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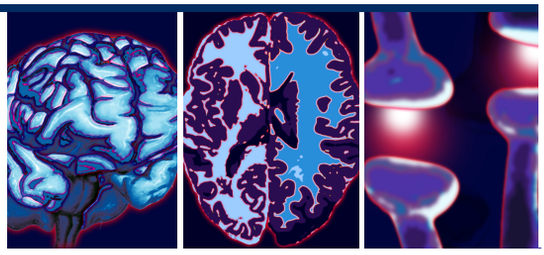

On the emergence of autism: neuroimaging findings from birth to preschool

Jason J Wolff*[1] & Joseph Piven[1]

Practice points

- Brain imaging early in development affords a unique opportunity to characterize brain development during the onset and consolidation of autism spectrum disorder (ASD).
- Due to the nonlinear effects of development, brain imaging findings from older children and adults may not inform the pathogenesis ASD.
- Neurobiological processes are not stable across development, and imaging findings should be interpreted with dynamic processes in mind.
- Although intersubject heterogeneity may confound studies of ASD, developmental brain imaging may be leveraged to clarify subtypes of autism.
- A number of reports now indicate that ASD is associated with increased cerebral brain volume and amygdala overgrowth starting as early as 2 years of age.
- Atypical patterns and trajectories of structural and functional connectivity have been observed across brain regions starting as early as 6 months of age.
- In contrast to diffusion tensor imaging findings from older children and adults with ASD, there is evidence of increased fractional anisotropy in infants and toddlers with the disorder.

SUMMARY By definition, autism spectrum disorder (ASD) emerges early in life. Core clinical symptoms generally appear after a child's first birthday, and most children receive a diagnosis by the age of 4 years. This relatively narrow window of birth to age of onset affords the opportunity to chart the neurodevelopmental processes that give rise to ASD. Although much remains unknown, magnetic resonance brain imaging studies centered around the emergence of the disorder have yielded important clues about its pathogenesis. Prominent findings include evidence of increased cortical gray and white matter volumes, increased amygdala volumes, aberrant structural and functional connectivity, and atypical neurodevelopmental trajectories. Findings to date suggest a disrupted pattern of early brain development during an interval typically characterized by dramatic experience-dependent neurobehavioral development. Developmentally informed neuroimaging studies of ASD have the potential to improve our knowledge pertaining to etiology and early intervention.

[1]Carolina Institute for Developmental Disabilities & Department of Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
*Author for correspondence: Tel.: +1 919 962 2652; jason.wolff@cidd.unc.edu

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On the emergence of autism: neuroimaging findings from birth to preschool

Autism spectrum disorder (ASD) is by far the fastest growing category of developmental disability, and recent estimates suggest that one in 161 children have the disorder worldwide [1]. While there is no evidence that ASD is differentially expressed across geographic, ethnic and socioeconomic boundaries, its prevalence in developed countries runs as high as one in 88 children in the USA [2] to a staggering one in 38 children in South Korea [3]. It is estimated that the average expense of lifetime care per individual with ASD is approximately US$3.2 million [4], representing a substantial and growing economic cost. Less easily quantified are the no less significant socioemotional costs and impediments to quality of life experienced by families and individuals impacted by ASD – costs that generally pervade across the life course [5–7].

By definition, ASD manifests early in life, and despite heterogenous patterns of symptom presentation and onset, the disorder is largely entrenched by school age save for subtle shifts in severity and adaptive behavior [8]. Findings from prospective studies of younger siblings of children with autism, who are at elevated risk for the disorder, suggest that children with ASD first diverge from typically developing (TD) peers on measures of core symptoms early in the second year of life [9–12]. Prior to this period, it is difficult to distinguish children who go on to develop ASD from those who do not, although an emerging body of evidence suggests that associated symptoms (e.g., motor delays) may be evident by as early as 6 months of age [13,14]. While such behaviors are not specific to ASD, these are nonetheless promising findings that underscore the importance of understanding how initially subtle delays may precipitate a dynamic unfolding of ASD over the first years of life.

For children who are TD, this early period is characterized by rapid brain development concomitant with the acquisition of increasingly sophisticated behaviors. MRI studies have provided rich *in vivo* data concerning the complex processes associated with early brain development [15–20]. While neurodevelopment clearly extends beyond early childhood, the alignment of timing between robust early brain change and the emergence of ASD provides a relatively narrow developmental target for study. It is during this time wherein a child transitions from not having clinically defined ASD to having it; we therefore know that ASD must arise from some process or set of processes that occur prior to the end of toddlerhood. This sets ASD apart from many other neuropsychiatric disorders, such as schizophrenia, where presymptomatic or prodromal periods are extended over wider stages of development.

The earliest brain imaging studies of infantile autism were, by and large, cross-sectional studies of adults with the disorder, or, in some cases, studies of children and adults with or without adjustment for age effects. Since brain development is neither linear nor uniform over time or across individuals, studies spanning wide age ranges or intervals implicitly treat the human brain as a static biological feature, and are potentially vulnerable to the influence of strong developmental rather than disorder-related effects [21]. These developmental events are sufficiently robust so as to possibly confound disorder-related signals. Interestingly, one of the first imaging studies of ASD, using computed tomography, identified significant cross-sectional age effects among children with autism of widely varying ages [22]. They suggested, nearly three decades ago, that brain differences associated with the disorder appear to be highly dynamic with regard to development.

To date, neuroimaging studies focused on the early developing brain in ASD remain a small fraction of a much larger body of work. Nonetheless, these studies have provided unique insights into the processes that give rise to the disorder. This article reviews MRI studies centered around the onset and early development of ASD, from birth through to preschool age. This includes MRI studies of any modality that focuses on children from birth to approximately 5 years of age, either in whole or as a subgrouping from a larger study. Studies included in this article are organized in **Table 1**.

Structural MRI

■ Brain overgrowth

The first structural MRI (sMRI) studies of individuals with ASD identified increased regional and total brain volumes [23,24], which was supported at the time by independent findings of increased head circumference [25–27] in older children and adults with the disorder. In the first sMRI studies to shift focus onto early development, Courchesne and colleagues identified higher total brain volumes and increased gray

and white matter volumes across cortical regions in a sample of 2–4-year-old children with autistic disorder versus TD controls as part of a larger cross-sectional study [28,29]. For older children and adolescents with ASD, no differences in gray and white matter volumes were identified, suggesting the possibility of developmental effects. Based on the confluence of these results, the authors suggested that overgrowth may be unique to early development in the disorder. Similar findings were later obtained by Carper and Courchesne in a study focused on frontal lobe parcellations in a refinement of their original sample of children with ASD and controls [30]. They found enlargement of the dorsolateral prefrontal and medial frontal cortices, and further identified that the brains of young children with ASD may show volume increases early on, but less change over time compared with TD children. While these combined findings suggest a pathogenic process coupled to a specific developmental interval, this conclusion is tempered by small sample sizes, particularly for the control groups, as well as a lack of longitudinal data.

Design considerations aside, independent lines of research have observed similar volumetric differences centered around the average age of onset in ASD. In a sample of 45 3–4-year-old children with ASD, Sparks *et al.* found significantly enlarged cerebral volumes, independent of IQ, in comparison with TD and developmentally delayed (DD) peers [31]. These findings are particularly compelling because they indicate that cerebral overgrowth in ASD may be specific to the disorder rather than a function of intellectual disability. This position has been further supported by the observation that underlying gray matter tissue composition differs in children with ASD aged 2–4 years, as shown by prolonged T_2 relaxation times compared with children with delayed or typical development [32]. Hazlett *et al.* replicated and downward extended these findings in a sample of 51 children with ASD, aged 1.5–3 years, and found significant enlargement of both gray and white matter volumes compared with children with developmental delay and typical controls [33]. Similar results were obtained by Hoeft and colleagues [34] in a study of 63 1–4-year-old boys with ASD relative to typical and DD controls using voxel-based morphometry. By contrast, Zeegers *et al.* found no differences in measures of both gray and white matter volumes in a clinically referred sample of 34 children aged 2–7 years with ASD compared with DD controls [35]. While this study employed close matching based on both age and developmental level, its null findings could be the result of the wider age range, which combines toddlers and school-age children, possibly washing out effects particular to early childhood.

It is notable that in the Hazlett study, brain volume differences were evident by 2 years of age, suggesting that divergence from typical development for children with ASD occurred prior to this time. Based on head circumference data obtained from a large sample of children with ASD and local controls, Hazlett and colleagues concluded that the onset of overgrowth associated with ASD occurs just prior to 1 year of age [33]. More recently, two longitudinal sMRI studies have bolstered findings concerning the timing and trajectory of early brain volume increases by examining individual change over early childhood. Schumann *et al.* tracked volumetric change in children with ASD from 1.5 to 5 years of age in comparison with TD controls [36]. They found enlargement was present, starting by 2.5 years of age in frontal, temporal and cingulate cortices, and that developmental patterns for all cortices with the exception of the occipital lobe were atypical for children with ASD. Hazlett *et al.* found significantly increased gray and white matter volumes beginning at 2 years of age, with rates of brain volume change similar for children with ASD compared with typical controls up to 4 years of age [37]. The authors also found that overgrowth was most likely linked to increased surface area rather than cortical thickness.

■ Substructure parcellations

In contrast to total brain or cerebral cortical volumes, where findings appear to converge around a common story of generalized enlargement, relatively few studies of substructures between birth and preschool age have been conducted, and conclusions remain unclear. This may be in part due to the unique methodological challenges associated with MRI segmentation in very young children, such as delineating gray and white matter boundaries, or the lack of off-the-shelf segmentation tools specific to pediatric imaging. In an early cross-sectional study of the cerebellum, Hashimoto and colleagues found that the brainstem and cerebellar vermis of children with ASD (n = 62) aged 6 months to 6 years were significantly smaller in comparison

Table 1. MRI studies of autism spectrum disorder centered on children from birth to preschool.

Study (year)	Subjects (n)	Age (years); mean (SD)	Prominent findings	Notes	Ref.
Structural MRI					
Akshoomoff *et al.* (2004)	52 ASD 15 TD	3.8 (0.8) 3.6 (1.1)	TBV: LFA > TD; cerebral GM: LFA > TD; total cerebellum: LFA > TD; cerebellar vermis I–V: TD > LFA, HFA and PDD-NOS; discriminant functional analysis classified > 90% ASD and TD cases based on MRI measures	ASD subgrouped by severity: LFA (n = 30); HFA (n = 12); and PDD-NOS (n = 10)	[41]
Bloss and Courchesne (2007)	9 female ASD 27 male ASD 14 female TD 13 male TD	3.7 (0.9) 3.7 (0.8) 3.8 (1.1) 3.6 (1.2)	ICV, cerebral GM, cerebellar GM and WM: female ASD > female TD; cerebral GM and cerebellar WM: male ASD > male TD	–	[40]
Carper *et al.* (2002)	12 ASD 8 TD	3.5 (0.4) 3.4 (0.6)	Frontal and parietal WM: ASD > TD; frontal and temporal GM: ASD > TD; parietal GM: TD > ASD	Age cohort from larger study	[29]
Carper and Courchesne (2005)	25 ASD 18 TD	5.2 (1.6) 5.1 (1.8)	Dorsolateral and medial PFC, age <5 years: ASD > TD	Subjects aged <5 years: ASD (n = 12) and TD (n = 9)	[30]
Courchesne *et al.* (2001)	30 ASD 12 TD	3.7 (0.7) 3.7 (0.7)	TBV: ASD > TD in total sample; cortical GM, WM and cerebellum: ASD > TD in 2–3-year-old subsample	Age cohort from larger study	[28]
Hashimoto *et al.* (1995)	102 ASD 112 TD	6.1 (4.7) 7.1 (5.4)	Cerebellar vermis, brainstem: ASD < TD; age-related trend for pons and cerebellar vermis: ASD > TD	Cross-sectional age effect study	[38]
Hazlett *et al.* (2005)	51 ASD 14 TD 11 DD	2.7 (0.3) 2.4 (0.4) 2.7 (0.4)	TBV, cerebral GM and WM: ASD > TD and DD; cerebral WM: ASD > TD; TBV, cerebral GM and WM: ASD > DD; cerebellum: not significant	Age and sex adjusted	[33]
Hazlett *et al.* (2011)	Time 1: 59 ASD 26 TD 12 DD Time 2: 36 ASD 15 TD 6 DD	 2.7 (0.3) 2.5(0.5) 2.8 (0.4) 5.0 (0.4) 4.6 (0.3) 5.0 (0.5)	TBV, cerebral GM and WM: ASD > TD and DD; cerebral GM and WM: ASD > TD; TBV, cerebral GM and WM: ASD > DD; cerebellum: not significant; parallel growth trajectories across groups. Overgrowth associated with increased cortical surface area	Longitudinal; age, sex and IQ adjusted	[37]
Hoeft *et al.* (2011)	63 ASD 52 FXS 31 TD 19 DD	2.8 (0.4) 2.9 (0.6) 2.6 (0.6) 3.0 (0.5)	Dissociable pattern across multiple GM and WM ROIs/lobes: ASD > TD and DD > FXS; cerebellar and fusiform gyri: TD and DD > ASD; caudate and cingulate: FXS > ASD	Voxel-based morphometry	[34]
Mosconi *et al.* (2009)	Time 1: 50 ASD 11 DD 22 TD Time 2: 31 ASD 6 DD 14 TD	 2.7 (0.3) 2.8 (0.4) 2.5 (0.5) 5.0 (0.4) 5.0 (0.5) 4.6 (0.5)	Amygdala (bilateral): ASD > TD and DD at times 1 and 2; growth rate: ASD = TD and DD; higher volume associated with less impaired joint attention in ASD	Longitudinal; age, sex and IQ adjusted	[43]

Time 1, 2 and 3 refers to a longitudinal study; participants were assessed at multiple time points.
ASD: Autism spectrum disorder; ASD+: With autism spectrum disorder; ASD-: Without autism spectrum disorder; DD: Developmentally delayed; FA: Fractional anisotropy; FXS: Fragile X syndrome; GM: Gray matter; HFA: High functioning autism; HR: High-risk infant sibling; ICV: Intercranial volume; IFG: Inferior frontal gyrus; LD: Language delay; LFA: Low-functioning autism; MA: Mental age; NR: Not reported; PDD-NOS: Pervasive developmental disorder not otherwise specified; PFC: Prefrontal cortex; ROI: Region of interest; SD: Standard deviation; STG: Superior temporal gyrus; TBV: Total brain volume; TD: Typically developing control; WM: White matter.

Table 1. MRI studies of autism spectrum disorder centered on children from birth to preschool (cont.).

Study (year)	Subjects (n)	Age (years); mean (SD)	Prominent findings	Notes	Ref.
Structural MRI (cont.)					
Sparks *et al.* (2002)	45 ASD 26 TD 16 DD	4.0 (0.4) 4.0 (0.5) 4.0 (0.5)	Total cerebral volume: ASD > TD and DD; amygdala volume: severe ASD > TD; IQ not associated with volume measures in ASD	Age, sex and total cerebral volume adjusted	[31]
Webb *et al.* (2009)	45 ASD 26 TD 14 DD	3.9 (0.3) 3.9 (0.5) 4.0 (0.5)	Cerebellar vermis I–V and VI–VII: TD > ASD > DD	Age, sex and total cerebral or cerebellar volume adjusted	[39]
Zeegers *et al.* (2009)	34 ASD 13 DD	3.7 (0.9) 3.4 (1.0)	ICV, TBV, cerebellum, amygdala, cerebral GM and WM: not significant	Age and ICV adjusted	[35]
Diffusion tensor MRI					
Ben Bashat *et al.* (2007)	7 ASD 41 TD	1.8–3.3 (range) 0.3–23 (range)	Increased FA, probability and displacement; multiple ROIs	Cross-sectional age curve estimation	[59]
Cascio *et al.* (2012)	33 ASD 17 TD 8 DD	4.6 (1.1) 3.2 (1.3) 3.9 (1.1)	Cortical/cerebellar FA distributions (variability): ASD < TD and DD	Age and sex adjusted	[64]
Weinstein *et al.* (2011)	22 ASD 28 TD	3.2 (1.1) 3.6 (1.2)	FA, genu and body of corpus callosum , right cingulum, left superior longitudinal fasciculus: ASD > TD	Age adjusted	[60]
Wolff *et al.* (2012)	Time 1: 28 HR ASD+ 64 HR ASD- Time 2: 17 HR ASD+ 49 HR ASD- Time 3: 17 HR ASD+ 33 HR ASD-	 0.6 (0.07) 0.6 (0.07) 1.1 (0.06) 1.1 (0.05) 2.0 (0.05) 2.1 (0.07)	FA growth trajectories significantly steeper for HR ASD- vs HR ASD+ for 12 WM tracts; HR ASD+ characterized by higher FA at 6 months but lower FA at 24 months relative to HR ASD-	Longitudinal; age and IQ adjusted	[61]
Functional MRI					
Dinstein *et al.* (2011)	29 ASD 13 language delay 30 TD	2.4 (NR) 1.6 (NR) 2.3 (NR)	Interhemispheric synchronization, STG and IFG: ASD < LD = TD; IFG correlated with expressive language in ASD	–	[74]
Eyler *et al.* (2012)	40 ASD 40 TD	2.7 (NR) 2.1 (NR)	Activation, division of STG (Broadmann 22): ASD < TD; middle occipital gyrus: ASD > TD; left anterior STG: negatively correlated with age in ASD but not TD	Passive auditory speech task; some age adjusted	[73]
Redcay and Courchesne (2008)	12 ASD 12 TD 11 MA match	2.9 0(0.6) 3.0 (0.4) 1.6 (0.4)	Multiregion activation: ASD < mental age match; left anterior cingulate, middle frontal, middle temporal and STG: TD > ASD; receptive language correlated with right hemisphere frontotemporal activation	Passive auditory speech task	[72]

Time 1, 2 and 3 refers to a longitudinal study; participants were assessed at multiple time points.
ASD: Autism spectrum disorder; ASD+: With autism spectrum disorder; ASD-: Without autism spectrum disorder; DD: Developmentally delayed; FA: Fractional anisotropy; FXS: Fragile X syndrome; GM: Gray matter; HFA: High functioning autism; HR: High-risk infant sibling; ICV: Intercranial volume; IFG: Inferior frontal gyrus; LD: Language delay; LFA: Low-functioning autism; MA: Mental age; NR: Not reported; PDD-NOS: Pervasive developmental disorder not otherwise specified; PFC: Prefrontal cortex; ROI: Region of interest; SD: Standard deviation; STG: Superior temporal gyrus; TBV: Total brain volume; TD: Typically developing control; WM: White matter.

with TD controls in a large imaging study [38]. More recently, Webb and colleagues identified reduced volumes across cerebellar vermis in toddlers with ASD relative to controls [39]. Bloss and Courchesne identified significantly lower cerebellar volume in girls with ASD, the only region for which this group was below that of TD controls [40]. In studies by Hazlett *et al.* [33,37] and Sparks *et al.* [31], however, the cerebellum was not significantly different between TD children and children with ASD when controlling for total cerebral volume. Meanwhile, Akshoomoff *et al.* identified increased cerebellar white matter and enlargement of anterior cerebellar vermis in 52 2–5-year-old children with ASD compared with TD controls [41]. In a discriminate function analysis, they found that children with ASD were well classified from one another (based on severity) and from controls using a combination of either cerebellar white matter volume, or cerebellar and cerebral white matter volumes and cerebellar vermus areas.

Sparks and colleagues identified enlargement of both the bilateral hippocampus and amydala in their sample of 3–4-year-old children with ASD [31]. However, controlling for total cerebral volume, only the amygdala remained enlarged relative to total cerebral volume, and only in the subgroup of children with more severe autism (autistic disorder). The finding of early amygdala overgrowth was replicated by Schumann and colleagues, who noted that bilateral amygdala volume increases were most pronounced in females with autism compared with controls [42]. Amygdala volume positively correlated with Autism Diagnostic Interview-Revised social scores from age 5 years, but were negatively correlated with communication as measured by the Vineland Adaptive Behavior Scales. In a longitudinal study, Mosconi *et al.* similarly found that the amygdala was enlarged in toddlers with ASD (n = 50) compared with controls (n = 33) starting at age 2 years, and that this difference remained stable at age 4 years [43]. Interestingly, they also found that amygdala enlargement was associated with better joint attention outcomes among children with ASD by preschool age.

A few common themes emerge from existing sMRI studies of ASD centered on early childhood. The findings that cortical gray matter volumes and, to a lesser extent, white matter volumes, are elevated as early as 2 years of age are the most consistent [28–31,33,34,36,37,40,41]. It is worth noting that these volumetric differences appear disorder-specific and are not linked to the sex of the individual [40] or familial risk/endophenotype [44]. The timing of volumetric increase in children with ASD offers what is perhaps the strongest indirect clue as to the underlying pathogenic process given that it occurs in tandem with a peak period of synaptic density and robust pruning in TD children [45,46]. While there is some evidence that the overgrowth associated with ASD may be secondary to atypical synaptogenesis or pruning [47], a direct link has yet to be established and it is possible that early increases in brain volumes stem from other processes, such as the hyperproliferation of progenitor cells [37,48].

Regarding the amygdala, Sparks and colleagues suggest that overgrowth may be linked to early socioemotional development underlying ASD onset, noting that null findings or findings of smaller amygdala among older individuals with the disorder [31,49,50] are consistent with a developmental framework wherein dynamic shifts in form and function are expected. In agreement with this view, a recent longitudinal study by Nordahl and colleagues identified initial enlargement and a steeper growth trajectory for the amygdala in 2–4-year-old boys with ASD compared with TD controls [51]. While not fully supported by the literature [52,53], converging evidence suggests that amygdala overgrowth in children with some variants of ASD may be unique to early development, with growth slowing by middle childhood to more closely match volumes and rates of change observed in children who are TD [54,55].

Diffusion tensor MRI

Diffusion tensor MRI (DTI) is an approach to structural imaging based on diffusion-weighted MRI data, which is itself based on the motion of water molecules through tissue [56,57]. DTI allows for the magnitude and direction of diffusion to be estimated and, in the living brain, this information may be used to derive the microstructural properties of white matter circuitry. Common measures derived from DTI data include mean diffusivity; axial and radial diffusivity, representing diffusion along the principle and transvere eigenvectors, respectively; and fractional anisotropy (FA), a scalar measure reflecting the magnitude of diffusion along the principle eigenvector relative to transverse directions. Rather than characterizing a single structural attribute, these measures reflect multiple facets of tissue composition, including axon size,

density, cohesion and degree of myelination. This imaging modality has been increasingly utilized in studies of ASD, and numerous findings from older children, adolescents and adults with the disorder generally point toward aberrant neural circuitry encompassing multiple brain regions [58]. However, because this relatively new body of work involves disparate approaches, measures and samples, it is difficult to characterize the precise nature of atypical white matter connectivity associated with the disorder.

Of the approximately 60 DTI studies of ASD, only a handful have centered on children with ASD prior to school age. Ben Bashat and colleagues first found evidence of atypical age effects in a cross-sectional study of seven children with ASD aged 1.8–3.3 years [59]. The authors further identified group differences in a variety of diffusion measures, including FA, across fiber tracts including the internal and external capsules, forceps minor and corpus callosum. Notably, the group of children with ASD had higher FA values than TD controls. Weinstein and colleagues similarly found increased FA in the cingulum bundle, left superior longitudinal fasciculus, and genu and body of the corpus callosum in 21 children with ASD aged 1.5–6 years using tract-based spatial statistics [60]. Once again, higher FA across a number of fiber pathways was indentified in a relatively narrow age band centered around the average age of diagnosis.

In a longitudinal DTI study of infant siblings of children with ASD, Wolff and colleagues found significant differences in developmental trajectories for multiple white matter pathways measured by FA starting at 6 months age between children who did (n = 28) versus those who did not (n = 64) show evidence of ASD at 2 years of age [61]. Twelve out of 15 fiber pathway trajectories were significantly different between groups and were in large part characterized by higher FA at 6 months of age followed by a blunted change over time such that children with ASD had lower FA values by the age of 2 years. These findings highlight the dynamic and shifting nature of early brain development in ASD and underscore the critical importance of accounting for such factors through design. The authors suggest that altered neural circuitry appears to precede the clinical onset of ASD, with its emergence concurrent with an atypically blunted neurodevelopmental pattern. In line with previous findings [59,60], Wolff *et al.* identified significant differences in pathways linked to a broad range of brain regions, suggesting that ASD may arise from a pervasive, global aberration rather than one initially limited to regions functionally linked to core domains such as social communication [61]. It is possible that findings of more localized differences associated with ASD later in life may be the result of development or, more specifically, functional specialization.

In contrast to findings from older children and adults, each study of infants and toddlers includes findings of increased FA associated with ASD. While the precise nature and timing of this phenomenon requires further investigation, it is nonetheless a potentially important clue concerning the emergence of ASD. Findings from early childhood suggest that by school age, children with ASD exhibit a pattern of decreased FA compared with TD children similar to that seen in adolescents and adults [58]. While higher FA in the adult brain may reflect more robust white matter connectivity, this conclusion may not hold for very young children, as the neurobiology underlying this metric is not isomorphic across development. During the first years of life, white matter pathways undergo a process of dynamic refinement, which includes substantial pruning in addition to growth and myelination [62,63]. Thus, the observation that FA is increased in infants and toddlers with ASD may reflect excess axonal fibers resulting from less responsive developmental elimination.

In partial agreement with this interpretation, Cascio *et al.* found less variability in multiregion white matter FA in 2–6-year-old children with ASD compared with combined typical and DD controls [64]. The authors posited that this relatively restricted variance may reflect decreased refinement during an interval typically characterized by robust alteration of white matter structure. A similar phenomenon has been observed in children with Williams's syndrome, wherein increased FA has been associated with functional impairment [65]. The notion that increased FA represents dampened axon elimination is consistent with sMRI findings of increased white matter volumes specific to this age interval [28,29,33,34,37] as well as histological findings [66,67]. In both the studies by Weinstein *et al.* [60] and Wolff *et al.* [61], there were no significant differences in most measures of axial or radial diffusivity alone, the latter of which is believed to be particularly sensitive to density.

Weinstein and colleagues did note, however, that FA differences appeared to be driven by developmental changes in radial diffusivity. This is consistent with evidence concerning the dynamic relationship between axial and radial diffusivities in the early developing brain [68], although it remains unclear which aspects of underlying neurobiology these measures reflect, and thus results based on DTI data must be interpreted with this limitation in mind [69].

Functional MRI

Given the practical limitations of conducting task-based functional MRI (fMRI) among young children [70], a small but expanding number of studies have examined blood oxygen level-dependent activation patterns during natural sleep in young children with ASD [71]. This approach is presumed to provide data concerning either resting state functional connectivity (e.g., co-activation across brain regions) or functional activation of specific regions in passive response to stimuli (e.g., auditory tones). In a preliminary study of toddlers with ASD (n = 12), Redcay and Courchesne found no significant differences in blood-oxygen level dependent response to spoken language in comparison with matched controls during natural sleep [72]. However, the authors pointed to a trend toward an unexpected pattern of right temporal response to language in the ASD group. Following this initial study, Eyler and colleagues examined a similar paradigm among 40 children aged 12–48 months with ASD [73]. They found less left lateralized response to a variety of speech sounds and atypical right lateralization in response to language. This same group has also recently identified reduced co-activation of bilateral inferior frontal gyri and bilateral superior temporal gyri in 29 toddlers with ASD compared with both children with language delay (n = 13) and TD controls (n = 30) [74]. The authors suggested that these findings point to dampened synchronization of regions implicated in language processing, a position supported, in part, by a significant and positive correlation between a measure of inter-hemispheric synchrony in the inferior frontal gyri and expressive language.

Sleep-based fMRI is a nascent, but potentially fruitful, inroad for investigating functional brain development among young children with or at risk of ASD. One study, to date, has identified atypical and less robust modular activation in response to speech stimuli in children with ASD [73], and follow-up work has linked interregional synchronization of putative language areas to verbal behavior [74]. The findings by Dinstein and colleagues also add support to the view that ASD may be characterized by atypical functional connectivity [74]. Whether and how such patterns of connectivity contribute to the emergence of ASD in early in life is fodder for future research.

Although well suited to studies of infants and toddlers, there are inherent limitations to sleep-based fMRI. Studies incorporating stimuli are bounded by the necessity to scan children during sleep or sedation, naturally limiting the scope of stimuli available. While feasible to examine responses to speech sounds, for instance, fMRI obtained during sleep rules out investigations of activation in response to visual and social stimuli. Regarding functional connectivity, this promising approach to neuroimaging should be carefully considered, as many questions remain as to its validity. For example, signal in fMRI is susceptible to very minor differences in autonomic functioning or micromovements present during natural sleep [75,76], the effects of which are variable across sleep stages. These factors are not fully addressed by common image processing procedures and are capable of producing misleading results. Assuming these artifacts can be ameliorated, however, fMRI data collected during sleep may reveal new knowledge apropos to an aberrant connectivity hypothesis of ASD [77] whereby local and distal connections are atypically organized. A multimodal approach that includes DTI to account for both structural and functional connectivity – particularly across regions over time – may be a particularly powerful means of answering whether the brains of children with ASD are wired differently early in life.

Conclusion & future perspective

A preponderance of behavioral findings point to a dynamic unfolding of the ASD phenotype over the first years of life, from birth until the average age of diagnosis between 3 and 5 years of age [2]. During this relatively narrow interval, a child transitions from appearing typical across multiple domains and developmental measures to fully manifesting ASD [9–12]. Implicated in this dramatic and critical shift from typical to grossly atypical development are the neurodevelopmental processes most active during this time. In just over 10 years, *in vivo* MRI has provided important clues as to the nature

and timing of such processes, and their role in the emergence of ASD. This includes evidence of brain overgrowth concurrent with the appearance of clinical symptoms encompassing cortical gray and white matter across brain regions [28–31,33,34,36,37,40,41] and the amygdala [31,42,43,51]. Emerging lines of research using DTI and fMRI have further provided evidence of atypical connectivity encompassing diverse fiber pathways, beginning as early as 6 months age [59–61], and aberrant functional organization as early as 12 months [73,74]. It is revealing that ASD arises during a unique period of robust experience-dependent development characterized by exuberant growth and sculpting through rapid refinement. One outstanding question is how the atypical trajectory culminating in ASD is linked to such early neurodevelopmental processes.

Neurodevelopment associated with ASD may be linked to atypical or interrupted experience-dependent refinement early in life. Functional specialization, the constructive process through which a typical and healthy brain is organized, requires that both individual regions and interconnected networks become more selective and efficient with regard to response to environmental stimuli [78]. In the developing brain, this dynamic process is characterized, in part, by changing patterns of connectivity that favor long-range networks over time [17,79], the disruption of which has been frequently associated with ASD [58,74,77,80]. If neurobehavioral feedback is not selectively transmitted in support of experience-dependent or experience-expectant processes, reciprocal brain–behavior refinement could stall. In infants and toddlers with hearing impairment, for example, decreased auditory feedback delays synaptic maturation as evidenced by increased gray matter density following less pruning [81], a phenomenon not unlike the increases in cortical volumes seen in young children with ASD. Interestingly, increased gray matter associated with hearing impairment is a developmentally dependent phenomenon; it is evident during childhood but not later in life [82], mirroring the pattern observed in some studies of ASD [29,55,83].

Even subtle delays present in early infancy could, through escalation, widen the gulf between environmental demands and a child's neurobehavioral capacity to meet them. Cohen and colleagues recently found that processing deficits involving sensory feedback may be implicated in the early emergence of ASD [84]. In their study, infants born preterm who went on to develop ASD showed greater insensitivity to auditory stimuli at 4 months of age, well in advance of clinical onset. Similarly, Cornish *et al.* found that low-level sensory deficits predicted later severity of autistic symptoms in children with fragile X syndrome, a genetic neurodevelopmental disorder commonly associated with ASD [85]. Elison and colleagues recently identified a disorder-specific deficit in visual orienting in 7-month-old infants who went on to develop ASD. Interestingly, the authors also found that expected brain–behavior relationships between ocular motor and visual orienting behaviors, present in TD infants, were absent in the ASD group [86]. Prominent among these findings is that the microstructure of the splenium of the corpus callosum, implicated in putative orienting networks, was only uniquely associated with visual orienting in children who were TD. Early aberrations in the neurobehavioral development of sensory motor response and subsequent visual orienting behavior could have downstream effects on the acquisition of more complex joint attention and related social communication skills. While hyposensitivity and orienting deficits are not themselves symptoms of ASD, they may be early links in a chain of events culminating in the core deficits associated with the disorder. **Figure 1** illustrates a hypothetical model of this constructive process.

At the cellular level, there is evidence that insensitivity to basic sensory feedback is associated with decreased refinement of dendritic spines in a mouse model of fragile X syndrome [87]. The apparent disconnect between external stimuli and neural refinement in this model culminates in cortical overgrowth relative to controls [88]. Atypical refinement during early development is associated with reduced synaptic response and generalized impairment of neural circuitry [89], which may precipitate a behavioral phenotype consistent with ASD [90]. While certainly promising, such findings may be limited in scope to an etiologically defined variant of ASD, that is fragile X syndrome. The noise from variable genetic and neurobehavioral trajectories is an ever-present confound to studies of ASD. While perhaps an overused justification for incongruent results in the field, it is increasingly clear that subtypes of autism, known and unknown, differ by ontogeny and neurobehavioral phenotype [91–93], the effect of which cannot be underestimated. For example, there is evidence suggesting that key neurobehavioral

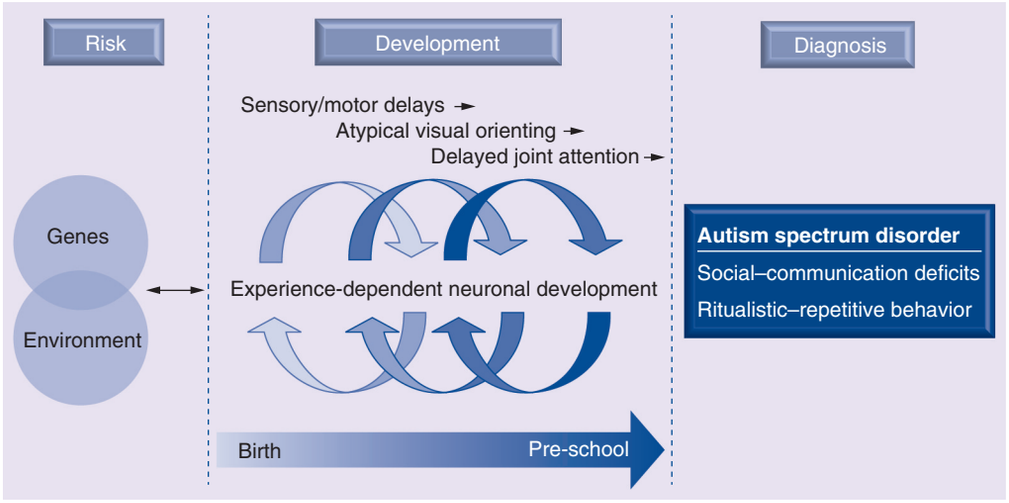

Figure 1. Hypothesized framework for the early development of autism spectrum disorder. Conferred genetic and environmental risk are reciprocal with neurobehavioral events. The constructive processes of neuronal and behavioral development proceed bidirectionally and in response to the increasingly sophisticated demands of the child's environment. In autism spectrum disorder, this early development may be progressively atypical, wherein minor delays early in life culminate in the primary deficits associated with the disorder during toddlerhood. As an example of such a cascade of events, early sensory-motor delays could engender atypical patterns of visual orienting to salient features of the infant's environment, giving rise to atypical or delayed joint attention during a sensitive period during which the development of foundational social-communication skills typically occur.

features of fragile X autism may not align with those characteristic of idiopathic autism [34,53,94]. To clarify endophenotypes of ASD, brain imaging studies might account for the interplay of background or modifier genes and neurobehavioral phenotype early in development. For instance, deletion of *CTNNB1* or GSK-3 has been associated with altered neurogenesis characterized by overgrowth secondary to increased cortical surface area [48,95], phenomenon that may be associated with specific variants of ASD [96]. Rudie and colleagues recently found that a *MET* risk allele was associated with altered structural and functional connectivity as well as specific neurobehavioral aspects of the ASD phenotype [97]. Neuroimaging studies have clear potential to clarify the heterogeneity of ASD by accounting for behavioral and genetic variations and their effects on the risk and manifestation of the disorder [97–99].

Although significant progress has been made in a relatively short time, many questions remain regarding the complex but crucial neurodevelopmental story of ASD. As increasingly advanced neuroimaging techniques become available, so too does the opportunity to find answers. Improved technology is a necessary, but insufficient, aspect of optimal neurodevelopmental research, and studies of ASD need to pay careful attention to behavioral and developmental phenomenon. For example, longitudinal findings have made clear the dynamic nature of early development [10,11,15,16,18–21,36,61,100], giving researchers strong empirical reasons to temper inferences based on cross-sectional data alone and perhaps abandon such approaches altogether in favor of a longitudinal design [21,101]. Given the nonlinear nature of development and the compounding effects of interindividual heterogeneity, a concerted effort to account for individual differences over time would help to clarify the many complexities associated with the emergence of ASD.

Perhaps the most compelling reason to focus such efforts on infancy and toddlerhood is the potential to identify targets for early or preventative intervention. Just as the early developing brain is vulnerable to pathoplastic risk factors, it is also highly resilient. For example, preclinical work has found that increased early socialization through peer interaction and play enhances neural plasticity, accelerating pruning and leading, ultimately, to decreased dendritic density [102]. In toddlers with

ASD, there is now evidence that early behavioral intervention improves neural response to social stimuli as measured by electroencephalography [103]. Such findings may be considered a bellwether of innovative efforts to integrate neuroscience with clinical research. Understanding how and when early risk for later deficits may be ameliorated by intervention is a clear and common goal for research efforts in ASD, and is one well served by the diligent use of neuroimaging.

Financial & competing interests disclosure

This work was supported in part by an Autism Center of Excellence Network grant to J Piven from the NIH (HD055741). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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