Chapter 3

The Neuroanatomy of ASD

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INTRODUCTION

The neuroanatomy of autism presents us with a diverse array of findings that are hard to encompass within a single explanatory framework. What we know about the neuroanatomy of autism has been derived from descriptive observations of imaging and tissue samples, hypotheses derived from neuropsychological inferences, and measures driven by prior observations, some of which could not have been predicted by any existing knowledge of brain–behavior correlation.

Out of this sprawling array of investigations has come evidence of the involvement of the limbic system, corpus callosum, basal ganglia, thalamus, cerebral cortex, white matter, cerebellum, brainstem, and ventricles—in short, pretty much the entire brain. Moreover, the brain as a whole appears to be affected in ways that cannot simply be reduced to discrete impacts on its individual parts. In order to make sense of these findings, it is important to be sensitive to the range of assumptions in various study designs and interpretations, and the capabilities and limitations of the technologies used to generate the data. This is particularly important in organizing the brain data with the intention of making sense of the behavioral features of autism spectrum disorders (ASD). Consequently, these considerations will be reviewed in order to bring them to bear on the findings.

Observing individuals with ASD, we note a range of features suggesting functions that are some combination of atypical (extraordinary as well as deficient), maladaptive (in relation to social norms or comfortable ranges of biopsychosocial regulation, or both), and dysfunctional (functions that cannot be performed as desired or needed). These features clearly suggest a role for nervous system function in the development of autism spectrum disorders. We also find neuroanatomical features that are different than those found in individuals who are not on the autism spectrum. To understand the anatomy findings and how they may contribute to functional features, we utilize what we know about how the brain controls and modulates the

functions we perform. This immediately confronts us with a problem: many of the functions that are so meticulously described at the psychological level as atypical in ASD do not have a clearly understood anatomical basis. Therefore, we cannot simply make an a priori selection of specific brain regions relevant to ASD features, dive into the brain, measure those regions, and come out with the answers. In fact, this "modular" strategy was dominant earlier in autism brain research but it was only mildly fruitful, in that neither initial nor subsequent efforts found any localized region with a clear abnormality that was specific to autism, present in everyone with autism, or present in the same way at different points in development or in different cohorts. Instead, unexpected findings have emerged, such as a tendency toward increased brain size and widespread alterations in functional connectivity, that challenge a modular approach to brain–behavior correlation (Herbert & Anderson, 2008).

The modular approach to brain–behavior correlations and neuroanatomical investigations of autism is further challenged by the question of how to anticipate anatomical findings in a condition with early-life onset. So much of our knowledge of neuroanatomy is based on research on the impact of brain lesions acquired after brain maturation (due to, for example, injuries or disease processes such as tumors). But how do we work back in developmental time from our knowledge of the impact of such lesions on behaviors in adults or older children to valid or useful inferences about the origins of the brain underpinnings of these behaviors? We are far from possessing a detailed picture of the stages of brain–neurofunction relationships through early development—indeed, our very capacity to measure such things is just emerging from its own early infancy. This issue can be framed in the context of the debate between "nativist" and "neuroconstructivist" positions regarding development (Karmiloff-Smith, 2006). A "nativist" position would impute the presence of all of the neurofunctional capabilities exercised in childhood and adulthood as innate, inborn features, almost as if there were a little "neurofunctional homunculus" imprinted on the fetus or the gene. By contrast, a neuroconstructivist position posits a dynamic interaction of genes, brain, cognition, and environment, so that development is a process of constructing emergent features—interactively building both neurofunctional capabilities and the underlying neural systems.

Investigations into the brain basis of autism can be, and have been, designed from both nativist and neuroconstructivist perspectives. A nativist study design might investigate direct correlations between genetic differences and altered neurocognitive functions, with the implicit or explicit assumption that functions emerging in mid-childhood or adulthood have direct correlates in both genes and brain, and that the genes and brain components contain these functions from the start. In a neuroconstructivist framework, the biological and developmental pathways from genes to brain development and neurocognitive function are seen as much more complex and interactive (Morton & Frith, 1995), so that genes are thought to "code for" something prior to neurocognitive function, rather than these functions themselves. This prior "something" (presumably at the level of protein and molecular signaling pathways) needs to interact with other factors in a dynamic and developmental interaction that creates emergent new capabilities in the brain and in neurofunctional capability—and creates these both simultaneously and in relation to each other. Neuroconstructivist investigators would therefore be less likely to infer innate capabilities from anatomy or function described in older individuals, but instead would look for trajectories of differentiation and maturation beyond the fetal and infant stages that could lead to what could be observed later in life. These distinctions are pertinent to the interpretation of neuroanatomical findings in autism.

One can also approach neuroanatomical investigation from a physical point of view: Can the nature or localization of the physical changes give any indication of the timing or characteristics of the agent that could have caused them? This is an approach much used in neuroteratology, the study of developmental brain malformations. But if we apply this approach to findings in autism brain research, we face a challenge. Some neurogenetic and neurotoxic syndromes present us with evidence of striking alterations in brain development where the features of the brain abnormalities are clearly associated with brain developmental processes known to occur in a certain interval of time, generally in utero. The time of action and the molecular targets of the genes, drugs, or toxins that disturb the brain have been inferred in this manner for a variety of agents (Slikker & Chang, 1998). But in ASD, brain changes have a number of features that in the aggregate make it difficult to use this methodology to locate the origin of the changes at one clearly

delineated point in development. These include: the subtlety of most of the observed findings; the distribution of these findings through many parts of the brain whose developmental timetables may vary, and; the involvement of processes at many different biological levels, so that it is not easy to encompass them all in a simple model of causation. It appears unlikely that any single gene, infectious agent, or xenobiotic exposure can uniquely account for the panoply of findings identified to date.

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Moreover, most of the anatomical changes identified to date in ASD, particularly as compared to the dramatic abnormalities identified in neurogenetic syndromes of brain malformation, are subtle. Thus some researchers have held that since the autistic brain lacks major dysmorphology, it is unlikely to have suffered significant insult prior to the late gestational or early postnatal period (Ciaranello, VandenBerg, & Anders, 1982; Coleman, Romano, Lapham, & Simon, 1985; Raymond, Bauman, & Kemper, 1996).

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The pervasiveness of neuroanatomical findings extends not only across the anatomical landscape, but also across biological levels, with differences from controls having been documented from genes, proteins, and other molecules, to the hierarchy of "levels of integration" ranging from subcellular assemblies to cells, tissues, brain regions, neural systems, and large brain units such as lobes and hemispheres (Salthe, 1985). This pervasiveness suggests the potential presence of a complex range of underlying mechanisms. While a nativist or genetic determinist might assume that all of the higher-order levels of integration derive their characteristics from the underlying genetic blueprint, this is more a belief than a demonstrated fact; physiological science suggests that the situation is much more complex and multidirectional (Noble, 2008). If one elaborates the neuroconstructivist position biologically, it becomes apparent that feedback back and forth across levels becomes a potentially important ongoing modulator of both development and function. In fact, gene expression can be modulated by both environment (including behavior and feedbacks to the organism from that behavior) and endogenous physiology. While the neuroanatomical literature generally contains investigations of one or just a few levels at a time, all are active simultaneously and in fundamental integration with each other. Thus, for example, higher cognitive functions can be traced back to the cellular level, and alterations in each of these levels ought to be related to the other. Observations are generally made at one or a few levels at a time, but usually then are subjected to interpretation regarding their pertinence to other levels; for example, a neuropathological or microanatomical measurement of alterations in a neurotransmitter receptor is likely to be interpreted as having implications for behaviors associated with that neurotransmitter, even though careful behavioral assessment could not have been performed on subjects contributing postmortem specimens under study. A major challenge not limited to the field of autism research is validating such inferences with careful experimental data.

METHODS AND MEASURES: SENSITIVITIES AND CONSTRAINTS

In addressing this complexity, it is important to be familiar with the means by which information about the brain is acquired—not only the methods and sensitivities of the techniques, but also the constraints on and limitations of what can be detected. Information about neuroanatomy is collected from living subjects and from postmortem tissue samples. Currently, the major methods for investigation of structural neuroanatomy in vivo (in living human beings) include measures of head circumference, ultrasound (USG), computed tomography (CT), and magnetic resonance imaging (MRI). With these techniques, we can take measures of size (area and volume), shape, tissue types (e.g. gray matter, white matter, cerebrospinal fluid, bone, blood), density, and tissue integrity. There are also dynamical measures of anatomy, including perfusion measures (which we will review, since they can be measured at rest and construed as indices of microanatomy); functional imaging techniques measuring surrogates of neural activity (such as functional MRI [fMRI], electroencephalography [EEG], and magnetoencephalography [MEG], as well as PET [positron emission tomography] and SPECT [single photon emission computed tomography]) are for the most part beyond the scope of this chapter.

In neuroanatomical investigations using postmortem tissue specimens, it is possible to report microscopic as well as gross (visible to the eye) measures. Grossly, one can report weight, size, and shape, as well as description of injury, atrophy, disease processes (e.g. bleeding, tumor, or macroscopically visible inflammation), and disproportion or malformation. Microscopic investigation opens the possibility of

utilizing stains sensitive to particular cellular characteristics; techniques for tracing the trajectories of fibers are available, and quantitative cell counting techniques (known as stereology) are advancing.

Because so many of the advances in neuroanatomical understanding in autism are both enabled and constrained by the limits of the resolution of the measurement methods employed, the following will review the major methods in a little more detail.

Methods: Macroscopic and In vivo

Volumetric imaging

Volumetric imaging, one of the most broadly used methods, can be performed on any type of scan that yields a physical picture of the brain (USG, CT, MRI), but the acquisition method with the greatest spatial resolution is CT, while that with the best contrast resolution is MRI. The size of a delineated piece of brain tissue reflects the influence of cell types, cell size, cell density, cell composition, intracellular and extracellular fluid characteristics, density of vasculature, and extracellular matrix, among other factors (Caviness, Lange, Makris, Herbert, & Kennedy. 1999). Size can be measured as the area on a single MRI slice by tracing the boundaries of a region—a task which can be accomplished computationally. When CT and MRI are used to image the whole brain or a large part of it, they produce a series of slices through the brain. In MRI, each slice captures a slab of a specific thickness. These slabs can be captured continuously without gaps, or with gaps between the slices. When the slices are continuous it becomes possible to calculate the volume of regions that are included in more than one slice. This is done by deriving the "area" of the region on each slice, turning it into a volume measurement by multiplying by the slice thickness, and adding up the volumes of the region on all of the slices in which that region appears. Limits to resolution are more prominent when the slice is thicker: since the surfaces in the brain are highly curved and convoluted, the boundaries of a region can cross the thickness of a slice at an oblique angle. The boundaries of a region in an MRI slice cannot capture these kinds of angles, but are instead perpendicular to the slice surfaces. This is potentially also true of each voxel (the unit volume of measurement in a scan, whose dimensions are specified by the scanning acquisition protocol parameters). This leads to a phenomenon called "partial voluming," in which a voxel (or region) as defined on a scan can contain elements of more than one tissue type—that is, it can have gray matter, white matter, or even cerebrospinal fluid, yielding a value that averages the signal characteristics of the different components, and is therefore ambiguous and difficult to interpret. As MRI technology has advanced, it has become possible to acquire thinner slices so that there is less partial voluming, and boundaries are clearer.

Several MRI-based morphometric methods have been developed that expand what one can learn about contributors to volume. One set of methods gives information about tissue: the other set of methods goes beyond the limitations of traditional volumetrics with regard to surfaces and shapes.

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Tissue information.

1). Tissue parameter mapping exploits the capabilities of certain volumetric imaging acquisitions by extracting further information from signal properties, in order to give indications of tissue composition (lipid, water, or protein) present in each voxel, or unit of imaging acquisition.

2). Quantitative T2 transverse relaxation time is an additional way to identify tissue abnormalities, with an increase in time measured largely reflecting tissue water.

3). Voxel-based morphometry (VBM; Ashburner & Friston, 2000) uses a voxel-based comparison of local tissue concentration—typically grey or white matter—between two groups of subjects, to generate metrics of gray- and white-matter "density." VBM is distinct from classical volumetric or morphometric techniques, which deal with regional or total tissue volume, because it localizes group differences in a brain that has been spatially normalized (aligning images from multiple subjects to increase validity of across-subject comparisons) against a standard template. The method outputs a three-dimensional statistical parametric map (SPM) showing regions where the concentration or the density of the tissue differs significantly between the groups.

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4). Deformation-based morphometry (DBM) is a complementary class of methods that analyzes the deformations used in the normalization process, as opposed to the resulting normalized images. These can be used to study differences in brain shapes at various scales, or to identify differences in relative positions of brain structures (Ashburner et al., 1998).

Surface and shape information. Partial voluming issues in volume-based normalizations illustrate the inability of this method to address adequately the complexity of the shape and folding of the cerebral cortex, which is extremely convoluted and quite variable across individuals, and even between identical twins (Eckert et al. 2002). Two regions can have the same volume and still have different shapes—for example, a longer, thinner region could have the same volume as a shorter, thicker one. Accurate alignment of sulci is difficult to achieve using volume-based techniques. Surface-based measures have been developed to resolve this problem (Anticevic et al. 2008). Surface-based analysis involves reconstruction and display of the cortical surface in multiple representations, including 3-D formats, 2-D slices, smooth surfaces, and ellipsoidal, spherical, and flat maps. These visualizations are often used to display functional data, because they are more sensitive and accurate than volume-based analysis, particularly in detecting neural activation in buried cortical regions, in analyzing geometrical and topological relationships, in assessing structural properties like cortical thickness, and in generating surface-based atlases (Van Essen, Drury, Joshi, & Miller, 1998).

Measures derived from raw brain-size data. There are several possible types of basic calculations that can be made with volumetric, tissue quantification, or shape quantification data. If both the left and right sides of a region are measured, it becomes possible to calculate an asymmetry index, allowing the comparison of size on both the left and right sides and an assessment of statistical significance of differences. If a measure of total brain volume is available, it becomes possible to calculate proportion, and consider such questions as, is a brain region really larger in autism cases than in control cases, or is it just larger because the whole brain is larger?) (O'Brien et al., 2006). If quantifications of several regions are available, it becomes possible to calculate ratios. This allows one to ask, for example, whether thalamus volume change is proportional to cortical volume change, or whether there is a disturbance in proportion that could be reflected in altered thalamocortical functional relationships. Another significant question of this type is whether corpus callosum size has the same ratio to white-matter size across different groups, or whether this relationship is different between groups. Volumetric and other tissue-quantification findings can be important indicators of issues that need further research, whether using the same or other methods. For example, a deviation in thalamocortical or corpus callosum-white matter proportion may suggest follow-up studies using functional MRI, EEG, or MEG to study the impact on brain function and connectivity, looking at thalamocortical dysrhythmia (Llinas, Urbano, Leznik, Ramirez, & Marle, 2005) or interhemispheric information transfer (Ringo, Doty, Demeter, & Simard, 1994).

Diffusion tensor imaging. Diffusion tensor imaging (DTI) is often thought of as a way to measure white-matter tracts, but it is important to remember that it does not really take a direct measurement of them. This is due to the nature of what it measures, and the limits to resolution in this imaging modality. It may be better described as a measure of "white-matter integrity." DTI measures restrictions to the free movement or diffusion of water in tissue. In a glass of water, the molecules are free to diffuse without restriction. This is called isotropy: "tropy" refers to direction and "iso" refers to the dispersion being the same in all directions. But in brain tissue, the properties of the cells in which the water molecules exist, or which border on water molecules in extracellular tissues, restrict the direction of diffusion; here, the water shows "anisotropy," that is, diffusion that is *not* the same in all directions. In white-matter tracts, the water movement appears to be restricted to the direction along the tract, because water cannot diffuse across the fatty lipids in the myelin that wraps the axons in these tracts. Quantitative data generated by DTI include measures of apparent diffusion coefficient (ADC) and fractional anisotropy (FA). ADC is a measure of diffusivity, or the degree with which water moves freely within the tissue, with high ADC suggesting increase in water or decrease in restriction to water motion. FA, on the other hand, is a measure of directional coherence, or the fraction or extent to which water motion is restricted in a given part of tissue, with a high FA indicating a large amount of restriction of water motion. Higher FA indicates more restriction and higher ADC less restriction.

DTI has a number of limitations. While DTI tractography can generate dramatically stunning multicolor pictures of fibers within the brain, these figures are of qualitative but not quantitative use. In addition, ambiguity arises in DTI imaging in areas where white matter tracts cross each other. This crossing

is especially prominent in the white matter right under the cerebral cortex, where fibers are going in many different directions. Standard DTI techniques cannot track a specific fiber through an area of crossing fibers and out the other side. However, recent advances in diffusion imaging allow us to resolve these "crossing" and "kissing" fibers.

DTI images are constructed from magnetic signals that make contact with the brain tissue from a number of directions. The more directions used, the greater is the resolution of the image. But the price for this increased resolution is a longer scan, which makes it hard to acquire the highest quality images on individuals who may have difficulty staying still. Recent formidable technical advances have considerably shortened the time needed for a high-resolution acquisition, but the application to fully awake children with attentional or neurodevelopmental impairment is still limited.

Magnetic resonance spectroscopy. Magnetic resonance spectroscopy (MRS) is a way of using MRI to quantify levels of substances in living individuals. To do this, MRS takes advantage of the way that different substances resonate differently in a uniform magnetic field. The substances measured are related to different tissues and physiological processes in the brain, so that the measured increases or decreases in a substance give an indication of underlying cellular or anatomical differences or disease processes.

However, MRS has a number of technical limitations. Proton magnetic resonance spectroscopy is the variant most commonly used, but other methods exist of potential relevance, particularly phosphorus magnetic resonance spectroscopy, which can offer insights into brain energy metabolism. Since these other methods often involve very lengthy imaging acquisitions, their practical utilization is limited, particularly with children or impaired individuals. Unlike the other MRI techniques discussed above, MRS cannot be performed on the whole brain at once, and a lot of the variability between studies is due to major inconsistencies between studies in choice of brain region scanned, and the approach taken to imaging the region in question (not to speak of cohort age ranges). In the method most commonly used, single voxel spectroscopy (SVS), the size of the one voxel imaged is so large that many different types of tissue, and/or a large area of tissue, are included in one measurement. Even so, this method has yielded intriguing insights into autism anatomy, which will be reviewed later in this chapter. When MRS is performed in more powerful MRI magnetic fields, it is possible to get much greater resolution; however, at the time of this writing, almost all studies have been performed on relatively low-field-strength 1.5 Tesla (1.5T) magnets. More powerful scanners are increasingly available, and this development, along with increased replication of choice of regions and approaches, may further improve the yield of this approach.

Perfusion imaging. Perfusion refers to blood flow in the brain, which has been studied nearly two dozen publications on autism (reviewed later in this chapter). Three major methods of measuring brain perfusion are single photon emission computed tomography (SPECT), positron emission tomography (PET), and arterial spin labeling (ASL). SPECT and PET are both invasive, involving injection of radioactive tracers that are detected directly (SPECT) or indirectly (PET). PET has higher resolution than SPECT due to its selection of photons for simultaneous arrival at the detection device, but SPECT is cheaper and uses longer-lasting and more readily available radioisotopes. Both of these methods, while valuable, are severely limited in clinical and research applicability, particularly in minors, given the need to inject radioactive material. Arterial spin labelling (ASL) is an attractive alternative because it is performed in an MRI scanner without isotopes or contrast. ASL uses endogenous blood water as a contrast agent, by magnetically tagging arterial blood, tracking the decay of the magnetization of the tag as it enters the tissue of interest, and computing perfusion maps by comparing images of tagged versus with untagged blood water (Deibler et al., 2008b). ASL is in widespread clinical use in the imaging of tumors, stroke, and other cerebrovascular disorders (Deibler et al., 2008a). Moreover, because it is MRI-based and noninvasive, this technique is potentially widely available. Yet while ASL is being advocated as a tool in psychiatric diagnosis, because of its capacity to make discriminations in a number of psychiatric disorders such as schizophrenia and depression (Theberge, 2008), its use at present has been limited to a small set of research studies, with no studies to date in autism.

Methods: Postmortem and Microscopic

While this review predominantly focuses on in vivo neuroimaging data, neuropathological findings will

be included when relevant. Neuropathological studies clearly have the potential for much finer resolution than microscopic imaging, but they also have a range of limitations. Most prominent among them is the great scarcity of brain tissue. Autism is not a fatal condition; deaths in childhood in individuals with autism are mainly from accidents, drowning, suffocation and seizures (Shavelle, Strauss & Pickett, 2001), and are relatively rare. Autopsies are performed much less commonly today than they were in the years preceding neuroimaging, and this contributes to the third problem, which is the difficulty in educating families about the importance of brain donation, and encouraging families to donate brains when they are dealing with the profound grief and massive stress associated with the death of a child (the Autism Tissue Program is one brain bank that is addressing this issue).

change "brain bank" to "resource"

An additional major limitation is unavoidable variability in the postmortem interval, or length of time between death and processing (fixing or freezing) of the brain tissue, which can have many types of impacts on the state of the tissue. In addition, the processing itself can alter cells and regional volumes in a fashion that is not uniform across the brain (Lodin, Mares, Faltin, & Karasek, 1969).

Overall, it is exceedingly important to remember that interpreting the significance of neuroanatomical findings in a developmental disorder is extremely complicated. Insofar as one is trying to reconstruct the past from present evidence, one needs to be aware that there might be trajectories other than the ones that easily come to mind that could have led to the present state of the tissue. One's interpretations also need to be mindful of the limitations of the study sample, the depth of characterization of the sample, and the resolution and sensitivity of the instrumentation used.

ANATOMY IN REVIEW

37: change ANATOMY to ANATOMICAL IMAGING 38: CHANGE "neuroanatomy" to "neuroanatomical imaging"

In this next section, autism neuroanatomy will be reviewed within a neuroconstructivist context, and with a sense of the historical development of our understanding as it is influenced by models, by data available at each point in time, and by the growing availability of technologies capable of producing new classes of data. A core theme is the discovery and elucidation of overall brain enlargement in autism. Although the investigations of regional abnormalities in autism predate a concerted attempt to understand brain enlargement in autism, these will be reviewed following the discussion of brain enlargement, so that the insights gained from the general enlargement can be brought to bear on the features noted in specific brain regions. The review will begin with some of the early neuroanatomical observations in autism. It will then move to observations and measures of differences between autistic and control brains at the largest scales, and will trace the exploration of these measures through a range of dimensions and the techniques used to elucidate these dimensions. The review will then turn to measures of a number of regions that have been explored in more detail, and describe the dimensions and techniques of some additional techniques used to study them. Finally, reflections will be shared regarding the linkages between large-scale and regional measures, and directions important to future progress will be shared, particularly those that hold the clearest relevance to clinical evaluation and intervention.

Clinical Imaging

Clinical neuroradiological imaging involves the acquisition of brain images from individual patients (rather than from individuals chosen to be members of a specifically defined cohort) to look for explanations for diseases and symptom complexes, and to seek targets for treatment interventions., Most of the findings in autism neuroanatomy have been generated using quantitative methods, because these findings are generally too subtle to be detected qualitatively by non-computational clinical radiological assessments which for the most part are qualitative rather than quantitative. On the other hand, the findings discerned quantitatively to date are group findings that substantially overlap with findings in non-autistic individuals, rather than pathognomonic findings that can be used for clinical diagnosis. Presently, therefore, while clinical assessment has not proven useful for identifying diagnostic features, research imaging has thus far not had much influence on clinical practice.

A number of publications have collected and tabulated clinical neuroradiological observations in autism. A 1983 study observed gross abnormalities in 26% of an autism cohort (Gillberg & Svendsen 1983). A 2006 study of imaging findings in children with autism and developmental delay reported that 15 of the 32 children with autism or pervasive developmental disorders (PDD) showed structural abnormalities

(Zeegers et al., 2006). A 2009 study found abnormalities in 48% of a cohort of 77 children with nonsyndromic autism (Boddaert et al., 2009). However, in all of these clinical studies, the abnormalities that were observed, while common in the aggregate, were quite variable in their nature and distribution. These included white-matter lesions in various locations, reduced corpus callosum size, wide or asymmetrical ventricles, arachnoid cysts, Chiari I malformations, cavum septum pellucidum, and dilated Virchow-Robin spaces. These findings are all nonspecific and do not obviously point to any clear avenues of medical intervention. Consequently, brain imaging is not considered to be an essential component of the medical or neurological evaluation of autism, and tends to be ordered only when additional abnormalities are found that suggest that imaging will assist with clinical management.

Early CT findings: Asymmetries and Ventricular Differences

The earliest macroanatomical studies of autism utilized computerized tomography (CT) scans, a method of using x-ray technology to generate a large series of two-dimensional images along a single axis through tissue, such as brain tissue. What distinguishes these studies from the above clinical studies is that consistent anatomical features were measured across all subjects. As these scans have very limited resolution within the gray and white matter, however, observations were confined to the level of overall size, overall asymmetries, and differences in the size of cerebral ventricles, which are easily discerned in this imaging modality (Gillberg & Svendsen, 1983; Hier, LeMay, & Rosenberger, 1979; Hoshino, Manome, Kaneko, Yashima, & Kumashiro, 1984). Some attempt at quantification was made in these images, but due to the level of resolution, these measures and indices were crude. While the findings in these early CT studies suggest differences in the physical status and/or development of the brain, they are not clear enough to offer etiological or functional implications.

41: change "implications" to "insight"

Brain size in autism

One of the most replicated findings in autism brain investigations has been the observation of a tendency toward increased brain size, particularly in younger autistic individuals. Although large head size was observed by Leo Kanner in his initial paper identifying autism as a syndrome (Kanner, 1943), this observation were initially buried in studies performed for other purposes; eventually its relevance was noticed, and the phenomenon investigated deliberately. The earliest measures were of brain weight. There is some source of artifact in brain weight measures because of variability due to the postmortem interval (time from death to removal and fixation of brain), or cause of death (e.g. an illness that involved swelling might change brain size and weight), but even so a trend toward larger brains was noted. In 1985 Bauman and Kemper observed that 8 out of 11 brains in autistic individuals less than 12 years of age showed a significant increase in weight as compared with controls, but 6 out of 8 of those over 18 years of age weighed less than expected (Bauman & Kemper, 1985). Bailey and colleagues (1998) noted that four of six brains in their sample (one four year old, remainder ages 20–27) were greater than the normal range for brain weight for age.

Head circumference

Head circumference (HC) has been an important measure in documenting head-size trends in autism. Davidovitch, Patterson, and Gartside (1996) reported that 18.2% of a group of 148 brain head circumference measures in autistic individuals were at or above the 98th percentile (though, interestingly, while this 1996 paper showed macrocephaly in an American cohort, a 2009 poster by the same author could not replicate this phenomenon in an Israeli autistic sample [Davidovitch, Golan, Vardi, Lev, & Lerman-Sagie, 2009]). Woodhouse and colleagues (1996) noted that 19.7% of a PDD cohort had macrocephaly and 48.7% had head circumference greater than the 90th percentile. Fidler and colleagues noted more macrocephaly in probands with autism and their first-degree relatives (Fidler, Bailey, & Smalley, 2000). Miles and colleagues (2000) reported increased HC in probands in a range of subgroups based on phenotype, onset, seizure status, and IQ, and also found macrocephaly in at least one parent in 45% cases. Dementieva and colleagues (2005) found macrocephaly in 19% of a cohort of 251 with autism.

87: change "45% cases" to "45% of cases"

MRI

Head circumference measures have made a great contribution to studies of autism, but they have obvious

limits, including not only an inability to address anything specific about the brain, but also potential inaccuracy stemming from inconsistent placement of the tape measure on the skull. Head circumference is insensitive to shape differences, such as wide versus long and narrow or taller heads; but shape may be relevant in autism, where a higher preponderance of wide heads has been noted (Deutsch & Joseph, 2003). Starting in the late 1980s and developing into the early 1990s, the advent of brain imaging utilizing structural MRI emerged and allowed a new kind of measure to contribute to our knowledge of brain size in autism. Filipek and colleagues (1992) reported on brain volumes in a cohort of 92 children with high-functioning autism (HFA, IQ > 80), developmental language disorder (DLD, IQ > 80), low-functioning autism (LFA, IQ < 80), and non-autistic low IQ (NALIQ, IQ < 80), as well as controls (CTRL). They found that for total brain volume, HFA > LFA = DLD > CTRL > NALIQ. Large brain size was also reported earlier on by several other research teams (Courchesne et al., 2001; Deutsch & Joseph 2003; Piven, Arndt, Bailey, & Andreasen, 1996; Sparks et al., 2002). By now, structural MRI measures have documented increased total brain volume multiple times; an excellent meta-analysis of these findings has been performed by Stanfield and colleagues (2007).

When does brain size increase occur?

Lainhart and colleagues (2003) generated longitudinal data by performing a retrospective study of head circumference of infants later diagnosed with autism. This brought to light the issue of a postnatal-head-size developmental trajectory. Lainhart found that most of the macrocephalic autistic subjects did not manifest macrocephaly at birth, but developed it during early childhood, as they manifested accelerated increase in head size during this period. This finding has since been replicated multiple times. Courchesne and colleagues (2003) performed a retrospective study of head circumference in children diagnosed with autism as compared with HC norms from the Centers for Disease Control (CDC) and found that, while mean head circumference at birth was at the 30th percentile in this group (as compared with Lainhart's group, where it was in the normal range), it increased 2 standard deviations in the first year and a half of life. Mraz and colleagues (2007) replicated this trajectory, finding a significantly smaller head circumference at birth to two weeks, and a significantly larger head circumference by 10–14 months of age, although when overall length and weight were controlled for, this difference disappeared. This group also found that children with an early history of autism who subsequently moved off the autism spectrum showed the same head circumference trajectory as did the stable autism group (Mraz, Dixon, Dumont-Mathieu, & Fein, 2009). Lainhart's group subsequently performed a retrospective study of fetal ultrasounds in children who had later been diagnosed with autism, and did not find abnormalities of fetal head circumference in autism (Hobbs et al. 2007). Hazlett and colleagues (2005) found enlargement of head circumference beginning at about 12 months of age. Of the 79 individuals in Dementieva's sample (2005) for which two consecutive HC measures were available (not necessarily starting at birth), 35% had accelerated head growth between the two available measures, while the remainder did not. Of the 37 of these individuals whose consecutive HC measures began at birth, 65% showed abnormal head growth starting at birth.

A number of researchers have been able to use volumetric MRI to document brain volume in small children. Courchesne and colleagues reported increased brain volume in 2–4-year-olds, with 90% above average and 37% meeting criteria for developmental macrocephaly (Courchesne et al., 2001). Hazlett and colleagues (2005) also found significantly increased brain volume in 2-year-olds. A large multicenter prospective at-risk infant MRI imaging study is presently underway at the National Institutes of Health that will greatly enlarge the data available, by documenting changes in total brain and regional volumes during the earliest postnatal development.

Does accelerated head growth have behavioral correlates?

While Courchesne and colleagues (2003) found that greater head size increase was associated with lower ADI scores, Dementieva (2005) did not replicate this correlation, finding that accelerated head growth, whether or not it started at birth, was associated with increases in several composite scores on the Vineland Adaptive Behavior Scales. Elder and colleagues (2008) found that large brain size at 12 months, followed by a more marked slowing of head growth, predicted the manifestation of autism symptoms. The impact of body weight and length on head circumference

83: there was supposed to be a citation of IBIS, Natl Inst of Health, www.ibis-network.org (FN on line 49 of page 74 of these proofs)

has not been consistent across studies (Mraz et al., 2007).

What parts of the brain are driving brain size increase?

To understand brain enlargement in autism, it has become important to understand the neuroanatomy of autism in much more detail. Given that autism is classified as a developmental disorder, theories about a range of developmental disruptions that could lead to this phenomenon have been advanced (Courchesne & Pierce, 2005a, 2005b; McCaffery & Deutsch, 2005). One prominent model is that brain enlargement early in life is due to persistence of the early exuberant proliferation of neurons in the developing brain, presumably because of a failure of pruning, or apoptosis. To understand what is happening with these brains during development, and to assess the merits of various models and hypotheses, it is necessary to measure subcomponents of the brain, in order to assess their respective contributions to this enlargement. Volumetric or morphometric profiling has been a major tool in getting this information, but it has also revealed its own limits; there are questions that can only be answered with other methodologies. To address the brain size question, volumetrics has been applied to quantifying sizes of large regions of the brain, such as the cerebral cortex, white matter, or cerebellum, and to comparing these measures between autistic and control populations. It has also involved measuring the whole brain and comparing proportional volumes between groups.

Gray matter and white matter during development

While the model of "failure of pruning" would predict a larger cerebral cortex, data on cerebral cortex volume are contradictory. One recent paper identified increased cortical thickness (Hardan, Muddasani, Vemulapalli, Keshavan, & Minshew, 2006), whereas Hadjikhani and colleagues (2006) found that cortical thickness of the right inferior frontal cortex was inversely correlated with autism severity. Some of the differences between cohorts seem to map in an age-dependent fashion, suggesting that reconciliation of contradictions may in part come from recognizing a developmental process where volumetric trajectories are non-uniform across brain regions. In early childhood there appears to be an increase in both gray and white matter volumes. Ben Bashat and colleagues (2007), using a form of DTI imaging, measured an accelerated maturation of white matter in 1.8–3.3-year-olds. In middle childhood the data vary. Cerebral cortex has been measured to be absolutely the same, but relatively smaller, than controls in school-aged, high-functioning autistic children (Herbert et al., 2003), but it has also been measured to be larger in the same age group (Palmen et al., 2005).

In a landmark study comparing cross-sectional findings from a large number of subjects ranging in age from 2 through 16 years, growth trajectories were presented for cerebral and cerebellar gray and white matter (Courchesne et al., 2001). Cerebral cortical gray matter was 12% greater in 2–3-year-old autistic subjects than in controls, but by middle childhood (6–9 years of age), distinct trajectories had led to different changes: the control volume had increased 12%, while the autistic group volume had decreased by 2%. Cerebral white matter also appears to have a different growth trajectory in autistic individuals than control subjects. In the autistic subjects, it grew in a linear fashion, starting out 18% larger in the 2–3-year-old autistic group, but having a much flatter slope of volume increase than in controls, and ending up in adolescence being only 10% larger than in early childhood. In controls there was a 12% increase in volume from the 2–3-year-old to the 6–9-year-old age group, and a 59% increase compared to early childhood in the adolescent group. Cerebellar white matter was, dramatically, 39% larger in 2–3-year-old autistic children than in controls; however, in the cross sectional 12–16-year-old adolescent comparison sample, cerebellar white matter was only 7% larger than in the 2–3-year-old autistic subjects, but 50% larger than in early childhood in the comparison control sample.

Distribution of white matter changes

To address the question of whether the white-matter changes were uniform throughout the brain or were non-uniformly distributed in different subregions, our own group utilized a white-matter parcellation technique designed to subdivide the white matter from volumetric images according to the white-matter tract architecture (Makris et al., 1999; Meyer, Makris, Bates, Caviness, & Kennedy, 1999). Although white-matter tracts cannot be discerned or discriminated utilizing the standard MRI acquisition techniques

used for volumetric imaging analysis, it is possible to identify their probable locations based on proximal visible gray matter landmarks. Using this technique, a division was made between outer (radiate), sagittal (long tracts such as those going from front to back), and descending tracts. This analysis revealed that high-functioning, autistic, school-aged children (from the same sample as Filipek et al., 1992) had enlargements in radiate white matter in all four lobes as compared to controls, but no enlargements in the deeper white matter in either the sagittal or descending tracts. Interestingly, the developmental language disorder sample showed a similar radiate white-matter involvement, but to a lesser degree, and not involving the parietal lobe. This localization of enlargement is intriguing in light of the neurodevelopmental myelination sequence: the radiate white matter myelinates late in development (late in the first and into the second postnatal year), with the prefrontal white matter myelinating last and for the longest duration. In this analysis, prefrontal white matter, a component of radiate white matter, showed the largest increase, being 36% larger in the autistic sample and 25% larger in the DLD sample than in controls; these marked increases suggest some kind of process affecting late-myelinating white matter, which approximately coincides with the period of postnatal brain enlargement described above.

Corpus callosum

If the overall white matter volume is larger in younger autistic children, and if this enlargement is non-uniform with the deep white matter not being involved, then is this also true for the corpus callosum, the major inter-hemispheric deep white matter structure? The answer is mostly yes. One research group found corpus callosum enlargement in autism, and this only in autistic individuals (as well as non-autistic individuals) with macrocephaly (Kilian et al., 2008). Most other researchers have found the corpus callosum to be reduced in size, and a few reported no differences in size between the two groups (Rice et al., 2005). Within this size reduction there has been some variability between studies in localizing corpus callosum size changes; for example, using mid-sagittal area measures, Egaas and colleagues (1995) found reduction in the mid-sagittal area, while Hardan and colleagues (2000) found anterior reductions. Piven and colleagues (1997) found the body and splenium (posterior) to be smaller.

Additional methods have been utilized, and have yielded generally consistent findings. Using voxel-based morphometry, Waiter and colleagues (2005) found reductions in anterior splenium and isthmus, and using surface-based methods, Freitag and colleagues (2009) discerned reduced posterior thickness. Vidal and colleagues (2006) showed that, while traditional morphometric techniques discerned a reduction in total area and anterior third area, spatial maps discerned significant reduction in both splenium and genus. Recently, additional imaging methods have been applied, adding new dimensions to our understanding of corpus callosum changes in autism. Alexander and colleagues (2007) utilized DTI, and found that higher diffusivity and lower FA were associated with slower processing speeds. Just and colleagues (2007) linked functional and anatomical connectivity measures, and found corpus callosum size reduction that correlated with a lower degree of integration of information. Mason and colleagues (2008) found a correlation between connectivity within the brain regions associated with Theory of Mind and the size of a portion of the anterior corpus callosum.

Asymmetry

Since the corpus callosum is critical to connections between the cerebral hemispheres, this area's alterations in autism might be expected to be associated with brain asymmetries that differ from those in neurotypical individuals. In autism spectrum disorders (ASD), abnormal brain asymmetries have been documented at a variety of levels. At the macroanatomical level, deviations from normal asymmetries have been documented in localized regions (De Fosse et al., 2004; Herbert et al. 2002; Hier, LeMay, & Rosenberger, 1979; Rojas, Camou, Reite, & Rogers, 2005; Rojas, Bawn, Benkers, Reite, & Rogers, 2002), widely distributed, volumetrically measured regions (Herbert et al., 2005), metabolism (Burroni et al. 2008; Chandana et al., 2005; Chiron et al., 1995; Chugani et al., 1997), functional activation (Muller et al., 2004; Takeuchi et al., 2004), neurophysiology (Bruneau et al., 2003; Dawson et al., 1989; Flagg et al., 2005; Khalfa et al., 2001; Lazarev, Pontes, & Deazevedo, 2008; Stroganova et al. 2007), and neurocognitive assessments (Dawson et al., 1986, 1988; Dawson, Warrenburg, & Fuller 1983; Ozonoff & Miller, 1996). Architectonic asymmetries, such as in mini-columns, as has been identified in schizophrenia (Buxhoeveden et al., 2001),

have apparently not been investigated in ASD (Chance et al., 2008). Brain asymmetry has been documented in a fairly widespread distribution (Herbert et al., 2005), and may involve a developmental trajectory into adolescence (Flagg et al., 2005). This developmental trajectory may be indirectly supported by the finding by Herbert and colleagues (2005) of greater cortical asymmetry in higher-order associational areas, but not primary sensory and motor areas, since the development of higher-order associational areas is more experience-dependent than that of the primary sensory and motor areas. Although right-hemisphere dysfunction may predominate in autism (McKelvey et al., 1995; Ozonoff & Miller, 1996;), it may not be universal, but rather a marker for specific subtypes distinguished by language impairment (De Fosse et al., 2004; Whitehouse & Bishop, 2008).

Magnetic resonance spectroscopy and neuronal integrity

If it is true that brain enlargement is due to a failure of pruning, it should follow that there would be a greater number of neurons in the cerebral cortex. The metabolite N-Acetyl-aspartate (NAA), detectable by proton magnetic resonance imaging (1H-MRS), is a measure of neuronal integrity or neuronal function, and is sometimes considered a measure of neuronal density. Several 1H-MRS studies have been performed to test this hypothesis, but their findings have contradicted their predictions. Out of 22 magnetic resonance imaging papers in the autism literature, 80 measures of NAA were performed in all studied brain regions combined; 25 found reductions in NAA, 1 found an increase, and 54 showed no change. The lack of change in the majority of measures may be in part a reflection of 19 out of 22 of the studies having been performed on a relatively low field-strength 1.5T rather than a 3T magnet; 3T was used in only one study to date (DeVito et al., 2007).

33: citation missing at end of sentence: Shetty et al., 2009

Despite the need for further studies at higher field strength, it is worth noting that when differences were found, the strongly predominant finding was of reduction, rather than increase, of this metabolite. This suggests either reduced neuronal density, a lower level of neuronal functioning, impaired mitochondrial function, or less elaborate neuronal architecture (e.g. dendrites). It is also notable that in the epilepsy-surgery literature, following surgical resection of epileptic foci, there has been documented reversibility of reduced NAA in secondarily affected brain tissue (Hugg et al., 1996). Moreover, in the overall body of autism spectroscopy literature in children, other metabolites measured are lower rather than higher in autism than controls, suggesting a lower rather than a higher density of cells and metabolic components; this matter is exceedingly well reviewed (Dager, Friedman, Petropoulos, & Shaw, 2008). A problem with drawing inferences from this literature is that a number of spectroscopy studies in autism encompass wide age ranges, some with cohorts ranging in age from early childhood to adulthood; this may potentially reduce the chance of detecting changes in metabolites whose concentrations change during development.

Diffusion tensor imaging, transverse relaxation imaging, and white matter integrity

If the prediction is correct that brain enlargement is due to a failure of pruning, it should also follow that there would be a greater density of axonal processes emanating from the predicted larger number of neurons. There are now a number of DTI papers with findings related to the testing of this model; these are summarized in Table 3-1. Almost all of the studies showed either reduced FA, increased ADC, or both; Ben Bashat and colleagues (2007), who studied the youngest cohort, were also the only ones to utilize a variant "high b value" diffusion tensor imaging study, which is more sensitive than the more standard DTI acquisition to diffusion in areas with high restriction; it is unclear whether the difference between the findings in the younger as compared with the older groups is age- or methodology-related.

The short-range fibers investigated by Sundaram and colleagues (2008) are quite consistent in distribution with the radiate white matter that was reported as enlarged in Herbert and colleagues (2004), as described above. Sundaram and colleagues note that their findings of reduced FA and increased ADC, which suggest reduced rather than increased white matter integrity in this area, are not consistent with the hypothesis that this white-matter volumetric enlargement is composed of a larger number of myelinated axons. Cheung and colleagues (2009) also explicitly ponder how to reconcile lower FA with greater white-matter volume, which they described as counterintuitive. They suggest that white-matter

Table 3-1 Diffusion Tensor Imaging Findings

Study	*Subject characteristics*	*FA*	*ADC*	*Regions*
Barnea-Goraly et al. (2004)	7 Aut 14.6±3.4 yo 9 TD 13.4±2.8 yo	-		Ventromedial prefrontal cortices, anterior cingulate gyri, temporal parietal sulcus, superior temporal sulci, near the amygdala bilaterally, in occipotemporal tracts and in the corpus callosum
Lee et al. (2007)	43 ASD 7-33 yo 34 TD 8-29 yo	-	+	Superior temporal gyrus, temporal stem
Thakkar et al. (2008)	12 ASD 30±11 yo 14 TD 27±2.8 yo	-		Right anterior cingulate cortex (associated with severity in repetitive behavior ratings)
Sundaram et al. (2008)	50 ASD 57.5 ± 29.2 months 16 TD 82.1 ± 41.4 months	-	+	All frontal fibers ADC: Mean ADC in ASD children was significantly higher FA: No significant differences but there was a trend to lower FA in ASD Long Range Association Fibers ADC:Mean ADC in ASD was higher FA : No changes Short Association fibers ADC: Mean ADC was higher in ASD than in TD FA: Mean FA was significantly lower in ASD Negative correlation between FA and GARS AQ and social isolation subscale . . . but not significant after Bonferroni correction
Cheung et al. (2009)	14 ASD 6-14 yo 14 TD 6-14 yo	-		*Reduced FA*: bilateral prefrontal and temporal regions, especially adjacent to the fusiform gyrus in the right ventral temporal lobe *Increased FA:* right inferior frontal gyrus and left occipital lobe Correlations of lower FA in subsets of these regions with higher ADI-R diagnostic algorithm subscale scores
Ben Bashat et al. (2007)	7 Autism (1.8-3.3 yo) 18 TD (4 months to 23 years)	+		Increased FA and probability and decreased displacement in left sides of all of the following: posterior limb of internal capsule, external capsule and forceps minor, corpus callosum genu, corpus callosum splenium and corticospinal tract

volumetric indices are rather nonspecific, and that given their findings, volume increase might even be due to non-neuronal proliferative processes, such as the activation and cell swelling of microglia and astroglia that have been reported by Vargas and colleagues (2005), which will be discussed shortly. The finding that increased motor cortex white matter predicts motor impairment in autism, not enhanced motor performance (Mostofsky, Burgess, & Gidley Larson, 2007), could conceivably be interpreted as consistent with these reflections on tissue underpinnings. These diffusion tensor imaging findings highlight the limitations of volumetric imaging: it is indeed true that a volumetric measure of increased white matter can by no means be attributed with any certainty to an increase in myelinated axons, since the imaging acquisition used for volumetric analyses simply measures the size of the total white matter compartment, and does not distinguish between different types of cells and materials that contribute to the volume. It is also important to remember here that increases or decreases in FA or diffusivity can only be considered consistent with, and not proof of changes in, tract or myelinated fiber density.

Neuropathology: Minicolumns; neuroinflammation

Two neuropathology findings of potential relevance to the phenomenon of brain enlargement in autism are altered minicolumns, and evidence of innate immune activation in postmortem tissue from individuals with autism. Minicolumns, a microscopic architectural feature of the brain, were described as being smaller but less compact in their cellular configuration using a number of methods (Casanova, Buxhoeveden,

& Brown, 2002; Casanova, Buxhoeveden, Switala, & Roy, 2002a, 2002b; This phenomenon is pertinent to interneurons that are an important part of the structure of minicolumns, which play a critical inhibitory role in neuronal activity, and that thereby may alter functional connectivity. Casanova has proposed a relationship between minicolumnar alterations and structural connectivity—specifically, increased short-corticortical white matter, suggesting that larger brains require more white matter, and in particular short-range associational fibers, to maintain connectivity (Casanova, 2004). If, as recent DTI studies reviewed just above suggest, there is not in fact increased short-range associational fiber density, it is not obvious what implications this may have for the connectivity impacts of such minicolumns.

While neither MRI scans nor neuropathological investigations have identified focal patches of inflammation in postmortem brain tissue specimens from autistic individuals, neuropathological evidence of innate immune activation has been identified using specific staining techniques (Vargas et al., 2005). This immune activation was identified in the cerebral cortex, white matter, and cerebellum, and consisted of activated astroglial and microglial cells, as well as altered cytokine profiles. More recently, an increase in pro-inflammatory cytokines in brain tissue has been identified by other groups (Li et al., 2009; Morgan et al., 2010). A much larger number of studies have identified a range of systemic immune abnormalities (Ashwood & Van de Water, 2004; Ashwood, Wills, & Van de Water, 2006), although the specific details of the immune profiles are not identical between the central nervous system and the organism systemically. Innate immune activation is a prominent feature of a variety of neurodegenerative diseases such as Alzheimer's but it is not detectable by MRI scan or by other in vivo imaging techniques, other than through use of an experimental PET scan ligand, PK11195, which is no longer in use due to side effects. Inflammation can affect the neuroanatomical milieu due to the swelling that accompanies astroglial activation, which may affect volume and may also compress capillary lumen by as much as 50% (Aschner, Allen, Kimelberg, LoPachin, & Streit, 1999), compromising blood perfusion (perfusion will be discussed below). Inflammation may also alter the neurochemical milieu by impairing the reuptake of glutamate and leading to excessive extracellular glutamate (Pardo & Eberhart, 2007), an excitatory neurotransmitter, which may secondarily affect connectivity through increasing the excitation/inhibition ratio which could also have cascading developmental effects (see Chapter 4 for a review of neurochemistry in autism).

The volume enlargement that may be related to swelling of immune-activated glial cells may also conceivably be related to DTI measures of reduced water diffusion and increased FA. Neuroinflammation and biochemical changes also belong to a set of factors that in the broader neuroscience literature have been shown to have some influence on brain asymmetry. A prediction of asymmetry can be made mathematically on the basis of efficiencies of cross-hemispheric communication, where lateralization becomes more efficient with larger brain size (Ringo, 1991; Ringo et al., 1994); however, this does not account for the specifically rightward predominance of this asymmetry. In the broader neuroscience literature, there are papers documenting an influence on brain asymmetry specifically toward rightward asymmetry from various visceral and regulatory factors; these include autonomic (Craig, 2005), neuropeptides (Ramirez, Prieto, Vives, de Gasparo, & Alba, 2004), gonadal steroids (Wisniewski, 1998), and immune (Kang et al. 1991; Shen et al., 2005; Wittling, 1995); but at present, although all these factors have some documented pertinence to autism, their connection to asymmetry in autism has not been pursued.

Cerebral perfusion abnormalities have been identified in at least 18 papers studying autistic cohorts. Cerebral perfusion refers to the quantity of blood flow in the brain. Abnormal regulation of cerebral perfusion is found in a range of severe medical conditions including tumors, vascular disease, and epilepsy. Cerebral hypoperfusion has also been found in a range of psychiatric disorders (Theberge, 2008). Of the 18 papers found in a recent literature search for positron emission tomography (PET) and single photon emission computed tomography (SPECT or SPET) studies of brain perfusion in autism or ASD, 15 were performed using SPECT, two with PET, and one with both SPECT and PET. Neurocognitive hypotheses and conclusions, as well as localization of perfusion changes, were heterogeneous across these papers. Hypoperfusion has been identified in frontal regions (Degirmenci et al., 2008; Galuska et al., 2002; George et al., 1992; Gupta & Ratnam, 2009; Ohnishi et al., 2000; Wilcox et al., 2002), temporal lobes (Boddaert et al., 2002; Burroni et al., 2008; Degirmenci et al., 2008; Galuska et al., 2002; George et al., 1992;

27: after "in pro-inflammatory cytokines" add "or glial activation"

57: change "reduced" to "increased" 58 change "increased FA" to "reduced FA"

Btwn 78 & 79 there should be a header saying "Perfusion" in same font/style as Neuropathology: minicolumns and neuroinflammation (on previous page)

Hashimoto et al., 2000; Ohnishi et al., 2000; Ryu et al., 1999; Starkstein et al., 2000; Zilbovicius et al., 2000), as well as a variety of subcortical regions including basal ganglia (Degirmenci et al., 2008; Ryu et al., 1999; Starkstein et al., 2000), cerebellum (Ryu et al., 1999), limbic structures (Ito et al., 2005; Ohnishi et al., 2000), and thalamus (Ito et al., 2005; Ryu et al., 1999; Starkstein et al., 2000)—i.e., in a widely distributed set of brain regions. It is interesting to note that even with this regional variation in localization, 17 of the 18 publications showed that cerebral perfusion was *reduced*; in the only study reporting some areas of localized hyperfusion, these areas were found in the middle of the frontal pole and temporal lobe, which were more broadly hypoperfused (McKelvey et al., 1995). Only one study showed no difference in perfusion between autistic and control subjects (Herold et al., 1988). It is interesting to note that the variably located small white matter hyperintensities identified in a fair number of clinical scans, as noted earlier, may possibly be attributable to localized areas of hypoperfusion (Brickman et al., 2009). Possibly because virtually all of the autism perfusion studies studies were oriented toward testing neuropsychological rather than pathophysiological hypotheses, there were no probes or tests reported to unearth the tissue-level alterations that might be underlying these reductions in blood flow in these brains.

Regional findings

One of the lessons of the above explorations of the tendency toward large brain size in autism is that bigger is not always better. Large is easily assumed to be constituted by more neurons performing more neural processing in a usefully organized fashion—but this may not be the case. Another way of stating this is that it is not size, so much as function and mechanism, that determine impact. This lesson is pertinent not only in addressing the phenomenon of large brains, but also in making sense of the findings that have emerged in investigating regions of interest in the autistic brain. Region-oriented neuroanatomical studies in autism have been challenged by three major factors: the heterogeneity of findings across subjects and cohorts, the subtlety of the bulk of the findings, and the technical challenges involved in measuring subtle findings (e.g. the greater degree of error in measuring the volume of small-brain regions). Because of these challenges, more advanced imaging techniques beyond volumetrics have increasingly been applied to extend the resolution and discernment of investigations.

Region-based investigations in autism are based both upon descriptive observations and on localized brain areas suggested by behavioral, communication, sensory, motor epileptic, immune-endocrine, and visceral-system characteristics of autism. An early review by Damasio and Maurer, written before the vast bulk of neuroanatomical investigations in autism, is still cogent for its clinically based predictions of the location of anatomical involvement, as well as its reflections on the reasons for, and potential causes of, this distribution of brain change (Damasio & Maurer, 1978). These authors posited that autism arose from abnormalities in the mesolimbic structures associated with neurotransmitter imbalance that might be a consequence of perinatal viral infection, insult to the periventricular watershed area, or genetically determined neurochemical abnormalities; they also posited basal ganglia circuitry abnormalities based on the presence of gait and movement abnormalities. This set of inferences covers a broad range of possibilities, all of which—and more—are still under investigation today.

Limbic System

An obvious set of brain regions to consider as potentially implicated in autism neuroanatomy is the limbic system, given its role in emotional and social processing—functions that are so prominently atypical in the phenotype. Limbic cortical areas are an evolutionarily ancient set of structures, with a less differentiated cortical layering structure but a much denser set of interconnections than most other parts of the cerebral cortex. The limbic area connections with multiple polymodal and premotor cortex regions as well as with subcortical structure (Barbas, 1995; Tucker, 1992) and the robust connections of orbitofrontal and medial prefrontal areas to the amygdala (Ghashghaei & Barbas, 2002) allows these areas to address representations of experience which are less differentiated than those processed by primary sensory and motor cortex. Such a heavily connected set of regions might be preferentially vulnerable to underlying pathophysiological processes that impact connectivity. Certain infectious processes, such as herpes encephalopathy, also are known to preferentially impact some limbic structures.

Neuropathology

The importance of abnormalities in limbic systems was given early support by neuropathological identification of smaller and more tightly packed cells in the hippocampus, subiculum, entorhinal cortex, amygdala, mamillary body, anterior cingulate cortex, and septum. In the amygdala, the most significant increases in cell-packing density were noted in the medial, cortical, and central nuclei, whereas the lateral nucleus did not manifest this phenomenon (Bauman & Kemper, 1994). A later study by this group identified differences in hippocampal CA4 neurons in two cases of infantile autism, with smaller perikaryon area and less dendritic branching (Raymond et al., 1996). This finding could indicate a cellular basis for impaired information processing. A recent study by members of the same group identified reductions in the limbic system of γ-Aminobutyric acid – i.e. GABAergic systems (Blatt et al., 2001; Guptill et al. 2006), but not of six other receptors studied (Blatt et al., 2001). Since GABA is an inhibitory neurotransmitter, a reduction in GABAergic systems could impact a range of functional domains vulnerable to excessive excitation, including sleep, anxiety, sensory processing, and seizures. A number of other neuropathological studies have not found amygdala or other limbic abnormalities (Bailey et al. 1998; Coleman et al., 1985; Guerin et al., 1996; Rodier et al., 1996; Williams et al. 1980).

Neuroimaging

In neuroimaging, the hippocampus and amygdala have been studied both separately and together. Neuroimaging is particularly challenged here not only due to the previously mentioned greater degree of error in measuring the volume of small brain regions, but also due to the difficulties, particularly in earlier imaging studies performed with older scanners and acquisition protocols, in making a clear delineation between the two structures. The volumetric studies of these regions have not yielded consistent results. Piven and colleagues (1998) showed no difference between 35 autistic and 36 control subjects in hippocampal volume. Saitoh (1995) and colleagues found no difference in the cross-sectional area of the posterior hippocampal formation between autistic and control subjects aged 6 to 42, but in a later study found smaller hippocampal volume by a measure of cross-sectional area of the area dentata, sibiculum, and CA1-CA3 in subjects 29 months to 42 years of age, with the smallest sizes noted in the youngest age group of 4 years and younger (Saitoh, Karns, & Courchesne, 2001). Alyward and colleagues (1999) found amygdala to be significantly smaller in non-retarded adolescents, with greater significance for absolute volume and lesser, but still significant, difference when volumes were adjusted for the impact of total brain volume. Herbert and colleagues (2003) found a trend toward proportional reduction, while Schumann and colleagues (2004) found hippocampal volume to be increased on the right in low-functioning autistic children and adolescents, and bilaterally in high-functioning autistic children and adolescents. Findings of increased size were reported in several other studies using a variety of methods (Abell et al., 1999; Howard et al., 2000; Sparks et al., 2002), with the last of these studies reporting a subgroup with proportional enlargement and another subgroup with greater than proportional enlargement. On the other hand, no amygdala volume differences were found by Haznedar and colleagues (2000).

A number of studies of amygdala volume have included suggestive correlations with other clinically pertinent variables. Schumann and colleagues (2004) showed an enlargement of the amygdala in children with autism ages 7.5–12.5 years, but not in adolescents as compared with controls; furthermore, whereas the amygdala at the start of the younger age range was already enlarged, in the control group it was smaller in the younger children and larger in the older children within that age group. Nacewicz and colleagues (2006) included psychological correlates in their measures of change with age in their 8- to 25-year-old subjects, showing an earlier and more pronounced increase in amygdala volume in those individuals who had normal eye fixation, but little difference in amygdala volume across the same age range in individuals whose level of eye fixation was low (Nacewicz et al., 2006).

Nacewicz and colleagues (2006) also showed that a smaller amygdala was associated with slowness in distinguishing emotional from neutral expression and reduced fixation of eye regions, as well as greater social impairment in childhood according to ADI-R, a parent report measure. Juranek and colleagues (2006) found that anxious/depressed symptoms were significantly correlated with increased total and right amygdala volume, utilizing the Child Behavior Checklist.

Shape

Shape measurement methodologies have provided another way to show differences between limbic structures in autistic, as compared to control, subjects. Several studies aimed at detecting hippocampal shape and thickness differences not detectable through standard volumetric approaches have recently been published. Dager and colleagues (2007) reported an inward deformation of the subiculum, accentuated in a more severe subgroup and associated with deficits in medial temporal lobe function but not in prefrontal function; this shape deformation had previously been reported in studies of medial temporal lobe epilepsy. Nicolson and colleagues used 3-dimensional surface meshes and discerned localized differences with right medial posterior hippocampus volume reduction, that might be consistent with specific abnormalities of the dentate gyrus or hippocampal CA1, CA3, or CA4 regions (Nicolson et al., 2006).

MRS

Proton magnetic resonance spectroscopy has been used to investigate underlying tissue changes in the amygdala-hippocampal region. There have been a number of findings, but the methodologies are not consistent, which as mentioned above is a function of the restriction of MRS to specific regions rather than whole brain, and the wide range of variability in voxel placement that exacerbates the differences produced by disparities in cohort phenotypes, cohort age, and imaging acquisition methodologies. The most consistent finding was lower n-acetyl-aspartate (NAA) (17 out of 49 measures, with 41 showing no change), and NAA/creatine ratios (4 out or 14 measures, with 10 showing no change) (Shetty et al., 2009). Endo and colleagues (2007) examined this with neuropsychological correlates, and found lower NAA/Cr ratios in the right medial temporal lobe particularly pronounced in the autistic, as compared with PDD-NOS and control, groups, which correlated with their performance on the Childhood Autistic Rating Scale-Tokyo Version. Additional findings include higher glutamine and creatine/phosphocreatine in the amygdala-hippocampus than in the parietal lobe (Page et al., 2006), an increase in myo-inositol/creatine in the amygdala-hippocampus as well as cerebellum, and an increase in choline/creatine in left hippocampus and left cerebellum (Gabis et al., 2008). Two papers by Kleinhans and colleagues (2007 & 2009) have identified a correlation between metabolic and functional abnormalities utilizing MRS and functional MRI in the same subjects. In the 2007 study, five voxels were placed, including three in regions that were centers of activation in functional MRI in a verbal fluency task in healthy controls. NAA was lower in all regions, particularly the left frontal cortex. Among the behavior-neuronal integrity (i.e., MRS) correlations reported, there was a significant positive correlation between the amount of fMRI signal change in the autistic subjects, but not in the controls. In the 2009 study by Kleinhans and colleagues of high-functioning autistic and Asperger adults, there were no group differences in metabolites, but those autistic or Aspergers individuals with the lowest NAA or Cre (creatine/phosphocreatine) levels were found by ADI to have had the most significant clinical impairment in childhood (Kleinhans et al., 2009).

DTI

Diffusion tensor tracking methodologies have been utilized to study hippocampal-fusiform and amygdala-fusiform pathways (Conturo et al., 2008). In this methodology the measures were subtle, with the macrostructural measures (pathway volume, length, and area) sensitive at the millimeter scale and the microstructural measures (maximum and minimum diffusion coefficients) sensitive at the micron scale; these measures clearly go beyond the sensitivity or resolution of volumetrics. The main finding, noted to be worse in individuals with poorer face recognition or lower IQ scores, was a micron-scale reduction in the diffusion perpendicular to the axis of the white-matter tracts in right hippocampal-fugal pathways. In contrast, there were no detectable macrostructural abnormalities, which led the authors to suggest that the appropriate "machinery" is in place, but its operations are abnormal—in essence inferring that their findings point to underlying mechanisms of autism that are functional rather than structural.

Cerebellum

Studies of the cerebellum have been motivated by a combination of empirical observations of abnormalities and a growing body of literature implicating the cerebellum not only in motor coordination, but in a range of cognitive, affective, and language functions

highly pertinent to autism (Schmahmann, 2004; Schmahmann & Caplan 2006; Schmahmann & Pandya, 2008).

Early neuropathological studies indicated abnormalities in the cerebellum. These included a reduction in the number of Purkinje cell alterations in cerebellar nuclei (Bauman & Kemper, 1996). Of the two dozen postmortem cases of autism in the literature, 19 showed decreased density of Purkinje cells (Amaral, Schumann, & Nordahl, 2008). Whether this is due to a loss of cells, or to an alteration of cell functions or properties affecting their binding to histopathological stains, has not been clarified. A recent study suggests that the latter might be contributory. This study used a different staining technique; whereas prior studies had used Nissl staining, here calbindin was chosen because it is a more reliable marker for Purkinje cells. When this method was used, no group differences between autistic and control tissue samples were found in the density of Purkinje cells, with three of the six showing normal density, and the other three showing reduced density, but no correlation of density reduction with severity of autism (Whitney, Kemper, Bauman, Rosene, & Blatt, 2008). The poor uptake of Nissl stain by Purkinje cells in tissue from individuals with autism might nevertheless have some significance, in that it could indicate impaired function of these cells, such as chromatolysis (a depletion of somatic rough endoplasmic reticulum) related to chronically diseased and weakened cells during life.

More recently, the same group has reported a series of microanatomical findings pertinent to the functional features of cerebellar circuitry. A 40% reduction in the level of glutamate decarboxylase 67 isoform (GAD67) was identified in Purkinje cells, suggesting that these cells were contributing less inhibitory input to neural circuitry, presumably leading to a net gain in excitation (Yip, Soghomonian, & Blatt, 2007). Interestingly, upstream of Purkinje cells, there appear to be alterations in cerebellar basket and stellate cell interneurons also related to the balance of excitation and inhibition: an upregulation of GAD67 in basket cells was identified, and in the same study a trend toward a small increase was also noted in stellate cells. This seems to suggest an increased feed-forward inhibition to Purkinje cells whose inhibitory GAD67 production is already reduced. This yields a combination of mutually reinforcing alterations: upstream cells are inhibiting the Purkinje cell, lessening its inhibitory functioning, and at the same time the Purkinje cell itself is also showing reduced intrinsic contribution to inhibitory circuitry. The net result is a loss of inhibition, reducing the denominator in the excitation/inhibition ratio and thereby increasing net excitation. This is consistent with a widely discussed model of an increase in the ratio of excitation to inhibition as underlying autism (Levitt, Eagleson, & Powell, 2004; Rubenstein & Merzenich, 2003). Kern has argued that Purkinje cell loss or dysfunction could result from injury and not necessarily developmental derangement, and presents literature evidence that Purkinje cells are selectively vulnerable to ischemia, hypoxia, excitotoxicity, G protein dysfunction, viral infections (e.g. thiamine), heavy metals, various toxins, and chronic malabsorption syndrome (e.g. celiac disease, inflammatory bowel disease; Kern, 2003).

There have been a variety of other cerebellar neuropathological findings. Abnormalities in the cerebellar cholinergic system have been identified. Lee and colleagues identified a reduction in the high=affinity a4 receptor in the granule cell, as well as the Purkinje and molecular layers, with a possibly compensatory increase in the a7 receptor subunit that was significant in the granule cell layer (Lee et al., 2002). The abovementioned Vargas study identifying innate immune activation (including microglial and astroglial activation and proinflammatory cytokines) in the brains of individuals with autism found that the cerebellum was a main focus of this neuroinflammation (Vargas et al., 2005). It has also been noted that serum from children with ASD contains autoantibodies to specific cells in the cerebellum (Wills et al., 2009), and that in animal models of mid-gestation respiratory infection, postnatal cerebellar pathology in the offspring resembles that observed in autism (Shi et al., 2009).

Vermis area

The vermal lobules are midline structures in the cerebellum, the areas of which can be measured by tracing their outline on a single mid-sagittal section of a scan. An early report of cerebellar vermal lobules VI-VII hypoplasia (Courchesne et al., 1988) was followed by a substantial number of further papers reporting measurements of this area. These have been reviewed and debated in detail elsewhere (Courchesne, 1999; Courchesne, Townsend, & Saitoh, 1995; Filipek, 1995; Piven & Arndt, 1995; Piven et al., 1999). While some of the papers replicated the findings of hypoplasia, other did not.

35: change (GAD67) to (GAD67, which catalyzes GABA synthesis)

70: change = to -

79 add "among the regions studied" (w/o quotes) after "neuroinflammation"

Smaller cerebellar vermal lobules VI-VII were found in several studies (Courchesne et al., 1988; Gaffney et al., 1987; Murakami et al., 1989; Saitoh et al., 1995), while some later studies discerned a subgroup with an area increase (Courchesne et al. 1994). However, several other studies found no difference in cerebellar vermal lobules (Elia et al., 2000; Filipek et al., 1992; Garber & Ritvo, 1992; Holttum et al., 1992; Kleiman, Neff, & Rosman, 1992;Nowell et al., 1990; Piven et al. 1992, 1997; Rumsey & Hamburger, 1988). It was also argued that vermal hypoplasia was found in a number of other neurodevelopmental disorders, and therefore was not specific to autism (Ciesielski & Knight, 1994; Schaefer et al., 1996).

Volume

Cerebellar volume is a less frequently performed measure because it requires assessment of slices throughout the entire structure (which in the earlier days of MRI scans was visualized with poor resolution); when performed it has generally yielded increased volume in autism, although to different degrees. Two studies (Piven et al., 1997; Sparks et al., 2002) showed cerebellar increase proportional to increased total brain volume. Herbert and colleagues (2003) similarly found that total cerebellar volume was greater in autism than in controls, but not different after adjustment for total brain volume; this finding was in the same set of brains where earlier analysis had found no difference in midline vermal lobule VI-VI areas (Filipek et al., 1992). However, Hardan and colleagues (2001) found cerebellar volumes to be both relatively and absolutely larger. Using voxel-based morphometry, the metrics of which (as discussed in the earlier methodology section) are not directly comparable to those used in the above studies, Abell and colleagues (1999) also found increased gray matter volume bilaterally in the cerebellum. Cerebellar volume increase was not found in a comparison of autistic subjects with and without macrocephaly to normocephalic and macrocephalic typically developing individuals (Cleavinger et al., 2008).

Cerebellar white matter

Improvements in posterior fossa resolution have more recently allowed the segmentation of cerebellar gray and white matter; this measure has led to findings suggesting that overall cerebellar volume increase may be due to an increase in cerebellar white matter. The above-mentioned neuroinflammation in the cerebellum (Vargas et al., 2005) was prominent in the cerebellar white matter. In the only study in which this was measured, cerebellar white matter was as much as 39% larger in autism than in controls aged 2 to 4 years (Courchesne et al., 2001). Boddaert's group found a mean decrease in the voxel-based morphometry measure discussed above of cerebellar "white matter concentration" in 21 school-age children with autism (Boddaert et al., 2004), though this measure cannot be mapped onto volume measurements, as it is a metric of different physical properties, and a metric of reduced density cannot distinguish between increased water and reduced size.

Thalamus

The thalamus is a critical relay station in brain information processing and is of great potential interest in autism research, due to its central role in connectivity, coordination, and sensory modulation. Abnormalities in the thalamus have been found by various measures. Volumetric findings are not entirely consistent. One study showed reduced thalamic volume relative to total brain volume (Tsatsanis et al., 2003), while another showed proportional increase in thalamic volume that lost significance when adjusted for overall brain size (Herbert et al., 2003); a third study showed a lack of linear relationship between the volumes of thalamus and brain (Hardan et al., 2006). Decreased "gray matter concentration" was found in the thalamus using voxel-based morphometry (Waiter et al., 2005). Perfusion abnormalities have been found by SPECT (Ito et al., 2005; Ryu et al., 1999; Starkstein et al., 2000) and by PET (Haznedar et al., 2006; Horwitz et al. 1988). MRS has shown reduced NAA in thalamus (Friedman et al., 2003). Hardan and colleagues investigated the possibility of a relationship between metabolic and functional abnormalities in the thalamus; their findings included lower levels of N-acetylaspartate (NAA), phosphocreatine and creatine, and choline-containing metabolites in the left thalamus autism group, compared with controls even in the absence of volumetric differences, and some limited relationships between questionnaire measures of sensory abnormalities and proton MRS metabolites (Hardan et al., 2008). Nicotinic abnormalities in the cholinergic system in the thalamus have been identified in autism (Ray et al., 2005). Brain-specific

antibodies to proteins in the thalamus and hypothalamus have been identified in plasma from a subset of children with autism (Cabanlit et al., 2007).

Basal Ganglia

The basal ganglia are of potential interest in autism because of their contributions to movement and movement abnormalities such as tics and stereotypies, as well as for to the role they play in motivation, repetitive behaviors, and obsessiveness. These regions have been investigated using volumetrics, spectroscopy, perfusion measures, immune measures, and animal models, with findings summarized in Table 3-2. MacFabe and colleagues (2007) identified histopathological abnormalities and electrophysiological spiking in the caudate associated with tics in an animal model of environmentally induced autistic-like behaviors (MacFabe et al., 2007). A relationship has been identified of larger caudate volume at times of episodes of symptoms of PANDAS (an acronym for Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptotoccus)(Giedd et al., 2000), but there is no direct evidence of an analogous immune-infectious syndrome with brain volume in the case of autism.

Table 3-2 Basal Ganglia Findings

Study	*Subject characteristics*	*Measure*	*Regions*
Hardan et al., 2003	40 Aut Non-MR, 41 TD 8-45 yo	Volume Motor	Weaker motor functioniong in autistic group not accompanied by basal ganglia volumetric differences
Hollander et al., 2005	18 ASD, 17TD 17-57 yo	Volume ADI	Larger right caudate volume Correlation of total caudate and putamen volumes with repetitive behaviors on the ADI-C subscale
Haznedar et al., 2006	17 ASD, 17 TD 17-56 yo	Volume ADI Glucose metabolism	Greater right caudate volume with reduced glucose metabolism in the ventral caudate
Rojas et al., 2006	24 Aut, 23 TD 7-44 yo	VBM	Enlarged caudate nucleus
Langen et al., 2007	Sample 1: 21 HFA, 21 TD, 10-14 yo Sample 2 21 HFA, 21 TD, 15-24yo	Volume	Increased caudate volume associated with repetitive behaviors Caudate changes localize to head of caudate Caudate grew bigger over time in autism but decreased in volume over time in controls
Degirmenci et al., 2008	10 Aut, 6.7±1.7 yo; 5 TD, 6.4±1.4 yo Relatives: (8 mothers, 39± 4 yo; 8 fathers 36 ± 5 yo; 7 siblings, 13 ± 5 yo Controls for Relatives: Parents: 5M, 5F, 37±3 yo 22 controls for siblings, 5.4-15.7yo	SPECT	Reduced right caudate perfusion Other findings reviewed in section on perfusion
Levitt et al., 2003	22 Aut + ASD 5.4-15.7 yo 20 TD 6.8-16.3 yo	MRS	Creatine/Phosphocreatine ratio higher in head of right caudate but lower in body of left caudate
Singh and Rivas, 2004	68 Aut, 4-12 yo 30 TD, 5-12 yo	Immuno-blotting	Serum antibodies to caudate nucleus in 49% of subjects studied
MacFabe et al., 2007	74 adult male Long–Evans	Animal model	Animal model of environmentally induced autistic features using proprionic acid Histopathological abnormalities and caudate spiking associated with tics

Cerebral lobes

While a growing body of functional imaging evidence suggests that the connectivity between frontal lobes and other parts of the brain is particularly affected in autism, this literature is beyond the scope of the present neuroanatomical review (see Chapter 21). What insights into lobar anatomy have emerged from anatomical investigations? The substantial impact upon frontal lobe volumes of overall brain enlargement has already been reviewed above (Carper et al., 2002; Herbert et al. 2004). Frontal lobe volume has been measured as inversely correlated with cerebellar volume (Carper & Courchesne, 2000), and localized enlargement of the frontal lobes early in autism development has been noted (Carper & Courchesne, 2005). Orbitofrontal cortical (OFC) gray matter volume was measured as reduced in the right lateral OFC, and correlations between social deficits and white matter OFC structures were observed.

Temporal lobe involvement has also been noted. Superior temporal lobe abnormalities have been noted in a number of studies (Boddaert et al., 2004; Herbert et al., 2002). While Lainhart's group did not observe overall differences in temporal lobe volume (Bigler et al., 2003), they measured abnormalities in the left fusiform gyrus, the right temporal stem, and the right inferior temporal gyrus gray matter, with similarities noted between subjects with autism and subjects with reading difficulties (Neeley et al., 2007). White matter microstructure was also noted to be abnormal in these subregions of the temporal lobe (Lee et al., 2007). Reduced left planum temporale volume (Rojas et al., 2002) and lack of normal planum temporale asymmetry (Rojas et al., 2005) have been measured.

Parietal lobe involvement has been variable, with reductions noted in a subgroup (Courchesne, Press, & Yeung-Courchesne, 1993) and in parietal white matter (Ke et al., 2008), enlargement noted by others (Carper et al., 2002; Herbert et al., 2004; Piven et al., 1996), and lack of difference by yet others (Hazlett et al., 2006). Occipital lobe changes have not been strongly observed, perhaps because of an apparent anterior-to-posterior gradient in the degree of hyperplasia (Carper et al., 2002).

SUMMARY AND CONCLUSION

Overall, we clearly have a massive amount of evidence that there are differences in anatomy between autistic and non-autistic individuals. However, these differences are not always consistent, and while there are many potential contributors and explanations, it is not always clear specifically how to account for the inconsistencies. Developmental trajectory undoubtedly plays a role in total brain volume and in the volumes of regions such as the caudate and the amygdala. Technical issues may complicate measures, such as the greater risk of error involved in quantifying volumes of small regions. Imaging analysis methods may yield somewhat different boundaries between laboratories, and regional naming conventions may be idiosyncratic and inconsistent across sites. A study may have hypothesis-driven reasons for focusing on a subset of regions, and may give the impression that the findings they report are localized, when in fact a more broadly focused study of the same sample might identify similar findings in other regions as well. For example, Herbert and colleagues (2002) reported altered brain asymmetry in language-associated regions; however, using the same set of brains but performing a whole-brain analysis, they reported a much more widespread set of asymmetry alterations (Herbert et al., 2005). There is also the issue of heterogeneity in autism. It is by now clear that it is possible to meet the behaviourally defined criteria of "autism spectrum disorders" through a wide range of underlying biological challenges to brain function. It is likely, for example, that brain connectivity may be challenged by a variety of different underlying mechanisms (e.g., various gene variants affecting synapses, various environmental challenges reducing mitochondrial efficiency at synapses, astroglial activation that compromises the quality of tripartite synapse function, and much more). Overall, this combination of inconsistent findings, poorly understood impact of development, methodological differences between labs and underlying heterogeneity undoubtedly contributes to the challenge of putting together a coherent picture of autism neuroanatomy.

An additional potential contributor to the heterogeneity of anatomical (and other) findings in autism is the nature of the impact of environmental or physiological factors, such as immune alterations, infectious exposures, and toxic exposures, upon the brain. These factors may alter cellular functioning in a fashion that is either weakly or not at all targeted to specific regions, and whose localization may thus be somewhat accidental rather than necessarily revealing of regional alterations intrinsic to autism. This (along with the

region-restricted focus of some of the studies) may contribute to explaining the greatly distributed localization of perfusion abnormalities in autism, even while almost every study reported that perfusion is reduced. Reduced perfusion is what would be expected in the setting of immune alterations or infectious or toxic exposures, given their impact on glial cells, membrane and transport function, and various other physiological factors. Such changes could further alter synaptic, oscillatory, and connectivity dynamics in a way that would change neural systems function. This set of considerations raises the possibility that localizations of anatomical and neural systems dysfunctions may be downstream of pathophysiological impacts, rather than direct outcomes of regionally targeted causal factors.

Despite all of these potential contributors to heterogeneity and inter-study inconsistencies, there are a number of anatomical findings that are consistent across at least a preponderance of studies. Brain size (head circumference, brain volume, brain weight) is on average larger in younger subjects, with an intriguing developmental trajectory, while the corpus callosum is not enlarged proportional to overall enlargement and is mostly measured as smaller. There is involvement in the cerebellum, limbic system, basal ganglia, thalamus, and white matter, though the specific character of this involvement varies markedly across studies and age groups. Perfusion is lower, though the distribution of this perfusion has varied in measures to date. Metabolic and diffusion tensor imaging measures mostly suggest some kind of reduction in density of metabolites and in fiber integrity. Seen together, this picture, even with all its remaining loose ends, still represents a substantial advance over what was known a decade ago, and a stronger pace of progress in the next decade can be reasonably expected.

The comprehensiveness of this discussion is limited by its restriction to physical anatomy. A fuller picture would come from integrating the structural and functional advances being made in the field, but for now that will need to be done by the reader in relation to other chapters in this volume. The fact that we are seeing growing number of studies integrating measures of volume, metabolism, or perfusion with measures of functional activation suggests that we are getting more systematic in probing for the pathophysiological mediators of neuropsychological dysfunction. Pathophysiology is a critical and active intermediary. As DTI, MRS, and perfusion imaging are more widely utilized, as neuropathology advances, and as more multimodal studies are performed, a more integrated set of empirically grounded linkages across the levels of autism will begin to emerge.

References

Abell, F., Krams, M., Ashburner, J., Passingham, R., Friston, K., Frackowiak, R., Happe, F. Frith, C., & Frith, U. (1999.) The neuroanatomy of autism: A voxel-based whole brain analysis of structural scans. *Neuroreport*, *10*(8), 1647–1651.

Alexander, A. L., Lee, J. E., Lazar, M., Boudos, R., Dubray, M. B., Oakes, T. R., Miller, J. N., Lu, J., Jeong, E. K., McMahon, W. M., Bigler, E. D. & Lainhart, J. E. (2007). Diffusion tensor imaging of the corpus callosum in autism. *Neuroimage*, *34*(1), 61–73.

Amaral, D. G., Schumann, C. M., and Nordahl, C. W. (2008.) Neuroanatomy of autism. *Trends Neurosci*, *31*(3), 137–145.

Anticevic, A., Dierker, D. L., Gillespie, S. K., Repovs, G.,Csernansky, J. G., Van Essen, D. C., & Barch, D. M. (2008). Comparing surface-based and volume-based analyses of functional neuroimaging data in patients with schizophrenia. *Neuroimage*, *41*(3), 835–848.

Aschner, M., Allen, J. W., Kimelberg, H. K., LoPachin, R. M. & Streit, W. J. . (1999). Glial cells in neurotoxicity development. *Annu Rev Pharmacol Toxicol*, *39*, 151–173.

Ashburner, J., & Friston, K. J. (2000). Voxel-based morphometry—the methods. *Neuroimage*, *11*(6 Pt 1), 805–821.

Ashburner, J., Hutton, C., Frackowiak, R., Johnsrude, I., Price, C., & Friston, K. (1998). Identifying global anatomical differences: Deformation-based morphometry. *Hum Brain Mapp*, *6*(5-6), 348–357.

Ashwood, P., & Van de Water, J. (2004). A review of autism and the immune response. *Clin Dev Immunol*, *11*(2), 165–174.

Ashwood, P., Wills, S. & Van de Water, J. (2006). The immune response in autism: A new frontier for autism research. *J Leukoc Biol*, 80(1), 1–15.

Aylward, E. H., Minshew, N. J., Goldstein, G., Honeycutt, N. A., Augustine, A. M., Yates, K. O., Barta, P. E., & Pearlson, G. D. (1999). MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. *Neurology*, *53*(9), 2145–2150.

Bailey, A., Luthert, P., Dean, A., Harding, B., Janota, I., Montgomery, M., Rutter, M., & Lantos, P. (1998). A clinicopathological study of autism. *Brain*, *121* (Pt 5), 889–905.

Barbas, H. (1995). Anatomic basis of cognitive-emotional interactions in the primate prefrontal cortex. *Neurosci Biobehav Rev*, *19*(3), 499–510.

Bauman, M. L., & Kemper, T. L. (1985). Histoanatomic observations of the brain in early infantile autism. *Neurology*, *35*, 866–874.

Bauman, M. L., & Kemper, T. L. (1996). Observations on the Purkinje cells in the cerebellar vermis in autism. *J Neuropathol Exp Neurol*, *55*, 613.

Bauman, M. L., & Kemper, T. L. (1994). Neuroanatomic observations of the brain in autism. In M. L. Bauman, T. L. Kemper (Eds.), *The Neurobiology of Autism* (pp. 119–145). Baltimore: The Johns Hopkins University Press.

Ben Bashat, D., Kronfeld-Duenias, V., Zachor, D. A., Ekstein, P. M., Hendler, T. Tarrasch, R., Even, A., Levy, Y., & Ben Sira, L. (2007). Accelerated maturation of white matter in young children with autism: A high b value DWI study. *Neuroimage*, *37*(1), 40–47.

Bigler, E. D., Tate, D. F., Neeley, E. S., Wolfson, L. J., Miller, M. J., Rice, S. A., Cleavinger, H., Anderson, C., Coon, H.,Ozonoff, S., Johnson, M., Dinh, E., Lu, J., Mc Mahon, W., & Lainhart, J. E. (2003). Temporal lobe, autism, and macrocephaly. *AJNR Am J Neuroradiol*, *24*(10), 2066–2076.

Blatt, G. J., Fitzgerald, C. M., Guptill, J. T., Booker, A. B., Kemper, T. L., &Bauman, M. L.(2001). Density and distribution of hippocampal neurotransmitter receptors in autism: An autoradiographic study. *J Autism Dev Disord*, *31*(6), 537–543.

Boddaert, N., Chabane, N. Barthelemy, C., Bourgeois, M., Poline, J. B., Brunelle, F., Samson, Y., & Zilbovicius, M.(2002). Bitemporal lobe dysfonction in infantile autism: Positron emission tomography study. *J Radiol*, *83*(12 Pt 1), 1829–1833.

Boddaert, N., Chabane, N., Gervais, H., Good, C. D., Bourgeois, M., Plumet, M. H., Barthelemy, C., Mouren, M. C., Artiges, E., Samson, Y., Brunelle, F., Frackowiak, R. S., & Zilbovicius, M. (2004). Superior temporal sulcus anatomical abnormalities in childhood autism: A voxel-based morphometry MRI study. *Neuroimage*, *23*(1), 364–369.

Boddaert, N., Zilbovicius, M., Philipe, A., Robel, L., Bourgeois, M., Barthelemy, C., Seidenwurm, D., Meresse, I., Laurier, L., Desguerre, I., Bahi-Buisson, N., Brunelle, F., Munnich, A., Samson, Y., Mouren, M. C., & Chabane, N. (2009). MRI findings in 77 children with non-syndromic autistic disorder. *PLoS One*, *4*(2), e4415.

Brickman, A. M., Zahra, A., Muraskin, J., Steffener, J., Holland, C. M., Habeck, C., Borogovac, A., Ramos, M. A., Brown, T. R., Asllani, I., & Stern, Y. (2009). Reduction in cerebral blood flow in areas appearing as white matter hyperintensities on magnetic resonance imaging. *Psychiatry Res*, *172*(2), 117–120.

Bruneau, N., Bonnet-Brilhault, F., Gomot, M., Adrien, J. L., & Barthelemy, C. (2003). Cortical auditory processing and communication in children with autism: Electrophysiological/behavioral relations. *Int J Psychophysiol*, *51*(1), 17–25.

Burroni, L., Orsi, A., Monti, L., Hayek, Y., Rocchi, R., & Vattimo, A. G . (2008). Regional cerebral blood flow in childhood autism: A SPET study with SPM evaluation. *Nucl Med Commun*, *29*(2), 150–156.

Buxhoeveden, D. P., Switala, A. E., Litaker, M., Roy, E., & Casanova, M. F. (2001). Lateralization of minicolumns in human planum temporale is absent in nonhuman primate cortex. *Brain Behav Evol*, *57*(6), 349–358.

Cabanlit, M., Wills, S., Goines, P., Ashwood, P., & Van de Water, J. (2007). Brain-specific autoantibodies in the plasma of subjects with autistic spectrum disorder. *Ann N Y Acad Sci*, 1107, 92–103.

Carper, R. A., & Courchesne, E. (2000). Inverse correlation between frontal lobe and cerebellum sizes in children with autism. *Brain*, *123*(Pt 4), 836–844.

Carper, R. A., & Courchesne, E. (2005). Localized enlargement of the frontal cortex in early autism. *Biol Psychiatry*, *57*(2), 126–133.

Carper, R. A., Moses, P., Tigue, Z. D., & Courchesne, E. (2002). Cerebral lobes in autism: Early hyperplasia and abnormal age effects. *Neuroimage*, *16*(4), 1038–1051.

Casanova, M. F. (2004). White matter volume increase and minicolumns in autism. *Ann Neurol*, *56*(3), 453; author reply 454.

Casanova, M. F., Buxhoeveden, D. P., & Brown, C. (2002). Clinical and macroscopic correlates of minicolumnar pathology in autism. *J Child Neurol*, *17*(9), 692–695.

Casanova, M. F., Buxhoeveden, D. P., Switala, A. E., & Roy, E. (2002a). Minicolumnar pathology in autism. *Neurology*, *58*(3), 428–432.

Casanova, M. F., Buxhoeveden, D. P., Switala, A. E., & Roy, E. (2002b). Neuronal density and architecture (Gray Level Index) in the brains of autistic patients. *J Child Neurol*, *17*(7), 515–521.

Caviness, V. S. Jr, Lange, N. T., Makris, N., Herbert, M. R., & Kennedy, D. N. (1999). MRI-based brain volumetrics: Emergence of a developmental brain science. *Brain Dev*, *21*(5), 289–295.

Chance, S. A., Casanova, M. F., Switala, A. E.,& Crow, T. J. (2008). Auditory cortex asymmetry, altered minicolumn spacing and absence of ageing effects in schizophrenia. *Brain*, *131*(Pt 12), 3178–3192.

Chandana, S. R., Behen, M. E., Juhasz, C., Muzik, O., Rothermel, R. D., Mangner, T. J., Chakraborty, P. K., Chugani, H. T., & Chugani, D. C. (2005). Significance of abnormalities in developmental trajectory and asymmetry of cortical serotonin synthesis in autism. *Int J Dev Neurosci*, *23*(2–3), 171–182.

Chiron, C., Leboyer, M., Leon, F., Jambaque, I., Nuttin, C., & Syrota, A. (1995). SPECT of the brain in childhood autism: Evidence for a lack of normal hemispheric asymmetry. *Dev Med Child Neurol*, *37*(10), 849–860.

Chugani, D. C., Muzik, O., Rothermel, R., Behen, M., Chakraborty, P., Mangner, T., da Silva, E. A., & Chugani, H. T. (1997). Altered serotonin synthesis in the dentatothalamocortical pathway in autistic boys. *Ann Neurol*, *42*(4), 666–669.

Ciaranello, R. D., VandenBerg, S. R., & T. F. Anders. (1982). Intrinsic and extrinsic determinants of

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neuronal development: relation to infantile autism. *J Autism Dev Disord*, *12*(2), 115–145.

Ciesielski, K. T., & Knight, J. E. (1994). Cerebellar abnormality in autism: A nonspecific effect of early brain damage? *Acta Neurobiol Exp (Warsz) 54*(2), 151–154.

Coleman, P. D., Romano, J., Lapham, L., & Simon, W. (1985). Cell counts in cerebral cortex of an autistic patient. *Journal of Autism and Developmental Disorders*, *15*, 245–255.

Conturo, T. E., Williams, D. L., Smith, C. D., Gultepe, E. Akbudak, E., & Minshew, N. J. (2008). Neuronal fiber pathway abnormalities in autism: An initial MRI diffusion tensor tracking study of hippocampo-fusiform and amygdalo-fusiform pathways. *J Int Neuropsychol Soc*, *14*(6), 933–946.

Courchesne, E. (1999). An MRI study of autism: the cerebellum revisited. *Neurology*, *52*(5), 1106.

Courchesne, E., Carper, R., & Akshoomoff, N. (2003). Evidence of brain overgrowth in the first year of life in autism. *JAMA 290*(3), 337–344.

Courchesne, E., Karns, C. M., Davis, H. R., Ziccardi, R., Carper, R. A., Tigue, Z. D., Chisum, H. J., Moses, P., Pierce, K., Lord, C., Lincoln, A. J., Pizzo, S., Schreibman, L., Haas, R. H., Akshoomoff, N. A., & Courchesne, R. Y. (2001). Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study. *Neurology*, *57*(2), 245–254.

Courchesne, E., & Pierce, K. (2005a). Why the frontal cortex in autism might be talking only to itself: local over-connectivity but long-distance disconnection. *Curr Opin Neurobiol*, *15*(2), 225–230.

Courchesne, E., & Pierce, K. (2005b.) Brain overgrowth in autism during a critical time in development: Implications for frontal pyramidal neuron and interneuron development and connectivity. *Int J Dev Neurosci*, *23*(2–3), 153–170.

Courchesne, E., Press, G. A., & Yeung-Courchesne, R. (1993). Parietal lobe abnormalities detected with MR in patients with infantile autism [see comments]. *Am J Roentgenol*, *160*(2), 387–393.

Courchesne, E., Saitoh, O., Yeung-Courchesne, R., Press, G. A., Lincoln, A. J., Haas, R. H., & Schreibman, L. (1994). Abnormality of cerebellar vermian lobules VI and VII in patients with infantile autism: Identification of hypoplastic and hyperplastic subgroups with MR imaging. *Am J Roentgenol*, *162*(1), 123–130.

Courchesne, E., Yeung-Courchesne, R., Pres, G. A., Hesselink, J. R., & Jernigan, T. L. (1988). Hypoplasia of cerebellar vermal lobules VI and VII in autism. *New England Journal of Medicine*, *318*(21), 1349–1354.

Courchesne, E., Townsend, J., & Saitoh, O. (1995). Reply from the authors to Piven & Arndt. *Neurology*, *45*, 399–402.

Craig, A. D. (2005). Forebrain emotional asymmetry: A neuroanatomical basis? *Trends Cogn Sci*, *9*(12), 566–571.

Dager, S. R., Friedman, S. D., Petropoulos, H., & Shaw, D. W. W. (2008). Imaging evidence for pathological brain development in Autism Spectrum Disorders. In A. Zimmerman (Ed.), *Autism: Current theories and evidence* (361–379). Totowa, NJ: Humana Press.

Dager, S. R., Wang, L., Friedman, S. D., Shaw, D. W., Constantino, J. N., Artru, A. A., Dawson, G., & Csernansky, J. G. (2007). Shape mapping of the hippocampus in young children with autism spectrum disorder. *AJNR Am J Neuroradiol*, *28*(4), 672–677.

Damasio, A. R., & Maurer, R. G. (1978). A neurological model for childhood autism. *Archives of Neurology*, *35*, 777–786.

Davidovitch, M., Golan, D., Vardi, O., Lev, D., & Lerman-Sagie, T. (2009). Head circumference of Israeli children with autism spectrum disorder. International Meeting for Autism, Chicago, Poster.

Davidovitch, M., Patterson, B., & Gartside, P. (1996). Head circumference measurements in children with autism. *J Child Neurol*, *11*, 389–393.

Dawson, G., Finley, C., Phillips, S., & Galpert, L. (1986). Hemispheric specialization and the language abilities of autistic children. *Child Dev*, *57*(6), 1440–1453.

Dawson, G., Finley, C., Phillips, S., Galpert, L., & Lewy, A. (1988). Reduced P3 amplitude of the event-related brain potential: Its relationship to language ability in autism. *J Autism Dev Disord*, *18*(4), 493–504.

Dawson, G., Finley, C., Phillips, S., & Lewy, A. (1989). A comparison of hemispheric asymmetries in speech-related brain potentials of autistic and dysphasic children. *Brain Lang*, *37*(1), 26–41.

Dawson, G., Warrenburg, S., & Fuller, P. (1983). Hemisphere functioning and motor imitation in autistic persons. *Brain Cogn*, *2*(4), 346–354.

De Fosse, L., Hodge, S. M., Makris, N., Kennedy, D. N., Caviness, V. S., Mcgrath, L., Steele, S., Ziegler, D. A., Herbert, M. R., Frazier, J. A., Tager-Flusberg, H., & Harris, G. J. (2004). Language-association cortex asymmetry in autism and specific language impairment. *Annals of Neurology*, *56*(6), 757–766.

Degirmenci, B., Miral, S., Kaya, G. C., Iyilikci, L., Arslan, G., Baykara, A., Evren, I., & Durak, H. (2008). Technetium-99m HMPAO brain SPECT in autistic children and their families. *Psychiatry Res*, *162*(3), 236–243.

Deibler, A. R., Pollock, J. M., Kraft, R. A., Tan, H., Burdette, J. H., & Maldjian, J. A. (2008a). Arterial spin-labeling in routine clinical practice, part 2: Hypoperfusion patterns. *AJNR Am J Neuroradiol*, *29*(7), 1235–1241.

Deibler, A. R., Pollock, J. M., Kraft, R. A., Tan, H., Burdette, J. H., & Maldjian, J. A. (2008b). Arterial spin-labeling in routine clinical practice, part 1: Technique and artifacts. *AJNR Am J Neuroradiol*, *29*(7), 1228–1234.

Dementieva, Y. A., Vance, D. D., Donnelly, S. L., Elston, L. A., Wolpert, C. M., Ravan, S. A., DeLong, G. R., Abramson, R. K., Wright, H. H., &. Cuccaro, M. L. (2005). Accelerated head growth in early development of individuals with autism. *Pediatr Neurol*, 32(2), 102–108.

Deutsch, C. K., &. Joseph, R. M. . (2003). Brief report: Cognitive correlates of enlarged head circumference in children with autism. *J Autism Dev Disord*, 33(2), 209–215.

DeVito, T. J., Drost, D. J., Neufeld, R. W., Rajakumar, N., Pavlosky, W., Williamson, P., & Nicolson, R. (2007). Evidence for cortical dysfunction in autism: A proton magnetic resonance spectroscopic imaging study. *Biol Psychiatry*, *61*(4), 465–473.

Eckert, M. A., Leonard, C. M., Molloy, E. A., Blumenthal, J., Zijdenbos, A., & Giedd, J. N. (2002). The epigenesis of planum temporale asymmetry in twins. *Cerebral Cortex*, *12*(7), 749–755.

Egaas, B., Courchesne, E., & Saitoh, O. (1995). Reduced size of corpus callosum in autism. *Arch Neurol*, *52*(8), 794–801.

Elder, L. M., Dawson, G., Toth, K., Fein, D., & Munson, J. (2008). Head circumference as an early predictor of autism symptoms in younger siblings of children with autism spectrum disorder. *J Autism Dev Disord*, 38(6), 1104–1111.

Elia, M., Ferri, R., Musumeci, S. A., Panerai, S., Bottitta, M., & Scuderi, C. (2000). Clinical correlates of brain morphometric features of subjects with low- functioning autistic disorder. *J Child Neurol*, *15*(8), 504–508.

Endo, T., Shioiri, T., Kitamura, H., Kimura, T., Endo, S., Masuzawa, N., & Someya, T. (2007). Altered chemical metabolites in the amygdala-hippocampus region contribute to autistic symptoms of autism spectrum disorders. *Biol Psychiatry*, *62*(9), 1030–1037.

Fidler, D. J., Bailey, J. N., & Smalley, S. L. (2000). Macrocephaly in autism and other pervasive developmental disorders. *Dev Med Child Neurol*, *42*, 11: 737–740.

Filipek, P. A., Richelme, C., Kennedy, D. N., Rademacher, J., Pitcher, D. A., Zidel S., &Caviness, V. S. (1992). Morphometric analysis of the brain in developmental language disorders and autism (abstract). *Ann Neurol*, 32, 475.

Filipek, P. A. (1995). Quantitative magnetic resonance imaging in autism: The cerebellar vermis. *Current Opinion in Neurology*, 8(2), 134–138.

Flagg, E. J., Cardy, J. E., Roberts, W., & Roberts, T. P. (2005). Language lateralization development in children with autism: insights from the late field magnetoencephalogram. *Neurosci Lett*, *386*(2), 82–87.

Freitag, C. M., Luders, E., Hulst, H. E., Narr, K. L., Thompson, P. M., Toga, A. W., Krick, C., & Konrad, C. (2009). Total brain volume and corpus callosum size in medication-naive adolescents and young adults with autism spectrum disorder. *Biol Psychiatry*, *66*(4), 316–319.

Friedman, S. D., Shaw, D. W., Artru, A. A., Richards, T. L., Gardner, J., Dawson, G., Posse, S., & Dager. S. R. (2003). Regional brain chemical alterations in young children with autism spectrum disorder. *Neurology*, *60*(1), 100–107.

Gabis, L., Huang, W., Azizian, A., DeVincent, C., Tudorica, A., Kesner-Baruch, Y., Roche, P., & Pomeroy, J. (2008). 1H-magnetic resonance spectroscopy markers of cognitive and language ability in clinical subtypes of autism spectrum disorders. *J Child Neurol*, *23*(7), 766–774.

Gaffney, G. R., Tsai, L. Y., Kuperman, S., & Minchin, S. (1987). Cerebellar structure in autism. *Am J Dis Child*, *141*(12), 1330–1332.

Galuska, L., Szakall Jr., S., Emri, M., Olah, R., Varga, J., Garai, I., Kollar, J., Pataki, I., & Tron, L. (2002). PET and SPECT scans in autistic children. *Orv Hetil*, *143*(21 Suppl 3), 1302–1304.

Garber, H. I., & Ritvo, E. R. (1992). Magnetic resonance imaging of the posterior fossa in autistic adults. *American Journal of Psychiatry*, *149*, 245–247.

George, M. S., Costa, D. C., Kouris, K., Ring, H. A., & Ell, P. J. (1992). Cerebral blood flow abnormalities in adults with infantile autism. *J Nerv Ment Dis*, *180*(7), 413–417.

Ghashghaei, H. T., & Barbas, H. (2002). Pathways for emotion: Interactions of prefrontal and anterior temporal pathways in the amygdala of the rhesus monkey. *Neuroscience*, *115*(4), 1261–1279.

Giedd, J. N., Rapoport, J. L., Garvey, M. A., Perlmutter, S., & Swedo, S. E. (2000). MRI assessment of children with obsessive-compulsive disorder or tics associated with streptococcal infection. *Am J Psychiatry*, *157*(2), 281–283.

Gillberg, C., & Svendsen, P. (1983). Childhood psychosis and computed tomographic brain scan findings. *J Autism Dev Disord*, *13*(1), 19–32.

Guerin, P., Lyon, G., Barthelemy, C., Sostak, E., Chevrollier, V., Garreau, B., & Lelord, G. (1996). Neuropathological study of a case of autistic syndrome with severe mental retardation. *Dev Med Child Neurol*, 38(3), 203–211.

Gupta, S. K., & Ratnam, B. V. (2009). Cerebral perfusion abnormalities in children with autism and mental retardation: A segmental quantitative SPECT study. *Indian Pediatr.*, *46*(2), 161–164.

Guptill, J. T., Booker, A. B, Bauman, M. L., Kemper, T. L., & Blatt, G. J. (2006). 3Hflunitrazepam-labeled benzodiazepine binding sites in the hippocampal formation in autism: A multiple concentration autoradiographic study. *Journal of Autism and Developmental Disorder*, 37(5), 911–920.

Hadjikhani, N., Joseph, R. M., Snyder, J., & Tager-Flusberg, H. (2006). Anatomical differences in the mirror neuron system and social cognition network in autism. *Cereb Cortex*, *16*(9), 1276–1282.

Hardan, A. Y., Minshew, N. J., Harenski, K., & Keshavan, M. S. (2001). Posterior fossa magnetic resonance imaging in autism. *J Am Acad Child Adolesc Psychiatry*, *40*(6), 666–672.

Hardan, A. Y., Minshew, N. J., & Keshavan, M. S. (2000). Corpus callosum size in autism. *Neurology*, 55(7), 1033–1036.

Hardan, A. Y., Minshew, N. J., Melhem, N. M., Srihari, S., Jo, B., Bansal, R., Keshavan, M. S., & Stanley, J. A. (2008). An MRI and proton spectroscopy study of the thalamus in children with autism. *Psychiatry Res*, *163*(2), 97–105.

Hardan, A. Y., Muddasani, S., Vemulapalli, M., Keshavan, M. S., & Minshew, N. J. (2006). An MRI study of increased cortical thickness in autism. *Am J Psychiatry*, *163*(7), 1290–1292.

Hashimoto, T., Sasaki, M., Fukumizu, M., Hanaoka, S., Sugai, K., & Matsuda, H. (2000). Single-photon emission computed tomography of the brain in autism: effect of the developmental level. *Pediatr Neurol*, *23*(5), 416–420.

Hazlett, H. C., Poe, M., Gerig, G., Smith, R. G., Provenzale, J., Ross, A., Gilmore, J., & Piven, J. (2005). Magnetic resonance imaging and head circumference study of brain size in autism: Birth through age 2 years. *Arch Gen Psychiatry*, *62*(12), 1366–1376.

Hazlett, H. C., Poe, M., Gerig, G., Smith, R. G., & Piven, J. (2006). Cortical gray and white brain tissue volume in adolescents and adults with autism. *Biol Psychiatry*, *59*(1), 1–6.

Haznedar, M. M., Buchsbaum, M. S., Hazlett, E. A., LiCalzi, E. M., Cartwright, C., & Hollander, E. (2006). Volumetric analysis and three-dimensional glucose metabolic mapping of the striatum and thalamus in patients with autism spectrum disorders. *Am J Psychiatry*, *163*(7), 1252–1263.

Haznedar, M. M., Buchsbaum, M. S., Wei, T. C., Hof, P. R., Cartwright, C., Bienstock, C. A., & Hollander, E. (2000). Limbic circuitry in patients with autism spectrum disorders studied with positron emission tomography and magnetic resonance imaging. *Am J Psychiatry*, *157*(12), 1994–2001.

Herbert, M. R., & Anderson, M. P. (2008). An expanding spectrum of autism models: From fixed developmental defects to reversible functional impairments. In A. Zimmerman (Ed.), *Autism: Current Theories and Evidence* (pp. 429–463). New York: Humana Press.

Herbert, M. R., Harris, G. J., Adrien, K. T., Ziegler, D. A., Makris, N., Kennedy, D. N., Lange, N. T., Chabris, C. F., Bakardjiev, A., Hodgson, J., Takeoka, M., Tager-Flusberg, H., & Caviness Jr., V. S. (2002). Abnormal asymmetry in language association cortex in autism. *Ann Neurol*, *52*(5), 588–596.

Herbert, M. R., Ziegler, D. A., Deutsch, C. K., O'brien, L. M., Kennedy, D. N., Filipek, P. A., Bakardjiev, A. I., Hodgson, J., Takeoka, M., Makris, N., & Caviness Jr., V. S. (2005). Brain asymmetries in autism and developmental language disorder: A nested whole-brain analysis. *Brain*, *128*(Pt 1), 213–226.

Herbert, M. R., Ziegler, D. A., Deutsch, C. K., O'brien, Lange, N., Bakardjiev, A., Hodgson, J., Adrien, K. T., Steele, S., Makris, N., Kennedy, D., Harris, G. J., & Caviness, V. S. (2003). Dissociations of cerebral cortex, subcortical and cerebral white matter volumes in autistic boys. *Brain*, *126*(Pt 5), 1182–1192.

Herbert, M. R., Ziegler, D. A., Makris, N., Filipek, P. A., Kemper, T. L., Normandin, J. J., Sanders, H. A., Kennedy, D. N., & Caviness Jr., V. S. (2004). Localization of white matter volume increase in autism and developmental language disorder. *Ann Neurol*, *55*(4), 530–540.

Herold, S., Frackowiak, R. S., Le Couteur, A., Rutter, M., & Howlin, P. (1988). Cerebral blood flow and metabolism of oxygen and glucose in young autistic adults. *Psychol Med*, *18*(4), 823–831.

Hier, D. B., LeMay, M., & Rosenberger, P. B. (1979). Autism and unfavorable left-right asymmetries of the brain. *J Autism Dev Disord*, *9*(2), 153–159.

Hobbs, K., Kennedy, A., Dubray, M., Bigler, E. D., Petersen, P. B., McMahon, W., & Lainhart, J. E. (2007). A retrospective fetal ultrasound study of brain size in autism. *Biol Psychiatry*, *62*(9), 1048–1055.

Holttum, J. R., Minshew, N. J., Sanders, R. S., & Phillips, N. E. (1992). Magnetic resonance imaging of the posterior fossa in autism. *Biol Psychiatry*, *32*(12), 1091–1101.

Horwitz, B., Rumsey, J. M., Grady, C. L., & Rapoport, S. I. (1988). The cerebral metabolic landscape in autism. Intercorrelations of regional glucose utilization. *Arch Neurol*, *45*(7), 749–755.

Hoshino, Y., Manome, T., Kaneko, M., Yashima, Y., & Kumashiro, H. (1984). Computed tomography of the brain in children with early infantile autism. *Folia Psychiatr Neurol Jpn*, *38*(1), 33–43.

Howard, M. A., Cowell, P. E., Boucher, J., Broks, P., Mayes, A., Farrant, A., & Roberts, N. (2000). Convergent neuroanatomical and behavioural evidence of an amygdala hypothesis of autism. *Neuroreport*, *11*(13), 2931–2935.

Hugg, J. W., Kuzniecky, R. I., Gilliam, F. G., Morawetz, R. B., Fraught, R. E., & Hetherington, H. P. (1996). Normalization of contralateral metabolic function following temporal lobectomy demonstrated by 1H magnetic resonance spectroscopic imaging. *Ann Neurol*, *40*(2), 236–239.

Ito, H., Mori, K., Hashimoto, T., Miyazaki, M., Hori, A., Kagami, S., & Kuroda, Y. (2005). Findings of brain 99mTc-ECD SPECT in high-functioning autism—3-dimensional stereotactic ROI template analysis of brain SPECT. *J Med Invest*, *52*(1–2), 49–56.

Juranek, J., Filipek, P. A., Berenji, G. R., Modahl, C., Osann, K., & Spence, M. A. (2006). Association between amygdala volume and anxiety level:

Magnetic resonance imaging (MRI) study in autistic children. *J Child Neurol*, *21*(12), 1051–1058.

Just, M. A., Cherkassky, V. L., Keller, T. A., Kana, R. K., & Minshew, N. J. (2007). Functional and anatomical cortical underconnectivity in autism: Evidence from an FMRI study of an executive function task and corpus callosum morphometry. *Cereb Cortex*, *17*(4), 951–961.

Kang, D. H., Davidson, R. J., Coe, C. L., Wheeler, R. E., Tomarken, A. J., & Ershler, W. B. (1991). Frontal brain asymmetry and immune function. *Behav Neurosci*, *105*(6), 860–869.

Kanner, L. (1943). Autistic disturbances of affective contact. *Nervous Child*, *10*, 217–250.

Karmiloff-Smith, A. (2006). The tortuous route from genes to behavior: A neuroconstructivist approach. *Cogn Affect Behav Neurosci*, *6*(1), 9–17.

Ke, X., Hong, S., Tang, T., Zou, B., Li, H., Hang, Y., Zhou, Z., Ruan, Z., Lu, Z., Tao, G., & Liu, Y. (2008). Voxel-based morphometry study on brain structure in children with high-functioning autism. *Neuroreport*, *19*(9), 921–925.

Kern, J. K. (2003). Purkinje cell vulnerability and autism: A possible etiological connection. *Brain Dev*, *25*(6), 377–382.

Khalfa, S., Bruneau, N., Roge, B., Georgieff, N., Veuillet, E., Adrien, J. L., Barthelemy, C., & Collet, L. (2001). Peripheral auditory asymmetry in infantile autism. *Eur J Neurosci*, *13*(3), 628–632.

Kilian, S., Brown, W. S., Hallam, B. J., McMahon, W., Lu, J., Johnson, M., Bigler, E. D., & Lainhart, J. (2008). Regional callosal morphology in autism and macrocephaly. *Dev Neuropsychol*, *33*(1), 74–99.

Kleiman, M. D., Neff, S., & Rosman, N. P. (1992). The brain in infantile autism: Are posterior fossa structures abnormal? *Neurology*, *42*, 753–760.

Kleinhans, N. M., Richards, T., Weaver, K. E., Liang, O., Dawson, G., & Aylward, E. (2009). Brief Report: Biochemical correlates of clinical impairment in high functioning autism and Asperger's disorder. *J Autism Dev Disord*, *39*(7), 1079–1086.

Kleinhans, N. M., Schweinsburg, B. C., Cohen, D. N., Muller, R. A., & Courchesne, E. (2007). N-acetyl aspartate in autism spectrum disorders: regional effects and relationship to fMRI activation. *Brain Res.*, *1162*, 85–97.

Lainhart, J. E. (2003). Increased rate of head growth during infancy in autism. *JAMA*, *290*(3), 393–394.

Lazarev, V. V., Pontes, A., & Deazevedo, L. C. (2008). EEG photic driving: Right-hemisphere reactivity deficit in childhood autism. A pilot study. *Int J Psychophysiol*, *71*(2), 177–183.

Lee, J. E., Bigler, E. D., Alexander, A. L., Lazar, M., DuBray, M. B., Chung, M. K., Johnson, M., Morgan, J., Miller, J. N., McMahon, W. M., Lu, J., Jeong, E. K., & Lainhart, J. E. (2007). Diffusion tensor imaging of white matter in the superior temporal gyrus and temporal stem in autism. *Neurosci Lett*, *424*(2), 127–132.

Lee, M., Martin-Ruiz, C., Graham, A., Court, J., Jaros, E., Perry, R., Iversen, P., Bauman, M., & Perry, E. (2002). Nicotinic receptor abnormalities in the cerebellar cortex in autism. *Brain*, *125*(Pt 7), 1483–1495.

Levitt, P., Eagleson, K. L., & Powell, E. M. (2004). Regulation of neocortical interneuron development and the implications for neurodevelopmental disorders. *Trends Neurosci*, *27*(7), 400–406.

Li, X., Chauhan, A., Sheikh, A. M., Patil, S., Chauhan, V., Li, X. M., Ji, L., Brown, T., & Malik, M. (2009). Elevated immune response in the brain of autistic patients. *J Neuroimmunol*, *207*(1-2), 111–6.

Llinas, R., Urbano, F. J., Leznik, E., Ramirez, R. R., & van Marle, H. J. (2005). Rhythmic and dysrhythmic thalamocortical dynamics: GABA systems and the edge effect. *Trends Neurosci*, *28*(6), 325–333.

Lodin, Z., Mares, V., Faltin, J., & Karasek, J. (1969). Studies on the effect of fixation on nervous tissue. IV. Volumetric changes of cerebellar structures due to preparation of the tissue for electron microscopy. *Acta Histochem*, *34*(1), 1–9.

MacFabe, D. F., Cain, D. P., Rodriguez-Capote, K., Franklin, A. E., Hoffman, J. E., Boon, F., Taylor, A. R., Kavaliers, M., & Ossenkopp, K. P. (2007). Neurobiological effects of intraventricular propionic acid in rats: Possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. *Behav Brain Res*, *176*(1), 149–169.

Makris, N., Meyer, J. W., Bates, J. F., Yeterian, E. H., Kennedy, D. N., & Caviness, V. S. (1999). MRI-Based topographic parcellation of human cerebral white matter and nuclei II. Rationale and applications with systematics of cerebral connectivity. *Neuroimage*, *9*(1), 18–45.

Mason, R. A., Williams, D. L., Kana, R. K., Minshew, N., & Just, M. A. (2008). Theory of mind disruption and recruitment of the right hemisphere during narrative comprehension in autism. *Neuropsychologia*, *46*(1), 269–280.

McCaffery, P., & Deutsch, C. K. (2005). Macrocephaly and the control of brain growth in autistic disorders. *Prog Neurobiol*, *77*(1–2), 38–56.

McKelvey, J. R., Lambert, R., Mottron, L., & Shevell, M. I. (1995). Right-hemisphere dysfunction in Asperger's syndrome. *J Child Neurol*, *10*(4), 310–314.

Meyer, J. W., Makris, N., Bates, J. F., Caviness, V. S., & Kennedy, D. N. (1999). MRI-Based topographic parcellation of human cerebral white matter I: Technical foundations. *Neuroimage*, *9*(1), 1–17.

Miles, J. H., Hadden, L. L., Takahashi, T. N., & Hillman, R. E. (2000). Head circumference is an independent clinical finding associated with autism. *Am J Med Genet*, *95*(4), 339–350.

Morgan, J. T., G. Chana, C. A. Pardo, C. Achim, K. Semendeferi, J. Buckwalter, E. Courchesne, and I. P. Everall. (2010). Microglial activation and increased microglial density observed in the

dorsolateral prefrontal cortex in autism. *Biol Psychiatry 68*(4), 368–76.

Morton, J., & Frith, U. (1995). Causal modelling: A structural approach to developmental psychopathology. In D. Cicchetti and D. J. Cohen (Eds.), *Manual of developmental psychopathology* (pp. 357–390). New York: John Wiley.

Mostofsky, S. H., Burgess, M. P., & Gidley Larson, J. C. (2007). Increased motor cortex white matter volume predicts motor impairment in autism. *Brain*, *130* (Pt 8), 2117–2122.

Mraz, K. D., Dixon, J., Dumont-Mathieu, T., & Fein, D. (2009). Accelerated head and body growth in infants later diagnosed with autism spectrum disorders: A comparative study of optimal outcome children. *J Child Neurol*, *24*(7), 833–845.

Mraz, K. D., Green, J., Dumont-Mathieu, T., Makin, S., & Fein, D. (2007). Correlates of head circumference growth in infants later diagnosed with autism spectrum disorders. *J Child Neurol*, *22*(6), 700–713.

Muller, R. A., Cauich, C., Rubio, M. A., Mizuno, A., & Courchesne, E. (2004). Abnormal activity patterns in premotor cortex during sequence learning in autistic patients. *Biol Psychiatry*, *56*(5), 323–332.

Murakami, J. W., Courchesne, E., Press, G. A., Yeung-Courchesne, R., & Hesselink, J. R. (1989). Reduced cerebellar hemisphere size and its relationship to vermal hypoplasia in autism. *Arch Neurol*, *46*(6), 689–694.

Nacewicz, B. M.,Dalton, K. M., Johnstone, T., Long, M. T., McAuliff, E. M., Oakes, T. R., Alexander, A. L., & Davidson, R. J. (2006). Amygdala volume and nonverbal social impairment in adolescent and adult males with autism. *Arch Gen Psychiatry*, *63*(12), 1417–1428.

Neeley, E. S., Bigler, E. D., Krasny, L., Ozonoff, S., McMahon, W., & Lainhart, J. E. (2007). Quantitative temporal lobe differences: Autism distinguished from controls using classification and regression tree analysis. *Brain Dev*, *29*(7), 389–399.

Nicolson, R., DeVito, T. J., Vidal, C. N., Sui, Y., Hayashi, K. M., Drost, D. J., Williamson, P. C., Rajakumar, N., Toga, A. W., & Thompson, P. M. (2006). Detection and mapping of hippocampal abnormalities in autism. *Psychiatry Res*, *148*(1), 11–21.

National Institutes of Health. A longitudinal MRI study of infants at risk for autism. *Brain Development in Autism: Infant Siblings*. Retrieved August 3, 2010, from http://www.ibis-network.org/default.html.

Noble, D. (2008). *The music of life*. New York: Oxford University Press.

Nowell, M. A., Hackney, D. B., Muraki, A. S., & Coleman, M. (1990). Varied MR appearance of autism: Fifty-three pediatric patients having the full autistic syndrome. *Magn Reson Imaging*, 8(6), 811–816.

O'brien, L. M., Ziegler, D. A., Deutsch, C. K., Kennedy, D. N., Goldstein, J. M., Seidman,L. J., Hodge, S, Makris, N., Caviness, V., Frazier, J. A., & Herbert, M. R. (2006). Adjustment for whole brain and cranial size in volumetric brain studies: A review of common adjustment factors and statistical methods. *Harv Rev Psychiatry*, *14*(3), 141–151.

Ohnishi, T., Matsuda, H., Hashimoto, T., Kunihiro, T., Nishikawa, M., Uema, T., & Sasaki, M. (2000). Abnormal regional cerebral blood flow in childhood autism. *Brain*, *123*(Pt 9), 1838–1844.

Ozonoff, S., & Miller, J. N. (1996). An exploration of right-hemisphere contributions to the pragmatic impairments of autism. *Brain Lang*, *52*(3), 411–434.

Page, L. A., Daly, E., Schmitz, N. A. Simmons, Toal, F., Deeley, Q., Ambery, F., McAlonan, G. M., Murphy, K. C., & Murphy, D. G. (2006). In vivo 1H-magnetic resonance spectroscopy study of amygdala-hippocampal and parietal regions in autism. *Am J Psychiatry*, *163*(12), 2189–2192.

Palmen, S. J., Hulshoff Pol, H. E., Kemner, C., Schnack, H. G., Durston, S., Lahuis, B. E., Kahn, R. S., & Van Engeland, H. (2005). Increased gray-matter volume in medication-naive high-functioning children with autism spectrum disorder. *Psychol Med*, 35(4), 561–570.

Pardo C. A., & Eberhart, C. G. (2007). The neurobiology of autism. *Brain Patho.*, *17*(4), 434–47.

Piven, J., Bailey, J., Ranson, B. J., & Arndt, S. (1998). No difference in hippocampus volume detected on magnetic resonance imaging in autistic individuals. *J Autism Dev Disord*, *28*(2), 105–110.

Piven, J., Nehme, E., Simon, J., Barta, P., Pearlson, G., and Folstein, S. E. (1992). Magnetic resonance imaging in autism: Measurement of the cerebellum, pons, and fourth ventricle. *Biological Psychiatry*, *31*(5), 491–504.

Piven, J., Saliba, K., Bailey, J., & Arndt, S. (1997). An MRI study of autism: The cerebellum revisited. *Neurology*, *49*, 546–551.

Piven, J., Saliba, K., Bailey, J., & Arndt, S. (1999). An MRI study of autism: The cerebellum revisited—reply. *Neurology*, 52(5), 1106–1107.

Piven, J., & Arndt, S. (1995). The cerebellum and autism. *Neurology*, *45*, 398–399.

Piven, J., Arndt, S., Bailey, J., & Andreasen, N. (1996). Regional brain enlargement in autism: A magnetic resonance imaging study. *Journal of the American Academy of Child and Adolescent Psychiatry*, 35(4), 530–536.

Piven, J., Bailey, J., Ranson, B. J. & Arndt, S.(1997). An MRI study of the corpus callosum in autism. *Am J Psychiatry*, *154*(8), 1051–1056.

Ramirez, M., Prieto, I., Vives, F., de Gasparo, M., & Alba, F. (2004). Neuropeptides, neuropeptidases and brain asymmetry. *Curr Protein Pept Sci*, *5*(6), 497–506.

Ray, M. A., Graham, A. J., Lee, M., Perry, R. H., Court, J. A., & Perry, E. K. (2005). Neuronal

49 this reference should be cited on page 55 of these proofs but citation was missing

nicotinic acetylcholine receptor subunits in autism: An immunohistochemical investigation in the thalamus. *Neurobiol Dis*, *19*(3), 366–377.

Raymond, G. V., Bauman, M. L., & Kemper, T. L. (1996). Hippocampus in autism: A Golgi analysis. *Acta Neuropathol (Berl,) 91*(1), 117–119.

Rice, S. A., Bigler, E. D., Cleavinger, H. B., Tate, D. F., Sayer, J., McMahon, W., Ozonoff, S., Lu, J., & Lainhart, J. E. (2005). Macrocephaly, corpus callosum morphology, and autism. *J Child Neurol*, *20*(1), 34–41.

Ringo, J. L. (1991). Neuronal interconnection as a function of brain size. *Brain Behav Evol*, *38*(1), 1–6.

Ringo, J. L., Doty, R. W., Demeter, S., & Simard, P. Y. (1994). Time is of the essence: A conjecture that hemispheric specialization arises from interhemispheric conduction delay. *Cereb Cortex*, *4*(4), 331–343.

Rodier, P. M., Ingram, J. L., Tisdale, B., Nelson, S., & Roma, J. (1996). Embryological origins for autism: Developmental anomalies of the cranial nerve motor nuclei. *Journal of Comparative Neurology*, *370*, 247–261.

Rojas, D. C., Bawn, S. D., Benkers, T. L., Reite, M. L., & Rogers, S. J. (2002). Smaller left hemisphere planum temporale in adults with autistic disorder. *Neurosci Lett*, *328*(3), 237–240.

Rojas, D. C., Camou, S. L., Reite, M. L., & Rogers, S. J. (2005). Planum temporale volume in children and adolescents with autism. *J Autism Dev Disord*, *35*(4), 479–486.

Rubenstein, J. L., and M. M. Merzenich. 2003. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav 2*, *5*: 255–267.

Rumsey, J. M., & Hamburger, S. D. (1988). Neuropsychological findings in high-functioning men with infantile autism residual state. *Journal of Clinical and Experimental Neuropsychology*, *10*, 201–221.

Ryu, Y. H., Lee, J. D., Yoon, P. H., Kim, D. I., Lee, H. B., & Shin, Y. J. (1999). Perfusion impairments in infantile autism on technetium-99m ethyl cysteinate dimer brain single-photon emission tomography: Comparison with findings on magnetic resonance imaging. *Eur J Nucl Med*, *26*(3), 253–259.

Saitoh, O., Courchesne, E., Egaas, B., Lincoln, A. J., & Schreibman, L. (1995). Cross-sectional area of the posterior hippocampus in autistic patients with cerebellar and corpus callosum abnormalities. *Neurology*, *45*(2), 317–324.

Saitoh, O., Karns, C. M., & Courchesne, E. (2001). Development of the hippocampal formation from 2 to 42 years: MRI evidence of smaller area dentata in autism. *Brain*, *124*(Pt 7), 1317–1324.

Salthe, S. (1985). *Evolving hierarchical systems*. New York: Columbia University Press.

Schaefer, G. B., Thompson, J. N., Bodensteiner, J. B., McConnell, J. M., Kimberling, W. J., Gay, C. T., Dutton, W. D., Hutchings, D. C., & Gray, S. B. (1996). Hypoplasia of the cerebellar vermis in neurogenetic syndromes. *Ann Neurol*, *39*(3), 382–385.

Schmahmann, J. D. (2004). Disorders of the cerebellum: Ataxia, dysmetria of thought, and the cerebellar cognitive affective syndrome. *J Neuropsychiatry Clin Neurosci*, *16*(3), 367–378.

Schmahmann, J. D., & Caplan, D. (2006). Cognition, emotion and the cerebellum. *Brain*, *129*(Pt 2), 290–292.

Schmahmann, J. D., & Pandya, D. N. (2008). Disconnection syndromes of basal ganglia, thalamus, and cerebrocerebellar systems. *Cortex*, *44*(8), 1037–1066.

Schumann, C. M., Hamstra, J., Goodlin-Jones, B. L., Lotspeich, L. J., Kwon, H., Buonocore, M. H., Lammers, C. R., Reiss, A. L., & Amaral, D. G. (2004). The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. *J Neurosci*, *24*(28), 6392–6401.

Shavelle, R. M., Strauss, D. J., Pickett, J. Causes of death in autism. *Journal of Autism and Developmental Disorders*, *31*(6), 569–576.

Shen, Y. Q., Hebert, G., Moze, E., Li, K. S., & Neveu, P. J. (2005). Asymmetrical distribution of brain interleukin-6 depends on lateralization in mice. *Neuroimmunomodulation*, *12*(3), 189–194.

Shetty, N., Ratai, E., Ringer, A., & Herbert, M. (2009). Magnetic resonance studies in ASD: Review of regions investigated, findings, potential influence of methodology, and directions for future research. *International Meeting for Autism Research*, Poster 4188.

Shi, L., Smith, S. E., Malkova, N., Tse, D., Su, Y., & Patterson, P. H. (2009). Activation of the maternal immune system alters cerebellar development in the offspring. *Brain Behav Immun*, *23*(1), 116–123.

Slikker, W., & Chang, L. W. (1998). *Handbook of developmental neurotoxicology*. San Diego, CA: Academic Press.

Sparks, B. F., Friedman, S. D., Shaw, D. W., Aylward, E. H., Echelard, D., Artru, A. A., Maravilla, K. R., Giedd, J. N., Munson, J., Dawson, G., & Dager, S. R. (2002). Brain structural abnormalities in young children with autism spectrum disorder. *Neurology*, *59*(2), 184–192.

Stanfield, A. C., McIntosh, A. M., Spencer, M. D., Philip, R., Gaur, S., & Lawrie, S. M. (2007). Towards a neuroanatomy of autism: A systematic review and meta-analysis of structural magnetic resonance imaging studies. *Eur Psychiatry*, *23*(4), 289–299.

Starkstein, S. E., Vazquez, S., Vrancic, D., Nanclares, V., Manes, F., Piven, J., & Plebst, C. (2000). SPECT findings in mentally retarded autistic individuals. *J Neuropsychiatry Clin Neurosci*, *12*(3), 370–375.

Stroganova, T. A., Nygren, G., Tsetlin, M. M., Posikera, I. N., Gillberg, C., Elam, M., & Orekhova, E. V.

(2007). Abnormal EEG lateralization in boys with autism. *Clin Neurophysiol*, *118*(8), 1842–1854.

Sundaram, S.K., Kumar, A.J., Makki, M.I., Behen, M.E., Chugani, H.T. & Chugani, D.C.(2008). Diffusion tensor imaging of frontal lobe in autism spectrum disorder. *Cerebral Cortex*, *18*(11), 2659–65.

Takeuchi, M., Harada, M., Matsuzaki, K., Nishitani, H., & Mori, K. (2004). Difference of signal change by a language task on autistic patients using functional MRI. *J Med Invest*, *51*(1–2), 59–62.

Theberge, J. (2008). Perfusion magnetic resonance imaging in psychiatry. *Top Magn Reson Imaging*, *19*(2), 111–130.

Tsatsanis, K. D., Rourke, B. P., Klin, A., Volkmar, F. R., Cicchetti, D., & Schultz, R. T. (2003). Reduced thalamic volume in high-functioning individuals with autism. *Biol Psychiatry*, 53(2), 121–129.

Tucker, D. M. 1992. Developing emotions and cortical networks. In M. R. Gunnar & C. A. Nelson (Eds.), *Developmental behavioral neuroscience*, *vol. 24* (pp. 75–128). Hillsdale, NJ: Lawrence Erlbaum Associates.

Van Essen, D. C., Drury, H. A., Joshi, S., & Miller, M. I. (1998). Functional and structural mapping of human cerebral cortex: Solutions are in the surfaces. *Proc Natl Acad Sci U S A*, 95(3), 788–795.

Vargas, D. L., Nascimbene, C. Krishnan, C., Zimmerman, A. W., & Pardo, C. A. (2005). Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol*, *57*(1), 67–81.

Vidal, C. N., Nicolson, R., DeVito, T. J., Hayashi, K. M., Geaga, J. A., Drost, D. J., Williamson, P. C., Rajakumar, N., Sui, Y., Dutton, R. A., Toga, A. W., & Thompson, P. M. (2006). Mapping corpus callosum deficits in autism: an index of aberrant cortical connectivity. *Biol Psychiatry*, *60*(3), 218–225.

Waiter, G. D., Williams, J. H., Murray, A. D., Gilchrist, A., Perrett, D. I., & Whiten, A. (2005). Structural white matter deficits in high-functioning individuals with autistic spectrum disorder: a voxel-based investigation. *Neuroimage*, *24*(2), 455–461.

Whitehouse, A. J., & Bishop, D. V.(2008). Cerebral dominance for language function in adults with specific language impairment or autism. *Brain*, *131*(Pt 12), 3193–3200.

Whitney, E. R., Kemper, T. L., Bauman, M. L., Rosene, D. L., & Blatt, G. J. (2008). Cerebellar Purkinje cells are reduced in a subpopulation of autistic brains: A stereological experiment using Calbindin-D28k. *Cerebellum*, *7*(3), 406–16.

Wilcox, J., Tsuang, M. T., Ledger, E., Algeo, J., & Schnurr, T.(2002). Brain perfusion in autism varies with age. *Neuropsychobiology*, *46*(1), 13–16.

Williams, R. S., Hauser, S. L., Purpura, D. P., DeLong, G. R., & Swisher, C. N. (1980). Autism and mental retardation: Neuropathologic studies performed in four retarded persons with autistic behavior. *Arch Neurol*, 37(12), 749–753.

Wills, S., Cabanlit, M., Bennett, J., Ashwood, P., Amaral, D. G., & Van de Water, J. (2009). Detection of autoantibodies to neural cells of the cerebellum in the plasma of subjects with autism spectrum disorders. *Brain Behav Immun*, *23*(1), 64–74.

Wisniewski, A. B.(1998). Sexually-dimorphic patterns of cortical asymmetry, and the role for sex steroid hormones in determining cortical patterns of lateralization. *Psychoneuroendocrinology*, *23*(5), 519–547.

Wittling, W. (1995). Brain asymmetry in the control of autonomic-physiologic activity. In R. J. Davidson & K. Hugdahl (Eds.), *Brain asymmetry* (pp. 305–357). Cambridge, MA: MIT Press.

Woodhouse, W., Bailey, A., Rutter, M., Bolton, P., Baird, G., & Le Couteur, A. (1996). Head circumference in autism and other pervasive developmental disorders. *J Child Psychol Psychiatry*, 37(6), 665–671.

Yip, J., Soghomonian, J. J., & Blatt, G. J. (2007). Decreased GAD67 mRNA levels in cerebellar Purkinje cells in autism: pathophysiological implications. *Acta Neuropathol*, *113*(5), 559–568.

Zeegers, M., Van Der Grond, J., Durston, S., Nievelstein, R. J., Witkamp, T., Van Daalen, E., Buitelaar, J., & Engeland, H. V. (2006). Radiological findings in autistic and developmentally delayed children. *Brain Dev*, *28*(8), 495–499.

Zilbovicius, M., Boddaert, N., Belin, P., Poline, J. B., Remy, P., Mangin, J. F., Thivard, L., Barthelemy, C., & Samson, Y. (2000). Temporal lobe dysfunction in childhood autism: A PET study. Positron emission tomography. *Am J Psychiatry*, *157*(12), 1988–1993.