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Two-dimensional analysis of the supragranular layers in autism spectrum disorder

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ABSTRACT

Neurons in the supragranular layers of the human cerebral cortex play an important role in long-range cortico-cortical connections. Alterations to these layers are of special interest in autism spectrum disorder (ASD) as they could play a significant role in altered connectivity between distal regions of cortex. The present study isolated sampling boxes through the use of an automated boundary identification technique. A two-dimensional analysis of the Nissl-stained tissue was then performed to examine whether differences in cell size and number are present in ASD tissue. The analysis focused on layers II and III of association cortex sampled from frontal (BA9), temporal (BA21), and parietal (BA7) regions. In previous studies, both BA9 and BA21 have been linked to alterations in cortical connectivity in ASD. Aside from the expected differences between cortical layers and regions, data analysis revealed that ASD tissue possessed a higher density of cells, the magnitude of which was layer dependent, and that the cell profiles were of a smaller size. The results of this study suggest that cellular abnormalities with respect to cell size and number are present in multiple areas of association cortex, specifically within layers that are involved in long-range connectivity. Additionally, the results comport with previous findings of altered cortical minicolumns in frontal and temporal areas and further suggest that similar irregularities may also be present in parietal areas.

1. Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disability that is defined by impaired social communication and social interaction as well as the presence of restricted, repetitive patterns of behavior, interests, or activities (American Psychiatric Association, 2013). Although the diagnosis of ASD relies on observable behavioral characteristics, it is regarded as having a substantial genetic component (see Geschwind, 2011 for a review) and can be marked by wide-spread neuroanatomical abnormalities within the brainstem, limbic system, and cerebellum, as well as regions of the cerebral cortex (see Bauman & Kemper, 2005; Hutsler & Casanova, 2015; Palmen, van Engeland, Hof, & Schmitz, 2004 for reviews). Across the population as a whole, neuroanatomical, as well as behavioral, characteristics of the disorder are notably quite variable (Amaral, Schumann, & Nordahl, 2008), with many pathological features present in only a subset of the cases thus far examined (Bailey et al., 1998; Tager-Flusberg, 2006; Willemsen-Swinkels & Buitelaar, 2002).

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Postmortem analysis of the cerebral cortex in ASD has identified the presence of several markers of atypical organization. Some of these observations include an increased number of cortical minicolumns that possess a greater dispersion of neurons (Casanova, Buxhoeveden, Switala, & Roy, 2002), decreased columnar width (Buxhoeveden et al., 2006; Casanova et al., 2006; Casanova, El-Baz, Vanbogaert, Narahari, & Switala, 2010), increases in cortical thickness, irregular orientation of cells (Bailey et al., 1998), an ill-defined gray to white matter transition (Avino & Hutsler, 2010), and the presence of heterotopias (Hutsler, Love, & Zhang, 2007; reviewed in Casanova, 2014).

In addition to the aforementioned abnormal cortical structure, both short- and long-range connectivity differences have been noted in ASD. Long-range connectional changes in ASD include frontal-posterior abnormalities (Just, Keller, Malave, Kana, & Varma, 2012). Functional data has demonstrated that decreased synchronization between frontal and posterior regions are present in individuals with ASD as compared to controls when performing tasks that require executive functions (Just, Cherkassky, Keller, Kana, & Minshew, 2007), perceptual processing (Damarla et al., 2010), sentence comprehension (Just, Cherkassky, Keller, & Minshew, 2004) and attributing mental states to others (Kana, Keller, Cherkassky, Minshew, & Just, 2009). Structural data have suggested that the transfer of interhemispheric information may also be compromised by demonstrating that the corpus callosum in individuals with ASD is smaller than in neurotypical controls (Alexander et al., 2007; Freitag et al., 2009; Hardan, Minshew, & Keshavan, 2000; Just et al., 2007). Finally, increases in synaptic spines on superficial layer pyramidal cells found within association cortices also suggest connectional alterations that likely influence corticocortical connectivity (Hutsler & Zhang, 2010).

Additional microanatomical alterations supporting connectivity differences have also been found within the cortex. One potential marker of connectional change is soma size. While many studies have found no differences in cell size within regions such as the dorsolateral mesial prefrontal cortex (Courchesne et al., 2011), superior temporal gyrus (Kim et al., 2015), subregions of the anterior cingulate cortex (ACC; Simms, Kemper, Timbie, Bauman, & Blatt, 2009), and amygdala (Schumann & Amaral, 2006), other studies have reported smaller pyramidal neurons in the inferior frontal cortex (Jacot-Descombes et al., 2012). In addition, smaller neurons of varying types have also been reported in the fusiform gyrus (van Kooten et al., 2008), hippocampus (Raymond, Bauman, & Kemper, 1996), cerebellum (Fatemi et al., 2002), and portions of the ACC (Kemper & Bauman, 1998; Simms et al., 2009). Reasons for these divergent results regarding neuronal cell sizes could be the result of the different regions examined, differing subject characteristics such as age, and the distinct developmental trajectories associated with cell size in different regions of the brain (Wegiel et al., 2014). Although the cause of smaller cell size is not fully understood, it likely reflects altered functional integrity of the cell. This could be due to pathological processes to these regions themselves, decreased neuronal efferent targets, altered cortical development, reduced complexity of dendritic arborization and/or axonal length, or any combination of these factors (Hayes & Lewis, 1993; Jacobs, Driscoll, & Schall, 1997; Lund, Lund, Hendrickson, Bunt, & Fuchs, 1975; van Kooten et al., 2008). Further, smaller cell size has been documented in regions that overlap with identified minicolumnar pathologies in ASD within inferior frontal regions (Brodmanns Area [BA] 44; Casanova et al., 2010; Jacot-Descombes et al., 2012) as well as primary motor (BA4), sensory (S1), visual (BA17), and frontal association cortices (BA9) (Casanova et al., 2006). In contrast, minicolumnar pathology has also been identified in BA21 and posterior BA22 (Casanova et al., 2002) and one study found no differences with respect to cell volume in the rostral segment of BA22 (Kim et al., 2015). This dissociation could, however, be due to the subregions within BA22 that were examined. Posterior BA22 regions were taken from the temporoparietal auditory cortex (Tpt), whereas anterior BA22 locations examined were from the transition that occurs between temporopolar area (TG) and BA22 to the dorso-caudal boundary between BA22 and the magnocellular supratemporal area simplex (TB/BA41). Cytoarchitectonically, the caudal and rostral portions of BA22 are distinct (von Economo & Koskinas, 1925), suggesting that the two regions are functionally different from one another. More research is needed to investigate whether BA21 and posterior regions of BA22, regions in which minicolumnar pathology has been identified (Casanova et al., 2002), possess alterations to cell size as well.

The present study seeks to further assess the neuronal patterning of the supragranular layers (layers II and III) of association cortex from frontal (BA9), temporal (BA21), and parietal (BA7) regions in ASD. Layers II and III were of particular interest as pyramidal cells that lie within these layers engage in long-range corticocortical, callosal, and corticoamygdaloid projections (Jones, 1984; Mufson, Mesulam, & Pandya, 1981). Further, morphological abnormalities have been found in these layers in similar regions in several previous studies (Casanova et al., 2002, 2006, 2010; Hutsler & Zhang, 2010). The present study utilized automatically-placed sampling frames and two-dimensional analysis to document cell profile size and density over relatively large expanses of cortex. It was predicted that if the presence of minicolumnar pathology is associated with a reduction in cell size, as both are found in BA44 and BA9 of ASD tissue, then decreased cell size would be observed in BA21 as well, since minicolumnar pathology has also been documented in this temporal region (Casanova et al., 2002, 2006, 2010; Jacot-Descombes et al., 2012). Additionally, it was predicted that cell density would be greater in BA9 for the ASD cases, as increased cell densities have been previously documented in this frontal region (Casanova et al., 2006; Courchesne et al., 2011). Further, it was predicted that cell size and cell density differences would emerge with respect to both layer and cortical region.

Several outcomes from this semi-automated approach can be checked against well-documented differences between both layers and cortical regions to confirm the performance of this methodological approach. Based upon previous reports, these predictions include: (1) smaller cell sizes, and higher cell densities, in layer II relative to layer III; (2) increased cell densities in layer II of the superior parietal area, relative to layer II of the middle temporal gyrus and dorsolateral prefrontal cortex; and (3) decreased cell densities in layer III of the dorsolateral prefrontal cortex relative to both the superior parietal cortex and the middle temporal gyrus (von Economo & Koskinas, 1925).