BRAIN RESEARCH 1380 (2011) 175–186

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BRAIN RESEARCH

Review

Bridging the gap between MRI and postmortem research in autism

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ARTICLE INFO

Article history:
Accepted 16 September 2010
Available online 22 September 2010

Keywords:
Neurodevelopment
Neuroimaging
Neuropathology
Amygdala
Volume
Cerebellum

ABSTRACT

Autism is clearly a disorder of neural development, but when, where, and how brain pathology occurs remain elusive. Typical brain development is comprised of several stages, including proliferation and migration of neurons, creation of dendritic arbors and synaptic connections, and eventually dendritic pruning and programmed cell death. Any deviation at one or more of these stages could produce catastrophic downstream effects. MRI studies of autism have provided important clues, describing an aberrant trajectory of growth during early childhood that is both present in the whole brain and marked in specific structures such as the amygdala. However, given the coarse resolution of MRI, the field must also look towards postmortem human brain research to help elucidate the neurobiological underpinnings of MRI volumetric findings. Likewise, studies of postmortem tissue may benefit by looking to the findings from MRI studies to narrow hypotheses and target specific brain regions and subject populations. In this review, we discuss the strengths, limitations, and major contributions of each approach to autism research. We then describe how they relate and what they can learn from each other. Only by integrating these approaches will we be able to fully explain the neuropathology of autism.

Published by Elsevier B.V.

Contents

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0006-8993/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.brainres.2010.09.061

1. Introduction

Investigators searching for the neuropathology of autism have typically taken one of two parallel paths: either a macroscopic approach with magnetic resonance imaging (MRI) or a microscopic approach with postmortem brain tissue. There are unique advantages and limitations to each approach; though the road forward would benefit from better communication across disciplines. MRI studies have contributed significantly to our understanding of how the brains in people with autism deviate from early typical development and function. We now know that the brain undergoes an abnormal developmental time course that appears to include a period of early overgrowth followed by a deceleration in age-related growth in some individuals with autism, which is particularly noted in the frontal and temporal cortices and the amygdala (for reviews see Courchesne et al. (2007) and Amaral et al. (2008)). But what is leading to this deviant developmental trajectory? Is there excessive prenatal neurogenesis due to genetic or environmental alteration? Do dendrites and synapses develop in an excessive, dysregulated manner which results in aberrant connectivity of neurons? Or is there perhaps an inflammatory response leading to excessive microglial activation? Currently, only postmortem brain studies can answer these questions, providing a critical link to the etiology of, and potential treatments for, autism. We will review the strengths and limitations of MRI and postmortem research and the contributions that each has made toward our understanding of autism. We propose that better communication between the two fields would greatly enhance our progress toward understanding this devastating disorder.

2. What can MRI studies tell us about autism?

Because MRI is safe and non-invasive, it can be used repeatedly in large numbers of living subjects. This allows MRI studies to provide a framework for when, where, and in whom there is a deviation from typical brain development. Common types of structural MRI images include T1-weighted images, which provide the greatest level of anatomical detail, and T2-weighted images, which highlight cerebrospinal fluid and edema. Volumetric MRI studies typically utilize high-resolution T1-weighted images and can identify whether a particular region (e.g. cerebral gray or white matter) or structure (e.g. amygdala or caudate nucleus) is larger or smaller in individuals with autism. T1-weighted images are also used for cortical surface and shape-based analyses to identify differences in cortical folding patterns, abnormalities in specific gyri and sulci, and cortical thickness. Diffusion tensor imaging (DTI) studies, meanwhile, describe the organization of white matter by evaluating the differential motion of water along fiber bundles (e.g. fractional anisotropy), although significant work remains to be done to achieve a full consensus interpretation of these measures.

Cross-sectional imaging studies that span toddlerhood through adulthood have provided valuable information about the neuropathology of autism at different ages for more than a decade (Courchesne et al., 2001; Schumann et al., 2010). These studies have revealed that there is brain enlargement during infancy and early childhood in the disorder, but normalization of volume in adolescence and adulthood (see Courchesne et al. (2007) and Amaral et al. (2008) for review). Recently, longitudinal studies have been conducted that have confirmed the cross-sectional findings in young children (Mosconi et al., 2009; Schumann et al., 2010). Additional longitudinal studies of other developmental periods are currently underway, including studies of very early childhood (Nordahl et al., 2010, *IMFAR abstract*) and later development (Lainhart et al., 2010, *IMFAR abstract*) and a study of infant siblings of subjects with autism that aims to identify brain abnormalities that may precede clinical diagnosis (Hazlett et al., 2010, *IMFAR abstract*).

Another important strength of MRI is its ability to acquire large enough samples of individuals to identify phenotype subgroups. Indeed, autism is increasingly recognized as an extremely heterogeneous disorder, with multiple phenotypes and etiologies (Geschwind and Levitt, 2007; Amaral et al., 2008). Recent studies have identified subgroups of brain phenotypes based on white matter microstructure in the corpus callosum (Alexander et al., 2007), frontal cortical volume and connectivity (Shen et al., 2010, *IMFAR abstract*), and the growth rate of the amygdala relative to total brain volume (Nordahl et al., 2010, *IMFAR abstract*). These studies are only the beginning of this important avenue of research, particularly given the number of common co-morbid conditions such as epilepsy, Fragile X, and intellectual disability.

A final major strength of MRI studies is that many analytic approaches can be applied to the same data set from the same

case. These range from volumetric analyses of specific anatomically-defined structures to exploratory whole brain, voxel-wise studies of gray and white matter alterations. Other features that may be examined include cortical shape and cortical thickness (Nordahl et al., 2007; Levitt et al., 2003; Ecker et al., 2010). As DTI methodologies and analytic approaches become more sophisticated, the organization of specific white matter tracts is becoming possible.

Despite the powerful advantages of MRI, there are several significant challenges as well. One challenge is that any movement during an MRI scan, which is typically 30 min to an hour, adversely affects the quality of the image and makes the volume measurement of any brain region less reliable. For this reason, most MRI studies to date have focused on adults and adolescents with high functioning autism despite the developmental nature of the disorder. Sedation or anesthesia has been utilized in some studies of young children and lower functioning individuals with autism, but scanning during natural nocturnal sleep is another option (Nordahl et al., 2008).

Another challenge is that careful advance planning is required to conduct multi-site MRI acquisition, which would be the most efficient way to acquire large sample sizes. MRI data from different scanners cannot be pooled together posthoc. Volumetric measurements of both total brain and subregions can differ significantly between acquisition locations (Lotspeich et al., 2004). This may be attributed to differences in individual scanners, pulse sequences, and even scanning room environment, which produce differences in hardware-induced geometric distortions (Fox and Freeborough, 1997; Nordahl et al., 2010, *IMFAR abstract*). Thus, multi-site studies require careful study design, including matching pulse sequences and the use of calibration phantoms to ensure consistent image quality. However, these difficulties are surmountable, and there is now at least one large scale multi-site study underway (Hazlett et al., 2010, *IMFAR abstract*).

MRI studies have provided important clues into the aberrant pattern of brain development in autism during early childhood. In addition, MRI studies have the potential to help explain some of the heterogeneity of autism by identifying different brain phenotypes or subgroups. Despite these contributions, it is important to keep in mind that the resolution of MRI is still so coarse that only gross, macroscopic changes can be detected. MRI researchers must look to postmortem research to understand the neurobiological underpinnings of their findings.

3. What can postmortem studies tell us about autism?

Postmortem brain tissue, acquired from individuals who had autism during life, is an important tool for understanding the underlying neurobiology and genetics of autism. Although postmortem techniques have been used for hundreds of years to probe the structure of the human brain, the use of systematic, quantitative tools in the field is still very much in its infancy. Indeed, it is largely only in the last five years that these tools have been applied to the study of autism.

It is common practice, following postmortem brain procurement, to section the brain down the midline through the corpus callosum in order to separate the right and left hemispheres. One hemisphere is often immersed in a fixative, such as paraformaldehyde or formalin, to preserve the tissue by disabling damaging enzymes and bacteria as well as maintaining the structural morphology. This preparation is ideal for histological studies of microscopic anatomy and cellular characteristics, such as estimating the number of neurons, quantifying the length of dendritic processes, or using antibodies to localize proteins in a particular brain region. The other hemisphere of the brain is generally sliced into coronal slabs (e.g. 1 cm thick) and rapidly frozen to protect the integrity of the cells from protein degradation without the use of a chemical fixative. This preparation allows for molecular studies aimed at identifying, measuring the level of, and localizing proteins, neurotransmitter receptors, or genes expressed in cells in a given region of the brain. One strength of using postmortem brain tissue to study the neuropathology of autism is that multiple factors, from cell structure to gene expression, can be evaluated within an individual brain and compared between diagnostic groups.

Efforts to carry out postmortem brain studies of autism have historically been hindered by poor tissue quality and small sample sizes, with fewer than 150 autism cases studied to date and a mean sample size of 5 per study. Due to the limited number of cases and documentation available, postmortem studies are currently not well positioned to deal with the heterogeneity of autism spectrum disorders. In addition, nearly all of the brains studied have been from adults with autism, well after the period of peak aberrant neurological growth described by MRI studies. Additionally, postmortem studies are obviously limited to observing the end result of each case's neuropathological course, which is influenced by their individualized exposure to environmental factors, medications, co-morbid symptoms, cause of death, and particular type of autism. Given the heterogeneity of autism spectrum disorders, apparent age-related changes in pathology, and limited quantities of brain tissue available, well-designed postmortem studies must control for confounding factors such as age and sex, and exclude or segregate co-morbid conditions such as epilepsy, in order to detect interpretable differences in brain structure.

Despite these limitations, the availability of more abundant, high-quality postmortem tissue is rapidly increasing. With the recent adoption of modern neuroanatomical techniques in the field such as stereological estimation of cell numbers and in situ hybridization for evaluating expression levels of genes, a more complete picture of the neuropathology of autism spectrum disorders may be assembled in the near future. Postmortem studies may bring us one step closer to discovering the etiology of autism by providing the critical link between macroscopic brain abnormalities reported in MRI studies and specific neuropathology, genetic alteration, or other neurobiological events in development.

4. The gap

4.1. *What's in an MRI voxel?*

The answer depends on many factors, including the resolution of the MRI scan, the age of the individual being scanned, and

the brain region being examined. Currently, the typical voxel resolution of a T1-weighted scan is 1 mm^3. To give some perspective, a typical pyramidal neuron in the cerebral cortex is 10–50 μm in diameter (a micron, μm, is 1/1000 of a millimeter). An interneuron is ~10 μm in diameter. Fig. 1 depicts the scale between a standard 1 mm^3 MRI voxel and the underlying neuroanatomy. In the amygdala of a typical 3 year old, there are ~7000 neurons in just a single 1 mm^3 voxel (Schumann and Amaral, unpublished data). In the cerebral gray matter (i.e. neocortex) of a 3 year old, there are ~20 billion neurons (Larsen et al., 2006; Pakkenberg and Gundersen, 1997) in ~600 cm^3, which is over 40 thousand neurons per 1 mm^3

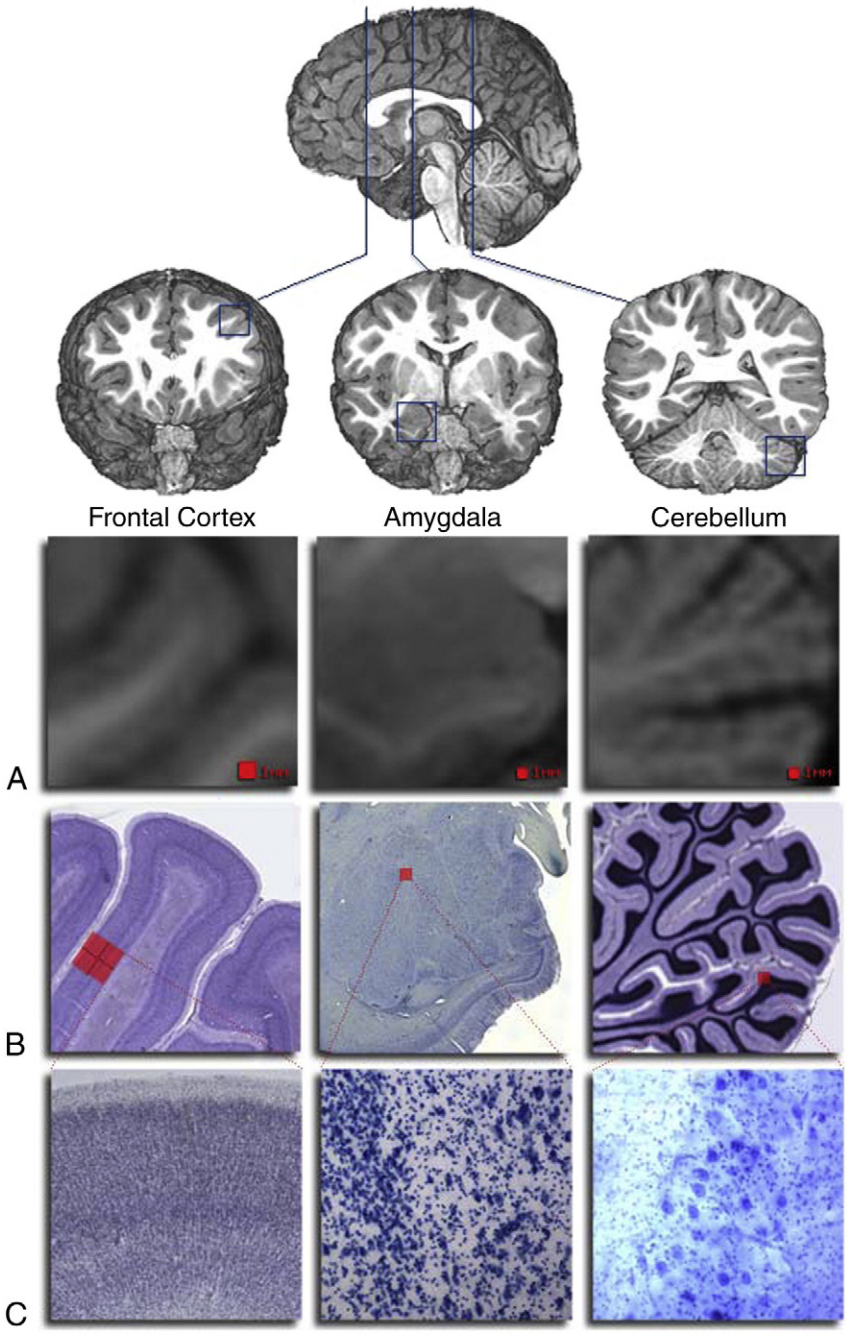

Fig. 1 – A depiction of the various levels of detail provided by MRI images and postmortem human brain tissue. At the top of the image, the sagittal rendering from a high-resolution T1-weighted MRI depicts the location of three coronal slices at the levels, moving in the rostral (left) to caudal (right) direction, of the frontal cortex, amygdala, and cerebellum. a) The voxels within the blue box on each coronal slice is magnified to depict the resolution of a single 1 mm^3 voxel (red box). b) Nissl stained sections from postmortem human brain tissue portrays the organization and morphology of cell bodies at approximately the same resolution as the MRI image above; red boxes indicate approximately 1 mm. c) At higher magnification; individual cell bodies can be seen. Note, though, that the 1 mm scale on the tissue section is only an estimate; since there is variable shrinkage during processing.

voxel! To understand the relationship between the gross macrostructural differences detectable by MRI and the cellular microstructural differences evaluated in postmortem research, a helpful exercise is to review the basic anatomical organization of gray and white matter in the human brain.

The mature brain is comprised of approximately 100 billion neurons, and perhaps three times as many glial cells (Pelvig et al., 2008; Azevedo et al., 2009). The cerebral cortex is a laminated sheet of gray matter that is 2–4 mm thick, and in most regions, neurons are organized into six horizontally arranged cellular layers (Fig. 2). In general, the input layer is layer IV, projections to other parts of the cortex arise primarily from layers II and III, and projections to subcortical regions arise from layers V and VI (Kandel et al., 2000). The proportional volume of the layers varies markedly across cortical region in a manner that is frequently speculated to reflect their function, e.g. early sensory cortices often display an enlarged input layer IV. Neurons in subcortical structures

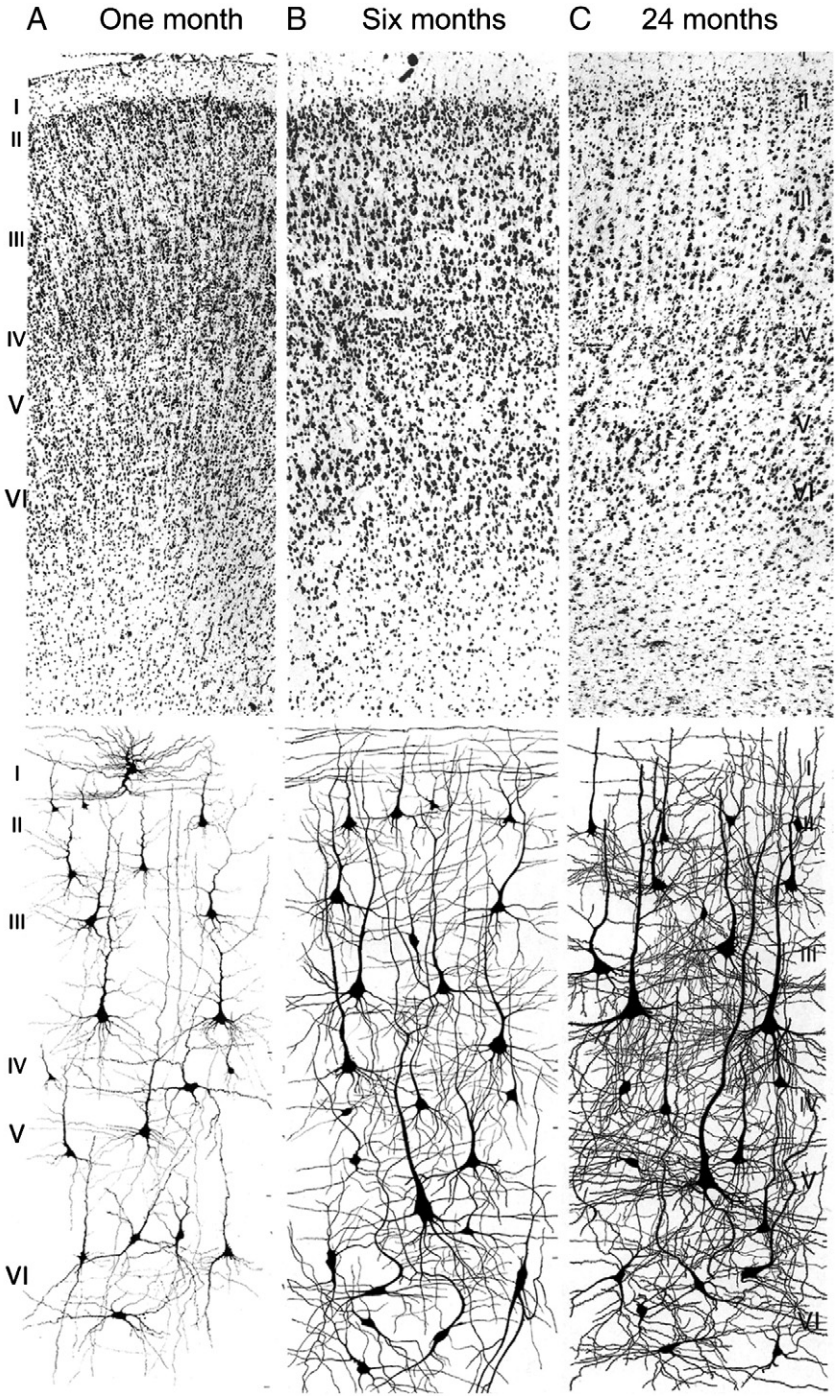

Fig. 2 – A–C depict cell body-stained sections of dorsolateral prefrontal cortex at 1, 6 and 24 months of age. Below each is a representative Golgi-stained section showing the extent of dendritic growth in this same cortical area over these same ages (from Conel (1939, 1947), *The Postnatal Development of the Human Cerebral Cortex*).

are typically clustered by size, shape, and function into bundles called nuclei rather than layers. As in the cortex, most nuclei are not homogeneous, but instead include a variety of cells, and the definition of nuclei may depend on the method by which the cells are visualized.

Neurons are extremely diverse in both size and function. Approximately 80% of neurons are excitatory. Many of these are projection neurons, such as pyramidal neurons, which are primarily located in layers III, V, and VI and send long distance connections to other parts of the brain. Smaller interneurons, located in all layers, are primarily inhibitory and project only locally (Kandel et al., 2000). The remaining cells are predominately glia, which are generally thought of as support cells involved in immunity and synaptic maintenance, but have also been found to play a wide array of other roles as well.

The cortex demonstrates vertical functional units that are arranged as radial columns, or cortical columns, that span across the six cortical layers (Mountcastle, 1997; Jones, 2000) (Fig. 2). Each cortical column comprises multiple narrower panlaminar 'minicolumns', each of which in turn contains 80–100 radially arranged neurons (Mountcastle, 1997). Minicolumn formation has been associated with early stages of cortical development when postmitotic neurons ascend in linear arrays along a radial glial scaffolding (Rakic, 1988). Neurons in each cortical column are believed to respond to similar stimuli and perform similar functions (Mountcastle, 1997).

The area between neuronal cell bodies is called the neuropil and contains neuronal dendrites and axons, synaptic connections, glia, and glial processes. Neurons communicate with one another through synapses; information is typically transmitted through axons and received through dendrites. The synaptic cleft, ~20–40 nm in size, is the space between an axon terminal of a presynaptic cell and the dendrite or soma of a postsynaptic cell (Kandel et al., 2000). It is estimated that in an adult, each neuron makes, on average, 1000 synaptic connections, and receives many more. During early postnatal development, the number of synaptic connections per neuron is much higher. Through a process of activity-dependent synaptic elimination, the number of synapses per neuron decreases during childhood and stabilizes during adulthood.

White matter is more homogenous than gray matter and is comprised primarily of myelinated axons. Within the cerebrum, there are both short distance (10–30 mm) and long distance fibers (30–170 mm). Currently, the highest voxel resolution for DTI studies of white matter tractography is about 2 mm^3, but voxel size may range up to 5 mm, sometimes with a gap in between slices. Again, to provide some perspective, axon diameter typically ranges between .2 and 20 μm (Kandel et al., 2000). Assuming an average diameter of 10 μm, one 2 mm^3 voxel contains about eight million axons. Thus, DTI studies are most useful in providing information about gross changes within large fiber bundles or fascicles. Significant work remains to achieve a consensus on the biological underpinnings of diffusion-weighted measures such as fractional anisotropy.

4.2. *Overlap (or lack thereof) of MRI and postmortem studies of autism*

Improved communication between MRI and postmortem research approaches is likely to generate a far more complete understanding of the neuropathology of autism than is possible at present. We briefly review major findings to date from each discipline, beginning with a review of total brain volume studies, then focusing on specific brain regions implicated in autism (Amaral et al., 2008). For some regions, such as cerebellum and amygdala, a significant amount of work has been carried out in both MRI and postmortem research. However, to date there is little overlap in the conclusions that can be drawn from each discipline; our intent is to identify areas in which one discipline can be informed by findings in the other.

4.3. *Total brain volume and cerebral lobes with MRI*

Collectively, studies of head circumference and MRI brain volume indicate a critical period of abnormal brain growth beginning in the first year of life that results in a persistent enlargement at least through early childhood. (Courchesne et al., 2001; Hazlett et al., 2005; Schumann et al., 2010). Whether the enlargement persists into adulthood is less certain (see Amaral (2008) for review). There is additional evidence suggesting that the frontal (Carper et al., 2002) and perhaps temporal gray matter may be especially affected during early childhood (Hazlett et al., 2005; Schumann et al., 2010). Hazlett et al. (2005) report an increase in temporal and frontal gray matter in two year olds with autism relative to a combined control group of typically developing and developmentally delayed children. In a more recent longitudinal study of cerebral cortical development in 2.5 year olds with autism, Schumann et al. (2010) also found selective enlargement in frontal and temporal lobes relative to typically developing controls. They extend the cross-sectional findings to report abnormal growth trajectories in these regions as well.

Several MRI studies have evaluated cortical thickness in adults with autism, but the findings are somewhat inconsistent. Hadjikhani et al. (2006) report cortical thinning in specific regions distributed across frontal, parietal, and temporal lobes while Hardan et al. (2006) report increased cortical thickness in the temporal lobes. More recently, Ecker et al. (2010) evaluated cortical thickness as one of five measures of cortical features in adults with autism and report a distributed pattern of relative increases in cortical thickness in temporal and occipital lobes and relative decreases in cortical thickness in frontal and parietal lobes. A look at the alterations reported by postmortem studies suggests that methodological differences, such as subject selection, may account for some of the disagreements between these studies.

4.4. *Frontal cortex with postmortem tissue*

Although MRI studies suggest that the peak of frontal cortical abnormalities is during early childhood development, no postmortem study has systematically explored the underlying neurobiology of the aberrant growth in children. Two studies indicate the presence of glial abnormalities in frontal cortex that might contribute to this volume increase. One reported qualitative microglial and astroglial activation (Vargas et al., 2005). A more recent study demonstrated increased microglial volume and number suggestive of

neuroinflammation in dorsolateral prefrontal cortex (Morgan et al., 2010).

Four studies of neuronal features have utilized frontocortical tissue from small groups of subjects. Two studies examined spindle neurons (a.k.a. Von Economo neurons) which are large cells that are localized to the frontal insular region and are unique in great apes and man. This uniqueness has led several researchers to suggest that spindle neurons may play a role in higher-order cognitive function and emotional behavior (Allman et al., 2001, 2005). The studies appear contradictory, finding either no difference in numbers in adults (Kennedy et al., 2007) or an increased ratio of spindle neurons to pyramidal neurons in children (Santos et al., 2010), but this disagreement may reflect the developmental trajectory of the disorder. Two studies of neuronal organization revealed ill-defined cortical layers in the dorsolateral prefrontal cortex (Mukaetova-Ladinska et al., 2004) as well as a poorly defined boundary between gray matter and white matter (Avino and Hutsler, 2010), suggesting the possibility of abnormalities in neurogenesis or neuronal migration.

The frontal cortex has also been the focus of studies of the minicolumnar organization of neurons. As described earlier, neurons in cortical gray matter are arranged in radial "minicolumns" during prenatal cortical development (Fig. 2). The space between the minicolumns is occupied by dendritic and axonal processes that extend throughout several layers of cortex (Jones, 2000; Rockland and Ichinohe, 2004), which dramatically increases with age in the first two years of life in typical development (Fig. 2). Three independent studies using varying techniques have suggested a reduction in intercolumnar width and increased cell density in dorsolateral prefrontal cortex in groups of subjects with autism not segregated by age (Casanova et al., 2002, 2006; Buxhoeveden et al., 2006). These findings imply that there should be a greater number of neurons and a decrease in dendritic arborization given early overgrowth followed by normal brain volume. However, a rigorous stereological study of neuron number in the frontal cortex has yet to be carried out.

4.5. *Anterior cingulate with postmortem tissue*

The anterior cingulate cortex (ACC) is classically considered to be associated with higher-order cognitive functions such as error detection. In the seminal postmortem studies carried out by Kemper and Bauman (1993), ACC was the only region of neocortex noted to show abnormalities; it was observed to be unusually coarse and poorly laminated in subjects with autism compared to controls. Abnormalities in the ACC have since been reported by others, including decreases in cell size (Simms et al., 2009) and decreases in the mean density of GABA(A) receptors and benzodiazepine binding sites (Oblak et al., 2009). This last finding suggests an alteration in GABAergic innervation that could potentially lead to a disturbance of the delicate balance between excitation and inhibition in this cortical area. Structural MRI studies have not highlighted the ACC as an area of obvious pathology in autism, however, there is significant evidence from functional imaging studies that suggests abnormal activation and connectivity in the ACC (see Minshew and Keller (2010) for review).

4.6. *Temporal cortex with postmortem tissue*

Postmortem studies designed to examine the underlying pathology of abnormal growth in the temporal cortex have yet to be carried out. The only such study to date and only one of two stereological studies of neuron number in the autism brain (besides Schumann and Amaral (2006) on the amygdala) was carried out on the fusiform gyrus, which is known to be involved in face processing, a notable deficiency in many subjects with autism. The autism group showed significantly lower neuronal densities within layer III, lower total neuron numbers in layers III, V and VI, and smaller mean perikaryal volumes of neurons in layers V and VI (van Kooten et al., 2008). This finding stands in notable contrast to longitudinal MRI studies indicating enlargement of the temporal cortex early in development (Hazlett et al., 2005; Schumann et al., 2010), although a postmortem study of temporal cortex in young children has yet to be carried out.

4.7. *Amygdala with MRI*

MRI studies indicate that the amygdala follows an aberrant trajectory of growth in individuals with autism, with amygdala enlargement present as early as age 2 (Sparks et al., 2002; Mosconi et al., 2009; Schumann et al., 2009). Schumann et al. (2004) demonstrated that, on average, amygdala enlargement remains present in 7–12 year old children with autism, but not in 12.5–18 year olds with autism. This general pattern of age-related changes in the amygdala has been observed in other cross-sectional studies covering a broad range of age intervals (Aylward et al., 1999; Haznedar et al., 2000a,b; Pierce et al., 2001; Sparks et al., 2002; Schumann et al., 2004; Nacewicz et al., 2006; Mosconi et al., 2009; Schumann et al., 2009). To date, there has only been one published longitudinal study of amygdala volume, reporting bilateral enlargement at ages 2–3 and 5 but no group differences in the rate of growth between the time points (Mosconi et al., 2009). More recently, Nordahl et al. found 3 distinct autism subgroups in the rate of growth between 2 and 3.5 years of age (Nordahl et al., 2010, *IMFAR abstract*). One subgroup demonstrated rapid amygdala growth and slow total brain volume growth, the second demonstrated slow amygdala growth and rapid total brain growth, and the final subgroup demonstrated growth trajectories consistent with those of typically developing children.

4.8. *Amygdala with postmortem tissue*

To date, no studies have evaluated younger postmortem cases with autism to determine what cellular properties account for the early overgrowth in amygdala volume. In contrast to MRI findings of early enlargement, the one quantitative stereological study conducted to date reported a ~15% decrease in the number of neurons in adolescents and adults with autism (Schumann and Amaral, 2006). This finding suggests that either neuron number does not account for the increase in amygdala size in young children with autism, or, there is considerable neuron loss later in life. Intriguingly, this alteration was particularly pronounced in the lateral nucleus, a subregion responsible for sensory integration.

4.9. *Cerebellum with MRI*

The cerebellum has long been an area of interest for both MRI and postmortem studies of autism. Five MRI studies of individuals with autism at a wide range of ages have found the cerebellum to be enlarged relative to controls (Minshew et al., 2005). However, this increase in cerebellar volume is generally proportional to total brain volume, with one exception in which the difference exceeded that of the cerebrum (Hardan et al., 2001). Only one published study to date has examined children younger than age 3, and this study did not find a difference in cerebellar size between the children at risk for autism and typically developing controls (Hazlett et al., 2005). In contrast to the total cerebellum, the size of the vermis appears to be slightly smaller in some individuals with autism (Kaufmann et al., 2003; Courchesne et al., 1994; Scott et al., 2009), though this may not be specific to autism but rather a common feature of neurodevelopmental disorders in general (Kaufmann et al., 2003; Piven et al., 1997).

4.10. *Cerebellum with postmortem tissue*

The cerebellum is the most studied postmortem structure to date. Of the 30 postmortem cases of autism in which the cerebellum has been studied, 22 (or 73%) cases showed lower density of Purkinje cells, particularly in the more lateral regions of the structure (Ritvo et al., 1986; Kemper and Bauman, 1993; Bailey et al., 1998a; Palmen et al., 2004; Whitney et al., 2008a). Even though this is one of the most striking and consistent descriptive findings in neuropathological analyses in the autistic brain, a comprehensive stereological study of the actual number of Purkinje neurons in the whole cerebellum has yet to be carried out. Recent studies from Blatt and colleagues have found that some, but not all, cases of autism show a reduction in the linear density of Purkinje neurons as well as decreases in basket and stellate cell densities (Whitney et al., 2008b, 2009) and substantial alterations in the GABAergic system (Yip et al., 2007, 2008, 2009). Others have reported no difference in the density of Purkinje cells in the cerebellum, but a 24% decrease in the size of Purkinje neurons in the autism group (Fatemi et al., 2002). Finally, neuroinflammation has been reported in the region (Vargas et al., 2005). This inflammation may be associated with the reported reductions in neuronal density. While these findings appear to conflict with MRI studies reporting an enlarged cerebellum in autism (Courchesne et al., 2007) they may simply reflect very different cohorts of subjects. In addition to differences in age, 22 of 24 subjects reported some degree of mental retardation and nearly half were taking medication for epilepsy (Ritvo et al., 1986; Kemper and Bauman, 1993; Bailey et al., 1998b; Palmen et al., 2004).

5. Bridging the gap — translating between MRI and postmortem research

Using as an example the notion that the brain grows too big too fast in autism, we explore how the MRI and postmortem research fields may look to one another for guidance. If the rate of brain growth is indeed accelerated, which factors are likely to account for this growth? If brain size is an indication of aberrant neurological development, what does this really tell us about the neuropathology of autism? Is it likely that volume differences reflect a single cellular alteration?

5.1. *The number of neurons*

An increase in the number of neurons is unlikely to be the sole factor in accelerated cortical growth in autism. Head circumference and MRI studies suggest that brain size at birth does not differ (see Redcay and Courchesne (2005) for review), and it is not until sometime between 6 and 12 months of age that brain enlargement becomes evident. Neurogenesis, the process by which neurons are generated, is, with a few notable exceptions, a prenatal event (Rakic (2002) for review). Other processes, such as disruption of programmed cell death (Haydar et al., 1999), could produce increases in neuron number relative to control subjects; however, this would not account for the accelerated brain growth as head circumference studies have proposed (Hazlett et al., 2005; Dawson et al., 2007).

It is possible that there are more neurons already present at birth in a newborn with autism, but without extensive neuronal arborization this increase does not translate into a significant change in head size. To give some perspective, if there are ~20 billion neurons in the neocortex from birth (Larsen et al., 2006), neuronal cell bodies on average have a ~20 μm diameter, and cerebral gray matter is ~600 cm^3 in a typical 2 year old, then the cell bodies of neurons only occupy 15% of the total neocortical volume. Therefore, even a 20% increase in the number of neurons, would only result in less than a 1% increase in cortical gray volume.

5.2. *The extent of neuronal dendritic growth and number of synapses*

If only one factor accounts for early overgrowth, this is the most likely candidate. In the typically developing newborn brain, dendritic arbors have barely sprouted and synaptic connections between neurons are sparse (Conel, 1939, 1947; Huttenlocher, 2002) (Fig. 2). Over the next few years, there is a dramatic increase in neuronal dendritic volume as dendrites establish synaptic connections with a wide array of neurons. During this "critical period" of development, synapses compete for neural growth factors to survive (McAllister, 2002). Connections that are inhibited, or underutilized, are subsequently pruned, resulting in a mature network of functioning synaptic connections between neurons. If dendrites grow in an excessive, dysregulated manner or do not undergo the same degree of efficient synaptic pruning, the result may be aberrant connectivity between neurons alongside overall brain enlargement. Although this is the most likely candidate, few postmortem studies have looked at neuronal dendritic arborization or synapses in autism (Raymond et al., 1996; Hutsler and Zhang, 2010); there is not a single study of dendritic arbors in children with autism to date.

5.3. *The number and size of glial cells*

There is an intermediate chance that the number of glia alone could account for increased brain size. The majority of

gliogenesis, the process by which new glial cells are generated, occurs prenatally. However, this rule is not absolute and has recently been challenged (Pelvig et al., 2008). The extent to which gliogenesis is prenatally limited also varies between the three main populations: oligodendrocytes, astrocytes, and microglia. Oligodendrocytes, whose main function is to insulate axons in a myelin sheath, comprise ~75% of the number of glial cells in gray matter (Pelvig et al., 2008); thus, there are ~25 billion in the neocortex. Although the number of oligodendrocytes does not increase dramatically after birth, the processes that form the myelin sheath around neurons do extend out and wrap axons throughout postnatal development. Astrocytes comprise ~17% of glia in the brain and, although there are subtypes of astrocytes, their main function is to regulate the external chemical environment of neurons. There are ~6 billion astrocytes in cerebral gray matter (Pelvig et al., 2008). Astrocytes are thought to be generated almost entirely prenatally from the same progenitor cell population as neurons, but they are capable of demonstrating significant changes in volume in response to inflammatory signaling, with swelling of both the soma and the inflammation-sensing processes.

If glia do account for some of the increased cerebral volume in children with autism, a neuroinflammatory response involving microglia may be a likely culprit. Microglia are resident phagocytes, constantly eliminating damaged neurons, accumulated debris, and infectious agents. Although microglia only comprise ~6% of the glia in the brain (~2 billion) (Pelvig et al., 2008), unlike neurons and the other glial populations, microglia readily increase in number in response to immune challenges. In addition, although they are normally small relative to other glia, they are capable of dramatic morphological changes if strongly stimulated with pro-inflammatory factors. Under these completely normal conditions, microglia become "activated," and the cell body may swell as much as 2–4 times its normal volume while processes thicken and then retract (Morgan et al, 2010). "Amoeboid" microglia in a fully activated state are commonly found in the typically developing brain in high concentrations in response to the large amounts of extracellular debris and neuronal apoptosis that is a consequence of programmed cell death (Rezaie and Male, 1999). If there is a chronic state of inflammation as some have proposed in autism (Pardo et al., 2005; Vargas et al., 2005; Morgan et al., 2010), perhaps in response to autoantibodies or infection (Shi et al., 2009; Singh, 2009; Goines and Van de Water, 2010) both microglia and astroglia may be larger and more abundant. Another possibility is a neuroinflammatory response leading to edema, resulting in an increase in overall brain size; however, edema has not been consistently detected in children with autism via MRI.

5.4. *A combination of all three cellular factors*

This appears most likely, particularly given the interaction between these cellular features during early development. One possible scenario is that excess neurons are generated prenatally, perhaps due to genetic abnormalities and/or environmental interference. For example, mutations in the PTEN gene, which normally regulates cell division, growth, and programmed cell death (Chu and Tarnawski, 2004), have been associated with large head size and autism (Zori et al., 1998; Goffin et al., 2001; McBride et al., 2010; Varga et al., 2009; Orrico et al., 2009). There is also evidence for an association between the short allele of the serotonin transporter gene (5-HTTLPR) and increased cerebral gray matter (Wassink et al., 2007). We predict, though, that excess neurons alone does not result in a detectable increase in overall brain size or head circumference at birth. However, this initial interference in otherwise typical development might set off a cascade of events over the next 2 years leading to enlargement and ultimately resulting in aberrant, dysfunctional communication between neurons. For instance, as mentioned earlier, there is an explosion of neuronal dendritic growth and synapse formation in the first few years of life (Fig. 2). This same process likely occurs in the brain of a child with autism, yet if there are more neurons, this would then lead to even more dendrites, synapses, myelin, etc. Glia could also be overproduced via similar genetic mechanisms, or become activated in an inflammatory response to neuronal overproliferation, contributing further to early volume increases. In addition, glial activation could bias maturation of dendritic arbors via release of neurotrophins and other modulatory factors (Christopherson et al., 2005; Bessis et al., 2007).

The scenario described is just one of several possible combinations of neuropathology that might occur in the autistic brain during this early critical period of development. Only by carrying out a comprehensive postmortem study of early brain development using modern quantitative methods will we unveil the process by which the brain becomes enlarged in autism. While MRI studies point to exuberant growth and enlargement in children, postmortem studies have found reductions in neuron number possibly related to neuroinflammatory and degenerative processes. However, it is critical to point out that there is not a single postmortem study focused on brain development in children with autism published to date; all studies have been carried out in adolescents and adults well past the peak time of aberrant growth described in MRI studies.

6. Closing the gap

Independently, MRI and postmortem studies have made significant contributions towards elucidating the neuropathology of autism. Greater communication and understanding between the fields could lead to even more significant progress being made. MRI and postmortem researchers working in the field of autism could learn much from each other's work and use findings from each field to better guide specific hypotheses about certain brain areas and periods of development. For example, MRI studies suggest that early childhood is a critical time of aberrant brain development in autism, yet there have been no postmortem studies of young children. Postmortem researchers might, therefore, use MRI studies to guide them where in the brain and when in development to focus. Specifically, it is important for postmortem researchers to keep in mind that MRI studies suggest that pathology changes with age in individuals with autism. Therefore, findings from postmortem studies done thus far on adult cases cannot explain early brain overgrowth in children with autism as reported by MRI studies.

On the flip side, MRI researchers need to look to postmortem studies to understand the neurobiological underpinnings of gross findings detectable by MRI. What, exactly, does the finding of a certain structure being bigger, smaller, thicker, or thinner mean? MRI researchers could also look to postmortem studies to guide hypotheses and theories. For example, a widely cited, but completely speculative theory in the imaging field is that there is a disruption in the process of synaptic elimination or 'pruning' that may account for early brain enlargement despite the fact that there is to date little or no postmortem evidence to support this notion.

As researchers begin to describe the developmental trajectory of autism and identify the likely heterogeneous subgroups in the disorder, the MRI and postmortem fields will benefit markedly from communicating with one another and generating hypotheses together. MRI studies can provide the framework by identifying different subgroups with distinct patterns of affected brain areas or the timing of aberrant development, while postmortem studies can tackle the neurobiological underpinnings and etiologies of the different subgroups. Together, the two fields will make significant advancements towards elucidating the neuropathology of this often devastating disorder.

Acknowledgments

Original research reviewed here was supported by NIMH grant MH 041479 and NINDS grant NS 16980 (CMS) and NIMH K99MH085099 (CWN). We are grateful to Aaron Lee, Jeff Bennett, and Brian Moon for their help with manuscript and figure preparation. Dr. John Morgan and Dr. Melissa Bauman provided helpful feedback on previous drafts of this manuscript.

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