

Chapter 2

The Neuropathology of Autism

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2.1 Introduction

Autism is a neurodevelopmental condition presently defined by operational criteria that, by themselves, lack in terms of construct validity. These operational criteria necessitate the screening of a variety of behavioral domains, e.g., communication, motor, and social skills. The diversity of behavioral domains that appear affected in autism makes this a Pervasive Developmental Disorder. By way of contrast, specific developmental disorders refer to explicit learning disabilities and other disorders affecting coordination. Specific developmental disorders may be subsumed under pervasive ones, and it is not unusual to have learning disorders, for example, in autism (Williams and Casanova 2012).

The term autism spectrum disorders (ASD) is used to describe three conditions with shared core symptoms: autism, Asperger, and Pervasive Developmental Disorders-Not Otherwise Specified (PDD-NOS). The existence of a PDD-NOS diagnosis prevents falsifiability for the diagnostic criteria of autism by sweeping atypical cases into this isolated category. The lack of construct validity to our classification schemes predisposes most studies to reaffirm the original observations upon which the criteria are based (Kanner 1943).

Autism is often seen in the presence of other medical/psychiatric conditions (for review, see Casanova 2007). Mental retardation and seizures are common comorbidities. Chromosomal abnormalities are frequent with certain genotypes having

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a significant higher incidence of manifesting autistic symptomatology. These genotypes include tuberous sclerosis, fragile X, Down, velocardiofacial, and Möbius syndrome. Much research has been done in regard to the possible link of tuberous sclerosis and autism. The coincidence in symptomatology should be expected as both tuberous sclerosis and autism share in widely distributed migrational abnormalities.

The comorbidity with chromosomal disorders attests to the neurodevelopmental nature of autism. The large number of comorbid conditions and susceptibility genes is a reflection of the clinical heterogeneity of autism and the potential for multiple underlying etiologies. Current medical understanding regards autism as a multifactorial or complex condition. Studies suggest that besides multiple susceptibility and protective genes, environmental influences play a significant role in its etiopathogenesis. As in many other multifactorial conditions, the risk of developing autism among first-degree relatives is higher than within the normal population.

The author has already suggested that, similar to other multifactorial conditions, autism offers a threshold phenomenon wherein three main factors impinge on each other to various degrees before the phenotype is able to supervene. The factors for the so-called triple-hit hypothesis are (1) a critical period of brain development, (2) an underlying vulnerability (e.g., genes), and (3) an exogenous stressor or stressors (Casanova 2007). The following sections will broach the subjects of gross and microscopic pathology before discussing the role of cortical modularity in autism.

2.2 Gross Neuropathology

Although a large number of structural abnormalities have been reported in autism, only a few have been reproduced by independent investigators. Among the more salient manifestation are increased brain size, complexity of gyrfication, and diminished size of the corpus callosum. Increased brain size occurs without concomitant signs of edema (Casanova 2007). The volumetric increase does not appear to be a postmortem artifact as it has been reported in vivo with neuroimaging techniques and, in addition, increased brain size has been reported in first-degree relatives of affected individuals (Woodhouse et al. 1996; Fidler et al. 2000). When present, brain enlargement appears to be generalized, with conflicting data regarding the putative role of the cerebellum within the volumetric increase (Courchesne et al. 2001; Sparks et al. 2002). The findings of either hyper- or hypoplasia of the vermian lobules in different subgroups of autistic individuals remain controversial (Courchesne et al. 1988, 1994). The cerebellar findings have not been reproduced by several groups and do not appear to be specific to autism as they have now been reported in fragile X syndrome (Schaefer et al. 1996). Piven et al. (1992), when correcting for IQ among their comparison groups, reported no difference in vermian lobule size.

In a postmortem study of 19 cases by Kemper and Bauman, eight of eleven subjects under 12 years of age had increased brain size as compared to controls

(Kemper 1988). By comparison, six of eight autistic individuals over 18 years of age showed reduced brain size. Cross-sectional MRI studies now suggest that brain volume in autistic individuals increases during early childhood with the rate decelerating by late childhood and adolescence when brain volumes of autistic and controls become similar (Courchesne et al. 2001; Hardan et al. 2001; Aylward et al. 2002).

The presence of macrocephaly in autism persists after controlling for height, gender, and other medical disorders including seizures (Piven et al. 1995; Fombonne et al. 1999). The relationship between macrocephaly and IQ is unclear (Piven et al. 1995; Lainhart et al. 1997; Stevenson et al. 1997). In a recent review of the neuroimaging literature, Goldberg et al. (1999) found few replicated findings and criticized published studies for not controlling for confounding variables. Goldberg et al. concluded that the only independently corroborated findings were macrocephaly and decreased size of the corpus callosum, primarily the splenium.

Gross inspection of the brain has revealed few abnormalities. The gyral pattern of the brain of autistic individuals appears normal. There is a report of increased gyrification in the frontal lobes of autistic subjects using a single anatomical level for comparison (Hardan et al. 2004). Similar studies using another anthropometric measurement, the gyral window, have revealed smaller dimensions to this compartment (Casanova et al. 2009b). The gyral window is the space that constrains the passage of cortical afferent and efferents. A smaller gyral window presumably biases the size of its fibers favoring short connections (e.g., arcuate) at the expense of longer ones (e.g., commissural).

Some morphometric studies suggest that the white matter is disproportionately enlarged in regard to the gray matter (Herbert et al. 2003). Independent studies have now corroborated that the outer radiate compartment, containing short projecting axons (e.g., arcuate fibers), accounts to a significant extent for the increased white matter (Herbert et al. 2004). The inner or deeper white matter consisting of longer projections, e.g., commissural fibers, is diminished as shown by the reduced size of the corpus callosum. Casanova has suggested that the increase in outer radiate white matter is the result of supernumerary minicolumns in need of short-range connections (Casanova 2004).

2.3 Microscopic Pathology

Bauman and Kemper's classic study surveyed whole-brain celloidin-embedded sections (Kemper 1988). Each section was Nissl stained and cut at 35 μm following a protocol originally designed for the Yakovlev Collection. Sections were examined with a stereomicroscope that allowed side-by-side comparisons of autistic and control slides. Mounting and staining the free-floating sections provided for a good number of artifacts primarily affecting the cortex. Most of the detailed examination was therefore spent studying subcortical structures. Of the cortical sections examined, Bauman and Kemper reported no abnormalities in neuronal morphometry, lamination, and cellular density. Reported findings were primarily within the limbic

system (e.g., hippocampus, amygdala, mammillary bodies, septal nuclei) and cerebellum (Bauman and Kemper 1985, 1994). Neuropathology found in these areas included increased cell-packing density and reduced neuronal size. Within the cerebellum, both Purkinje and granule cells were found to be decreased in numbers throughout the hemispheres without any evidence of reactive gliosis. Furthermore, the olivary nuclei failed to show atrophy as expected with Purkinje cell loss. Four of the six autistic patients suffered from seizures, but the reported abnormalities were said to be similar regardless of the presence or absence of this comorbidity (Bauman and Kemper 1994). Bauman and Kemper concluded that the described features were characteristic of a curtailment of normal development.

Bauman and Kemper also examined Golgi-impregnated hippocampal sections of two autistic subjects and an equal number of controls (Raymond et al. 1995). Only one autistic subject was of good-enough quality to allow for analysis. The patient showed smaller somas in the CA4 hippocampal subfield. This report as well as the previous one with Nissl were based on subjective appraisals that relied on biased (non-stereological) assumptions. The small neurons reported by Bauman and Kemper in various regions of the limbic system may represent, as they stated, a “developmental phenotype.” Other possibilities include *apoptosis* (cell withering usually associated with neurodegenerations) or a type of non-apoptotic dark degenerating cell. More recent studies using stereological techniques and well-defined anatomical criteria to define the subnuclei of the amygdala failed to reproduce the cell-packing results originally claimed by Bauman and Kemper (Schumann and Amaral 2005, 2006).

The nature of Purkinje cell loss in autism remains disputed. Although Bauman and Kemper insisted that the cell loss was part of a neurodevelopmental condition, the cerebellar foliar pattern remained normal and without additional evidence of disorganization of the remaining cellular elements (Harding and Copp 1997). This is the case even for the patches within the Purkinje cell layer where cell loss has been noted. More recent studies using immunocytochemistry (Bauman and Kemper used a Nissl stain) have shown marked glial proliferation as a reaction to Purkinje cell loss. Both the nature of the gliotic response and the use of GFAP staining denote a reactive process still undergoing at the time of death. The Purkinje cell loss may therefore be an acquired (postnatal) phenomenon explainable by seizures or the use of medications that exhibit neurotropism for the cerebellum, such as phenytoin (Dilantin) (Bailey et al. 1998; Pardo et al. 2005; Vargas et al. 2005).

Bailey et al. (1998) investigated the brains of six autistic cases (all mentally handicapped and three with epilepsy). Three of the brains were swollen, probably as a result of postmortem edema, one of which showed evidence of putrefaction. One case showed increased cell packing in all cornu ammonis subfields. Four cases showed areas of cortical abnormalities primarily involving the frontal lobes. This was the first report within the existing literature to incriminate a role for the cortex in the neuropathology of autism. The abnormalities reported by Bailey et al. (1998) included irregular laminar patterns, thickened cortex, increased number of neurons within the white matter, and heterotopias. The overall pattern was suggestive of cortical dysgenesis.

Similar to Bailey's report (see above) scattered postmortem and radiological data points to the presence of heterotopias in autism. Few magnetic resonance imaging (MRI) reports have indicated the presence of unidentified bright objects (Nowell et al. 1990; Bailey et al. 1998). Postmortem studies indicate their presence within the white matter and germinal zone (Bailey et al. 1998). All brain regions appear to be affected. Their presence, in terms of location, is highly variable among cases (Wegiel et al. 2010). The findings are suggestive of so-called epigenetic heterotopias as opposed to a genetically dictated condition. Previous authors have suggested that the presence of heterotopias in autism may help explain the link to seizures and tuberous sclerosis.

Several reports have suggested the presence of neuroinflammation in autism (Vargas et al. 2005). These reports are based primarily on the presence of reactive astrocytes and microglia. The classical inflammatory response involves a vascular component leading to the accumulation of hematopoietic cells and fluid within the extravascular space; vessels are engorged with margination of cells and the blood-brain barrier disrupted (Casanova 2007). As of present, there is no evidence that a classical inflammatory response is occurring in the brains of autistic individuals. Cerebrospinal fluid samples from live patients show normal results, including cellular components, protein electrophoresis, and levels of quinolinic acid and neopterin (Zimmerman et al. 2005). The reported findings do not support a role of tissue repair or recovery in the pathogenesis of autism.

The glial reaction observed in the brains of some autistic individuals may reflect, in part, their agonal conditions. A recent survey of available brains within the Autism Tissue Program (ATP) showed that the majority of patients died by drowning or incurred in other hypoxic conditions, e.g., seizures, sepsis, and anoxic encephalopathy (Casanova 2007). Reoxygenation of damaged tissue procreates a free radical cascade focusing on the rupture of double bonds as found primarily in membranes within the neatly arranged stacks of axonal bundles within the white matter. The end result is a gliotic response preferentially targeting the white matter. Agonal and preagonal conditions involving hypoxia and ischemia-reperfusion injury may therefore help explain some of the cellular response and the production of cytokines.

Hutsler et al. (2007) evaluated cortical thickness and lamination as proxy measurements of organization in eight ASD patients and a similar number of age-/sex-matched controls. There were no significant findings; i.e., average cortical thickness for any examined lobe was never greater than 3 % those of controls, and there was evidence of cell clustering in lamina I and subplate with little evidence of a defect in the lamination of the cerebral cortex. The same patients were later on used to study the gray-white matter boundary (Avino and Hutsler 2010). The results indicated an indistinct boundary in autistic patients believed to represent the presence of supernumerary neurons as a result of a migrational abnormality or failed apoptosis.

Courchesne et al. (2011) quantitated the total number of neurons in the dorsolateral and mesial prefrontal cortex from seven children with autism and six controls. Autistic children had 67 % more neurons as compared to controls. An interesting

observation by the authors was that autistic patients had more neurons than predicted from the large brain weights. Studies focusing on cortical modularity have attempted to explain findings of increased neuronal density based on the presence of supernumerary minicolumns.

2.4 Minicolumnar Findings

The best-known architectural motif of the cortex is its lamination. However, a vertical organization has also been recognized both anatomically and physiologically. Several anatomical elements have been used to describe morphometric features of the vertical organization. These anatomical elements include pyramidal cell arrays, dendritic bundles, axonal bundles, and the location of double bouquet cells (Casanova 2007). These elements can be used interchangeably as studies have shown their correspondence to each other (Casanova 2008). The most often used method for studying minicolumnarity employs pyramidal cell arrays. Processing conditions, thickness, and staining that allow visualization of pyramidal cell arrays are well known and can be obtained from the classical studies of the Vogts and Yakovlev.

Minicolumns, as defined by pyramidal cell arrays, vary in thickness from 25 to about 55 μm depending on brain region (DeFelipe 2005). They usually have some 80–100 cellular elements spanning layers II through VI. Studies by the Hungarian anatomist Szentágothai showed a preferred placement for pyramidal cells to be located at the center of the minicolumn with interneurons at its periphery forming a so-called shower curtain of inhibition (Szentágothai and Arbib 1975). The dimension of the core space of minicolumns seems conserved among multiple species. Variability in width throughout evolution is primarily ingrained within the outer peripheral space, a compartment housing inhibitory anatomical elements (Casanova et al. 2009a).

In the first study of minicolumnarity in autism, Casanova et al. (2002c) surveyed the morphometry of these modular structures in nine subjects and an equal number of controls. Photomicrographs were taken of Brodmann areas 9, 21, and 22 and studied by computerized image analysis (Buxhoeveden et al. 2000). The algorithm used had been tested against physiologically derived measurements, by 3D modeling and scatter (cell translation around the main axis of the minicolumn), to correct for curvature in case a flat face of a gyrus was not obtainable. The results showed significant reduction in the width of minicolumns primarily attributable to loss within their peripheral neuropil space. The same series was later on analyzed by using a different algorithm, the gray level index (GLI), modified from the method developed by Schleicher and colleagues (Schlaug et al. 1995; Casanova et al. 2002b). The original finding of diminished minicolumnar width was validated by the GLI method. These and other studies have found minicolumnar abnormalities as being widely distributed, but affecting principally, and most severely, the frontal lobes (Casanova 2006; Casanova et al. 2006a).

The minicolumnar findings appear to be quite specific being absent when correcting for mental retardation as in the case of Down syndrome. The morphometric findings also differ from those of other conditions expressing autistic-like behaviors, e.g., rubella babies and tuberous sclerosis. The only condition of similar neuronomorphometry is Asperger where differences are of degree rather than kind (Casanova et al. 2002a). In this regard, the two brain specimens of Asperger individuals examined by Casanova et al. (2002a) gave similar findings to those of autistic subjects but were less severely affected.

In a study sponsored by the Autism Tissue Program, an international group of researchers attempted to reproduce the minicolumnar findings (Casanova et al. 2006b). Different individuals were in charge of various parts of the analysis including patient/tissue selection, photomicrography, computerized image analysis, and statistical analysis of the results. The analysis was performed blind to diagnosis from coded slides. Results were provided to a third party in order to break the blind and perform the preliminary analysis. The initial results, based on an algorithm of the Euclidean minimum spanning tree, were corroborated by using the GLI method. Minicolumnar width, measured as tangential distances between pyramidal cell arrays, was significantly narrower in autistic individuals. In addition, a Delaunay triangulation was implemented to determine the distribution of distances between pyramidal cells (interneurons were thresholded based on size). No significant differences were noted in intracolumnar distances; rather, the results indicated reduced intercolumnar widths. The authors concluded that the total number of pyramidal cells per minicolumn was the same in both the autistic and control groups but that there were an increased number of these modular structures in autism. Finally, reduced measurements of pyramidal cell size as well as their nucleoli suggested a bias in connectivity favoring short axons vs. longer projections. The smaller neurons observed in this study are best suited at maintaining short connections of the type observed in arcuate fibers.

Minicolumnar width reduction in autism spans supragranular, granular, and infragranular layers (Casanova et al. 2010). The most parsimonious explanation to the findings is the possible abnormality of an anatomical element in common to all layers. Compartmentalization of the minicolumn (i.e., studying peripheral neuropil vs. core space) in autism has shown the largest width reduction in its peripheral compartment. This space provides, among others, for inhibitory elements: the so-called shower of inhibition to the minicolumn (see above). The findings have prompted the possibility of an inhibitory/excitatory imbalance in autism and a possible explanation to the multifocal seizures often observed in this condition (Casanova et al. 2003). Casanova has suggested that in autism there is an environmental factor that forces mitosis of periventricular germinal cells in susceptible individuals (Casanova 2012). The migration of daughter cells from the ventricular zone to the cortex then occurs at an inappropriate time when the radially migrating cells (pyramidal neurons) are not integrated with tangentially derived interneurons (see the triple-hit hypothesis at the beginning of the chapter). The end result is an inhibitory excitatory imbalance causing abnormalities in the flow of information through the minicolumn.

It thus appears that the periventricular germinal cells offer a *locus minoris resistentiae* to the expression of pathology in autism. Heterochronic periventricular cell divisions provide for nodular heterotopias and similar migratory abnormalities within the white matter. These changes resemble those observed in tuberous sclerosis, a condition that exhibits marked comorbidity with autism. Viruses that provide an autism phenotype either exhibit neurotropism for periventricular germinal cells or cause cystic damage within the same. Similarly, extreme prematurity is a major risk factor for autism that is usually associated to germinal cell hemorrhages.

2.5 Summary

In comparison with the rest of the literature on autism, few articles have been published on the subject of neuropathology. Given the limited resources, it is unsurprising to find that only 40 or so cases have so far been studied and reported. Characteristically, these reports describe a lack of gross findings and acute changes. The blood–brain barrier appears to be intact. There is no evidence of contusions, hemorrhage, or edema. Although extremely large brain weights have been reported for some autopsied specimens (e.g., more than 1,800 g), this probably represents postmortem edema wherein the fresh postmortem tissue enters in contact with a hyposmolar solution. Microscopic examination in some of these cases shows corresponding evidence of edema not found in properly preserved cases. These specimens need to be eliminated when acquiring a series for quantitative morphometric studies.

Although neuropathological studies suggest a large number of positive findings, few have been corroborated in independent populations. Among the more reproducible findings is Purkinje cell loss. However, the coexistence of Purkinje cell loss with acute reactive gliosis indicates an ongoing process rather than a neurodevelopmental one. Other positive findings like neuroinflammation need to be studied by controlling comparison series for agonal and preagonal conditions. Otherwise astrocytic and microglial activation, primarily affecting the white matter, is expected from specimens suffering from ischemia-reperfusion injury. A significant percentage of autism donor specimens in brain banks have suffered from ischemia-reperfusion injuries during their agonal state. This lesion characterizes the way the patients died rather than the core pathology of the condition.

In the field of neuropathology, findings bear importance when they have explanatory as well as predictive abilities. Certain gross and microscopic findings appear well established, e.g., larger brain size on average, smaller corpus callosum, and heterotopias. It should be clear that the importance of additional findings depends on how much they help explain the neuropathological phenotype already ascertained. Furthermore, the element of predictability should help assign importance to any new findings. We should always ask ourselves, what do the findings help us explain that wasn't previously known?

Autism is a neurodevelopmental condition whose symptoms denote abnormalities of the gray matter. The involved higher cognitive processes and seizures in a

significant portion of patients suggest a cortical localization. Described minicolumnar abnormalities comply with this location. Since supernumerary minicolumns are the mechanism of corticogenesis, their increased number could help explain abnormalities of brain volume as well as connectivity. Longer projections require an increased metabolic load and attendant time delays in transmission; new minicolumns are selectively pressured to provide a small-world network biasing connectivity within networks towards shorter projections. Emergence of this topology optimizes connectedness while minimizing wiring costs within networks. Desynchronization of maturing excitatory (pyramidal cells) and inhibitory (interneurons) elements helps explain the presence of seizures and other higher cognitive impairments in autism.

Postmortem Brain Imaging

By Jane Pickett, Ph.D.

The application of MR imaging to the postmortem brain closes the gap between the macroscopic and microscopic view of this complicated organ (Schumann and Nordahl 2011). A pioneering study comparing postmortem brain MRI and histology “slices” of the same brains showed the direct relationship between atrophy of the hippocampal formation and neuronal loss in Alzheimer’s disease (Bobinski et al. 1999). This seemingly simplistic explanation of both ante- and postmortem imaged volume loss in Alzheimer’s belies the numerous possible explanations (variable shrinkage, reduction in neuronal volume, etc.) instead of actual reduction in cell numbers found using unbiased stereology.

In autism research, classical neuropathology techniques were augmented by a new postmortem MRI protocol designed to give a three-dimensional representation of the intact, formalin-fixed brain (Schumann et al. 2001). Improvising on a protocol to optimize the *imaging* parameters for postmortem MRI (Blamire et al. 1999), this technique applied a proton density-weighted imaging sequence for optimal differences in gray and white matter contrast in fixed whole brains or hemispheres shown in Fig. 2.1.

The MRI data on the first 39 scanned brains (23 autism and 16 unaffected control) were used in proof-of-principle experiments using a new shape analysis of cerebral white matter gyrifications to classify autistic and control brains (El-Baz et al. 2007; Fahmi et al. 2007). MRI scan data records were made available on an FTP site at the UC Davis MIND Institute from 2001 to 2005 when they were integrated into the informatics platform of the Autism Speaks’ Autism Tissue Program (<http://www.autismtissueprogram.org>).

A Resource for Science: Autism Tissue Program MRI Records

Autism Speaks, an organization dedicated to autism research, is likewise dedicated to the investigation of postmortem brains as a fundamental part of autism research. Its Autism Tissue Program (ATP) has advocated and supported brain donation for research since 1998. MRI DICOM data is stored on the ATP informatics portal (<http://www.atpportal.org>). The records are accessible to researchers internationally

Fig. 2.1 3D reconstruction of MRI data from scan of the whole postmortem brain (Image courtesy of Cindi Schumann, M.I.N.D. Institute, University of California at Davis)



and federated with other autism research programs via the National Database for Autism Research (NDAR, <http://www.ndar.org>).

Beginning in 2005, formalin-fixed brains were routinely transferred from brain banks affiliated with the ATP to the New York Institute for Basic Research for a specialized neuropathologic examination that begins with MRI and often DTI scanning. A parallel process of MRI, DTI, and neuropathological examination was started on brain specimens in the brain bank for autism in the UK (BBA and RDR) based in Oxford. The imaging data records provide a permanent reference for brains that are further processed and dissected for distribution to many investigators exploring many brain regions—operations that are also tracked on the portal.

By 2012 there were MRI records on the ATP portal representing 63 donors (39 had a diagnosis of autism spectrum disorder (ASD), 20 are unaffected individuals, and others represented related disorders like tuberous sclerosis and chromosome 15q duplication). Antemortem scan records are rarely available; there is one case with two antemortem scan records (age 8, 11) and a postmortem scan record of the donor at age 15.

Diffusion Tensor Imaging (DTI)

In addition to conventional images that depict the macroscopic structure of the brain based on tissue types, “diffusion-weighted” MRI can be used to define the white matter tracts that provide the major connections in the brain (Miller et al. 2011, 2012). This technique faces considerable challenges in postmortem tissue. First, the diffusivity is lower in fixed brains at room temperature. Furthermore, any degradation of the tissue, or air pockets in the sample, can lead to signal distortions.

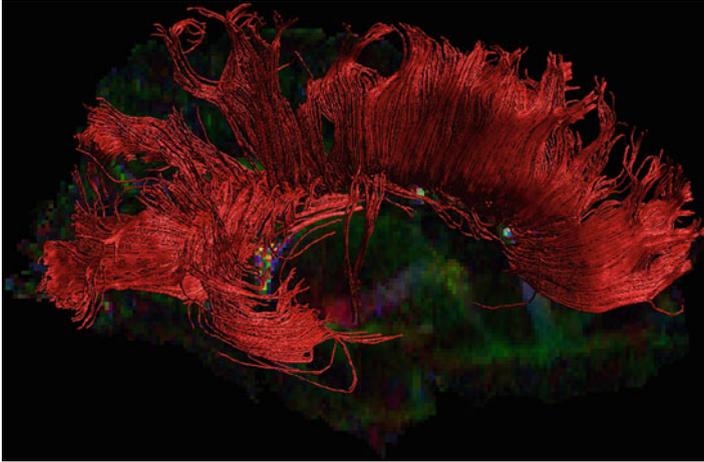


Fig. 2.2 Tractography generated by image analysis of a postmortem brain of a donor with autism shows tracts of the corpus callosum (Image courtesy of Derek Jonest, Cardiff)

Finally, the long-scan times place demands on the stability of the scanner hardware. However, with appropriate alterations to data acquisition and analysis, the virtual reconstruction of tracts is possible. Figure 2.2 shows the corpus callosum of an ASD brain. Connectivity in the ASD brain is a current topic of discussion, and this technology will contribute important information about the integrity of these of inter-hemispheric connections.

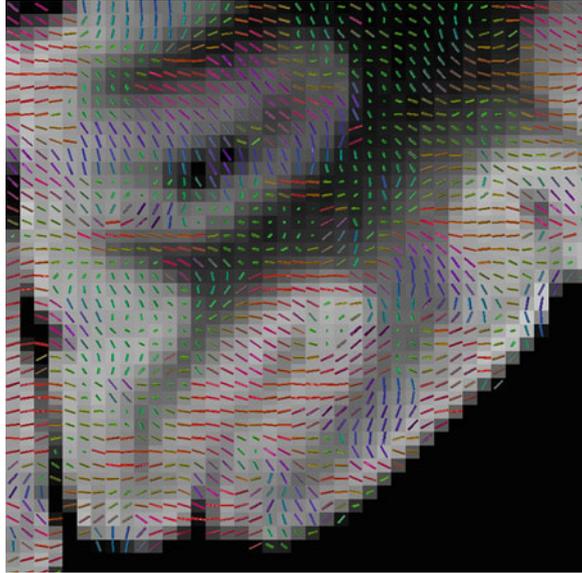
The combination of brain scanning and postmortem tissue dissection enables validation of imaging measurements against the original tissue at the microscopic scale. There is uncertainty about the underlying microanatomical basis of many aspects of the MRI signal, and some of these may be especially relevant to autism research. For example, anisotropy of the diffusion signal in the cerebral cortex (see Fig. 2.3) may relate to the density of cells, myelinated axon bundles, dendrites, and the microscopic minicolumn structure in the cortex.

Summary and New Imaging Frontiers

Postmortem (sometimes called “ex vivo”) scanning means that anatomical images and data can be preserved and shared again and again, even while the actual physical brains are gradually dissected and used by those researching the brain tissue itself. These scans can be reanalyzed as new analysis methods arise in the future.

The promise of diagnostic specificity using an imaging approach validated by histological methods has led to a growing literature where imaging is incorporated into brain banking protocols. It has even been suggested that postmortem imaging can replace autopsies to determine cause of death. This possibility was tested in the UK due to public aversion to autopsies. Roberts et al. (2012) used whole-body CT and MRI followed by full autopsy on 182 cases and compared findings with coroner

Fig. 2.3 Image of diffusion signal in the cortex of postmortem brain (Image courtesy of Karla Miller, Rebecca McKavanagh, and Steven Chance, all of Oxford University)



reports. They concluded that “The error rate when radiologists provided a confident cause of death was similar to that for clinical death certificates, and could therefore be acceptable for medico-legal purposes. However, common causes of sudden death are frequently missed on CT and MRI, and, unless these weaknesses are addressed, systematic errors in mortality statistics would result if imaging were to replace conventional autopsy.”

Imaging of fixed brains should be part of the autopsy process at least as a permanent record of the very rare autism donors and available as an open source when possible. It is unlikely that such a complicated structure can be adequately depicted with imaging alone. On the other hand, new chemical engineering technology called CLARITY (Cross-scale and *Locally*-precise Anatomy, *wi*Ring, and *Immunophenotype* in *Tissue Hydrogel*) might bring the field closer to deconstruction without “disassembly” (Deisseroth 2012).

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