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EFFECTS OF DEVELOPMENTAL HYPERSEROTONEMIA ON THE MORPHOLOGY OF RAT DENTATE NUCLEAR NEURONS

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Abstract—Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social cognition, disordered communication, restricted interests and repetitive behaviors. Furthermore, abnormalities in basic motor control, skilled motor gestures, and motor learning, are common in ASD. These characteristics have been attributed to a possible defect in the pre- and postnatal development of specific neural networks including the dentate-thalamo-cortical pathway, which is involved in motor learning, automaticity of movements, and higher cognitive functions. The current study utilized custom diolistic labeling and unbiased stereology to characterize morphological alterations in neurons of the dentate nucleus of the cerebellum in developing rat pups exposed to abnormally high levels of the serotonergic agonist 5-methyloxytryptamine (5-MT) pre-and postnatally. Occurring in as many as 30% of autistic subjects, developmental hyperserotonemia (DHS) is the most consistent neurochemical finding reported in autism and has been implicated in the pathophysiology of ASD. This exposure produced dramatic changes in dendritic architecture and synaptic features. We observed changes in the dendritic branching morphology which did not lead to significant differences ($p > 0.5$) in total dendritic length. Instead, DHS groups presented with dendritic trees that display changes in arborescence, that appear to be short reaching with elaborately branched segments, presenting with significantly fewer ($p > 0.001$) dendritic spines and a decrease in numeric density when compared to age-matched controls. These negative changes may be implicated in the neuropathological and functional/behavioral changes observed in ASD, such as delays in motor learning, difficulties in automaticity of movements, and deficits in higher cognitive functions.

Key words: Autism Spectrum Disorder, hyperserotonemia, dentate nucleus, stereology, diolistic labeling, neuronal morphology.

INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social cognition, disordered communication, restricted interests and repetitive behaviors (Association, 2000). Furthermore, abnormalities in basic motor control (Vilensky et al., 1981; Hughes, 1996; Ghaziuddin and Butler, 1998; Teitelbaum et al., 1998; Noterdaeme et al., 2002), skilled motor gestures (DeMeyer et al., 1972; Williams et al., 1980; Jones and Prior, 1985; Ohta et al., 1987; Smith and Bryson, 1994; Rogers et al., 1996; Mostofsky et al., 2006), and motor learning (Mostofsky et al., 2000; Rinehart et al., 2001), are common in ASD and have been attributed to a possible defect in the pre- and postnatal development of specific neural networks including a fronto-cerebello-thalamo-frontal pathway (Mostofsky et al., 2000; Coutinho et al., 2007).

The most consistent neurochemical finding reported in ASD is an observed 40–70% increase in platelet serotonin (5-hydroxytriptamine, 5-HT) (Veenstra-VanderWeele et al., 2002; Janusonis, 2005; Ramoz et al., 2006; Coutinho et al., 2007; McNamara et al., 2008; Azmitia et al., 2011), occurring in as many as 30% of autistic subjects and possibly implicated in the pathophysiology of ASD (Azmitia et al., 2011). Serotonin plays an active role in brain development, by promoting dendritic elaboration (Faber and Haring, 1999; Mazer et al., 1997; Yan et al., 1997; Kondoh et al., 2004), synaptogenesis (Okado et al., 1993), neurogenesis (Lauder et al., 1981, 1983), cortical organization (Bennett-Clarke et al., 1994; Cases et al., 1996; Janusonis et al., 2004) and autoregulation of the serotonergic system (Whitaker-Azmitia, 2001). Accordingly, it has been hypothesized that during the early stages of neural development, preceding the maturation of the blood–brain barrier, the diffusion of abnormally increased amounts of serotonin into the central nervous system, could induce developmental abnormalities in various neural networks relevant to autistic behaviors (Whitaker-Azmitia, 2001).

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Abbreviations: 5-HT, 5-hydroxytriptamine; 5-MT, 5-methyloxytryptamine; AI, anterior interposed nuclei; ANOVA, analysis of variance between groups; ASD, Autism Spectrum Disorder; CE, coefficient of error; DHS, developmental hyperserotonemia; GD, gestational day; h, hours; HPLC, High-Performance Liquid Chromatography; LN, lateral nucleus of the cerebellum; MBF, MicroBrightField; min, minutes; ms, milliseconds; PBS, phosphate-buffered saline; PCPA, para-chlorophenylalanine; PET, positron emission tomography; PND, postnatal day; PVP, polyvinylpyrrolidone; s, seconds; S-100β, S100 calcium binding protein B.

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To test this hypothesis, an animal model of developmental hyperserotonemia (DHS) was implemented by mimicking an increase in 5-HT in the peripheral blood through the subcutaneous administration of the serotonin agonist 5-methyloxytryptamine (5-MT) to dams and by extension to their developing rat pups (from gestational day (GD) 12 to postnatal day (PND) 20) (Winslow and Insel, 1990a,b; Shemer et al., 1991; Whitaker-Azmitia et al., 1996; Whitaker-Azmitia, 2001; Kahne et al., 2002; McNamara et al., 2008). The resulting increase in blood serotonin and its hypothetical accumulation in the developing pup brain are thought to perturb serotonergic autoregulatory mechanisms through binding of exogenous serotonin to 5-HT1A autoreceptors, which may alter normal mechanisms of brain development and ultimately lead to an inhibition in the outgrowth of serotonergic nerve fiber terminals (Kahne et al., 2002). Such a decrease in serotonergic terminals could potentially affect various serotonin-dependent mechanisms of brain maturation. Parenthetically, positron emission tomography (PET) studies in ASD patients have reported a decrease in serotonin synthesis in cortical and subcortical structures, which may correlate to altered serotonergic autoregulatory mechanisms during development (Muller et al., 1998; Chugani et al., 1999; Sundaram et al., 2008).

Previous studies with the rat hyperserotonemia model have focused on cellular changes in limbic regions and yielded data which support the hypothesis of serotonin driven developmental defects (Haring et al., 1991; Bennett-Clarke et al., 1994; Durham and Russo, 1998; Kahne et al., 2002; McNamara et al., 2008; Winslow and Insel, 1990b; Shemer et al., 1991; Whitaker-Azmitia et al., 1996; Whitaker-Azmitia, 2001). For example, in the amygdala, hyperserotonemia has been correlated with an increase in calcitonin-gene related peptide, which is normally suppressed by serotonin, thus suggesting a loss of serotonin terminals in this area through a putative negative feedback mechanism (Whitaker-Azmitia, 2001; McNamara et al., 2008). Furthermore, the stunting or absence of visible processes in serotonergic neurons in the raphe nucleus has been identified, although no obvious quantitative differences in neuronal numbers were reported (Mazer et al., 1997; Whitaker-Azmitia, 2001). Given these putative toxic effects of hyperserotonemia on the development of limbic neurons, it is conceivable that other neural systems could also be vulnerable. One such network involves the regulatory mechanisms of the motor system.

A neuronal network, which has been implicated in the abnormal motor behaviors characteristic of ASD (delays in motor learning and language acquisition) is the dentate-thalamo-cortical pathway, which is involved in motor learning, automaticity of movements, and higher cognitive functions (Chugani et al., 1999). Parenthetically, in serotonin receptor PET studies conducted in autistic populations, this network was shown to express decreased serotonin synthesis thus suggesting that serotonin mechanisms may account for the delays in motor learning and language acquisition found in these patients (Chugani et al., 1999). Furthermore, in brains of patients with autism examined post-mortem, the dentate nucleus was found to exhibit abnormal features, such as cerebellar heterotopia, and dysplasia, suggesting that the developmental mechanisms of neuronal migration and neuronal maturation are defective (Wegiel et al., 2010).

Although these findings suggest a hyperserotonemia-driven underpinning for brain developmental defects that may lead to abnormal motor behaviors in autism, there has been little research on this topic and a strong body of evidence in support of this hypothesis is still missing. The normal development and neuronal morphology of the rat cerebellar nuclei and efferent pathways is well characterized although the effects of exposure to increased levels of serotonin are not entirely known.

Our overarching hypothesis posits that during brain development, serotonin, which has a marked role as a developmental signaling molecule, can influence the resulting neuronal morphology in specific cell populations, which include the cerebellum. In particular, the experiments presented here test the hypothesis that DHS inhibits the elaboration of dendritic processes and dendritic spines characteristic of mature cerebellar dentate/lateral nucleus[†] (LN) neurons. This study provides new information relative to one of the possible putative mechanisms and known behavioral expressions of ASD.

EXPERIMENTAL PROCEDURES

To test the proposed hypotheses we modified the experimental protocol described by Whitaker-Azmitia (2001) for induced DHS. The procedure utilized in this study will be described in detail below. All procedures are in accordance with Animal Care and Use Committee guidelines and approved by the Saint Louis University Animal Care and Use Committee.

Animals and experimental design

Six timed-pregnant Sprague–Dawley rats were obtained from Charles River Laboratories (Wilmington, MA) and housed in the Saint Louis University Medical School vivarium under the care of the Department of Comparative Medicine. Animals were housed separately under identical conditions in light (on from 0800–2000 h) and temperature (21–24 °C)-controlled rooms, with food and water available *ad libitum*. Dams remained housed individually and with their subsequent pup litters until PND 20. Tissue collection was conducted from both experimental and control populations on PND 5, PND 10, and PND 20. The PND 5 and PND 10 samples were for exploratory purposes only and thus will only be described briefly in this report. Determination and confirmation of pregnancy was performed by Charles River Laboratories by the observation of a visible vaginal plug. The date of vaginal plug observation was considered to be day zero of gestation (GD 0), and was used for the subsequent experimental timeline.

[†] Though classical form only uses the term ‘‘lateral nucleus of the cerebellum” when referring to the distinct cellular population in the rat, and ‘‘dentate nucleus” when referring to primates, more contemporary literature uses the terms interchangeably (LN) as they will be in this report.