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Research Report

Abnormal microglial–neuronal spatial organization in the dorsolateral prefrontal cortex in autism

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ABSTRACT

Microglial activation and alterations in neuron number have been reported in autism. However, it is unknown whether microglial activation in the disorder includes a neuron-directed microglial response that might reflect neuronal dysfunction, or instead indicates a non-directed, pro-activation brain environment. To address this question, we examined microglial and neuronal organization in the dorsolateral prefrontal cortex, a region of pronounced early brain overgrowth in autism, via spatial pattern analysis of 13 male postmortem autism subjects and 9 controls. We report that microglia are more frequently present near neurons in the autism cases at a distance interval of 25 μm, as well as 75 and 100 μm. Many interactions are observed between near-distance microglia and neurons that appear to involve encirclement of the neurons by microglial processes. Analysis of a young subject subgroup preliminarily suggests that this alteration may be present from an early age in autism. We additionally observed that neuron–neuron clustering, although normal in cases with autism as a whole, increases with advancing age in autism, suggesting a gradual loss of normal neuronal organization in the disorder. Microglia–microglia organization is normal in autism at all ages, indicating that aberrantly close microglia–neuron association in the disorder is not a result of altered microglial distribution. Our findings confirm that at least some microglial activation in the dorsolateral prefrontal cortex in autism is associated with a neuron-specific reaction, and suggest that neuronal organization may degrade later in life in the disorder.

1. Introduction

The alterations in cellular microstructure that underlie macrostructural and functional abnormalities in autism are only beginning to be characterized. Surprisingly, despite the presence of regionally focused early brain overgrowth in some subjects with autism (Carper et al., 2002; Courchesne et al., 2001; Dawson et al., 2007; Dementieva et al., 2005;

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Dissanayake et al., 2006; Hazlett et al., 2005; Redcay and Courchesne, 2005; Schumann et al., 2010; Sparks et al., 2002; Webb et al., 2007), two groups have reported reductions in neuron number in groups comprised primarily of adolescent and adult subjects (Schumann and Amaral, 2006; van Kooten et al., 2008). Abnormalities in neuron organization have been qualitatively reported in a variety of brain regions, but limited subject availability has led to significant variability in the nature and location of the reported alterations (Bailey et al., 1998; Bauman, 1996; Bauman and Kemper, 1994; Kemper and Bauman, 1998). Additionally, a loss of neuropil volume, the space between neurons which is largely composed of neuronal connective elements, has been suggested by minicolumn analyses showing reduced column width, suggestive of possible axonal and dendritic degeneration (Buxhoeveden et al., 2006; Casanova et al., 2002a; Casanova et al., 2006). Despite these prior findings, it has yet to be determined whether there are quantifiable alterations in neuronal organization in the cortex in autism.

In addition to neuronal alterations, changes in microglial morphology and population density that are indicative of activation in autism have been observed (Morgan et al., 2010; Vargas et al., 2005). Normally functioning microglia are known to be intimately involved in supporting, functionally regulating, and if necessary removing aberrantly functioning neurons (Bessis et al., 2005; Christopherson et al., 2005; Ransohoff and Perry, 2009). Therefore, one possibility is that microglial activation in autism reflects or results in a response to abnormally functioning neurons, whether protective, deleterious, or an attempt to correct abnormal neuronal functioning, perhaps due to altered neuronal connectivity. However, it might instead be the case that the reported alterations reflect alterations in the innate immune system or blood–brain barrier, or genetic immune abnormalities, that produce moderate microglial activation without a neuron-directed response.

To address these questions regarding microglial and neuronal organization, we recorded the cellular co-ordinates of both neurons and ionized calcium-binding adapter molecule-1 (iba-1) positive microglia in the dorsolateral prefrontal cortex (dlPFC), a region of pronounced early macrostructural overgrowth in autism (Carper and Courchesne, 2005). The organization of both neuronal and microglial populations, as well as their interaction, was analyzed in a density-independent, cortical layer-corrected fashion via spatial pattern analysis using the k-function, a technique that has previously been adapted to assess alterations in cellular organization in human postmortem tissue in several

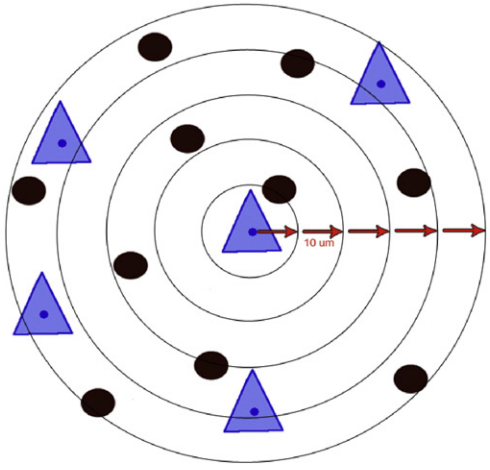

Fig. 1 – Spatial pattern analysis methodology. The number of cells of the population of interest is assessed at distance increments from each analyzed cell. In this example, neurons (blue triangles) and microglia (brown circles) are assessed at distance intervals of 10 μm from a neuron. The population number at each distance interval is compared to 200 simulations in which cell location is randomized by layer in order to produce a density-corrected spatial clustering ratio. Ratios greater than 1 indicate a greater presence of the population of interest (clustering) than would be expected in the spatially randomized condition, while ratios below 1 indicate anti-clustering.

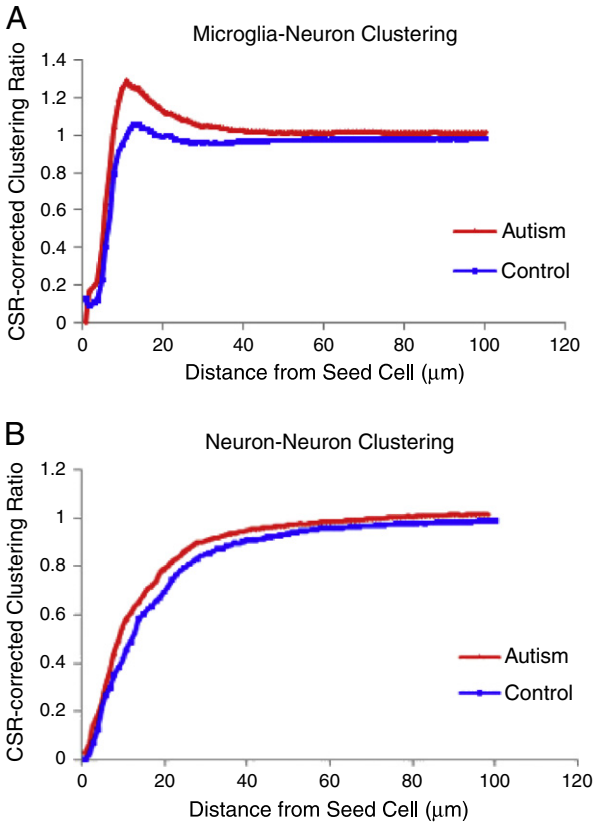

Fig. 2 – Features of cellular spatial clustering over the range of analysis. A. Microglia–neuron interaction is significantly increased in autism at 25 μm ($p = .006$), 75 μm ($p = .005$), and 100 μm ($p = .005$). The profile is marked by a spike in spatial clustering in the 10–30 μm range that is more pronounced in the autism group. B. Neuron–neuron spatial clustering is not significantly different at any distance interval, and lacks prominent features across the distance range in either diagnostic group. Abbreviations: CSR, Complete Spatial Randomness.