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Understanding Autism and Related Disorders: What Has Imaging Taught Us?

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Synopsis

Structural imaging studies documented increased total brain volume and early acceleration in brain growth. FMRI studies in autism have also led to the development of methods for analyzing connectivity and in viewing autism as a disorder of functional connectivity among brain regions but also as a disorder in which cortical activity is disturbed and cortical specialization somewhat atypical. The functional underconnectivity appears to be a universal characteristic of the brain in autism, reflecting a disturbance in synchronization.

Keywords

autism; brain development; cognition; neuroimaging; fMRI; functional connectivity

OVERVIEW

This review focuses on the valuable and unique contributions that structural and functional imaging studies of autism have made to the understanding of the brain and mind. Structural imaging studies have documented increased total brain volume and early acceleration in brain growth, particularly conspicuous in cerebral white matter but also impacting cortical gray matter, highlighting the role of white matter in neural connectivity and this presentation as a developmental disorder compared to cerebral palsy. The stark contrast between increased white matter volume and poor clinical and brain function highlighted the dissociation between white matter tract overgrowth and dendritic and synaptic underdevelopment. Hence, white matter tract overgrowth is not necessarily synonymous with connectivity at all histological levels. The next lesson learned was that white matter was not the whole story. A series of recent 1H magnetic resonance spectroscopy (MRS) studies have documented the involvement of cerebral cortex and in fact perhaps its primacy as the site of origin of the neurological abnormalities in addition to alterations in the amount, synchrony, and location of cortical activation with fMRI. These studies have served as a reminder that white matter tracts have an origin in the neuron cell body. The lack of increased white matter in the corpus callosum, rather the opposite, suggests that the primary pathophysiology is intrahemispheric and not specific to white matter. More sensitive imaging measures of brain tissue volume from voxel-based morphometry have

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demonstrated a consistent pattern of cortical gray and white matter volume alterations across autism, Asperger's disorder, British and Chinese populations, using standardized methods and automated processing that can be used reliably across different laboratories. The final lesson so far of structural imaging in a developmental disorder is the importance of large samples in small age bins and age, gender, IQ-matched control groups if structural anatomy is to be defined across the age span of development and brain maturation that is prolonged.

FMRI studies of autism have brought many insights about the brain and mind. Although there was not space to review all such literature, autism has been instrumental in defining many neural systems related to social interaction (theory of mind and the mirror neuron system), gaze and motion processing, face identity, face emotion and emotion processing, visual perception and higher order perceptual processing and, importantly, the difference between automatic processing and deliberate conscious or cognitive processing among others. These neural systems and others have slowly helped us to understand how people think and how the mind works, and with it has come a much improved understanding of behavior in autism and an emerging generation of intervention strategies that focus on cognitive interventions aimed at changing neural circuitry. FMRI studies in autism have also led to the development of methods for analyzing connectivity and in viewing autism as a disorder of functional connectivity among brain regions but also as a disorder in which cortical activity is disturbed and cortical specialization is somewhat atypical. The first level of connectivity analyses revealed a decrease in the connectivity of all lobes, with the frontal lobe and an increase in right posterior activity compensating for under-functioning regions. A related lesson to come from the latter studies was that brain localization cannot be inferred from a cognitive or neuropsychological task, because individuals with autism were shown on fMRI to use alternate regions to compensate for dysfunctional brain regions such as dorsolateral prefrontal cortex during verbal working memory. The inability to do so during spatial working memory had resulted in a dissociation in performance between clinically intact verbal working memory and impaired spatial working memory that was not understood until fMRI studies were completed. The functional underconnectivity appears to be a universal characteristic of the brain in autism, reflecting a disturbance in synchronization. The slightly atypical cortical localization of regions on fMRI suggests that the basic mechanisms of inhibition-excitation may also be disturbed. Much remains to be learned about the mind and brain from autism and the knowledge of the future will be even more exciting.

INTRODUCTION

In the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV) [1], autism is the prototype disorder for the category called Pervasive Developmental Disorders (PDD). Of the PDDs, Autistic Disorder, Asperger's Disorder, and Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS) are informally referred to as the Autism Spectrum Disorders (ASDs), which may affect up to 1% of children born [2]. PDDs are defined by impairments in reciprocal social skills, in the comprehension and communicative use of verbal and nonverbal language, and by restricted and repetitive behaviors [3]. The latter cluster of behaviors share a focus on the details of an object or category in conjunction with a failure to grasp the conceptual meaning of the object or category as a whole. All of the impairments that define the ASDs are the result of the failure to process information in the same way as typical people producing a different perception and view of the world. PDD-NOS and Asperger's Disorder are likely milder consequences of whatever causes autism, although some argue for them being biologically distinct disorders.

Neuroimaging studies have contributed substantially to the understanding of the neurobiology of autism and, in particular, to understanding the important distinction between developmental neurobiological disorders and acquired disorders. Findings in autism have given proof to the

statement that in the brain “The way things are broken is not necessarily the way they are made” (Antonio Damasio, personal communication, 1990). The study of autism has also contributed to the understanding of neural systems supporting higher order cognitive and neurologic processes, and of the neural basis of complex behavior, automatic thought, and the experience of emotions (feelings), e.g. the mind.

The path to the present day understanding of the neural basis of the ASDs was initially very slow, but has rapidly accelerated over the past 15 years. The first thirty years of neurobehavioral research in autism (1950's-1970's) was marked by erroneous analogies to adult neurological conditions secondary to acquired brain damage, and by simple cognitive models for the abnormal complex behavior of autism without supporting data. The emergence from this phase began with computerized axial tomographic (CT) studies that demonstrated the connection between gross structural brain abnormalities and the co-existence of associated causes of brain damage in subjects being diagnosed with autism at that time [4,5]. Thereafter, it became standard research practice to exclude subjects from research studies with causes for brain dysfunction other than autism. Of equal importance, structured research instruments, e.g., the Autism Diagnostic Interview (ADI) [6] and the Autism Diagnostic Observation Schedule (ADOS) [7], were developed and introduced with world-wide uniformity in training and reliability procedures, which substantially improved the specificity and sensitivity of autism and autism spectrum diagnoses and standardized diagnosis across research sites. These contributions to subject characterization provided the foundation for the emergence of the contemporary neurobiology of autism.

The imaging contributions to the understanding of the brain and mind in autism can be divided into those arising from structural imaging studies and those from functional imaging research. Whereas these advances have come from the combined contributions of multiple methodologies, imaging has certainly made valuable and unique contributions. These contributions are the focus of this chapter.

THE ENLARGED BRAIN AND EARLY BRAIN OVERGROWTH: THE WAKEUP CALL

The contemporary neurobiology of autism began with the recognition that the brain in most young children with autism was too large. The initial characterization of brain size and growth in autism took a decade or more, and relied heavily on head circumference data. Ultimately, head circumference studies in autism defined a group mean head circumference (HC) at the 60th-70th percentile relative to population norms and disproportionate to height (HT) and weight (WT); 15-20% of the autism group were found to have macrocephaly (HC >99th percentile) [8]. Although larger HC for HT was the most common finding and true for the group as a whole, it was not universal: HC proportionate to HT, and HC less than HT was also frequent in autism [8]. Thus, many head growth trajectories were found to be consistent with autism. Retrospective and prospective head circumference data from very young children with autism found onset of accelerated head growth by or before 12 months of age [9,10] and macrocephaly in 15-20% by 4 to 5 years of age [10,11,12]. Growth abnormalities have not been detected at birth relative to population norms.

Structural MRI studies confirmed the increase in total supratentorial brain volume (TBV) in autism suggested by the increase in head circumference. The increase in TBV was documented as early as 2 to 4 years of age [10,13], the earliest age of clinical recognition, and found to persist into childhood but not adolescence [12]. The *rate of brain growth* in autism accelerated early in life then decelerated and plateaued, resulting in changes in brain and head size; but these data are not sufficient to define the age of cross-over to normal HC and TBV in autism or to adequately define the heterogeneity in growth trajectories and TBV within the autism

population. Cross-sectional data suggested that increased TBV likely persisted until the end of the first decade of life [13,14]. However, longitudinal studies of preschool children with autism have suggested that increased TBV may not persist past 5-6 years of age [15]. There are a subset who are macrocephalic throughout life; however, this appears to be a separate phenomenon.

The onset of brain overgrowth precedes or coincides with the presentation of the signs and symptoms of autism, indicating that the overgrowth was part of a pathologic process that disrupted brain structure and function and thus led to autism. The actual acceleration of growth suggests the underlying process is related to active growth of central nervous system tissue, rather than a passive process, such as inhibition of pruning. Early in life, increased brain volume is clearly associated with loss of function. However, it is difficult to interpret the significance of changes in regional brain volumes reported at later ages.

DEVELOPMENTAL DIFFERENCES IN WHITE MATTER

The tissues contributing to the overall increased TBV were total cerebral white matter and total cortical gray matter, with the latter contribution varying with the parcellation program used. One small but very important study for its impact on the understanding of the white matter volume changes in autism involved 6 to 11-year-old boys with (n=13) and without (n=28: 14 developmental language disorder, 14 typically developing) autism, and the parcellation of cerebral white matter into an outer zone of radiate white matter composed of intra-hemispheric corticocortical connections and an inner zone of bridging and sagittal components [16]. The inner zone of white matter, including the corpus callosum and internal capsule, showed no volume differences. The volume of the outer radiate white matter was increased in all cerebral lobes but with a frontal predominance. These findings were interpreted as evidence of overgrowth of short- and medium-range intra-hemispheric corticocortical connections (fiber pathways) with no detectable involvement of inter-hemispheric connections or connections between cortex and subcortical structures. It is important to recognize that the white matter fiber pathways may be larger without actual dendritic or synaptic connections having been established or, if established, being functional. Hence, the appropriate interpretation of the structural findings is that the white matter pathways are enlarged, not that connectivity per se is increased. It could be inferred that connectivity is likely affected, but its functional status and the status of synapses cannot be discerned from this gross measurement. It is possible that there was an overgrowth of white matter projections because functional synapses were not being established.

Another recent study examined cortical connectivity by comparing gyral and sulcal thickness as indices of short and longer distance cortical connections [17]. This study found an overall increase in cortical thickness in a sample of high functioning 8 to 12-year-old boys with autism compared to typical boys. Cortical thickness was greater (analogous to increased volume of outer radiate white matter) in sulci (long connections) than gyri (short vertical connections), analogous to the findings of Herbert and colleagues [18].

The broader importance of these white matter findings is that they demonstrated that *developmental* pathology could present very differently from damage to white matter, which traditionally manifested as cerebral palsy and prominence of long tract signs. Autism became a lesson in the expression of developmental pathology of white matter as alterations in brain connectivity, absence of long tract signs, and increased brain size.

Cross-sectional imaging studies in autism have reported a *reduction* in the size of the corpus callosum, although the segment reported to be affected has varied [19]. In some studies, the decrease in cross-sectional area was only present relative to total brain volume in the autism group; in other studies, there was an absolute decrease in corpus callosum area independent of

brain volume. The contrast between the *increase* in intra-hemispheric white matter volume and the *lack of change* or *decrease* in corpus callosum size is notable and indicated that the neurobiological process affecting intra-hemispheric white matter was not impacting inter-hemispheric white matter. A key question for structural imaging studies is: To what brain structure should the corpus callosum be compared to determine if its size is abnormal? Since TBV in autism is influenced by its largest component (cerebral white matter), which has no relation to the origin of the corpus callosum, is this an appropriate comparison or the most appropriate comparison?

A study using diffusion tensor imaging (DTI) also found abnormalities in the corpus callosum in individuals with high-functioning autism and typically developing controls [20]. Functional anisotropy (FA) values were significantly lower in the overall corpus callosum, genu and splenium of individuals with high-functioning autism. Additionally, the autism group displayed higher mean diffusivity measurements relative to controls in the overall corpus callosum, genu and midbody. This study also found that a cluster of individuals with autism that had the highest mean diffusivity and lowest FA also had the slowest processing speeds. The relationship of structure and processing speed to cognitive deficits was not demonstrated, but this was a first step toward relating corpus callosum structure to interhemispheric processing alterations in autism. Although there is ample evidence of *intra-hemispheric* processing deficits in autism, the status of *inter-hemispheric* processing mediated by corpus callosum remains an unanswered but significant research question. It is not a simple question to address because the degree of intra-hemispheric processing deficit makes it difficult to tease out any additive affect of inter-hemispheric processing.

LONGITUDINAL STUDIES

To our knowledge, there are currently three *longitudinal* studies of preschoolers with autism [10,13,21]. Although the sample sizes for these studies are still relatively small {n=30 with autism [13]; n=51 with ASD [10]; n=29 with autism plus n=16 with Pervasive Developmental Disorder, Not Otherwise Specified (PDD-NOS) [21]}, all three studies reported increased TBV or total cerebral volume (TCV) at the 2 to 4 year age point. Two of the studies have reported an increase in cerebellar volume but the study of the youngest cohort did not find an increase in cerebellar volume; however, definitive diagnosis of the subjects in the latter study has not been completed and so subjects with PDD-NOS have not been separated from those with autism. The first study reported increased cerebral white (18%) and gray (12%) matter and increased cerebellar white matter (39%) volumes [13]. The study involving the ASD group reported increased TCV and increases in total cerebellar volume and in bilateral hippocampal and amygdala volumes that were proportionate to the increase in TCV [21]. In the subgroup with autism in this study, the amygdala enlargement was disproportionate to the increase in TCV. At follow-up at 6 to 7 years of age, an increase in amygdala volume was still present but hippocampal volume and TCV were the same as controls [15]. The third study reported a significant increase in cerebral volume in their ASD group, but not cerebellar volume [10]. The increase in volumes in the cerebral hemispheres involved both gray and white matter and was generalized rather than regional. Although informative, these three studies have reported data on only a few structures and only one study has reported data at the first follow-up. It will be most interesting to find out about total and regional cortical gray and white matter volumes, total and regional area measurements of the corpus callosum, the status of the basal ganglia in all three samples, and the findings at the first and second follow-up times.

INDIVIDUAL STRUCTURES AND NON-UNIFORM GROWTH

The increase in brain volume in children with autism is not uniform. One study of seventeen 7 to 11-year-old non-retarded boys with autism and 15 typical boys approached this issue by

examining clusters of structures that co-varied in size, revealing a cluster that was increased in volume compared to matched controls (cerebral white matter), a cluster that was no different in volume from controls (caudate, globus pallidus-putamen, diencephalon, cerebellum, brainstem), and a cluster that was decreased in volume (cerebral cortex, hippocampus-amygdala). The findings highlighted an apparent dissociation between white and gray matter and between cerebral cortex-hippocampus-amygdala and other gray matter structures. The approach was interesting and the results provocative. It would be enlightening to see the same approach applied to imaging data from pre-schoolers with autism, and adolescents, adults and an age-matched, mentally retarded group with autism to determine age and severity influences on these associations.

STRUCTURAL IMAGING OF HIPPOCAMPUS, AMYGDALA, AND BASAL GANGLIA

Many brain structures have been measured in autism but most have been the subject of only a few small studies, and outcomes have been inconsistent [22-24]. The hippocampus, amygdala, and basal ganglia are three brain structures that have particular relevance to the symptoms of autism and have at least some consistency in reported findings.

The hippocampus is especially relevant to memory and learning, and neuropsychologic studies of autism have consistently demonstrated abnormalities in memory when use or detection of an organizing strategy was required to support memory. The amygdala has been of particular interest in autism because of its role in emotion and its potential relationship to attachment, emotion dysregulation, affect, and the experience of emotion. Cross-sectional volumetric MRI studies of the hippocampus and amygdala have been confined largely to individuals over the age of 6 years, and results over the age of 12 years have represented all possible outcomes. A *cross-sectional study* [25] of 7 to 18-year-olds with ASD, [69 males with ASD (19 low-functioning autism; 27 high-functioning autism; 25 Asperger's disorder) and 27 controls] reported no difference in TBV but bilateral enlargement of the amygdala in the 7 to 12-year-old low-functioning autism, high-functioning autism, and Asperger's disorder groups of the same magnitude as that reported by a similar study [21]. They reported no differences from controls in the 13 to 18-year-old groups. It is also notable that this study found that the ASD groups started with an amygdala volume that was 13 to 17% larger than controls, but did not exhibit the 40% increase in volume seen in the second decade in the normal controls in this and other studies [25]. Hence, developmental dynamics of the amygdala in autism from childhood to adulthood did not follow a normal pattern. The study also reported a 9 to 12% increase in the volume of the left and right hippocampus in the autism groups, which persisted across the age spans (while there was no developmental increase in the size of this structure in the normal control group as was seen with the amygdala).

Eight other cross-sectional studies have reported amygdala volumes. One of these studies reported a decrease in amygdala-hippocampal volume in 17 individuals with autism, aged 7 to 11 years [26]. Six of these studies, in individuals with autism over the age of 12, reported either an increase in amygdala volume (two studies) [27,28], a decrease (two studies) [29,30], or no difference (two studies) [31,32]. The eighth study bridged the first and second decade [25].

Five structural imaging studies reported data on hippocampal volume in subjects over the age of 12 years. Three of these studies found no difference [28,31,33], one study found an absolute and proportional decrease in bilateral hippocampal volume [30], and one study found an absolute increase proportional to TBV [32]. A sixth study spanned the first two decades and found a relative increase in right and left hippocampal volume in individuals with autism [25]. A more recent study, individuals with autism, ages 6 to 16 years (mean 9.5 ± 3.3 years), demonstrated a decrease in the right medial posterior hippocampus [34].

The 13 studies of the hippocampus and/or amygdala were frequently limited by small samples (8 with < 20 autism subjects), a broad age range, lack of control and subject group matching for males and females, inconsistent anatomic measurement methods across studies, and lack of IQ-matched control groups. However, all three studies of preschoolers have reported increased TBV and it seems likely that the remaining two will confirm an increase in amygdala if not hippocampal volume in their preschool samples. The age at which volumes “normalize” will not be clear until the longitudinal studies report data on subsequent time points and limitations of their control groups are addressed at follow-up.

The basal ganglia have been of interest because of their proposed relationship to repetitive behaviors. One study (n = 35 with autism) reported an increase in caudate volume proportionate to total brain volume that correlated with complex repetitive motor behavior scores from the ADI [35]; this finding was replicated in a second smaller sample (n=13 with autism). A second study (n=15 with autism) found an increase in the volume of globus pallidus but not caudate [26] and another (n=40) found no difference in size compared to normal controls [36].

VOXEL-BASED MORPHOMETRY

Most structural imaging studies to date have used region-of-interest manual tracing methods that have low inter-laboratory reliability [37,38,39]. A few laboratories have switched to automated voxel-based morphometry, which is more sensitive to subtle differences and can be standardized across laboratories.

A comparison MRI study of 21 adults with Asperger's disorder and 24 age- and gender-matched controls found no differences in regional brain volume differences *using manual tracing methods* [37]. However, when voxel-based morphometry methods were applied to these same subjects, significant reductions in gray matter volume in frontostriatal and cerebellar regions were identified. In addition, there was an increase in white matter around the basal ganglia and in the left hemisphere. A third finding was that controls exhibited age-related changes in the volume of the cerebral hemispheres and in gray matter but the Asperger's group did not. A follow-up study extended this study substantially and demonstrated a consistent pattern of findings across age, severity of autism, race and ethnicity once sensitive standardized methods were used, e.g., voxel-based morphometry.

A second study of 17 Chinese children with autism and 17 age matched typical controls, all with normal IQ scores, mapped regional gray and white matter volumes across the brain [39]. Predicting that volumes of interconnected regions would correlate positively, they analyzed connectivity using correlational analyses. The autism group had significantly less total gray matter volume but no change in whole brain volume. They also exhibited regional brain reductions within the fronto-striatal gray matter and parietal-temporal cortices. Analyses revealed fewer and less positive cortico-cortical and cortico-subcortical gray matter correlations in the autism group compared to the control group, similar to an early report based on positron emission tomography in autism [40]. Together, the two studies demonstrate a consistent pattern of imaging alterations in ASD across age, IQ, race, and ethnicity.

MAGNETIC RESONANCE SPECTROSCOPY AS A TOOL FOR UNDERSTANDING BIOLOGIC PROCESSES

In vivo proton MRS has been used to investigate hypotheses about the cellular histopathology of autism, resulting in an interesting series of results. One study set out to test the hypothesis that the increase in total cerebral volume observed in pre-school children with autism was the consequence of reduced apoptosis or reduced synaptic pruning early in brain development [41]. This hypothesis predicted increased chemical concentrations and shorter relaxation times

due to decreased mobility of densely packed brain molecules. The study involved 45 three to four year old children with ASD, 13 children with typical development, and 15 children with developmental delay. All groups were age matched; the ASD and developmentally delayed groups were matched on mental age but not gender and the ASD and typical development groups were matched on gender but not mental age. The study revealed a generalized pattern of reduced (not increased) brain chemical concentrations and prolonged (not shortened) T_2 relaxation times. These results did not support reduced apoptosis or pruning as the mechanism for increased brain volume. Rather the findings were more consistent with reports of increased minicolumns with reduced cellularity as described in the many regions of cerebral cortex by Casanova and colleagues [42,43]. However, because the study was unable to determine whether gray or white matter was specifically affected, two additional studies were completed.

In a subsequent analysis of the data from these same three to four year old cohorts, using cerebral volume as a covariate, the study found a distinct pattern of specific chemical alterations in gray matter in ASD that were distinct from both the typical development and developmental delay groups [44]. The ASD group exhibited decreased gray matter concentrations of choline – containing compounds (Cho), creatine and phosphocreatine (Cre), n-acetylaspartate (NAA), and myoinositol (mI), and prolonged Cho T_2 relaxation time compared with the typical development group. Compared to the developmentally delayed group, the ASD group had decreased gray matter, Cho, and mI concentrations. The ASD and developmentally delayed children exhibited the same pattern of decreased NAA and mI concentrations in white matter, suggesting that the white matter findings were non-specific effects of developmental delay. These findings provided evidence of abnormalities in cortical gray matter early in life in autism that were distinct from abnormalities in children with non-autism developmental delay. The findings suggested decreased cellularity or density. Interestingly, because synaptosomes express high levels of chemicals by MRS and shorter T_2 times, these findings were consistent with a reduction in dendritic arborization and accompanying decreased synaptosome density.

A third study of these 3 and 4-year-old children examined T_2 relaxation in cortical gray matter proposing that if enlarged cerebral volume were due to an acceleration of normal brain growth, then gray and white matter T_2 would be decreased in the ASD children relative to the typical development children, reflecting a more advanced stage of brain development, and the developmentally delayed children would be expected to have prolonged T_2 consistent with more immature development [45]. The study revealed prolonged whole-brain cortical gray matter T_2 in the ASD children but no differences in whole-brain white matter T_2 compared to the typical development children. The developmentally delayed children exhibited prolonged T_2 in both gray and white matter. These findings did not support an acceleration of the normal brain maturation processes as the cause of the early increase in brain volume in autism. Furthermore, these findings suggested that the triggering or primary event might have been neuronal, e.g., in cerebral cortical, and that the white matter changes were an expression or consequence of that event.

A recently published study provided confirmation of cortical involvement and also implicated glutamatergic mechanisms in autism [46]. Using ^1H MRS methods similar to those of Friedman et al. [44], DeVitro and colleagues [46] used partial volume regression (all valid voxels used for calculating metabolite concentrations) in a pilot study of 26 boys aged 6 to 17 years with autism, with IQs above 70, group matched to 29 typical 6 to 16 year old boys. Using a multivariate linear regression model to determine if abnormalities were localized to specific lobes of the brain, the autism group exhibited reduced NAA and glutamine-glutamate (Glx) levels in cortical gray matter and no differences in metabolite levels in overall cortical white matter. Metabolite levels were examined in frontal, temporal occipital gray matter, cerebral white matter and cerebellum. These analyses revealed lower NAA concentrations in frontal and occipital gray matter and a trend for temporal cortex (0.07), cerebral white matter (0.06)

and cerebellum (0.06). Of particular interest, there were lower G1x levels in frontal and occipital gray matter and in cerebellum, and a trend in temporal lobe (0.07). Also of note, the controls had a strong negative correlation between age and gray matter and G1x, but the autism subjects did not. Similarly, controls showed significant negative correlations between age and concentrations of frontal, temporal, occipital and cerebellar NAA, but autism subjects only showed negative correlations between age and NAA in cerebellum. The widespread cortical gray matter reduction in NAA concentrations in this 6 to 17-year-old-group of individuals with autism supported continuation of neuronal dysfunction or reduction in neuronal number past the preschool age despite “normalization” of brain volume. This finding provided further support for the involvement of cerebral cortex. This study was also important for the attention it drew to abnormalities of the glutamatergic system, although others had hypothesized a role for disturbances in this system in the developmental neurobiology of autism [47] and some groups had provided evidence of its involvement [46,48,49].

THE GAPS

Much has been accomplished in defining the gross anatomy of autism. However, structural imaging research in autism has yet to fulfill its original mission of defining developmental trajectories for TBV and for individual structures in relation to each other, consistent with a neural systems model. These trajectories also need to be determined within the three decades during which disturbances of brain development in autism are unfolding. Suggestions of premature aging extend the time frame of interest across the life span. Above all, structural imaging has not yet achieved the goal of accounting for the heterogeneity so characteristic of autism, nor has it tapped the richness of imaging genetics that would enrich the understanding of individuals with ASD and improve their treatment.

FUNCTIONAL IMAGING IN AUTISM

On the basis of behavioral neuropsychological profile studies, we have proposed a model of autism as a disorder of complex information processing with unimpaired processing of simple information [50,51]. A central implication of this model is that autism is not simply a disorder of social functioning but involves a range of deficits across the cognitive domains. Another implication is that autism is dynamically realized as an individual with autism processes information. Therefore, an imaging method that allows examination of neurofunctioning during the performance of cognitive tasks, functional neuroimaging using MRI and PET methods, has proven fruitful in further understanding the neural bases of autism. Thus far, the functional imaging work that has been completed in autism has primarily focused on areas of deficiency – in language, auditory processing, social cognition, face processing, and executive function -- that have been studied extensively behaviorally and found to be characteristic of autism. Limited examination of the mirror neuron system, a system that is thought to be linked to imitation and perspective-taking has also been completed.

LANGUAGE PROCESSING IN AUTISM

The examination of language processing was an obvious area for the use of neuroimaging in autism because, whereas it is well established that individuals with autism have difficulty processing language [52] and impairment in verbal communication is part of the DSM-IV diagnostic triad for autism, the nature of the difficulty in language processing in autism has not been clearly established. The presentation of the language problems can vary from no or little productive expressive language with significant comprehension problems to intact syntactic and semantic skills with impairments in pragmatics or the functional use of language [53]. Individuals with high-functioning autism, that is, those that have IQs of 70 or above, have a language-processing pattern that allows them to decode written language at the word level,

but even they experience greater challenges with the comprehension of text that requires more integration of levels of language, memory, and experiential knowledge [50].

Functional neuroimaging studies have yielded findings for older adolescents and young adults with high functioning autism thought to be consistent with the behavioral pattern associated with autism of good word reading with poor integration of syntactical and semantic information. An initial fMRI study of sentence comprehension found that individuals with autism had relatively greater activation in Wernicke's area [posterior left superior and middle temporal gyri (BA21, 22)] than in Broca's area (BA44, 45) as compared to the age and IQ-matched controls [54]. The functional connectivity, i.e., the degree of synchronization or correlation of the time series of the activation, between these two important language-processing areas was also reduced in the autism group. A separate fMRI study that used a single-word semantic judgment task resulted in a similar pattern of reduced Broca's area activation and increased Wernicke's activation in males, who met criteria for a clinical diagnosis of an autism spectrum disorder [55].

Other investigations have provided more clues as to how individuals with autism process verbal and associated visual information. A visually presented *n*-back letter task during fMRI was used to examine the verbal working memory processes of individuals with autism [56]. In the 1-back version of this task, letters are presented one at a time and the respondent pressed a button when two letters in a row are the same (H-G-G). In the 2-back version, the respondent presses a button when two letters separated by another letter are the same (H-G-A-G). Analysis of the imaging data indicated that the control group had more activation in the left than the right parietal regions as compared to the autism group; whereas the autism group had more activation in the right prefrontal and parietal regions. Rather than activation in the frontal regions as expected in a working memory task, the autism group had more activation than the control group in the posterior inferior temporal and occipital regions. This posterior activation was thought to represent low level visuospatial feature analysis rather than higher level cognition. A factor analysis of functional connectivity for the regions-of-interest in the working memory network was interpreted as indicating less synchronization of frontal activity and greater synchronization of the posterior visual network in the autism group. The pattern of results suggested that the individuals with autism performed the tasks using visual/object-processing areas by treating letter codes as visual stimuli; whereas the control group used language-processing areas, by translating the letter codes into their word names and using a strategy of verbal rehearsal to perform the task. Individuals with autism thus showed a bias toward remaining at the level at which the information was received and initial meaning attached (visual decoding/encoding). They did not incorporate an additional level of processing (verbal encoding) in which language was used to provide a scaffold for task performance. This use of a primary visual decoding/encoding strategy was effective for the *n*-back task, and the participants with autism did not differ from the normal controls on the behavioral measures. However, this strategy may not be effective when the amount or complexity of the visual information increases.

A similar tendency to process language using visual areas was demonstrated in another study [57] that used sentences that either invited or did not invite visual imagery during comprehension (e.g., Low Imagery: Addition, subtraction, and multiplication are all math skills; High Imagery: The number eight when rotated 90 degrees looks like a pair of spectacles). Whereas the controls only activated parietal and occipital cortical regions assumed to be involved in visual imagery when comprehending sentences with high imagery, the autism group activated these areas when processing both types of sentences. Once again, the individuals with autism drew more heavily on visual areas than language areas when completing a language task. The authors interpreted their results as suggesting that it may be

true that some individuals with autism “think in pictures,” a common assumption about individuals with autism.

Comprehension of irony is of interest because it is a form of language that requires a common ground between speaker and addressee in shared beliefs and knowledge, demanding the integration of regions associated with language processing and social cognition. An investigation of the neural basis of language comprehension in male children 7 to 16 years of age with high-functioning autism or Asperger syndrome used auditorily presented stimuli in which the speaker was sincere or ironic [58]. In comparison to gender, age, and IQ-matched controls with typical development, the ASD group had significantly greater brain activation in the right inferior frontal gyrus and bilateral temporal regions. In addition, Verbal IQ was reliability positively correlated with activation in these regions for the ASD group but not for the controls. These results were interpreted as suggesting that the children with ASD were using similar neural mechanisms to comprehend utterances but in a more effortful manner, with the more verbally able ASD participants more likely to use this compensatory strategy.

AUDITORY PROCESSING

Individuals with autism exhibit abnormal auditory processing that affects their ability to comprehend social cues and to learn language. Children with autism demonstrate a lack of preference for their mother's voice [59] and have absent involuntary orienting to vowel changes as measured by evoked response potentials [60]. Individuals with autism also are impaired in extracting mental states from voices [61]. An fMRI study of adults with autism and age-matched controls during passive listening to speech and nonspeech vocal sounds revealed that the autism group failed to activate bilateral superior temporal sulcus regions, which are considered to be voice-selective areas, in response to vocal sounds [62]. Conversely, no difference was found between the two participant groups with respect to the activation pattern in response to non-vocal sounds and the autism group had equivalent cortical activation during vocal and non-vocal stimuli. Individuals with autism appear to have abnormal cortical processing of vocal sounds such that they are processed the same as non-vocal sounds. They demonstrate a lack of saliency for vocal stimuli.

Additional evidence that the left hemispheric language processing areas do not function efficiently in individuals with autism is provided by two studies — one conducted in children and one in adults with autism -- that investigated function in temporal region specialized in the perception and integration of complex sounds, using positron emission tomography (PET) [63,64]. When passively listening to complex auditory speech-like stimuli, children and adults with autism had a different pattern of regional cerebral blood flow (rCBF) than controls. Both the autism groups and the control groups had bilateral activation in the auditory cortex in the superior temporal gyrus (BA 22). However, control children with mental retardation and normal control adults had a left hemispheric asymmetry in the superior temporal cortex, whereas no asymmetry was observed for either the children or the adults with autism. The left middle temporal gyrus (BA 21, 39) and the left precentral gyrus (BA 6, 43) also were significantly less activated in the children with autism. The conclusion from these studies was that children and adults with autism had a dysfunction in the temporal regions that was specialized for the perception and integration of complex sounds.

THEORY OF MIND

A large step forward in understanding why individuals with autism have difficulty with social communication/interaction was the realization that they have difficulty with theory of mind, or understanding that other people have thoughts and making inferences of what those thoughts might be [65]. Children and adults with autism have been shown to have difficulty with first-order (knowing what someone is thinking), second-order (knowing what someone knows about

what someone else is thinking) theory of mind, or with inferencing of what an individual is thinking or feeling in more complex scenarios [66]. A PET [67] and an fMRI [68] study both used stimuli of animations of triangles that performed goal-directed or random movements, or interacted with each other in ways that evoked social interpretations. There were no overt cues that the individuals with autism were to assign social meaning to the movements. Both studies reported that the autism group did not engage the so-called “theory of mind” network (superior temporal sulcus, medial frontal gyrus, right temporal pole) to the same extent as typically developing controls when performing tasks that have a theory of mind component.

EYE GAZE SHIFTS

Eye gaze is used to establish joint attention and to comprehend the mental states and intentions of other people, both of which are important skills for successful social communication that are well documented as impaired in individuals with autism [69,70]. Recent neuroimaging work has examined activation differences in individuals with autism in brain regions involved in gaze processing [71]. In an event-related fMRI task, adults with high-functioning autism and age, gender, and IQ-matched controls watched as an animated character either looked toward (congruent) a checkerboard that appeared and flickered in the character's visual field or looked toward empty space rather than the checkerboard (incongruent). As expected, the autism group had significant activation in the right hemisphere superior temporal sulcus region, an area previously identified in normal subjects as sensitive to gaze shifts [72]. However, whereas the neurologically normal group had greater activation in the right posterior superior temporal sulcus, superior temporal gyrus, middle temporal gyrus and inferior parietal lobule in the incongruent than the congruent condition, the autism group had a markedly different activation pattern. For the autism group, the significant clusters of activation of incongruent > congruent activity were in the left inferior frontal gyrus, the right insular cortex/claustrum, the right posterior middle temporal gyrus, and the left middle and inferior occipital gyrus. Furthermore, in the autism group, a greater incongruent > congruent difference was significantly correlated with greater impairment in reciprocal social interaction. In the autism group, the perceptual processing of the eye gaze movements in the superior temporal sulcus region was disconnected from the attribution of significance of these movements.

FACE PROCESSING

Numerous behavioral studies have demonstrated impairment in the processing of faces in children and adults with autism [73,74], and face processing in individuals with autism also has been studied repeatedly using functional imaging. Whereas reduced or absent activation of the face-fusiform area during tasks that require processing of faces is a well-replicated finding [75], a small number of neuroimaging studies report fusiform activation in autism when comparing faces to non-face stimuli [76] associated with differentiation of familiar faces [77] and correlated with amount of eye gaze fixation to faces for the autism group [78]. Some neuroimaging studies indicate that during face processing individuals with autism activate the inferior temporal gyrus [79] or the medial occipital gyrus [80] (brain regions more typically associated with object processing) or the superior parietal lobe and medial frontal gyrus (regions associated with visual search). [80]. What is clear across these studies is that the brain activation in individuals with autism during the processing of faces is atypical when compared to age and IQ-matched controls.

EXECUTIVE FUNCTION

Executive dysfunction in autism has been suggested by numerous neuropsychological studies [81]. One large behavioral study of 61 children, adolescents, and adults with high-functioning autism and 61 individually-matched typical controls [82], using response inhibition and spatial working memory tasks, revealed the presence of executive dysfunction throughout

development in autism; however, some developmental improvements were found in speed of sensorimotor processing and voluntary response inhibition. Thus, behavioral studies suggest that examination of the neural basis of executive function tasks might shed light on the cognitive impairments that are characteristic of the disorder of autism.

An fMRI investigation of the neural basis of response inhibition in adults with high-functioning autism compared to age and IQ-matched controls used two types of inhibition tasks: a simple response inhibition task (e.g., press a button for every letter, presented one at a time, except A) and an inhibition task using working memory (e.g., only Fs and Gs were presented; press for every letter except for the second of two consecutive letters) [57]. No behavioral differences were obtained between the participant groups; however, analysis of the imaging data indicated that during both inhibition tasks the autism group had less brain activation than the controls in the anterior cingulate cortex, a region typically associated with response inhibition tasks. In the inhibition task with the working memory demand, the autism group demonstrated greater activation in the premotor areas relative to controls. Furthermore, the study findings indicated that during both tasks, the autism group had lower functional connectivity between the inhibition network (anterior cingulate gyrus, middle cingulate gyrus, and insula) and the right middle and inferior frontal and right inferior parietal regions. These results were interpreted as indicative of atypical circuitry for inhibition in the autism group, which required the autism group to use strategic control to accomplish the task.

As described above in an fMRI study of verbal working memory [56], the autism group successfully performed the task behaviorally but did not use frontal and language areas to the same extent as the control group. In an fMRI study of spatial working memory in which adults with high-functioning autism and age and IQ-matched controls performed an oculomotor delayed response task [83], the autism group had significantly less activation in the bilateral dorsolateral prefrontal cortex areas (BA 9, 46) and in the right posterior cingulate cortex (BA 23) relative to controls. However, activation in other areas that are part of the widely-distributed circuitry underlying spatial working memory (the cortical eye fields, posterior parietal cortex, MT/V5, temporal regions, anterior cingulate cortex, pre-supplementary motor area, superior temporal sulcus, inferior frontal gyrus pars opercularis, insula, basal ganglia, and thalamus) did not differ between the autism and control groups.

A fourth fMRI study of executive function measured the brain activation of a group of high-functioning adult participants with autism during the performance of a Tower of London task, in comparison to an age, IQ, and gender-matched control group [84]. Little difference was found between the groups with respect to activation of cortical areas. However, there was evidence of underconnectivity between frontal and parietal areas in the group with autism including a lower degree of synchronization between the frontal and parietal areas of activation, smaller cross-sectional areas in relevant regions of the corpus callosum, and correlation between the size of the genu of the corpus callosum with the frontal-parietal functional connectivity measure for the autism group, but not the control group. These findings were interpreted as suggesting that in autism, a lower degree of information integration across certain cortical regions is the result of reduced intra-cortical connectivity.

The results of these studies suggest that, even with executive function tasks that can be behaviorally performed by individuals with autism, the underlying neurofunctioning differs either in the pattern of brain activation or in measures of cortical synchronization. These differences suggest that individuals with autism are accomplishing the tasks by using alternate cognitive processes or strategies.

MIRROR NEURON SYSTEM

Based on deficits in imitation, theory of mind, and social cognition, a deficit in the mirror neuron system (MNS) in autism has been proposed [85]. Some support for this proposal has been provided by a structural imaging study that reported cortical thinning in areas belonging to the MNS correlated with ASD symptom severity in high-functioning adults with autism compared to controls matched on gender, age, IQ and handedness [86]. First identified in the ventral premotor cortex of macaque monkeys, a human MNS located in the pars opercularis of the inferior frontal gyrus has been associated with imitation, observation of the action of others, and understanding of the intentions and emotional states of others. Event-related fMRI was used to investigate neural activity in the MNS in high-functioning children with autism and age and IQ-matched controls during imitation and observation of facial emotions [87]. The controls activated areas that were consistent with previous research with typical adults including bilateral activation of the pars opercularis of the inferior frontal gyrus (BA 44), a site previously identified with mirror properties. The children with autism did not show activation in these regions; furthermore, they had relatively greater activation than the controls in left anterior parietal and right visual association areas. Both groups had reliable activation in the face processing areas of the fusiform gyrus and amygdala, indicating that the children with autism had focused on the faces. However, the children with autism appear to interpret the data at a visual level rather than attributing emotional and intentional meaning to the face stimuli.

SUMMARY AND FUTURE DIRECTIONS

This review has focused on the valuable and unique contributions that structural and functional imaging have made to the understanding of autism, a neurodevelopmental disorder that has a broad impact on cognitive and neurologic functioning. Structural imaging confirmed the increase in TBV suggested by studies of head circumference and have indicated an abnormal trajectory of neurodevelopment in autism, with an early acceleration in brain volume that is no longer apparent by adolescence. Further structural evidence suggests an increase in intra-hemispheric white matter with a lack of change or decrease in the size of the corpus callosum. The results of *in vivo* proton MRS studies are consistent with cortical gray matter abnormality involvement in the neurobiology of autism. FMRI studies generally have indicated that, although brain regions generally accomplish the same cognitive processing tasks in autism as in healthy controls, the activation patterns are somewhat atypical and thus suggestive of an underlying disturbance or processing abnormalities. Furthermore, the cortical networks used to accomplish cognitive tasks are smaller and functionally under-connected, particularly between regions that are central to the cognitive processing demands of that task. In some studies, functional measures have been correlated to size of relevant regions of the corpus callosum and to behavioral verbal IQ measures for the autism group but not the control group. Autism appears to be a disorder of connectivity, particularly intra-hemispheric connectivity, although structural and functional imaging evidence suggests additional disturbance in intra-cortical connectivity.

The neurobiology of autism is currently in the midst of a third phase of studies and evolving findings, based on diffusion tensor imaging and tracking methods; voxel-based morphometry; MRS; the longitudinal study of first-diagnosed preschool children with ASD; just started or to-be started studies of infant siblings of children with autism; and large-scale, multi-site imaging, phenotype, and genotype studies. The challenge of this work will be to produce an accounting of the behavioral heterogeneity in cognitive, structural, functional, and genetic terms to link cognitive and imaging findings to cellular and molecular histopathology and then to developmental neurobiological, genetic and epigenetic mechanisms.

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