

Respondent's Exhibit I

CORTICAL NEUROPATHOLOGY AND AUTISM

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The following report summarizes research into the neuropathology of autism, including some of my own work. The opinions in this report are based on knowledge I have gained through my education, training and professional experience as a medical doctor and researcher, and are expressed to reasonable degree of scientific probability.

Qualifications

I am a Professor in the Department of Psychiatry and Behavioral Sciences at the University of Louisville, where I hold the Gottfried and Gisela Kolb Endowed Chair in Psychiatry and Associate Chair for Research. I received my M.D. degree at the University of Puerto Rico School of Medicine in 1979, and subsequently went on to become Chief Resident in Neurology at the University District Hospital in Rio Piedras, Puerto Rico. I received further training as a Clinical Fellow in the Neuropathology Clinic at The Johns Hopkins University School of Medicine in Baltimore, Maryland. I was a Major in the Medical Corps for the U.S. Army Reserves from 1984 through 1990, and served as the Director of the Brain Bank Unit in the Neuropathology Laboratory of the Clinical Brain Disorders Branch at the National Institutes of Health from 1987 to 1991. During that same time, I held several positions consulting, lecturing and practicing in the Washington, D.C. area. In 1991, I became a faculty member at the Medical College of

Georgia. I was at that institution until 2003, when I moved to the University of Louisville. I am board certified in neurology, and am a reviewer for numerous scientific journals. I am also a member of several professional societies, and have served on committees including the National Alliance for Autism Research Scientific Advisory Board, the Center for Scientific Review Special Emphasis Panel for the National Institute of Health, and the Tissue Advisory Board of the Autism Tissue Program. My qualifications are further detailed in my *curriculum vitae*.

Introduction

Autism is a neurodevelopmental condition defined by clinical criteria in three primary behavioral domains: communication skills, social interaction and restricted interests and activities (APA 2000). Diagnostic criteria require that symptoms appear within the first three years of life. While some patients receive a diagnosis of autism as early as 18 months of age, many others are not formally diagnosed until age five (Filipek et al. 1999). The broader term “autism spectrum disorder” (ASD) encompasses autism and two other conditions that share core autistic symptoms: Asperger disorder and Pervasive Developmental Disorders-not otherwise specified (PDD-NOS) (CDC 2007). Throughout this report I will interchangeably use the terms “autism” and “ASD” when discussing findings and hypotheses about these three conditions.

Autism often occurs in the presence of other medical conditions such as seizures, mental retardation and chromosomal abnormalities, such as tuberous sclerosis complex (TSC), fragile X, Down, William-Beuren, Angelman/Prader-Willi, velocardiofacial and Möbius syndromes (Filipek 2005). None of these comorbidities invariably accompanies

autism, so the presence or absence of any these conditions will not require or exclude a diagnosis of autism. Nevertheless, as I will discuss throughout my report, our knowledge of how these concomitant conditions develop, as well as our understanding of autistic neuropathology, indicates that autism originates early in the first trimester of gestation.

Three Components in the Development of Autism

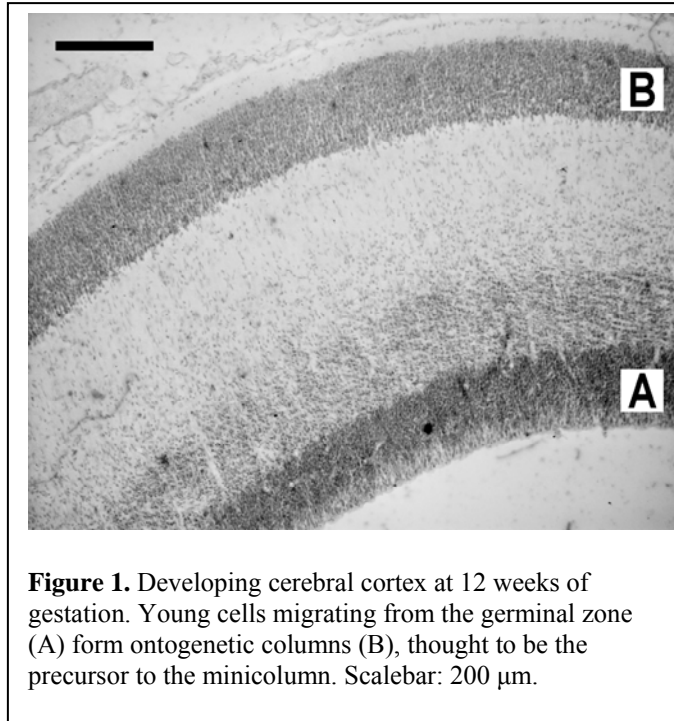
The current clinical consensus views autism as a multifactorial condition involving primarily one organ, the brain. Research suggests that three elements play roles of varying importance in the development of autism. First, and most importantly, several lines of research, including my own, indicate that autism manifests after a developmental insult or error that occurs during a specific critical period for brain development, primarily the first trimester. Second, genetic factors are known to play a significant role in the development of autism. Lastly, exogenous stressors (i.e., anything other than genetics) also may play a role in causing autism. In this regard, studies have investigated the possible role, for example, of *in utero* exposures to valproic acid and thalidomide at very specific times early in gestation as a cause of autism (Strömmland & Miller 1993; Strömmland et al. 1994; Ingram et al. 2000; Moore et al. 2000; Williams et al. 2001; Rodier, 2002; Schneider & Przewłocki 2005). In addition, the first trimester is the time of development for multiple congenital anomalies that may produce autistic manifestations, for example Möbius syndrome, which is possibly caused by genetic abnormalities, vascular deficits, maternal trauma or the use of certain drugs during pregnancy (Towfighi et al. 1979). Thus, both the genetic and prenatal environmental components associated with autism point to the first trimester as the period during which autism originates.

Research into autism has uncovered differing histopathological manifestations (i.e., patterns of tissue damage) in autistic brains and varied clinical expression in autistic patients. These variations suggest that the timing of the underlying insult, which appears to be a constant against the background of different etiologies, may tell us as much as, if not more than, the anatomical loci affected. In other words, when considering the developing brain and its orchestrated sequential maturation of neurons, synapses and cortical maps, “when is as important as what” (Ben-Ari 2006). While findings by others have implicated a possible vulnerability period during the second and third trimesters of gestation, there is a growing body of evidence for vulnerability in the early stages of gestation (Coleman & Betancur 2005).

The Cortex and Mini-columnar Pathology in Autism

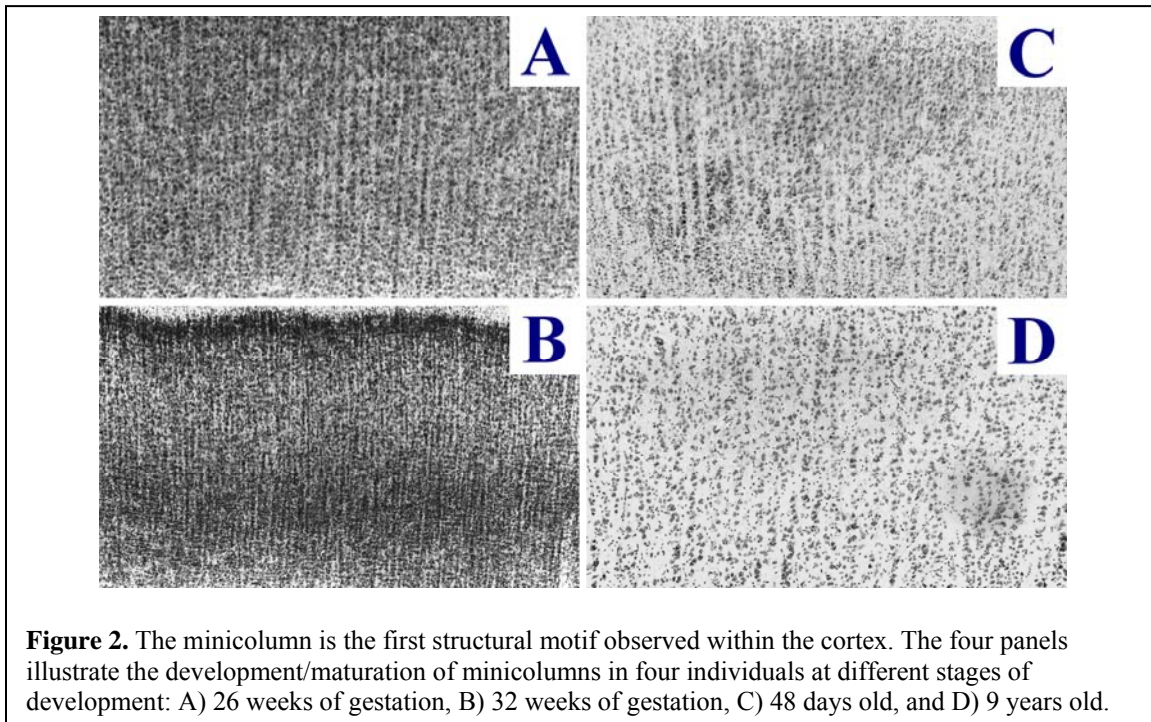
Neurologists traditionally view autism as a disorder of the cortex due to evidence of seizures in a significant proportion of cases and the absence of either spasticity or vision loss. Clinically, the dysfunction of higher cognitive functions (e.g., social behavior and expressive language) pinpoints the putative deficit to the isocortex. More specifically, autism appears to arise from a defect in the modular organization of the cortex that provides for the emergence of cognitive properties.

The cortex consists of a highly complex network of neural connections in which the activity of single neurons or small groups of neurons contributes to larger patterns of activity throughout the cortical network. Maintaining a constant degree of connectedness—the average number of connections per neuron



within a network—means that the number of connections must grow geometrically relative to growth in number of neurons. This growth, as well as selective constraints to minimize the consumption of energy and space, leads to a “small world” network in which neurons maintain short connection length within clusters, which in turn are linked by longer-range projections (Watts & Strogatz 1998; Chklovskii et al. 2002). These constraints influence neuronal networks in the developing isocortex to assemble in the form of the ontogenetic cell column, a radially oriented linear array of pyramidal neurons which extends through multiple layers of the cortical plate (**Figure 1**) (Rakic, 1988; Kriegstein & Noctor 2004). Hypothetically, the migration and development of neurons along a radially oriented framework should maximize the efficient use of brain space and facilitate close-range connections among many neurons.

After 30 weeks gestation in humans, the pervasive columnar organization of the cortical plate is obscured to varying degrees by the migration of glia and interneurons and

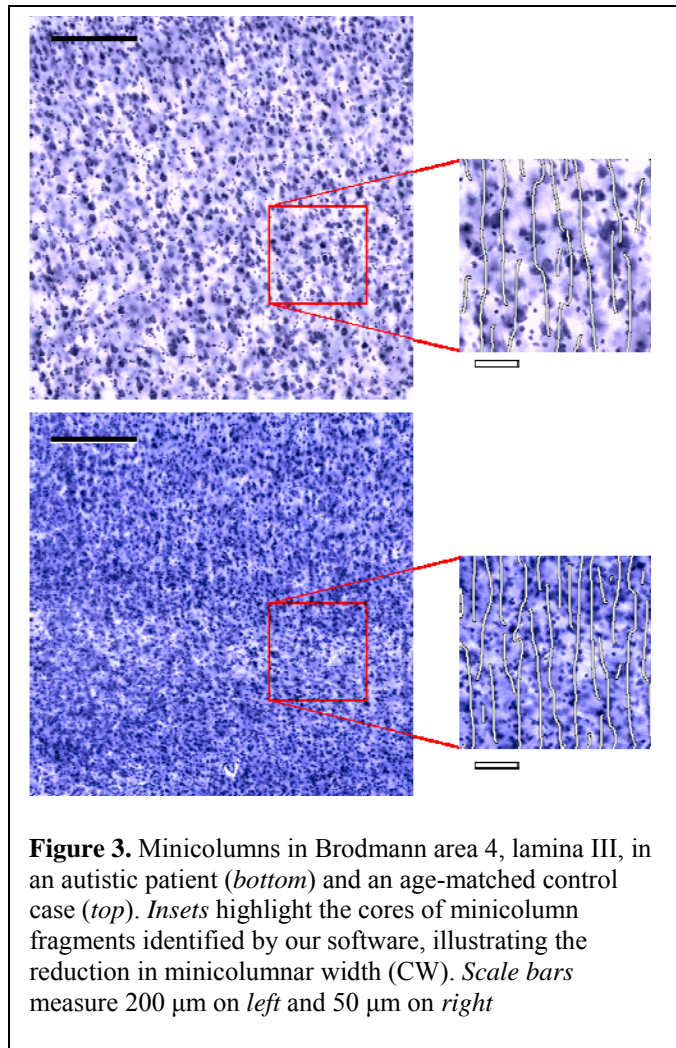


the growth of dendritic and axonal branches. However, the underlying radial organization of these pyramidal cell columns remain intact (**Figure 2**). Imaging studies of post-mortem cortical tissue from individuals of various ages have demonstrated the continuity of columnar morphometry during fetal and postnatal development and throughout the lifespan (Casanova et al. 2006b). As these fundamental radial circuits within the pyramidal cell column core mature and develop their synaptic connections, mini-columns emerge within the original columns to provide further structure to the isocortex.

Four principal cellular features have been studied to assess mini-columnar morphometry and morphology: the core pyramidal cell column, the apical dendritic and vertical myelinated axon bundles arising from the pyramidal cells, and radially oriented translaminar axon bundles of double-bouquet inhibitory cells situated in peripheral neuropil (Von Bonin & Mehler 1971; Seldon, 1981; DeFelipe et al. 1990; Ong & Garey 1990; Viebahn, 1990; Peters & Sethares 1991, 1996, 1997; Ferrer et al. 1992; Del Río & DeFelipe 1997). Morphometric linkages between these four components suggest that they

provide complementary information from which the general structure of mini-columns can be derived (Casanova et al. 2006b).

To date, no pathological entity has been conclusively and systematically identified with autism at the cellular level. However, recent post-mortem studies performed by me and others have shown area-specific changes in the mini-columns of autistic individuals. Specifically, in brains of autistic individuals, minicolumnar width has been found to be significantly narrower, with most of that decrease attributable to reduction of peripheral neuropil space (**Figure 3**). At the same



time, mini-columns appeared to be more numerous within the images of brain tissue that we examined (Casanova, 2006; Casanova et al. 2002a, b, 2006a, b).

What are the functional implications of increased numbers of narrower mini-columns containing smaller projection neurons? These modular microcircuit assemblies are interconnected by thousands of collateral projections within larger networks. Each

mini-column is linked to local networks through myelinated bundles in superficial, or radiate, white matter, and to more distant cortical areas via deeper white matter tracts. Additive increase in mini-column numbers would entail a geometric increase in short- and long-distance projection fibers in order to maintain a constant degree of transcortical connectivity among modules (Hofman, 2001). Longer white matter fibers occupy more space, require disproportionately larger soma to support increased metabolic costs and result in signal processing delays. Selection pressure would therefore be expected to have given rise to modules internally linked by radially oriented processes and integrated into local networks by short collaterals. Proportionately less white matter would be devoted to longer-range connections, encouraging regional functional specialization.

Neuropathological descriptions of decreased cell size and narrow mini-columns (Casanova et al. 2006a), studies revealing increased superficial white matter (Herbert et al. 2004), and functional imaging studies revealing decreases in activity linking prefrontal and posterior areas (Just et al. 2004) support this view. On a functional level, a putative increase in local interconnectivity and reduced long-distance connections in areas subserving cognitive flexibility and prioritizing and emotional and social cognition, such as the prefrontal cortex, is consistent with the clinical picture of stereotypy, rigidity and interpersonal deficits characterizing autism.

The number of mini-columns is established within the first 40 days of gestation, indicating that any environmental influence that might have the capacity to interfere with mini-columnar development would have to act within that timeframe. The findings, as described above, are not consistent with mercury's toxic affects in the brain. An increased number of minicolumns can explain the larger than normal brains of autistic

individuals. In contrast, mercury intoxication causes brains to be smaller (atrophic) with corresponding cell loss in certain areas of vulnerability, e.g., granule cell layer, depths of sulci, and calcarine cortex (Graham & Montine 2002; Nelson & Bauman 2003). In autism we have not found this selective vulnerability (Casanova et al. 2006b) and instead of cell death we have reported increased neuronal density (Casanova et al. 2006a).

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