gaze is associated with later emerging autism. Curr Biol. 2012; 22:338–342. [PubMed: 22285033]

Emery NJ. The eyes have it: the neuroethology, function and evolution of social gaze. Neurosci Biobehav. 2000; 24(6):581–604.

Falk-Ytter T, Rehnberg E, Bölte S. Lack of visual orienting to biological motion and audiovisual synchrony in 3-year-olds with autism. PLoS One. 2013; 8(7):e68816. [PubMed: 23861945]

Farroni T, et al. Eye contact detection in humans from birth. PNAS. 2002; 99(14):9602–9605. [PubMed: 12082186]

Farroni T, et al. Mechanisms of eye gaze perception during infancy. J Cogn Neurosci. 2004; 16(8): 1320–1326. [PubMed: 15509381]

Farroni T, et al. Direct gaze modulates face recognition in young infants. Cognition. 2007; 102(3): 396–404. [PubMed: 16540101]

Field J, et al. Infants' orientation to lateral sounds from birth to three months. Child Dev. 1980; 51:295–298. [PubMed: 7363744]

Field T, et al. Changes in imitative behavior during early infancy. Infant Behav Dev. 1986; 9:415–421.

Freeman SM, et al. The neuroanatomical distribution of oxytocin receptor binding and mRNA in the male rhesus macaque (Macaca mulatta). Psychoneuroendocrinology. 2014; 45:128–141. [PubMed: 24845184]

Fox R, McDaniel C. The perception of biological motion by human infants. Science. 1982; 218:486–487. [PubMed: 7123249]

Fries P, et al. Modulation of oscillatory neuronal synchronization by selective visual attention. Science. 2001; 291(5508):1560–1563. [PubMed: 11222864]

Frith CD, Frith U. Interacting minds: a biological basis. Science. 1999; 286(5445):1692–1695. [PubMed: 10576727]

Garey LJ, de Courten C. Structural development of the lateral geniculate nucleus and visual cortex in monkey and man. Behav Brain Res. 1983; 10(1):3–13. [PubMed: 6639728]

Geschwind DH. Genetics of autism spectrum disorders. Trends Cogn Sci. 2011; 15(9):409–412. [PubMed: 21855394]

Ghazanfar A, Santos LR. Primate brains in the wild: the sensory bases for social interactions. Nat Rev Neurosci. 2004; 5:603–616. [PubMed: 15263891]

Gillman SR, et al. Rare de novo variants associated with autism implicate a large functional network of genes involved in formation and function of synapses. Neuron. 2011; 70(5):898–907. [PubMed: 21658583]

Glessner JT, et al. Autism genome-wide CNV reveals ubiquitin and neuronal genes. Nature. 2009; 459(7246):569–573. [PubMed: 19404257]

Gottesman II, Gould TD. The endoiphenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry. 2003; 160:636–645. [PubMed: 12668349]

Gould, SJ. Ontogeny and Phylogeny. Belkap Press; Cambridge, MA: 1977.

Gould BR, Zingg HH. Mapping oxytocin receptor gene expression in the mouse brain and mammary gland using an oxytocin receptor-LacZ reporter mouse. Neuroscience. 2003; 122(1):155–167. [PubMed: 14596857]

Gould TD, Gottesman II. Psychiatric endophenotypes and the development of valid animal models. Genes Brains Behav. 2006; 5(2):113–119.

Green AE, et al. Using genetic data in cognitive neuroscience: from growing pains to genuine insights. Nat Rev Neurosci. 2008; 9:710–720. [PubMed: 19143051]

Gregorious GG, et al. High-frequency, long-range coupling between prefrontal and visual cortex during attention. Science. 2009; 324(5931):1207–1210. [PubMed: 19478185]

Grelotti DJ, et al. fMRI activation of the fusiform gyrus and amygdala to cartoon characters but not to faces in a boy with autism. Neuropsychologia. 2005; 43(3):373–385. [PubMed: 15707614]

Grigorenko EL, et al. Annotation: hyperlexia: disability or superability? J Child Psychol Psychol. 2003; 44(8):1079–1091.

Grossmann T, Johnson MH. The development of the social brain in human infancy. Eur J Neurosci. 2007; 25(4):909–919. [PubMed: 17331189]

Gupta AR, State MW. Recent advances in the genetics of autism. Biol Psychiatry. 2007; 61(4):429–437. [PubMed: 16996486]

Haith MM, et al. Eye contact and face scanning in early infancy. Science. 1977; 198(4319):853–855. [PubMed: 918670]

Halit H, et al. Cortical specialization for face processing: face-sensitive event-related potential components in 3- and 12-month-old infants. NeuroImage. 2003; 19(3):1180–1193. [PubMed: 12880843]

Herb BR, et al. Reversible switching between epigenetic states in honeybee behavioral subcastes. Nat Neurosci. 2012; 15:1371–1373. [PubMed: 22983211]

Hitchcock PF, Hickey TL. Prenatal development of the human lateral geniculate nucleus. J Comp Neurol. 1980; 194(2):395–411. [PubMed: 7440807]

Hoffman HW, Ratner AM. A reinforcement model of imprinting: implications for socialization of monkeys and men. Psychol Rev. 1973; 80:527–544.

Horn G, et al. An autoradiographic study of the chick brain after imprinting. Brain Res. 1979; 168:361–373. [PubMed: 445146]

Horn G, McCabe BJ. Predispositions and preferences: effects on imprinting of lesions to the chick brain. Anim Behav. 1984; 32:288–292.

Horn G, et al. Changes in the structure of synapses associated with learning. J Neurosci. 1985; 5:3161–3168. [PubMed: 4078621]

Horn G, Johnson MH. Memory systems in the chick: dissociations and neuronal analysis. Neuropsychologia. 1989; 27(1):1–22. [PubMed: 2565559]

Horn G. Visual imprinting and the neural mechanisms of recognition memory. Trends Neurosci. 1998; 21:300–305. [PubMed: 9683322]

Horn G. Pathways of the past: the imprint of memory. Nat Rev Neurosci. 2004; 118:108–120. [PubMed: 14735114]

Huttenlocher PR, et al. Synaptogenesis in human visual cortex: evidence for synapse elimination during normal development. Neurosci Lett. 1982; 33(3):247–252. [PubMed: 7162689]

Insel TR, Fernald RD. How the brain processes social information: searching for the social brain. Annu Rev Neurosci. 2004; 27:697–722. [PubMed: 15217348]

Iossifov I, et al. De novo gene disruptions in children on the autistic spectrum. Neuron. 2012; 74(2): 285–299. [PubMed: 22542183]

Itier RJ, Batty M. Neural bases of eye and gaze processing: the core of social cognition. Neurosci Biobehav Rev. 2009; 33:843–863. [PubMed: 19428496]

Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. Nat Rev Genet. 2007; 8:253–262. [PubMed: 17363974]

Johansson G. Visual perception of biological motion and a model for its analysis. Percept Psychophys. 1973; 14:201–211.

Johnson M. Functional brain development in humans. Nat Rev Neurosci. 2001; 2:475–483. [PubMed: 11433372]

Johnson MH. Subcortical face processing. Nat Rev Neurosci. 2005; 6(10):766–774. [PubMed: 16276354]

Johnson MH. Biological motion: a perceptual life detector? Curr Biol. 2006; 16(10):R376–R377. [PubMed: 16713949]

Johnson MH, Horn G. Development of filial preferences in dark-reared chicks. Anim Behav. 1988; 36:675–683.

Johnson, MH.; Karmiloff-Smith, A. Neuroscience perspectives on infant development. In: Bremner, G.; Slater, A., editors. Theories of Infant Development. Blackwell; Malden, MA: 2004.

Johnson MH, et al. A sensitive period for the development of a predisposition in dark-reared chicks. Anim Behav. 1989; 37:1044–1046.

Johnson MH. Cortical maturation and the development of visual attention in early infancy. J Cogn Neurosci. 1990; 2:81–95. [PubMed: 23972019]

Johnson MH, et al. Newborns' preferential tracking of face-like stimuli and its subsequent decline. Cognition. 1991; 40(1-2):1–19. [PubMed: 1786670]

Johnson MH, et al. The effects of movement of internal features on infants' preference for face-like stimuli. Infant Behav Dev. 1992; 15(1):129–136.

Johnson MH, et al. The emergence of the social brain network: evidence from typical and atypical development. Dev Psychpathol. 2005; 17(3):599–619.

Jokish D, et al. Differential involvement of the cerebellum in biological and coherent motion perception. Eur J Neurosci. 2005; 21(12):3439–3446. [PubMed: 16026481]

Jones W, et al. Absence of preferential looking to the eyes of approaching adults predicts level of social disability in 2-year-olds with autism spectrum disorder. Arch Gen Psychiatry. 2008; 65(8): 946–954. [PubMed: 18678799]

Jones W, Klin A. Heterogeneity and homogeneity across the autism spectrum: the role of development. J Am Acad Child Adolesc Psychiatry. 2009; 48(5):471–473. [PubMed: 19395902]

Jones W, Klin A. Attention to eyes is present but in decline in 2-6 month-olds later diagnosed with autism. Nature. 2013; 504(7480):427–431. [PubMed: 24196715]

Jordan H, et al. Intact perception of biological motion in the face of profound spatial deficits: Williams syndrome. Psychol Sci. 2002; 13:162–167. [PubMed: 11934001]

Just MA, et al. Autism as a neural systems disorder: a theory of frontal-posterior underconnectivity. Neurosci Biobehav Rev. 2012; 36(4):1292–1313. [PubMed: 22353426]

Kaiser MD, et al. Neural signatures of autism. Proc Natl Acad Sci U S A. 2010; 107(49):21223–21228. [PubMed: 21078973]

Kampe KKW, et al. Reward value of attractiveness and gaze. Nature. 2001; 413(6856):589–590. [PubMed: 11595937]

Kana RK, et al. Atypical frontal-posterior synchronization of theory of Mnd regions in autism during mental state attribution. Soc Neurosci. 2009; 4(2):135–152. [PubMed: 18633829]

Kang HJ, et al. Spatio-temporal transcriptome of the human brain. Nature. 2011; 478(7370):483–489. [PubMed: 22031440]

Kanner L. Autistic disturbances of affective contact. Nerv Child. 1943; 2:217–250.

Kanwisher N, Yovel G. The fusiform face area: a cortical region specialized for the perception of faces. Philos Trans R Soc London, Ser B: Biol, Sci. 2006; 361(1476):2109–2128. [PubMed: 17118927]

Keehn B, et al. Functional connectivity in the first years of life in infants at-risk for autism: a preliminary near-infrared spectroscopy study. Front Hum Neurosci. 2013; 7:444. [PubMed: 23964223]

Keown CL, et al. Local functional overconnectivity in posterior brain regions is associated with symptom severity in autism spectrum disorders. Cell Rep. 2013; 5:567–572. [PubMed: 24210815]

Khan AA, et al. Development of human lateral geniculate nucleus: an electron microscopic study. Int J Dev Neurosci. 1994; 12(7):661–672. [PubMed: 7900548]

Kliemann D, et al. The role of the amygdala in atypical gaze on emotional faces in autism spectrum disorders. J Neurosci. 2012; 32(28):9469–9476. [PubMed: 22787032]

Klin A. Understanding early infantile autism: an application of G.H. Mead's theory of the emergence of mind. London Sch Econ Q. 1989; 4(3):336–356.

Klin A. Young autistic children's listening preferences in regard to speech: a possible characterization of the symptom of social withdrawal. J Autism Dev Disord. 1991; 21(1):29–42. [PubMed: 1828067]

Klin A. Attributing social meaning to ambiguous visual stimuli in higher functioning autism and Asperger syndrome: the social attribution task. J Child Psychol Psychol. 2000; 41(7):831–846.

Klin A, et al. Visual fixation patterns during viewing of naturalistic social situations as predictors of social competence in individuals with autism. Arch Gen Psychiatry. 2002a; 59(9):809–816. [PubMed: 12215080]

Klin A, et al. Defining and quantifying the social phenotype in autism. Am J Psychiatry. 2002b; 159(6):895–908. [PubMed: 12042174]

Klin A, et al. The enactive mind—from actions to cognition: lessons from autism. Philos Trans R Soc London, Ser B. 2003; 358:345–360. [PubMed: 12639332]

Klin A, Jones W. Attributing social and physical meaning to ambiguous visual displays in individuals with higher functioning autism spectrum disorders. Brain Cognit. 2006; 61:40–53. [PubMed: 16497422]

Klin A, et al. Social and communication abilities and disabilities in higher functioning individuals with autism spectrum disorders. J Autism Dev Disord. 2007; 37(4):748–759. [PubMed: 17146708]

Klin A, Jones W. Altered face scanning and impaired recognition of biological motion in a 15-month-old infant with autism. Dev Sci. 2008; 11(1):40–46. [PubMed: 18171365]

Klin, A., et al. Opportunities for research: concepts and future directions. In: Chawarska, K.; Klin, A.; Volkmar, FR., editors. Autism Spectrum Disorders in Infants and Toddlers: Diagnosis, Assessment and Treatment. Guilford Press; New York, NY: 2008.

Klin A, et al. Two-year-olds with autism fail to orient towards human biological motion but attend instead to non-social, physical contingencies. Nature. 2009; 459:257–261. [PubMed: 19329996]

Komissarova NV, Anokhin KV. Effects of an imprinting procedure on cell proliferation in the chick brain. Neurosci Behav Physiol. 2008; 38(3):289–296. [PubMed: 18264776]

Kobayashi H, Kohshima S. Unique morphology of the human eye. Nature. 1997; 387(6635):767–768. [PubMed: 9194557]

Kröger A, Bletsch A, Krick C, Siniatchkin M, Jarczok TA, Freitag CM, Bender S. Visual event-related potentials to biological motion stimuli in autism spectrum disorders. Soc Cogn Affective Neurosci. 2014; 9(8):1214–1222.

Lakatos P, et al. Entrainment of neuronal oscillations as a mechanism of attentional selection. Science. 2008; 320(5872):110–113. [PubMed: 18388295]

Lamb ME, et al. The development of infant social expectations in face-to-face interaction: a longitudinal study. Merrill Palmer Q. 1987; 33:241–254.

LaSalle JM. Epigenomic strategies at the interface of genetic and environmental risk factors for autism. J Hum Genet. 2013; 58:396–401. [PubMed: 23677056]

Lederhendler I, Schulkin J. Behavioral neuroscience: challenges for the era of molecular biology. Trends Neurosci. 2000; 23:451–454. [PubMed: 11006460]

Lloyd-Fox S, et al. Reduced neural sensitivity to social stimuli in infants at risk for autism. Proc Biol Sci. 2013; 280(1758):20123026. [PubMed: 23486434]

Lockett GA, et al. DNA methylation changes elicited by social stimuli in the brains of worker honey bees. Genes Brain Behav. 2012; 11:235–242. [PubMed: 22098706]

Lorenz KZ. Der Kumpan in der Umwelt des Vogels (Companions as factors in the bird's environment). J Ornithol. 1935; 83:137–215.

Lord, C., et al. Autism Diagnostic Observation Schedule—WPS (ADOS-WPS). Western Psychological Services; Los Angeles, CA: 2000.

Lord C, et al. Regression and word loss in autistic spectrum disorders. J Child Psychol Psychiatry. 2004; 45:936–955. [PubMed: 15225337]

Luyster RJ, et al. Neural correlates of familiar and unfamiliar face processing in infants at risk for autism spectrum disorders. Brain Topogr. 2011; 24(3-4):220–228. [PubMed: 21442325]

Maratos, O. Trends in the development of early imitation in infancy. In: Beaver, TG., editor. Regressions in mental development: basic phenomena and theories. Lawrence Erlbaum; Hillsdale, NJ: 1982.

Martin E, et al. Visual processing in infants and children studied using functional MRI. Pediatr Res. 1999; 46(2):135–140. [PubMed: 10447104]

Mash C, et al. Brain dynamics in young infants' recognition of faces: EEG oscillatory activity in response to mother and stranger. NeuroReport. 2013; 24(7):359–363. [PubMed: 23470433]

Maurer D, Barrera M. Infants' perception of natural and distorted arrangements of a schematic face. Child Dev. 1981; 52(1):196–202. [PubMed: 7238143]

McCabe BJ, et al. Effects of restricted lesions of the chick forebrain on the acquisition of filial preferences during imprinting. Brain Res. 1981; 205:29–37. [PubMed: 7470865]

Meek SE, Lemery-Chalfant K, Jahromi LB, Valiente C. A review of gene-environment correlations and their implications for autism: a conceptual model. Psychol Rev. 2013; 120(3):497–521. [PubMed: 23915084]

McPartland JC, et al. Recent advances in understanding the neural bases of autism spectrum disorder. Curr Opin Pediatr. 2011; 23(6):628–632. [PubMed: 21970830]

Messinger D, Fogel A. The interactive development of social smiling. Adv Child Dev Behav. 2007; 35:327–366. [PubMed: 17682330]

Meyer-Lindenberg A, Weinberger DR. Intermediate phenotypes and genetic mechanisms of psychiatric disorders. Nat Rev Neurosci. 2006; 7:818–827. [PubMed: 16988657]

Miklóski Á, et al. A simple reason for a big difference: wolves do not look back at humans, but dogs do. Curr Biol. 2003; 13(9):763–766. [PubMed: 12725735]

Morita T, et al. Difference in the metabolic response to photic stimulation of the lateral geniculate nucleus and the primary visual cortex of infants: a fMRI study. Neurosci Res. 2000; 38(1):63–70. [PubMed: 10997579]

Morrow EM, et al. Identifying autism loci and genes by tracing recent shared ancestry. Science. 2008; 321(5886):218–223. [PubMed: 18621663]

Moy SS, et al. Mouse models of autism spectrum disorders: the challenge for behavioral genetics. Am J Med Genet C: Semin Med Genet. 2006; 142C(1):40–51. [PubMed: 16419099]

Mundy, P.; Neal, R. Neural plasticity, joint attention and autistic developmental pathology. In: Glidden, L., editor. International Review of Research in Mental Retardation. Academic Press; New York NY: 2000. p. 141-168.

Myowa-Yamakoshi M, et al. Preference for human direct gaze in infant chimpanzees (*Pan troglodytes*). Cognition. 2003; 89:53–64.

Nauta, WJH.; Karten, HJ. A general profile of the vertebrate brain with sidelights on the ancestry of the cerebral cortex. In: Schmitt, FO., editor. The Neurosciences: second Study Program. Rockefeller Press; New York, NY: 1970.

Neri P, et al. Seeing biological motion. Nature. 1998; 395(6705):894–896. [PubMed: 9804421]

Omori E, Watanabe S. Discrimination of Johansson's stimuli in pigeons. Intl J Comp Psychol. 1996; 9:92.

Oram MW, Perrett DI. Integration of form and motion in the anterior superior temporal polysensory area (STPa) of the macaque monkey. J Neurophysiol. 1996; 76(1):109–129. [PubMed: 8836213]

O'Shea AG, et al. Source memory in children with autism spectrum disorders. Dev Neuropsychol. 2005; 27(3):337–360. [PubMed: 15843101]

Oyama, S. The Ontogeny of Information: Developmental Systems and Evolution. Duke University Press; Durham, NC: 2000.

Panksepp, J. Affective Neuroscience: The Foundations of Human and Animal Emotions. Oxford University Press; New York NY: 1998.

Parr JR, et al. Early developmental regression in autism spectrum disorder: evidence from an international multiplex sample. J Autism Dev Disord. 2011; 41:332–340. [PubMed: 20711649]

Parsons CH, Rogers LJ. NMDA receptor antagonists extend the sensitive period for imprinting. Physiol Behav. 2000; 68(5):749–753. [PubMed: 10764906]

Pasley BN, et al. Subcortical discrimination of unperceived objects during binocular rivalry. Neuron. 2004; 42(1):163–172. [PubMed: 15066273]

Pavlova M, Sokolov A. Orientation specificity in biological motion perception. Percept Psychophys. 2000; 62(5):889–899. [PubMed: 10997036]

Pelphrey KA, et al. Functional anatomy of biological motion perception in posterior temporal cortex: an fMRI study of eye, mouth and hand movements. Cerebr Cortex. 2005; 15(12):1866–1876.

Pelphrey KA, Carter EJ. Brain mechanisms for social perception: lessons from autism and typical development. Ann N Y Acad Sci. 2008; 1145:283–299. [PubMed: 19076404]

Provencal N, et al. The signature of maternal rearing in the methylome in rhesus macaque prefrontal cortex and T cells. J Neurosci. 2012; 32(44):15626–15642. [PubMed: 23115197]

Redcay E. The superior temporal sulcus performs a common function for social and speech perception: implications for the emergence of autism. Neurosci Biobehav Rev. 2008; 32(1):123–142. [PubMed: 17706781]

Regolin L, et al. Visual perception of biological motion in newly hatched chicks as revealed by an imprinting procedure. Anim Cogn. 2000; 3(1):53–60.

Reid VM, et al. The perception of biological motion by infants: an event-related potential study. Neurosci Lett. 2006; 395:211–214. [PubMed: 16298485]

Rogers SJ. What are infant siblings teaching us about autism in infancy? Autism Res. 2009; 2(3):125–137. [PubMed: 19582867]

Rosa Salva O, et al. The evolution of social orienting: evidence from chicks (*Gallus gallus*) and human newborns. PLoS One. 2011; 6(4):e18802. [PubMed: 21533093]

Rose SPR. God's organism? The chick as a model system for memory studies. Learn Mem. 2000; 7:1–17. [PubMed: 10706598]

Rutishauser U, et al. Single-neuron correlates of atypical face processing in autism. Neuron. 2013; 80(4):887–899. [PubMed: 24267649]

Sanders SJ, et al. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature. 2012; 485(7397):237–241. [PubMed: 22495306]

Sato W, et al. Amygdala integrates emotional expression and gaze direction in response to dynamic facial expressions. NeuroImage. 2010; 50(4):1658–1665. [PubMed: 20096793]

Schanen NC. Epigenetics of autism spectrum disorders. Hum Mol Genet. 2006; 15(Spec No 2):R1138–R1150.

Scherer SW, Dawson G. Risk factors for autism: translating genomic discoveries into diagnostics. Hum Genet. 2011; 130(1):123–148. [PubMed: 21701786]

Shic F, Macari S, Chawarska K. Speech disturbs face scanning in 6-month-old infants who develop autism spectrum disorder. Biol Psychiatry. 2014; 75(3):231–237. [PubMed: 23954107]

Schultz RT. Developmental deficits in social perception in autism: the role of the amygdala and fusiform face area. Int J Dev Neurosci. 2005; 23(2-3):125–141. [PubMed: 15749240]

Schultz RT, et al. Abnormal ventral temporal cortical activity during face discrimination among individuals with autism and Asperger syndrome. Arch Gen Psychiatry. 2000; 57(4):331–340. [PubMed: 10768694]

Shultz S, et al. Inhibition of eye blinking reveals subjective perceptions of stimulus salience. Proc Natl Acad Sci U S A. 2011; 108(52):21270–21275. [PubMed: 22160686]

Sewards TV, Sewards MA. Innate visual object recognition in vertebrates: some proposed pathways and mechanisms. Comp Biochem Physiol A: Mol Integr Physiol. 2002; 132(4):861–891. [PubMed: 12095868]

Siegel M, et al. Neuronal synchronization along the dorsal visual pathway reflects the focus of spatial attention. Neuron. 2008; 60(4):709–719. [PubMed: 19038226]

Simion F, et al. A predisposition for biological motion in the newborn baby. Proc Natl Acad Sci U S A. 2008; 105(2):809–813. [PubMed: 18174333]

Skuse DH, et al. Common polymorphism in the oxytocin receptor gene (OXTR) is associated with human social recognition skills. Proc Natl Acad Sci U S A. 2014; 111(5):1987–1992. [PubMed: 24367110]

Solomonia RO, et al. Neural cell adhesion molecules, learning, and memory in the domestic chick. Behav Neurosci. 1998; 112(3):646–655. [PubMed: 9676980]

State MW, Sestan N. Neuroscience: the emerging biology of autism spectrum disorders. Science. 2012; 227(6100):1301–1303. [PubMed: 22984058]

Südhof TC. Neuroligins and neurexins link synaptic function to cognitive disease. Nature. 2008; 455(7215):903–911. [PubMed: 18923512]

Sullivan RM, Wilson DA. Molecular biology of early olfactory memory. Learn Mem. 2003; 10:1–4. [PubMed: 12551958]

Supekar K, et al. Brain hyperconnectivity in children with autism and its links to social deficits. Cell Rep. 2013; 5:738–747. [PubMed: 24210821]

Szyf M, et al. The social environment and the epigenome. Environ Mol Mutagen. 2008; 49:46–60. [PubMed: 18095330]

Tager-Flusberg, H., et al. Communication in autism. In: Volkmar, F.; Klin, A.; Paul, R.; Cohen, D., editors. Handbook of Autism and Pervasive Developmental Disorders. third. Wiley & Sons; New York, NY: 2005.

Thompson, DW. On growth and form. University Press; Cambridge, UK: 1942.

Thompson JC, et al. Configural processing of biological motion in human superior temporal sulcus. J Neurosci. 2005; 25(39):9059–9066. [PubMed: 16192397]

Trevarthen, C. Communication and cooperation in early infancy: a description of primary intersubjectivity. In: Bullowa, M., editor. Before Speech: The Beginning of Interpersonal Communication. Cambridge University Press; Cambridge, MA: 1979. p. 321-347.

Troje NF, Westhoff C. The inversion effect in biological motion perception: evidence for a life detector? Curr Biol. 2006; 16:821–824. [PubMed: 16631591]

Turati C, et al. Three-month-olds' visual preference for faces and its underlying visual processing mechanisms. J Exp Child Psychol. 2005; 90(3):255–273. [PubMed: 15707862]

Tronick EZ, et al. The infant's response to entrapment between contradictory messages in face-to-face interaction. J Am Acad Child Psychiatry. 1978; 17:1–13. [PubMed: 632477]

Tzourio-Mazoyer N, et al. Neural correlates of woman face processing by 2-month old infants. NeuroImage. 2002; 15(2):454–461. [PubMed: 11798279]

Vallortigara G, et al. Visually inexperienced chicks exhibit spontaneous preference for biological motion patterns. PLoS Biol. 2005; 3(7):e208. [PubMed: 15934787]

Varela F, et al. The brain web: phase synchronization and large-scale integration. Nat Rev Neurosci. 2001; 2:229–239. [PubMed: 11283746]

Vinter A. The role of movement in eliciting early imitations. Child Dev. 1986; 57:66–71.

Volkmar FR, Pauls D. Autism. Lancet. 2003; 362(9390):1133–1141. [PubMed: 14550703]

Volkmar FR, et al. Autism and pervasive developmental disorders. J Child Psychol Psychiatry. 2004; 45(1):1–36.

Vuilleumier P. How brains beware: neural mechanisms of emotional attention. Trends Cogn Sci. 2005; 9(12):585–594. [PubMed: 16289871]

Wang K, et al. Common genetic variants on 5p14.1. Nature. 2009; 459(7246):528–533. [PubMed: 19404256]

Wang Y, et al. The control of mimicry by eye contact is mediated by medial prefrontal cortex. J Neurosci. 2011; 31(33):12001–12010. [PubMed: 21849560]

Wolff, PH. The Development of Behavioral States and the Expression of Emotions in Early Infancy: New Proposals for Investigation. The University of Chicago Press; Chicago, IL: 1987.

Wolff JJ, et al. Differences in white matter fiber tract development present from 6 to 24 months in infants with autism. Am J Psychiatry. 2012 Jun; 169(6):589–600. [PubMed: 22362397]

Yakovlev, PI.; Lecours, AR. The myelogenetic cycles of regional maturation ofthe brain. In: Minkowski, A., editor. Regional development ofthe brain in early life. Blackwell; Oxford: 1967. p. 3-70.

Yamaguchi S, et al. Gene expression profile in cerebrum in the filial imprinting of domestic chicks (*Gallus gallus domesticus*). Brain Res Bull. 2008; 76(3):275–281. [PubMed: 18498941]

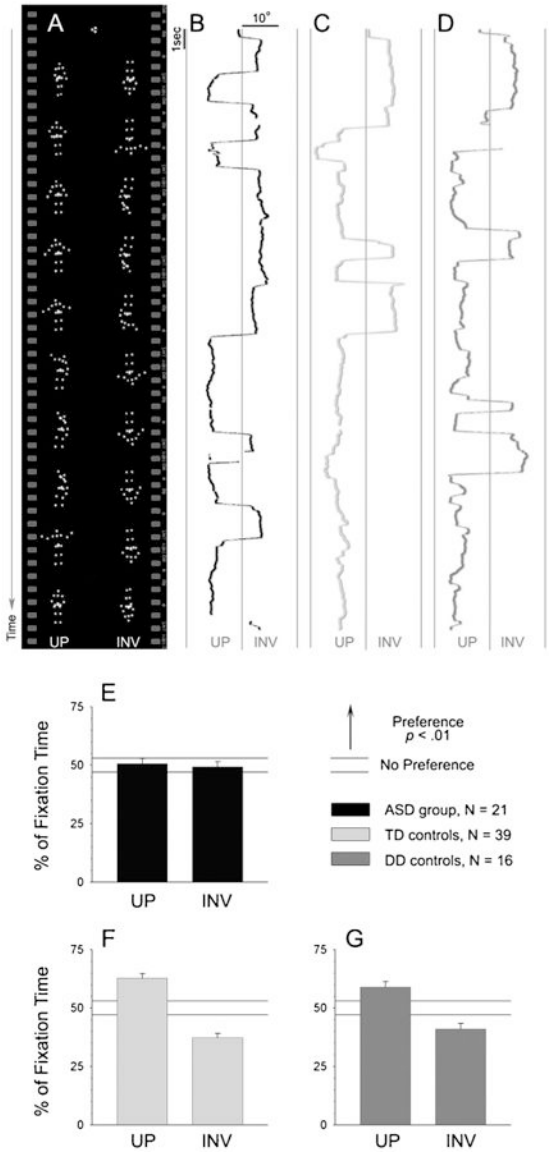

Fig. 1.

Two-year-olds with autism exhibit no preferential attention to biological motion, while control children show significant preferences. (A) Example still images from point-light biological motion stimuli, with centering cue at start. Each animation showed an upright (UP) and inverted (INV) figure with accompanying soundtrack matching the actions of the upright figure. The upright figure enacted childhood games. Figures were identical except that the inverted figure was rotated 180 and its movements played in reverse order. In (B–D), visual scanning data of individual children are plotted as horizontal location by time. Breaks in data occur for blinks or offscreen fixations. (B) Visual scanning data from one toddler with autism (ASD), for one animation. (C) Data from one typically-developing toddler (TD). (D) Data from one developmentally-delayed but non-autistic toddler (DD). (E) For the ASD group, fixation to upright and inverted biological motion occurs at chance levels. (F) TD toddlers give preferential attention to upright animations. (G) DD toddlers

also give preferential attention to upright animations. Horizontal guidelines denote percentages not significantly different from chance. Error bars are SEM.

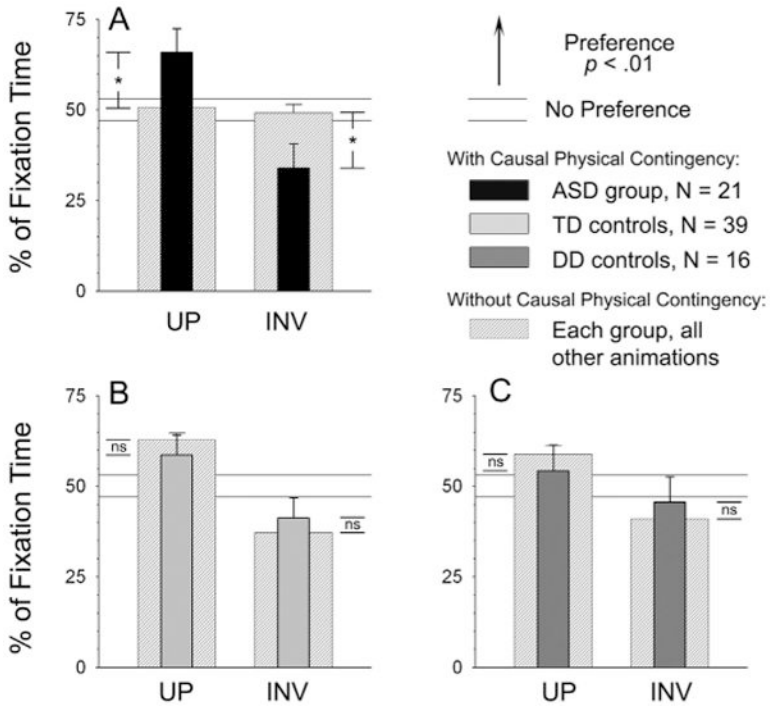

Fig. 2.

When the animation contains a physical contingency, two-year-olds with autism do show significant viewing preferences. (A) During other biological motion animations, ASD toddlers show no preference; but when a physical contingency is present on the upright side, these toddlers show significant preference for the upright figure: different from chance ($p <$ .01), and different from their viewing behavior to other animations ($p = .044$). While other animations presented only moving point-lights and human voice, one type of animation contained an additional cue: as two point-lights, representing the actor's hands, collided, the sound of clapping could be heard (playing “pat-a-cake”). The collision of point-light “hands” actually caused a noise (the clap) to occur, localized to the upright figure and absent from the inverted (the inverted figure's movements were not synchronous with the claps). (B) TD toddlers show no significant change in preferential viewing. (C) DD toddlers also show no significant change in preferential viewing. Horizontal guidelines denote percentages not significantly different from chance. Error bars are SEM. * $p < .05$.

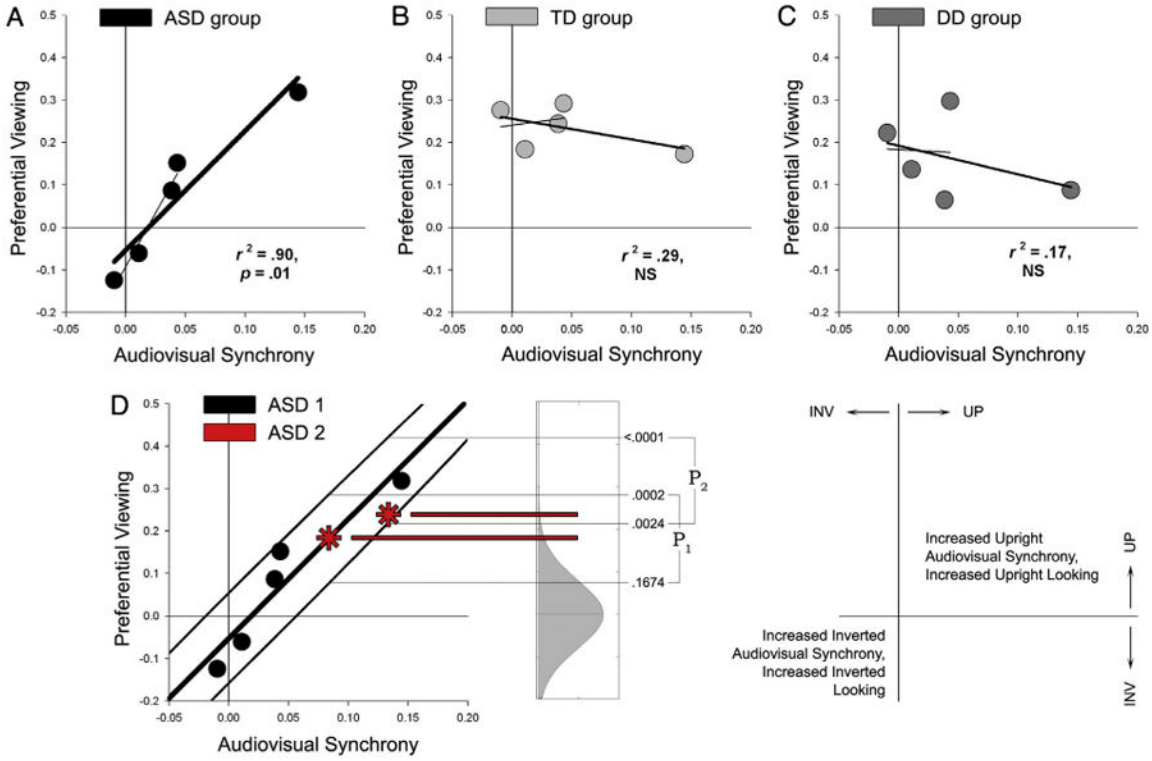

Fig. 3.

Level of audiovisual synchrony is highly correlated with preferential viewing in two-year-olds with autism; is uncorrelated with viewing in control children; and can predict ASD viewing patterns in novel animations. (A) Preferential viewing is significantly correlated with audiovisual synchrony in ASD toddlers. Plots pair preferential viewing and audiovisual synchrony. When the animation with greatest upright audiovisual synchrony (pat-a-cake) is withheld from analysis, audiovisual synchrony is still significantly correlated with viewing behavior in ASD toddlers: $r^2 = .95$, $p = .018$ (plotted as thin regression line through remaining 4 data points). (B) Preferential viewing by TD toddlers is uncorrelated with audiovisual synchrony, across either 4 or 5 animations. (C) Preferential viewing by DD toddlers is also uncorrelated with audiovisual synchrony. (D) To test whether audiovisual synchrony could predict looking behavior in new animations, we created two additional animation types. The regression from the original data, with weighted binomial prediction intervals, provided a model for expected behavior. P_1 and P_2 denote prediction intervals for the new animations. Probability of obtaining results in these intervals is noted to the right of the regression plot. For an independent cohort of toddlers with autism, matched to the original cohort, preferential viewing was predicted on the basis of audiovisual synchrony ($p = .0004$). In all plots, axis *Y* shows preferential viewing as a difference score: percentage of fixation time to upright (UP) minus percentage of fixation time to inverted (INV). Positive values indicate increased looking at the upright. Likewise, axis *X* shows audiovisual synchrony as synchrony of the upright (as percentage of total synchrony) minus synchrony of the inverted (also as percentage of total). Positive values indicate greater synchrony in the upright figure.

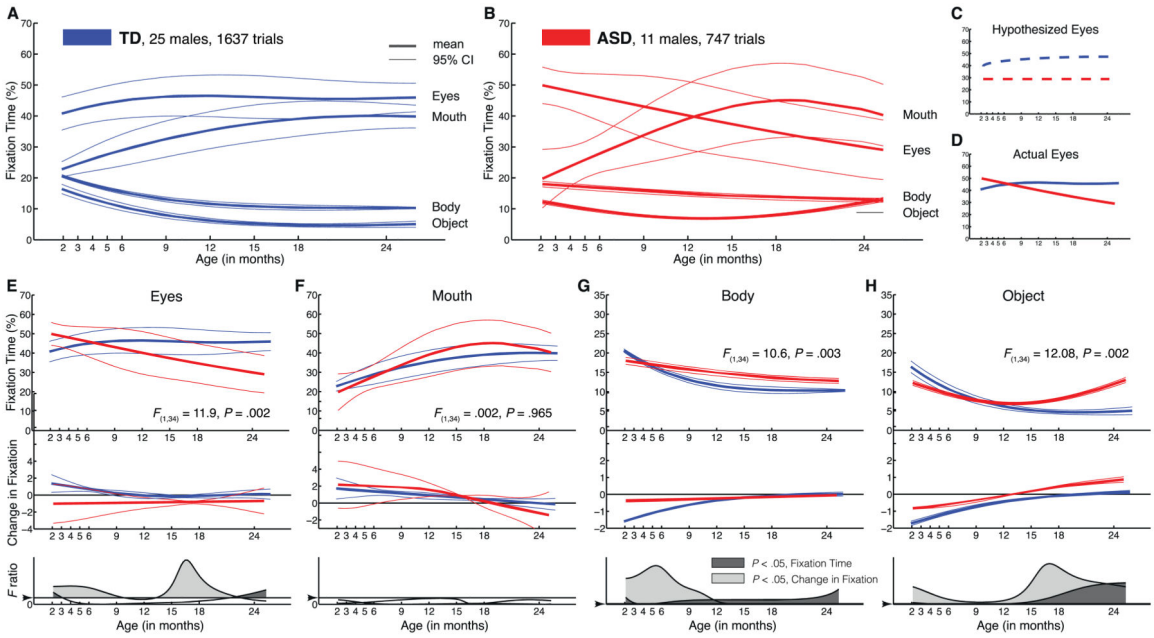

Fig. 4.

Growth charts of social visual engagement for typically-developing children relative to children diagnosed with ASD. (A) Fixation to eyes, mouth, body, and object from 2 until 24 months in TD males (in blue) and (B) in males with ASD (in red). Contrary to a hypothesis of congenital reduction in preferential attention to the eyes in ASD, infants with ASD exhibit mean decline in eye fixation from 2 until 24 months of age. Hypothesized (C) and actual (D) mean eye fixation curves are plotted in blue for TD children and in red for children with ASD. Longitudinal change in fixation to (E) eyes; (F) mouth; (G) body; and (H) object regions. Dark lines of each color represent mean growth curves, while light lines indicate pointwise 95% confidence intervals. Top panel in each section plots percent fixation overtime; middle panel plots change in fixation overtime (the first derivative, in units of % change per month); and the bottom panel plots *F* value functions for pointwise comparisons of fixation and change in fixation between groups. Pointwise comparisons with *F* values greater than F_{crit} (for 1.34 dof=4.13, p = .05, marked by arrowhead on *F* ratio axis) are shaded in medium gray (for comparison of fixation data) and light gray (for comparison of change-in-fixation data). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)