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REVIEW

Structural, Functional, and Molecular Imaging of Autism Spectrum Disorder

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Abstract Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder associated with both genetic and environmental risks. Neuroimaging approaches have been widely employed to parse the neurophysiological mechanisms underlying ASD, and provide critical insights into the anatomical, functional, and neurochemical changes. We reviewed recent advances in neuroimaging studies that focused on ASD by using magnetic resonance imaging (MRI), positron emission tomography (PET), or single-positron emission tomography (SPECT). Longitudinal structural MRI has delineated an abnormal developmental trajectory of ASD that is associated with cascading neurobiological processes, and functional MRI has pointed to disrupted functional neural networks. Meanwhile, PET and SPECT imaging have revealed that metabolic and neurotransmitter abnormalities may contribute to shaping the aberrant neural circuits of ASD. Future large-scale, multi-center, multimodal investigations are essential to elucidate the neurophysiological underpinnings of ASD, and facilitate the development of novel diagnostic biomarkers and better-targeted therapy.

Keywords Autism spectrum disorder · Positron emission tomography · Magnetic resonance imaging · Molecular imaging · Functional connectivity · Serotonin · Oxytocin

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Introduction

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder characterized by impaired social communication and restricted, repetitive behaviors [1], typically emerging at about 24 months of age. The global prevalence rate of ASD is about 1% [2], and its prevalence rate in Chinese children is estimated at 0.7% [3]. According to the latest report, ASD is associated with an annual economic burden of >250 billion dollars in the USA, mainly due to special education costs, higher medical costs, and loss of parental labor [4].

The etiologies of ASD are complex and are thought to be attributable to both genetic and environmental risk factors [5]. Evidence from genetic research has associated >100 genetic variants with ASD risk [6]. Currently, the clinical diagnosis of ASD is mainly based on observation of behaviors [7], and there is no valid biomarker that can aid in its diagnosis, or predict the onset, progression, or

severity. Furthermore, it is primarily treated through behavioral and educational interventions with no available medication for treating its core neurobiological determinants [8]. Hence, it is a priority to better understand the neurobiological mechanisms in order to provide early biologically-based diagnosis and more effective therapeutic interventions for ASD.

Neuroimaging provides a non-invasive window into the human brain. Over the past few decades, neuroimaging modalities including positron emission tomography (PET) and single-positron emission computed tomography (SPECT) combined with structural and functional magnetic resonance imaging (MRI) have been increasingly used to explore the neural anatomical, functional and molecular bases underlying ASD. Molecular imaging techniques such as PET and SPECT allow the mapping of biological processes *in vivo* at the cellular and molecular levels, and facilitate the non-invasive visualization of ASD-specific metabolic and neurochemical changes *in vivo* [9, 10]. MRI provides anatomical and functional information underlying ASD.

In the current review, we present the recent advances in structural, functional, and molecular neuroimaging for ASD. We included original neuroimaging articles on ASD published between 2010 and June 30, 2020, particularly structural MRI, resting-state functional MRI, PET, and SPECT, and briefly summarize the main findings followed by discussion of future directions. We did not cover task-related functional MRI due to space limitations. We searched PubMed using the following search terms: "autism", "magnetic resonance imaging", "connectivity", "resting state", "perfusion", "positron emission tomography", and "single photon emission computed tomography" alone and in combination. Neuroimaging studies examining ASD and typically developing (TD) subjects were included. We screened each retrieved article by the relevance of its abstract, and checked the reference list of each article for further relevant publications.

MRI

MRI is a versatile imaging modality that is capable of probing extensive physiological processes based on the special spin properties of protons and neutrons [11]. One key strength of MRI is its superb spatial resolution (micrometers), enabling the *in vivo* detection of subtle changes in brain morphology. Using specialized techniques, diffusion tensor MRI interrogates the microstructure of white matter based on free water diffusion, and arterial spin labeling (ASL) allows the quantitative measurement of tissue perfusion. In addition, functional MRI enables the investigation of functional connectivity patterns based on the blood-oxygen-level-dependent signal [12]. In particular, resting-state functional MRI and structural MRI have been widely employed to investigate the neural correlates of ASD (Fig. 1).

MR Imaging of Brain Morphology

Numerous structural MRI studies on ASD have indicated alterations in brain morphology, mainly in cortical surface area and thickness, gray matter volume, and white matter connectivity, particularly in the frontal cortex, temporal cortex, and amygdala [13–17]. Table 1 summarizes the main findings of structural MRI studies in ASD.

Cortex

Voxel-based morphometry is an objective and pragmatic approach to assessing anatomical abnormalities (for methodology, see [18]). By using voxel-based approaches, gray matter volume abnormalities have been identified throughout the brain in ASD [15, 16]. Recent meta-analysis of voxel-based studies in ASD has provided evidence of age-related cerebral enlargement, particularly gray matter overgrowth in the prefrontal cortex (PFC) [13, 14]. The PFC is involved in multiple cognitive and social functions, such as social cognition, inhibition [19, 20], working memory [21], language [22], motivation, and reward-based learning [20]. Thus, structural abnormalities in the PFC may be associated with the social impairment and language deficits in ASD.

Emerging evidence from longitudinal neuroimaging studies has indicated age-specific anatomical changes and atypical neurodevelopmental trajectories in ASD [16, 23–25]. For instance, autism is marked by brain overgrowth during infancy and the toddler years, followed by an accelerated rate of decline in size and perhaps degeneration from adolescence to adulthood when decreases in structural volume are observed. This has led to the theory of age-specific anatomic abnormalities in autism [23], which may be related to age-specific changes in gene expression, and molecular, synaptic, and cellular as well as circuit abnormalities. To address the original cause driving these age-specific changes in anatomical abnormalities is a challenge in autism research, and it has been suggested that the optimum age for studying the pathophysiology of autism is the first three postnatal years and prenatal life [23].

A number of longitudinal studies focused on infants and toddlers offer critical insights into the atypical neurodevelopmental trajectories of ASD in early postnatal life [17, 24–33]. A recent prospective study on infants at risk for ASD (106 high-risk and 42 low-risk) has revealed that children who go on to develop ASD show cortical surface

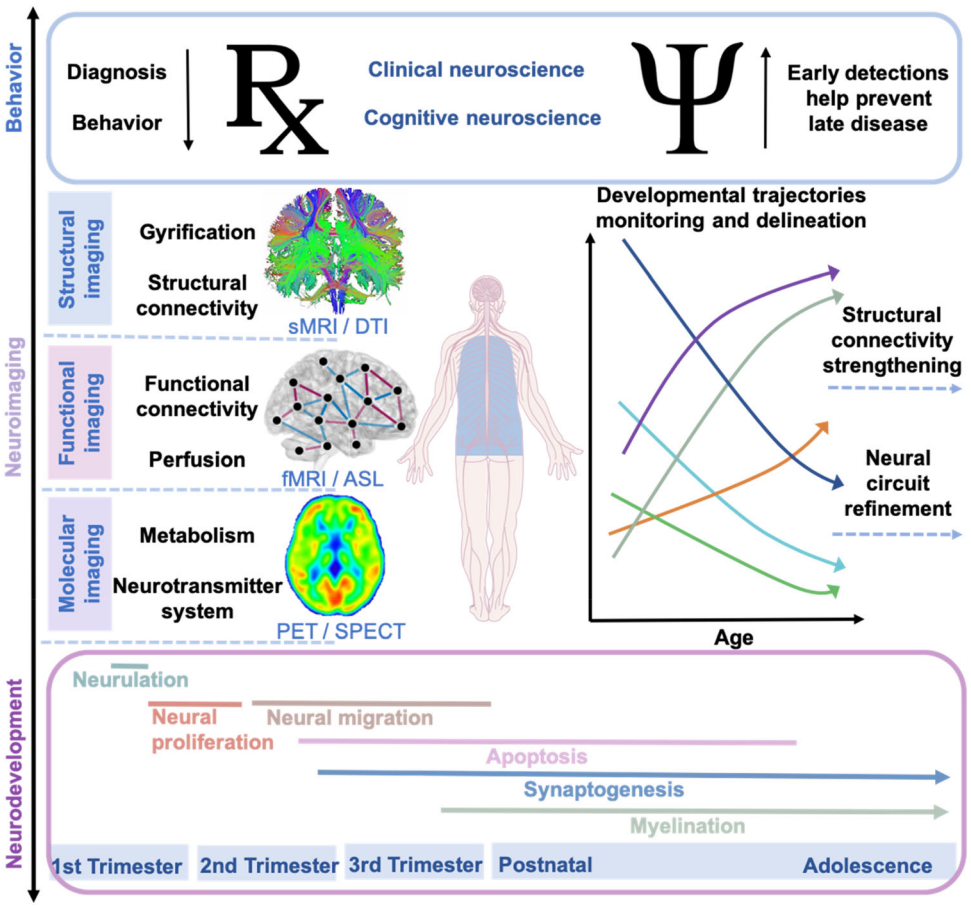

Fig. 1 Schematic of multilevel imaging-based studies on ASD brain development in the context of the temporal dimension (horizontal axis) at different scales (vertical axis). Different indices are derived from structural, functional, and molecular imaging, such as gyrification, structural connectivity, functional connectivity, perfusion, metabolism, and neurotransmitter systems. Longitudinal neuroimaging studies monitor and delineate brain developmental trajectories in ASD, which are postulated to be related to cascading neurodevelopmental processes. Bottom panel, prominent processes relevant to ASD during fetal and post-natal brain development. Adapted from [163].

area hyper-expansion from 6 months to 12 months of age. This hyper-expansion starts in domains mediating auditory and visual processing and is highly predictive (a positive predictive value of 81%) of the diagnosis of ASD in high-risk infants [29]. The presence of cortical surface area hyper-expansion precedes the onset of brain volume overgrowth in the second year after birth [26, 29, 33]. Besides, several studies found no differences in cortical thickness in ASD toddlers [29, 30, 33]. Thus, it has been suggested that the early cortical overgrowth in ASD may be driven by accelerated cortical surface area expansion rather than increased cortical thickness [29, 30, 33], since distinct developmental mechanisms may account for the radial expansion that produces the multilayered cortex of mammals and for the tangential expansion of cortical surface area [34]. Specifically, tangential expansion of the neocortical surface area is closely associated with the number of radial units formed by symmetrical divisions along the ventricular zone, whereas cortical thickness is controlled by the number of asymmetric radial glial cell divisions [35, 36]. Since each round of mitosis results in an exponential increase in the number of progenitor cells, small changes affecting the duration of symmetric growth will have a dramatic impact on surface area. Thus, cortical surface area hyper-expansion in ASD individuals is possibly attributed to dysregulated neural progenitor cell proliferation and differentiation, and this has been supported by the findings from ASD patient-derived neural progenitor cells showing excessive proliferation compared with normal controls [37–39]. On the other hand, incomplete synaptic pruning could also contribute to the brain overgrowth in ASD [40], but the underlying mechanisms are not conclusive and need to be further elucidated in future studies.

Besides, atypical cortical folding has been indicated in ASD [41, 42], as measured by the local gyrification index (lGI). Gyrification, the process by which the brain forms sulcal and gyral regions, allows for optimized compact wiring of neuronal fibers that promotes efficient neural processing in the brain [43]. In individuals with ASD aged 7 years–19 years, lGI is increased in the left parietal and temporal and right frontal and temporal regions compared with TD [42]. lGI declines with age, but more steeply in ASD aged 41 years–61 years; compared with TD, lGI is decreased bilaterally in insular and anterior cingulate cortex (ACC), left postcentral, and orbitofrontal and supramarginal regions. Gyrification is postulated to be influenced by axonal tension [44] or differential expansion

Table 1 MR imaging of brain morphology.

Reference	Age range	Brain regions	Main findings in ASD group
Schumann *et al.* [15]	Longitudinal, 1.5-5 years	Cerebrum	↑ GMV and WMV in cerebrum; notably in frontal, temporal, and cingulate cortices
Hazlett *et al.* [17]	18-35 months	Cerebrum; cerebellum	↑ GMV and WMV in in frontal, temporal, and parietal-occipital lobes
Sparks *et al.* [70]	3-4 years	Cerebrum; cerebellum; hippocampus; amygdala	↑Volumes in cerebrum, cerebellum, amygdala, and hippocampus
Courchesne *et al.* [24]	2-16 years	Cerebrum; cerebellum; cerebellar vermis	↑ Cerebral GMV and WMV; cerebellar WMV; ↓ cerebellar GMV, cerebellar vermis lobules VI–VII in 2- to 3- year-olds
Carper *et al.* [25]	2-9 years	Cerebrum; frontal lobe	↑ Volumes in dorsolateral and medial frontal regions in those under age 5
Shen *et al.* [26]	(Longitudinal, prospective) 6-9, 12–15, and 18–24 months	Cerebrum; extra-axial CSF	↑ Extra-axial CSF, particularly over the frontal lobes (6-9 mo); ↑ total cerebral volume (12-15 and 18–24 mo)
Hazlett *et al.* [29]	(Longitudinal, prospective) 6-7, 12-13, 24-25 months	Global brain tissue; surface area; cortical thickness	↑ Cortical surface area (6-12 mo); ↑ TBV (12-24 mo); no difference in cortical thickness
Ohta *et al.* [30]	3-3.5 years	Cortical grey matter; cortical thickness; surface area	↑ Cortical surface area (3 y); no difference in cortical thickness
Shen *et al.* [31]	(Longitudinal, prospective) 6-7, 12-13, 24-25 months	Extra-axial CSF; lateral ventricle	↑ Extra-axial CSF (6-24 mo); no difference in lateral ventricle volume
Shen *et al.* [32]	2-4 years	Extra-axial CSF; cerebrum	↑ Extra-axial CSF (2-4 y)
Hazlett *et al.* [33]	(Longitudinal, prospective) 2-3, 4-5 years	Cerebrum; cortical thickness	↑ Volume in cerebrum, particularly in temporal lobe white matter; no difference in cortical thickness
D'Mello *et al.* [49]	8-13 years	Cerebrum; cerebellum	↓ GM in cerebellar lobule VII (Crus I/II)
Pierce *et al.* [50]	3-8 years	Cerebellar vermis; cerebrum	↓ Area of cerebellar vermal lobules VI–VII
Foster *et al.* [51]	6-17 years	Global brain tissue; cortical thickness; surface area	↑ GM concentration in frontal, temporal lobes, putamen, and caudate nucleus; ↓ GM concentration in cerebellum
Wolff *et al.* [52]	(Longitudinal, prospective) 6-7, 12-13, 24-25 months	Corpus callosum (CC); global brain tissue	↑ Area and thickness in CC, particularly in the anterior CC (6-12 mo); correlation between CC area and thickness (1 y) and repetitive behaviors (2 y)
Haar *et al.* [53]	6-35 years	Global brain tissue; cortical thickness; surface area	↑ Ventricular volume; cortical thickness in several area; ↓ CC volume; no difference in intracranial volume, cerebellar and amygdala volume
Schumann *et al.* [55]	1-5 years	Amygdala	↑ Amygdala volume
Barnea-Goraly *et al.* [58]	8-12, 11-15	Amygdala; hippocampus	↑ Right hippocampus volume (8 y); ↓ right hippocampus volume (15 y)
Pote *et al.* [59]	4-6 months	Global brain tissue; CSF; lateral ventricle	↑ Cerebellar and subcortical volumes (4–6-mo)
Kohli *et al.* [41]	41-61 years	Cortical thickness; surface area; cortical folding	↓ lGI bilaterally in insular and ACC, left postcentral, and middle frontal and right orbitofrontal and supramarginal regions; positive correlations between lGI in the bilateral insula and right orbitofrontal cortex and executive function scores
Kohli *et al.* [42]	7-19 years	Cortical thickness; surface area; cortical folding	↑ lGI in left parietal and temporal and right frontal and temporal regions

ACC, anterior cingulate cortex; CT, cortical thickness; CSF, cerebrospinal fluid; GM, grey matter; GMV, grey matter volume; lGI, local gyrification index; ROI, region of interest; SA, surface area; SBM, surface-based morphometry; TBV, total brain volume; VBM, voxel-based morphometry; WMV, white matter volume.

rates of cortical layers [45]. In ASD, altered gyrification patterns can be impacted by abnormal neuronal proliferation and migration. Further investigations of cortical folding would deepen our understanding of cortical development, and allow us to better define the neurobiological mechanisms associated with ASD.

Recently, the largest study to date of brain asymmetry in ASD has mapped the differences in brain asymmetry between participants with ASD and TD [46], suggesting altered lateralization in ASD. Cerebral lateralization is a prominent feature of the brain in organizing certain motor and cognitive functions, such as handedness and language [47]. Individuals with ASD exhibit generally reduced asymmetry compared to TD, which suggests abnormal hemispheric specialization in autistic individuals. Interestingly, many of the regions that show significant alterations in asymmetry, including medial frontal, anterior cingulate, and inferior temporal regions, overlap with the default mode network (DMN), which further supports a role of abnormal functional lateralization of the DMN in ASD [48].

Cerebellum and Subcortical Areas

Other morphometric studies of ASD have also characterized abnormalities in the cerebellum [49–51], corpus callosum [52–54], amygdala [16, 55], caudate nucleus [51, 56], and cerebrospinal fluid (CSF) [26, 31, 32]. The posterior lobe of the cerebellum (lobules VI and VII) is functionally coupled to the PFC and the ACC [57]. It is thought to be engaged in cognitive-affective functions, and thus may play a critical role in the pathophysiology of ASD [49, 50]. Similarly, given the important role of the amygdala in emotion processing, the aberrant amygdala structure may underlie the social-emotional deficits in individuals with ASD [24, 58]. There is evidence that children with ASD have an enlarged amygdala that shows a significant correlation with the severity of their social and communication impairments [55]. Moreover, accumulating evidence has implicated enlargement of the caudate nucleus within the cortico-striatal circuits, and enlargement of the caudate is correlated with the repetitive behaviors in children with ASD [56, 59]. Notably, a disproportionately large midsagittal corpus callosum relative to total brain volume has been identified in a longitudinal study on ASD infants, particularly in the anterior region, which mediates sensory-motor functions and behavioral inhibition [52]. Besides, the authors reported that the increased area and thickness of the corpus callosum are significantly correlated with the severity of restricted, repetitive behaviors in ASD toddlers at age 2. In contrast, decreased corpus callosum volume has been reported in older children and adults with ASD compared to TD subjects [53, 54]. This atypical trajectory of the corpus callosum fits the theory of age-specific anatomical abnormalities in ASD, and could be related to the early excessive thin axons and subsequent insufficient axon growth and refinement in ASD patients [52, 60]. In addition, patients with ASD have elevated extra-axial CSF (defined as CSF in the subarachnoid space surrounding the cortex) relative to TD children from infancy to age 3 [26, 31, 32]. Given the important role of normal CSF circulation for the delivery of neural tropic factors as well as the clearance of neurotoxins and metabolites [61, 62], it has been suggested that the abnormal cortical development in ASD may be attributable to a dysfunction of CSF circulation [32].

Notably, abnormal white matter connectivity has also been indicated in ASD (Table 2). Diffusion tensor imaging (DTI) is a specialized MRI technique for non-invasive detection of fiber orientation and white matter connectivity by assessing the diffusion of water molecules in nervous system tissue [63]. DTI-derived metrics like fractional anisotropy (FA) and mean diffusivity are used to measure the orientation and the magnitude of diffusion, respectively, indicative of the white-matter microstructural properties including axon composition and myelination. A longitudinal DTI study has characterized increased FA and volume of fiber tracts (12 out of 15) in infants who later developed ASD at 6 months of age [27], whereas decreased FA and volume were identified in older children with ASD compared with TD controls [27, 64, 65], suggesting that axonal plasticity is implicated in the development of ASD. The changes of white matter connectivity properties over time could be associated with the dynamic processes of axonal pruning and myelination [66], e.g. early excessive thin axons and subsequent insufficient axon refinement in ASD patients. Another longitudinal study in ASD toddlers has reported axonal over-connectivity, as indexed by FA, in the frontal white matter tracts including the uncinate fasciculus connecting the frontal cortex and the amygdala as well as the arcuate fasciculus that is involved in language transmission [28], in agreement with the findings of Wolff *et al.* [27]. Elevated FA has also been reported in the corpus callosum and superior and inferior longitudinal fasciculi, as well as the inferior frontal-occipital fasciculus in tract-based spatial statistical studies of preschool-aged children and adults with ASD [67, 68]. Intriguingly, one recent study using the high angular resolution diffusion-weighted imaging method, which is more sensitive to deep brain structures, has provided evidence of abnormal structure in the mesolimbic reward pathway which connects the nucleus accumbens and the ventral tegmental area in ASD children; this supports the hypothesis that impaired reward processing circuitry might be a mechanism underlying ASD [69].

Table 2 Diffusion tensor imaging studies.

Reference	Age range	Brain regions	Main findings in ASD group
Wolff *et al.* [27]	(Longitudinal, prospective) 6-7, 12-13, 24-25 months	Global main fiber tracts	↑ FA in the body of corpus callosum, left fornix, inferior longitudinal fasciculus, uncinate fasciculus at 6 months; ↑ FA in anterior thalamic radiations, anterior internal capsule at 24 months
Solso *et al.* [28]	(Longitudinal, prospective) 1-4 years	Frontal tracts	↑ FA and volume in forceps minor, inferior frontal superior frontal tract, uncinate, frontal projection of the superior corticostriatal tract; ↑ FA in arcuate fasciculus portion of the superior longitudinal fasciculus; ↑ volume in inferior frontal occipital fasciculus, inferior longitudinal fasciculus (12 months)
Nordahl *et al.* [54]	(Longitudinal) 2-4,3-5, 5-7 years	Corpus callosum (CC)	↓ CC regions with fibers directed to superior frontal cortex and midsagittal CC area in both males and females with ASD, ↓ CC region with fibers directed to the orbitofrontal cortex in males with ASD; ↓ CC region associated with the anterior frontal cortex in females with ASD; ↑ MD, AD and RD in females with ASD
Sundaram *et al.* [64]	2-7 years	Association fibers in frontal lobes	↑ MD in short- and long-range fibers; ↓ FA in short-range fibers
Langen *et al.* [65]	19-39 years	Fronto-striatal tracts; global brain volume	↓ FA of white matter tracts connecting putamen to frontal cortical areas; ↑ MD of white matter tracts connecting accumbens to frontal cortex; ↓ total brain WM volume
Andrews *et al.* [67]	3-5 years	Global main fiber tracts	↑ FA in CC, inferior frontal-occipital fasciculi, inferior and superior longitudinal fasciculi, middle and superior cerebellar peduncles, and corticospinal tract
Catani *et al.* [68]	18-41 years	Global main fiber tracts	↓ FA in regions that include frontal lobe pathways; ↑ MD in the left arcuate fasciculus, cingulum, uncinated and anterior portions of the CC connecting left and right frontal lobes

AD, axial diffusivity; FA, fractional anisotropy; MD, mean diffusivity; ROI, region of interest; PT, probabilistic tractography; RD, radial diffusivity; WM, white matter.

Taken together, MRI studies employing voxel–based morphometry and DTI have reported age-related alterations in surface area, cortical thickness, cortical folding, brain lateralization, white matter connectivity, and gray matter volume in the prefrontal and temporal cortex, cerebellum [49–51], corpus callosum [52–54], amygdala [55, 70], caudate nucleus [51, 56], and CSF [26, 31, 32] in ASD patients, which may partly account for the emotional, behavioral executive, and language impairments. Although demographic features, such as sex and intelligence quotient can to some extent explain these age-specific findings [71], individual differences in ASD, such as the level of impairment, and the presence of medical and behavioral comorbidities in the selected groups may be another important factor in these inconsistent patterns of abnormality related to age. Longitudinal studies have delineated several atypical developmental trajectories as early as 6 months, and provide critical insights into the atypical neurodevelopment of ASD in early postnatal life [17, 24–33]. The presence of several prodromal abnormalities, such as greater cerebellar and subcortical volumes at 4–6 months, elevated extra-axial CSF level at 6 months, increased thickness of the corpus callosum and cortical surface area hyper-expansion at 6 months–12 months, total brain volume overgrowth between 12 months and 24 months, and hyper-connectivity in the frontal white matter tracts in high-risk infants at 6 months–24 months of age, may aid in pre-symptomatic diagnosis and progression prediction of ASD.

MR Imaging of Resting-State Functional Connectivity

An increasing number of resting-state functional MRI investigations have concentrated on the alterations in the cerebral functional connectivity of patients with ASD by measuring spatiotemporal patterns of blood-oxygen-level-dependent signals (Table 3). The upsurge of interest in this field stems from the theory that the intrinsic activity of the brain may play a pivotal role in higher-order cognition [72]. Diverse methodologies such as independent component analysis, seed-based correlation analysis, graph-theoretical analysis, and regional homogeneity have been widely used to analyze the functional connectivity (reviewed in [73]). Taking advantage of these methodologies, atypical functional connectivity has been characterized in ASD, including in the salience network (SN) [74–77], DMN [75–78], executive control network (ECN)

[75–77, 79], and dorsal attention network [77, 79, 80]. The SN, primarily composed of the anterior insula and dorsal ACC, is thought to be involved in detection and the allocation of attention to internal and external stimuli, and coordination between large-scale networks such as the DMN and the ECN to guide appropriate behaviors [81]. Disruption of the SN may account for reduced attention to social stimuli in ASD individuals. The DMN, comprising the medial PFC, posterior cingulate cortex, precuneus, and temporo-parietal junction, is implicated in autobiographical memory, introspective thought, and theory of mind [82–84]. A majority of studies have reported a trend of both global hypo-connectivity and local hyper-connectivity of the DMN in children with ASD [85–87]. It has been speculated that excess neurons may cause early brain overgrowth and produce a mis-wired brain with exuberant local and short-distance cortical interactions impeding the function of large-scale, long distance interactions between brain regions [88]. Dysfunction of the DMN may explain the disturbance of self-referential thought in ASD [87]. The ECN, predominantly anchored in the dorsal lateral PFC and parietal cortex, plays a crucial role in decision-making, working memory, and cognitive control [89]. Disruption of this network may account for the impairment in cognitive flexibility in ASD [79, 89]. The dorsal attention network, mainly consisting of the middle temporal area complex, intraparietal sulcus, and the frontal eye fields, underlies top-down control of attention [90], thus dysfunction of this network may contribute to the attention shift deficits in ASD.

One study investigated the DMN, SN, and ECN simultaneously using resting-state functional MRI [75] and revealed that all three large-scale networks showed atypical intrinsic connectivity in children and adolescents with ASD relative to TD controls. Specifically, the DMN and ECN had age-related over-connectivity in young children with ASD but not in adolescents with ASD, so this may reflect delayed network segregation in ASD. In addition, the SN was under-connected internally and with the ECN, and the connectivity within the SN was associated with socio-communicative impairment, indicating that reduced SN functional integrity may compromise its role in switching between DMN and ECN. Watanabe *et al*. [91] found that high-functioning adults with ASD showed fewer neural transitions than neurotypical controls, and such atypically stable brain dynamics underlay general cognitive ability and core symptoms in ASD. This study of brain network dynamics revealed that functional stability in neural circuits and atypical functional coordination among networks may underpin the aberrant decreases in the flexibility of dynamics in the brains of adults with ASD.

Besides the above networks, several other functional connections have also been reported to be compromised in the development of ASD. Hahamy *et al*. [92] examined both intra- and inter-hemispheric functional connectivity in children with ASD using resting-state functional MRI. They found topographically distorted inter-hemispheric functional connectivity in ASD when compared to TD children, and indicated that the level of distortion in homotopic inter-hemispheric functional connectivity was correlated with autism severity. This study demonstrated that individualized differentiation of functional connectivity patterns might be a core neural characteristic of ASD, in accordance with the findings of Dickie *et al*. [93]. In addition, Shou *et al*. have reported alterations of functional connectivity in children with ASD in the vasopressin-related neural circuits that are critically implicated in social behaviors [94].

Notably, a growing number of neuroimaging studies have explored the diagnostic (that is, predictive) value of various measures of brain anatomy, functioning, and connectivity for ASD [29, 95]. To explore whether the pre-symptomatic pattern of functional connectivity can be used to predict the diagnosis in ASD, Emerson *et al.* [95] carried out a prospective investigation in 59 infants at high risk for ASD using resting-state functional MRI. The authors reported that a machine learning algorithm based on the functional connections (selected as those that correlated with 24-month ASD-related behaviors) of 6-month-old infants at high familial risk for ASD is highly predictive of an ASD diagnosis at 24 months (positive predictive value of 100%). This study indicates that atypical brain connectivity patterns precede the emergence of behavioral anomalies and functional MRI could facilitate the detection of ASD at the prodromal stage.

Taken together, patients with ASD exhibit patterns of under- and over-connectivity compared to TD in multiple brain regions and networks: the SN [74–77], DMN [75–78], ECN [75–77, 79], dorsal attention network [77, 79, 80], and corticostriatal [79] and vasopressin-related neural circuits [94]. Aberrant homotopic connectivity [92] and atypical brain dynamics [91] in ASD compared to controls have also been reported. Long-range under-connectivity and short-range over-connectivity have been hypothesized as brain abnormalities in autism[96]. Other hypotheses suggest abnormal segregation and integration of resting-state networks [97] and idiosyncratic connectivity [75, 85, 92]. Moreover, the functional connectivity pattern evaluated with a machine learning algorithm may be valuable in predicting a diagnosis before the onset of ASD.

MR Imaging of Perfusion

ASL is a noninvasive MR-based imaging technique using endogenous water in arterial blood as a freely-diffusible

Table 3 Resting-state functional MRI studies in ASD.

Reference	Age range (mean)	Brain regions examined	Main findings in ASD group/connectivity
Uddin *et al.* [74]	7-12 (9.9) years	Networks: SN (frontal-insular, ACC); ECN (dlPFC, PPC); DMN (medial PFC, PCC); dorsal attention network (intraparietal sulcus and frontal eye fields)	↑ Connectivity in SN and posterior DMN (precuneus, PCC, and left angular gyrus)
Abbott *et al.* [75]	9-17 (13.9) years	Networks: SN; DMN; ECN	↑ Connectivity between DMN (PCC seed) and rECN (right IPL seed); ↓ connectivity in SN internally (right anterior insular seed) and with lECN (left IPL seed)
Plitt *et al.* [76]	(17.9) years	Networks: SN; DMN; frontoparietal task control network	Connectivity involving SN, DMN, and frontoparietal task control network are highly predictive of future autistic traits and the change in autistic traits and adaptive behavior over the same time period; functional connectivity involving the SN predicted reliable improvement in adaptive behaviors with 100% sensitivity and 70.59% precision
Elton *et al.* [77]	6-18 (13.2) years	Networks: SN; DMN; ECN; dorsal attention network	↑ Connectivity between DMN and middle frontal gyrus, bilateral IPL, and right insula; ↑ connectivity between dorsal attention network and the precuneus, cerebellum, and right precentral gyrus; ↓ connectivity between dorsal attention network and medial frontal gyrus and lateral temporal cortices; ↑ connectivity between SN and dorsal ACC; ↓ connectivity between SN and the medial frontal gyrus, left middle frontal gyrus, and left postcentral gyrus; ↑ connectivity between ECN and the left cerebellum; ↓ connectivity between ECN and the medial PFC, right superior frontal gyrus, right precentral gyrus, left middle frontal gyrus, left postcentral gyrus, and medial frontal gyrus
Doyle-Thomas *et al.* [78]	6-17 (12.3) years	DMN; whole brain	↓ Connectivity between PCC-L and the left medial frontal gyrus, left and right angular gyri, and right inferior temporal gyrus; ↓ connectivity between PCC-R and the left medial frontal gyrus, left PCC, left and right angular gyrus, and right inferior temporal gyrus; ↑ connectivity between PCC-L and the left IPL, left superior frontal gyrus, left precentral gyrus, right middle frontal gyrus, right superior parietal lobule, and right IPL; ↑ connectivity between PCC-R, and the left and right IPL, right middle frontal gyrus, left precentral gyrus, left superior frontal gyrus
Holiga *et al.* [79]	7-12, 12-18, 18-30 (17.5) years	Whole brain	↓ Connectivity in sensory-motor regions and right temporal regions, insula, amygdala, and hippocampus; ↑ connectivity in PFC, ACC, PCC, and parietal cortices
Oldehinkel *et al.* [80]	7-30 years	Networks: SN; sensory and motor networks	↑ Connectivity of the cerebellum with sensory and motor networks; ↓ connectivity of the visual association network with somatosensory, medial and lateral motor networks
Yerys *et al.* [85]	8-13 years	Networks: DMN; SN	↓ Connectivity within DMN (PCC-MPFC); ↑ connectivity between DMN and SN
Hahamy *et al.* [92]	(26.6) years	Whole brain	↓ Homotopic interhemispheric connectivity, particularly in the primary somatosensory and motor cortices; ↑ connectivity in frontal and temporal cortex

Table 3 continued

Reference	Age range (mean)	Brain regions examined	Main findings in ASD group/connectivity
Dickie *et al.* [93]	6-65 years	DMN; dorsal attention; ventral attention; frontoparietal; sensory motor; and visual network	↓ Connectivity in DMN, dorsal attention, ventral attention, frontoparietal, sensory motor, and visual network; ↑ connectivity in DMN and ventral attention network
Jann *et al.* [99]	(13.8) years	DMN	Frontotemporal hyperperfusion and hypoperfusion in the dorsal ACC; increased local FC in the anterior module of the DMN accompanied by decreased CBF in the same area
Peterson *et al.* [100]	5-60 (24.9) years	Global brain tissue	↑ rCBF values throughout frontal white matter and subcortical gray; negative correlation with NAA metabolite levels throughout frontal white matter

ACC, anterior cingulate cortex; CBF, cerebral blood flow; dlPFC, dorsolateral prefrontal cortex; DMN, default mode network; ECN, executive control network; FC, functional connectivity; IPL, inferior parietal lobules; NAA, N-acetylaspartate; PCC, posterior cingulate cortex; PCC-L, left posterior cingulate cortex; PCC-MPFC, posterior cingulate cortex medial prefrontal cortex; PCC-R, right posterior cingulate cortex; PFC, prefrontal cortex; PPC, posterior parietal cortex; rCBF, resting cerebral blood flow; rECN, right executive control; Seed, seed-based correlation analysis; SN, salience network.

tracer for the measurement of tissue perfusion with high reproducibility [98] (Fig. 1). Altered cerebral perfusion has been indicated in both gray and white matter in ASD using ASL. One study explored the resting cerebral blood flow and functional connectivity simultaneously in high-functioning children with ASD using ASL MRI. The authors found frontotemporal hyper-perfusion as well as hypoperfusion in the dorsal ACC in children with ASD compared to TD children [99]. While the functional connectivity was positively associated with the perfusion in TD children, this association was abnormal in children with ASD [99]. The increased functional connectivity in the ACC was accompanied by hypo-perfusion in this same area in children with ASD [99], possibly reflecting neurovascular decoupling, which impairs the function of the ACC and contributes to the social impairments in ASD. It has been speculated that possible factors driving neurovascular decoupling in ASD could be changes of inhibitory gamma-aminobutyric acid (GABA) neurotransmitters, which has been well replicated in ASD. However, the specific neural mechanism still needs to be further clarified. Furthermore, the functional connectivity strength between the anterior and posterior modules of the DMN is reduced in ASD compared to TD children, indicating long-range hypo-connectivity. These results suggest that ASD children experience cerebral energetic inefficiency. More recently, Peterson *et al.* [100] measured both cortical and subcortical perfusion in ASD patients using ASL, and identified hyper-perfusion in cortical white and subcortical gray matter. Interestingly, the regional cerebral blood flow throughout the frontal white matter in the ASD group was inversely correlated with the N-acetylaspartate metabolite levels, a marker for neuronal density and mitochondrial metabolism [101]. These results suggest increased myelin synthesis in ASD patients and that elevated cerebral blood flow might represent a compensation for maintaining the energy status of axons.

Taken together, these ASL-based studies indicate aberrant resting functional connectivity and altered resting perfusion in both gray and white matter, and significantly enhance our knowledge of brain network organization and energetic efficiency in patients with ASD. The ability of ASL to quantify the cerebral metabolic changes non-invasively with excellent reproducibility renders it a valuable imaging technique to inform the pathophysiology of ASD.

PET and SPECT

PET and SPECT are molecular imaging techniques that use radiolabeled tracers to probe molecular interactions of biological processes *in vivo,* with high sensitivity and specificity [102]. They offer critical insights into biological events *in vivo*, such as glucose metabolism [103], gene expression, blood flow, oxygen consumption, neurotransmitter release and receptor occupancy [104] (Fig. 1). PET, a representative mode of molecular imaging and transpathology [105], exhibits higher sensitivity and temporal resolution compared with SPECT, as well as the potential for quantitative and dynamic imaging [106]. Table 4 summarizes the main findings of PET/SPECT studies in ASD.

PET Imaging of Brain Metabolism

Using ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET, previous studies have characterized changes in cerebral glucose metabolism in patients with ASD while performing tasks. A recent study assessed cerebral glucose metabolic rates in ASD patients using ^{18}F-FDG PET [107]. The results showed decreased task-dependent glucose metabolism in the amygdala, frontal premotor and eye-field areas, and the parietal lobe in ASD patients relative to TD controls, which could be related to hypoactivation of these regions during a cognitive task [108]. Increased glucose metabolism has been detected in the hippocampus, occipital cortex, posterior cingulate cortex, and basal ganglia. The increased metabolic rate in the posterior cingulate cortex could reflect decreased cognitive task engagement of subjects with ASD, which in healthy participants typically leads to decreased metabolic activity in this central-to-DMN area [109]. Another possibility is that it could represent inefficient functioning of the DMN in ASD, e.g., a greater metabolic cost is devoted to the maintenance of a cohesive sense of self, a putative role of the posterior cingulate cortex [110]; this is thought to be the central impairment in autism.

In addition, other investigations have also characterized increased metabolism in white matter structures including the corpus callosum, internal capsule, and the white matter in the frontal and temporal lobes of patients with ASD compared to those of TD controls [111]. Theoretically, the metabolic and wiring costs in connections among anatomically adjacent brain areas are lower than those among distant brain regions [112]. The increased glucose metabolism in white matter structures of ASD patients might be associated with metabolically inefficient transfer of information in the autistic brain, possibly due to inefficient axonal wiring.

However, it is worth noting that metabolic abnormalities in these regions have also been indicated in schizophrenia [107], major depression [111], and bipolar disorder [113], thus further investigations are needed to identify specific metabolic alterations underlying the unique clinical features of ASD.

PET and SPECT Imaging of Neurotransmitter Systems

Serotoninergic System

Multiple lines of evidence have indicated that serotonergic system dysfunction is implicated in ASD (reviewed in [114, 115]). For instance, the serotonin 5-HT$_{1A}$ receptor agonist buspirone has been shown to ameliorate the obsessive–compulsive behaviors in children with ASD [116]. However, there is also evidence indicating that selective serotonin (5-HT) reuptake inhibitors failed to show additional benefit for repetitive behaviors in ASD as compared to placebo [117]. The disparity in results may suggest only a subset of autistic patients benefit from modulation of the serotonergic system.

Several studies have focused on the changes of 5-HT synthesis in ASD. α-^{11}C-methyl-L-tryptophan (AMT) is a radiolabeled tryptophan analogue that allows for the non-invasive visualization of 5-HT synthesis (Fig. 2). Using AMT PET, Chugani *et al.* [118–120] found global abnormalities [118], as well as asymmetric regional abnormalities of brain 5-HT synthesis in the dentato-thalamo-cortical pathway [119]; these were later suggested to be related to handedness and language function in ASD children [120].

Serotonin transmission abnormalities have also been indicated in ASD. ^{18}F-setoperone is a radioligand for visualization of 5-HT$_2$ receptors through PET (Fig. 2). Beversdorf *et al.* [121] explored 5-HT$_2$ receptor density in high-functioning autistic adults using ^{18}F-setoperone PET. They characterized lower 5-HT$_2$ receptor binding in the thalamus in high-functioning autistic adults compared to controls, and this was associated with language impairment. The lower 5-HT$_2$ receptor binding may occur as a consequence of increased synthesis of 5-HT [118], or inadequate activity of 5-HT$_2$ receptors in autism may result in increased synthesis in autism. This needs to be further clarified.

The serotonin transporter (SERT) regulates serotoninergic signaling through the reuptake of released 5-HT into presynaptic neurons [122] (Fig. 2). Genetic studies have proposed links between SERT polymorphisms and autism in some families [123]. The SERT is also the target of the widely-used selective serotonin receptor inhibitors in ASD for symptom management. Iodine-123-labelled N-(2-fluoroethyl)-2β-carbomethoxy-3β-(4-iodophenyl)-nortropane ([^{123}I] nor-β-CIT) is a radioligand that binds specifically to the SERT (Fig. 2). Using [^{123}I] nor-β-CIT SPECT, Makkonen *et al.* [124] evaluated the SERT availability in ASD children. They found significantly lower SERT binding in ASD patients compared to controls in various areas, including the medial frontal cortex, the midbrain, and the temporal lobe, indicating diminished SERT binding capacity in autistic individuals. These results have been supported by the findings of Nakamura *et al.* [125], who found markedly reduced SERT binding in the whole brain in adults with high-functioning autism compared to controls using carbon 11 (^{11}C)–labeled trans-1,2,3,5,6,10-β-hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-a]isoquinoline ([^{11}C](+)McN-5652) PET to measure SERT availability (Fig. 2). Specifically, the reduction of SERT binding in the thalamus was correlated with the repetitive,

Table 4 PET/SPECT studies in ASD.

Reference	Modality	Target	ASD age: mean± SD, range	Number of participants (N)	ASD diagnosis	ASD IQ: mean± SD, range	ASD sedation	Control group	Main findings in ASD
Mitelman *et al.* [107]	PET	Glucose	31.5 years ± 11.6	ASD: N = 25; CON: N= 55	ASD [DSM-IV and ADI-R]	IQ: 108.80 ± 20.25	No	Matched by similar age (31.48 years ± 11.57, 33.36 years ± 12.85)	↓ Metabolic rates in the parietal lobe, frontal premotor and eye-fields areas, and amygdala; ↑ Rates in the posterior cingulate, occipital cortex, hippocampus and basal ganglia
Mitelman *et al.* [111]	PET	Glucose	31.5 years ± 11.6	ASD: N = 25; CON: N= 55	ASD [DSM-IV and ADI-R]	IQ: 108.80 ± 20.25	No	Matched by similar age (31.48 years ± 11.57, 33.36 years ± 12.85)	↑ Metabolic rates across the white matter regions assessed, including internal capsule, corpus callosum, and white matter in the frontal and temporal lobes
Park *et al.* [158]	PET	Glucose	14 years	ASD: N = 1; CON: N = 0	ASD	IQ: 60 at 8 years of age	No	N/A, case study	↓ Metabolism in the prefrontal and frontal cortex as well as the occipital cortex was marked
Chugani *et al.* [118]	PET	Serotonin synthesis	6.41 years ± 3.3	ASD: N = 30; CON: N = 8	Autism [ADI-R and DSM-IV]	OAB: 49 ± 9.3	No	Matched by age	↑/↓ Serotonin synthesis in autistic boys but not in autistic girls
Chugani *et al.* [119]	PET	Serotonin synthesis	4.1-11.1 years	ASD: N = 8; CON: N = 5	ASD [DSM-IV]	OAB: 22 (mean)	Nembutal or midazolam for 8 autistic children and 3 siblings	Matched by age	↓ Serotonin synthesis in the left frontal cortex and thalamus in 5 boys; ↑ AMT accumulation in the right dentate nucleus of the cerebellum; ↓ AMT accumulation in the right frontal cortex and thalamus and elevated in the left dentate nucleus in the remaining 2 boys
Beversdorf *et al.* [121]	PET	5-HT_{2A}	31.0 years ± 8.0	ASD: N = 8; CON: N = 12	Autism [DSM-IV and ADI-R]	IQ: 114.7 ± 14.7	No	Matched by age and FISQ	↓ 5-HT_{2A} binding in thalamus
Nakamura *et al.* [125]	PET	SERT and DAT	21.2 years ± 2.0 18–26 years	ASD: N = 20; CON: N = 20	Autism [DSM-IV-TR, ADI and ADOS]	IQ: 99.3 ± 18.1	No	Matched by sex and similar age: 21.9 years ± 2, 18–26 years, IQ not significantly different	↓ SERT binding in AC and posterior cingulate correlated w/ASD symptomatology
Ernst *et al.* [128]	PET	Presynaptic DA activity	13 years ± 2	ASD: N = 14; CON: N = 10	Autism [DSM-III-R]	IQ: 74 ± 23.1, range 46–123	Propofol	Similar age: 14 years ± 2	↓ FDOPA uptake in mPFC
Mori *et al.* [133]	SPECT	$GABA_A$	7.0 years ± 3.7	Autism: N = 9; AS: N = 15; CON: N = 10	ASD classified as autism or AS [DSM-IV]	IQ < 70 (N = 7); IQ > 70 (N = 17)	Triclofos sodium	Non-symptomatic partial epilepsy patients w/o intellectual delay, similar age: 7.8 years ± 3.6	↓ Binding in superior and medial frontal cortex in ASD
Fung *et al.* [134]	PET	$GABA_A$	26.6 years ± 8.3	ASD: N = 28; CON: N = 29	ASD [DSM-5, ADI-R and ADOS-2)]	IQ: 102.1 ± 16.5	No	Matched by IQ, sex and age	No differences in $GABA_A$ receptor density in bilateral thalami and left dorsolateral prefrontal cortex between ASD and TD group

Table 4 continued

Reference	Modality	Target	ASD age: mean± SD, range	Number of participants (N)	ASD diagnosis	ASD IQ: mean± SD, range	ASD sedation	Control group	Main findings in ASD
Fatemi *et al.* [137]	PET	mGluR5	20 years ± 2.1	ASD: N = 6; CON: N = 3	Autism [ADOS, ADI-R, ASSQ, CGI, ABC, SCQ and GAF]	Not specified	No	Matched by sex and race	↑ mGluR5 binding in cerebellum, postcentral gyrus, entorhinal area, and the precuneus
Zürcher *et al.* [141]	MR-PET	TSPO	24.1 years ± 5.5	ASD: N = 15; CON: N = 18	ASD [DSM-IV-TR, ADI-R, ADOS-2 and DSM-5]	IQ: 86.1 ± 19.2	No	Matched by sex and age	↓ TSPO expression in the bilateral insular cortex, putamen, precuneus/ posterior cingulate cortex, orbitofrontal cortex, lateral occipital cortex, superior temporal gyrus, angular gyrus, supramarginal gyrus, and left postcentral gyrus

5-HT_{2A}, serotonin 2A receptor; ABC, Autism Behavior Checklist; AC, Anterior Cingulate; ADI, Autism Diagnostic Interview; ADI–R, ADI- Revised; ADOS, Autism Diagnostic Observation Schedule; ADOS-2, Autism Diagnostic Observation Schedule, Second Edition-2; AMT, methyl-L-tryptophan; AS, Asperger Syndrome; ASSQ, Autism Spectrum Screening Questionnaire; CARS, Childhood Autism Rating Scale; CGI, Clinical Global Impression; DA, dopamine; DAT, Dopamine Transporter; DSM-III-R, DSM Third Edition Revised; DSM-IV, DSM Fourth Edition; DSM-IV-TR, DSM Fourth Edition Text Revised; DSM-5, DSM Fifth Edition; FDOPA, fluorine-18-labelled fluorodopa; FISQ, Full Scale Intelligence Quotient; $GABA_A$, Gamma-aminobutyric Acid Type A Receptor; GAF, Global Assessment of Functioning; ICD-10, International Classification of Diseases Tenth Revision; IQ, Intelligence Quotient; mGluR5, metabotropic glutamate receptor 5; mPFC, medial prefrontal cortex; OAB, overall adaptive behavior composite from Vineland Adaptive Behavior Scale in age equivalents (months of age); SCQ, Social Communication Questionnaire; SERT, Serotonin Transporter; TD, Typically Developing; TSPO, translocator protein.

obsessive behaviors, and the reduction in the anterior and posterior cingulate cortex was associated with the social cognition in ASD, compatible with previous studies [121, 126]. Taken together, these studies indicate a disturbed serotonergic system in ASD patients and highlight its involvement in social cognition, and the restricted, repetitive behaviors in ASD.

Dopaminergic System

Dopamine (DA) is a catecholamine neurotransmitter involved in reward and social motivation that may be central to the social deficits in autism [127]. Previous studies have characterized abnormalities in DA synthesis and DA transporters in ASD patients [124, 125, 128]. ^{18}F-labelled fluorodopa (^{18}F-FDOPA) is an L-DOPA analogue that allows the evaluation of DA synthesis. Using ^{18}F-FDOPA PET, Ernst *et al.* [128] have found decreased ^{18}F-FDOPA accumulation in the anterior medial PFC in 14 drug-naïve ASD children compared to age-matched healthy controls, indicating that prefrontal dopaminergic deficits may account for the cognitive impairment in ASD. Nakamura *et al.* [125] measured DA transporter binding in 20 adults with high-functioning autism and 20 age- and intelligence quotient-matched TD adults using 2-β-carbomethoxy-3-β-(4-fluorophenyl) tropane (^{11}C-WIN-35,428) PET. In the autistic group, they found significantly higher DA transporter binding in the orbitofrontal cortex, a key region in the network underlying emotional regulation [129]. Over-functioning of the dopaminergic system in the orbitofrontal-limbic circuit could be associated with the impulsive and aggressive behaviors in ASD.

GABAergic System

Gamma-aminobutyric acid (GABA) is the most prevalent inhibitory neurotransmitter in the mature central nervous system, mainly acting on $GABA_A$ and $GABA_B$ receptors. But GABA-mediated signaling also plays a central role in regulating key developmental processes, such as cell proliferation, neuron differentiation, and circuit refinement (reviewed in [130]). Emerging evidence has suggested that impaired GABA-mediated signaling lead to an imbalance of excitation and inhibition that may contribute to the pathogenesis of autism (reviewed in [131, 132]). To investigate the $GABA_A$ receptor in ASD patients, Mori *et al.* [133] performed ^{123}I-iomazenil (^{123}I-IMZ) SPECT in children with ASD and found decreased ^{123}I-IMZ accumulation in the superior and medial frontal cortex, a region that is thought to be associated with theory of mind, in the ASD group compared to the control group. A more recent study examined the GABAergic system in 28 high-functioning adults with ASD [134]. While they did not find altered $GABA_A$ receptor density in high-functioning adults with ASD using ^{18}F-flumazenil (^{18}F-FMZ) PET, region-dependent and sex-specific differences in GABA concentrations were indicated. Notably, they discovered a higher GABA concentration in the left dorsal-lateral PFC in the autistic group than in TD adults, which may underlie the hypo-activation of dorsal-lateral PFC during working memory tasks in adults with ASD [135]. Another possibility is that higher cortical GABA levels occur in compensation for primary defects in GABAergic signaling.

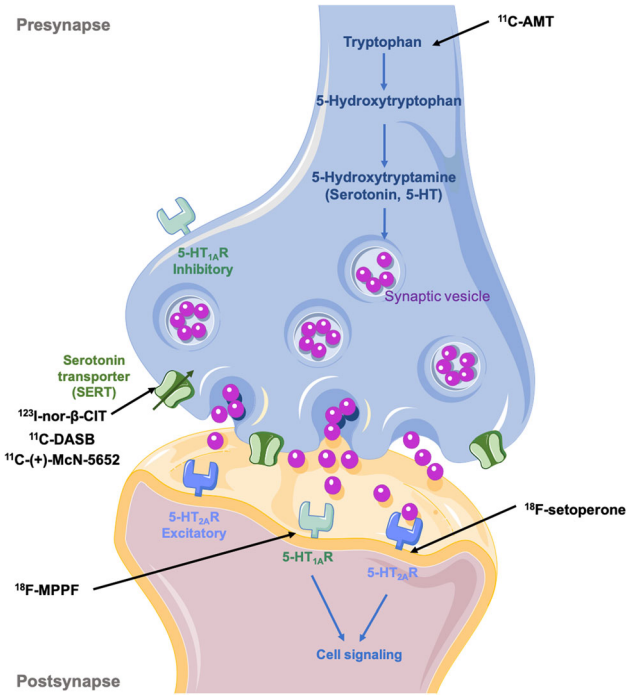

Fig. 2 Schematic of PET and SPECT modalities for assessing presynaptic and postsynaptic serotonergic targets. 5-Hydroxytryptamine (5-HT, serotonin) is synthesized from the amino-acid tryptophan *via* hydroxylation (forming the intermediate precursor 5-hydroxytryptophan), and then stored in synaptic vesicles. α-^{11}C-methyl-L-tryptophan (^{11}C-AMT) is a radiolabeled tryptophan analogue that allows for non-invasive visualization of 5-HT synthesis. Serotonin reuptake into presynaptic neurons occurs *via* a serotonin transporter (SERT). [^{123}I] nor-β-CIT, ^{11}C-DASB and [^{11}C](+)McN-5652 are radioligands that bind specifically to SERTs. 5-HT_{1A} receptors are located on both presynaptic and postsynaptic neurons. ^{18}F-MPPF allows the visualization of 5-HT_{1A} receptors *in vivo*. Two different types of 5-HT receptors are expressed on postsynaptic neurons: 5-HT_{1A} and 5-HT_{2A}. ^{18} F-setoperone is a radioligand for mapping 5-HT_2 receptors.

Glutamatergic System

Glutamate is the predominant excitatory neurotransmitter in the brain and plays vital roles in brain development and neural plasticity. Deficiency in *Shank 3* gene coding the postsynaptic scaffold protein located in glutamatergic neurons, has been associated with ASD [136]. A recent

study reported significantly higher metabotropic glutamate receptor 5 in the cerebellum and postcentral gyrus in ASD adults compared to those in the TD group, using ^{18}F-3-fluoro-5-[(pyridin-3-yl)ethynyl]benzonitrile (^{18}F-FPEB) PET to measure metabotropic glutamate receptor 5 density [137]. The cerebellum is crucial to the control of action through the integration of sensory and motor signals necessary for skilled movement [138]. Morphological changes in the cerebellum of subjects with autism have been identified, such as changes in total cerebellar volume, altered Purkinje cell density, and abnormal deep cerebellar nuclei [138]. Changes of glutamate signaling in the cerebellum may contribute to abnormalities in these somatosensorimotor-cerebellar circuits, leading to the motor and cognitive deficits associated with autism. Intriguingly, positive correlation between [^{18}F]-FPEB binding and autistic symptomatology as measured by the autism behavior checklist has been found in the precuneus, a principal component of the DMN. Changes in glutamate signaling, leading to an excitatory/inhibitory imbalance, coupled with structural and functional abnormalities in the DMN, potentially impact the functioning of the DMN.

PET Imaging of Neuroinflammation

Increasing evidence has suggested involvement of the immune system in the pathophysiology of ASD. The ^{11}C-PBR28 is a radiotracer that is able to detect subtle changes of 18-kDa translocator protein (TSPO) expression [139]. The putative roles of TSPO include apoptosis, steroidogenesis, neuroinflammation, energy production, cell metabolism, and oxidative stress [140]. Changes in TSPO may reflect abnormalities in these processes. Using ^{11}C-PBR28 PET-MR, Zürcher *et al.* [141] reported significantly lower TSPO levels in young male adults with ASD compared to those of age- and sex-matched controls in multiple brain regions, including the precuneus/posterior cingulate cortex, insular cortex, and temporal, angular, and supramarginal gyri bilaterally. Decreased TSPO in ASD could reflect changes in glia, neurons, or endothelial cells. Additional research is warranted to elucidate the specific mechanism behind the abnormal TSPO expression in ASD.

Taken together, impaired serotonergic, dopaminergic, glutamatergic, and GABAergic systems are critically involved in shaping the disturbed neural circuitry of ASD. However, the specificity of such abnormalities remains to be elucidated, since similar alterations in neurotransmitter systems have also been shown in other psychiatric disorders, such as schizophrenia [107]. In addition, glucose metabolic abnormalities and neuroinflammation may contribute to the pathogenesis of autism. Thus, the onset and progression of ASD is a complex process that involves multiple mechanisms. Additional research is warranted to identify the ASD-specific molecular basis and to further our understanding of the neural correlates of ASD.

Intervention-Related Neuroimaging Biomarkers

To date, psychological and behavioral interventions are still the predominant treatment for ASD. However, behavioral therapies are typically expensive and difficult to access, and can put substantial strain on families and caregivers. To provide on-demand, personalized interventions for ASD is urgently needed. Lately, technology-based interventions, such as wearable digital intervention and robot-assisted therapy, have shown potential to improve socialization in ASD and hold great promise for augmenting the current standard of care [142, 143]. However, effective pharmacological treatments for the core symptoms of ASD are still lacking. Oxytocin, a neuropeptide mediating social affiliation, is emerging as a promising medical therapy for ASD [144]. Neuroimaging techniques, such as resting-state MRI and PET have become important methods for measuring the therapeutic effect of oxytocin in patients with ASD [145–151] (Table 5). Using resting-state functional MRI, researchers found that oxytocin enhanced the functional connectivity of the DMN [152], and cortical-striatal circuits [153]. Specifically, 6-week intranasal administration of oxytocin enhanced the functional connectivity between the ACC and dorsal medial PFC in ASD patients, and significantly attenuated the impaired reciprocal social interactions [152]. Moreover, this functional connectivity enhancement was robustly correlated with symptom improvement. However, these findings cannot be extrapolated to female patients with ASD due to the unbalanced sex representation (all male participants). Another study focused on the effect of oxytocin on intrinsic functional connectivity in females [83] and found that oxytocin increased cortical-striatal connectivity and this was positively associated with autistic traits. Future work might include efforts to quantify how therapeutically effective treatments remediate topologically sub-optimal network configurations in ASD patients using graph theoretical measures.

Using 2′-methoxyphenyl-(N-2′-pyridinyl)-p-[18F]fluoro-benzamidoethylpiperazine (^{18}F-MPPF) PET to assess 5-HT$_{1A}$ receptors (Fig. 2), Lefevre *et al.* [154] investigated the therapeutic effect of oxytocin on the serotoninergic system in ASD patients. In TD controls, they found an oxytocin-serotonin interaction which was absent in patients with ASD. Hirosawa *et al.* [155, 156] investigated the serotonergic modulation after long-term administration of oxytocin in ASD patients using (^{11}C)-3-amino-4-(2-[(demethylamino)methyl]phenylthio)benzonitrile (^{11}C-

Table 5 Intervention-related neuroimaging studies.

Reference	Diagnosis	ASD age mean± SD, range	Medication	Major drug target(s)	Symptom treated in ASD	Therapeutic effect
Ajram *et al.* [145]	ASD (N=37)	33 years ± 2.5	Riluzole	GABA and glutamate targets	Anxiety	↑ PFC inhibitory index in ASD; ↓ PFC inhibitory in controls
Hegarty *et al.* [148]	ASD (N=13)	15-35 years	Propranolol vs. placebo	Beta-adrenergic antagonist	Attention deficit/ hyperactivity disorder (ADHD) or anxiety	↓ Connectivity in the dorsal medial prefrontal cortex subnetwork of the DMN; ↑ connectivity in the medial temporal lobe subnetwork
Alaerts *et al.* [149]	ASD (N=40)	Oxytocin: 24.76 years ± 4.85; Placebo: 24.06 years ± 5.54	Oxytocin vs. placebo	Resting-state functional connectivity between key regions of the central oxytocinergic system (amygdala, hippocampus, nucleus caudatus, nucleus accumbens, and hypothalamus).	ASD symptom	↓ Amygdala–hippocampal connectivity
Watanabe *et al.* [152]	High-functioning ASD (N = 20)	24-42 years	Oxytocin vs. placebo	Intrinsic functional connectivity in the medial PFC	Social reciprocity	↑ Functional connectivity between ACC and dorso-medial PFC
Chugani *et al.* [116]	ASD (N = 166)	2-6 years	Buspirone vs. placebo	5-HT_{1A} receptor	Restricted and repetitive behavior	↑ AMT SUV in basal ganglia, thalamus, cerebellum, and brainstem
Lefevre *et al.* [154]	ASD (N = 18)	34.3 years ± 7.6	Oxytocin vs. placebo	5-HT_{1A} receptor	NA	No changes in MPPF binding potential or serum-free serotonin concentration
Fukai *et al.* [155]	ASD (N = 10)	23-41 years	Oxytocin vs. placebo	Serotonergic system	Emotional response to human faces	↑ ^{11}C-DASB binding potential in the striatum; positive correlation with increased negative emotional response to human faces
Hirosawa *et al.* [156]	ASD (N = 10)	23-41 years	Oxytocin vs. placebo	Serotonergic system	ASD symptom	↑ ^{11}C-DASB binding potential in the left inferior frontal gyrus extending to the left middle frontal gyrus

5-HT_{1A}, serotonin 1A receptor; ACC, anterior cingulate cortex; AMT, methyI-L-tryptophan; ^{11}C-DASB, (^{11}C)-3-amino-4-(2-[(demethylamino) methyl] phenylthio) benzonitrile; DMN, default mode network; GABA, gamma-aminobutyric acid; MPPF, 2′-methoxyphenyl-(N-2′-pyridinyl)-p-[^{18}F]fluoro-benzamidoethylpiperazine; PFC, prefrontal cortex; SUV, standard uptake value.

DASB) PET to measure SERT availability (Fig. 2). The authors found significantly elevated ^{11}C-DASB binding in regions including the left inferior frontal gyrus and striatum in patients with ASD following oxytocin administration. Given that oxytocin-based therapy can modulate the serotonergic system and enhance the functional connectivity in frontal-striatal neural circuits that are enriched in dopaminergic neurons [153, 157] in patients with ASD, further studies are needed to investigate the interaction of oxytocin with the dopaminergic system.

One study has investigated the therapeutic effect of deep brain stimulation for an ASD patient with self-injurious behaviors using ^{18}F-FDG PET [158]. The authors reported that the glucose metabolism in the occipital cortex as well as the prefrontal and frontal cortex was significantly decreased with symptomatic improvement 2 years after bilateral nucleus accumbens deep brain stimulation. Moreover, the reduction in glucose metabolism was associated with decreased volumes in these regions as revealed by volumetric MRI after deep brain stimulation. These results indicate that ASD patients with life-threatening self-injurious behaviors have organic lesions associated with structural and functional alterations, and such lesions can be modulated by deep brain stimulation to achieve symptomatic improvement, suggesting the therapeutic potential of nucleus accumbens-targeted deep brain stimulation for these patients.

Taken together, neuroimaging studies significantly increase our understanding of the treatment outcome in ASD at the circuit level, and offer objective biomarkers for the evaluation of novel therapies for ASD.

Conclusions and Future Perspectives

Accumulating evidence has suggested that ASD encompasses alterations of brain structural and functional connectivity, particularly in regions and networks implicated in social-cognition. Longitudinal neuroimaging studies have delineated atypical developmental trajectories of ASD that are associated with cascading neurobiological processes (Fig. 1). Meanwhile, impaired serotonergic, dopaminergic, glutamatergic, and GABAergic systems as well as neuroinflammation may be critically involved in the pathogenesis of ASD. Still, further research is warranted to explore how imaging biomarkers are related to the treatment effect, symptomology, and genetic variants of ASD.

While most neuroimaging findings show extensive variability [159], the progress of finding clinically-relevant biomarkers is likely to be facilitated by identifying homogeneous ASD subgroups based on neuroimaging features, referred to as neurosubtyping (for review, see [160]). Future neurosubtyping approaches would benefit from leveraging findings from the larger literature of candidate biomarkers. Examining the totality of attributes in the context of each other is crucial for understanding the developmental course of individuals. Big data and data-driven methods may be promising approaches to decomposing the extensive heterogeneity in ASD [161]. Advanced analytical models that are tailored to capture the categorical and dimensional nature of ASD heterogeneity will be important to delineate biologically and clinically meaningful subgroups [162] and model complex growth- and time-related courses. In addition, the high comorbidity rate underscores the need for a trans-diagnostic framework for deepening our understanding of the heterogeneity within and beyond autism.

Future longitudinal studies covering younger patients may help identify the causative mechanisms and vulnerable developmental stages of ASD. Big data approaches coupled with data-driven methods will likely facilitate efforts to decompose the ASD heterogeneity. In parallel, large-scale, multi-center, multidisciplinary collaborations are increasingly important to elucidate neurophysiological underpinnings of ASD and facilitate the development of objective diagnostic biomarkers and ASD-targeted therapy. Future advances in imaging techniques and the development of new tracers may further our understanding of the pathophysiology and promote drug development by revealing novel mechanistic or therapeutic targets for ASD.

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