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ORGANIZATION OF THE HUMAN SUPERIOR OLIVARY COMPLEX IN 15Q DUPLICATION SYNDROMES AND AUTISM SPECTRUM DISORDERS

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Abstract—Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by a number of behavioral and social features. Although the etiology of most cases of ASD is idiopathic, a significant number of cases can be attributed to genetic causes, such as chromosome 15q duplications [dup(15q)]. Recent neuropathological investigations have provided evidence for distinct patterns of heterotopias and dysplasias in ASD and subjects with both ASD and dup(15q). Individuals with ASD characteristically have hearing difficulties and we have previously demonstrated significant and consistent hypoplasia in a number of auditory brainstem nuclei in subjects with ASD. Herein, we compare results from a morphometric investigation of auditory brainstem nuclei in subjects with ASD, dup(15q) and controls. Our observations in subjects with ASD support our previous reports. However, in subjects with dup(15q), we find significantly fewer neurons and in many nuclei, neurons were significantly smaller than in ASD subjects. Finally, we find a notably higher incidence of ectopic neurons in dup(15q). These results suggest that in the brainstem, these neuropathological conditions may evolve from some of the same developmental errors but are distinguished on microscopic features.

Key words: brainstem, ectopic, auditory.

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by difficulties with communication and social interactions, restricted, repetitive behaviors and sensory abnormalities (Allen, 1988; Wing, 1997; American Psychiatric Association, 2013). The vast majority of individuals with ASD have some degree of auditory dysfunction (Greenspan and Wieder, 1997; Tomchek and Dunn; 2007; Gomes et al., 2008; Bolton et al., 2012). The degree to which hearing is affected in ASD varies by subject but ranges from deafness to hyperacusis and includes difficulty listening in noisy environments (Rosenhall et al., 1999; Roper et al., 2003; Alcántara et al., 2004; Khalfa et al., 2004; Szelag et al., 2004; Kellerman et al., 2005; Lepistö et al., 2005; Teder-Sälejärvi et al., 2005; Gravel et al., 2006; Tharpe et al., 2006; Russo et al., 2009). Testing of auditory brainstem responses and the stapedial reflex in subjects with ASD provide evidence implicating dysfunction of lower auditory brainstem neurons (Skoff et al., 1980; Gillberg et al., 1983; Rumsey et al., 1984; McClelland et al., 1992; Klin, 1993; Maziade et al., 2000; Rosenhall et al., 2003; Kwon et al., 2007; Roth et al., 2012; Lukose et al., 2013). Accordingly, there is evidence of consistent and severe hypoplasia in the superior olivary complex (SOC), an essential component of the auditory pathway, in subjects with ASD (Rodier et al., 1996; Kulesza and Mangunay, 2008; Kulesza et al., 2011).

Presently, the majority of ASD cases are idiopathic, although up to 20% are attributed to genetic disorders (e.g. Fragile X syndrome), genetic mutations, or chromosomal copy number variations, such as chromosome 15q duplication (Gillberg and Coleman, 1996; Sebat et al., 2007; Boddaert et al., 2009; Pinto et al., 2010). The incidence of chromosome 15q duplication syndrome [dup(15q)] is quite low; isodicentric chromosome 15 duplications affect only about 1 in 30,000 (Schinzel and Niedrist, 2001; Battaglia, 2008). This syndrome refers specifically to duplications of the 15q11–13 region where maternally derived duplications are associated with developmental problems (Martinsson et al., 1996; Browne et al., 1997; Cook et al., 1997; Schroer et al., 1998; Repetto et al., 1998; Mao et al., 2000; Bolton et al., 2004; Roberts et al., 2002; Battaglia et al., 2010). Approximately 1–3% of ASD cases are associated with chromosome 15 abnormalities and duplications in the 15q region are of the most common chromosomal duplications associated with ASD (Cook et al. 1998; Schroer et al., 1998; Bolton et al., 2004; Battaglia, 2005; Vorstman et al., 2006; Abrahams and Geschwind, 2008; Depienne et al., 2009; Moreno-De-Luca et al., 2013). In fact, ASD is diagnosed in nearly 70% of subjects with maternal 15q11.2–q13 duplications (Rineer et al., 1998; Kent et al., 1999; Borgatti et al., 2001). Patients with

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Abbreviations: ASD, autism spectrum disorder; CN, cochlear nucleus; dup(15q), chromosome 15q duplication syndrome; FN, facial nucleus; LNTB, lateral nucleus of the trapezoid body; LSO, lateral superior olive; MNTB, medial nucleus of the trapezoid body; MSO, medial superior olive; SOC, superior olivary complex; SPON, superior paraolivary nucleus; tz, trapezoid body; VNTB, ventral nucleus of the trapezoid body.

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dup(15q) characteristically display hypotonia, motor and cognitive delays, autistic behaviors and seizures (Battaglia, 2008; Park et al., 2013; Urraca et al., 2013). Furthermore, social, communicative and behavioral features are similarly affected in subjects with ASD and combined dup(15q) and ASD (Wisniewski et al., 1979; Gillberg et al., 1991; Rineer et al., 1998; Wolpert et al., 2000). Finally, deafness and hearing loss have been reported in subjects with dup(15q) syndrome (Bingham et al. 1996; Simic and Turk, 2004).

Throughout the brain of subjects with ASD, there is evidence for dysregulated neurogenesis, stunted neuronal maturation, including aberrant neuronal migration (ectopic neurons) and alterations in cell body size, number and dendritic morphology (Bauman and Kemper, 1985; Ritvo et al., 1986; Gaffney et al., 1988; Arin et al., 1991; Piven et al., 1992; Hashimoto et al., 1993; Raymond et al., 1996; Palmen et al., 2004; Schumann and Amaral, 2006; Kulesza and Mangunay, 2008; Whitney et al., 2008; Weigiel et al., 2010; Kulesza et al., 2011; Stoner et al., 2014; Wegiel et al., 2014). Brains from subjects with dup(15q) syndrome reportedly weigh less than brains from both ASD and control subjects and microcephaly is more commonly found in dup(15q) subjects (Wegiel et al., 2012b). Additionally, there are more heterotopias and significantly more dysplasias in the hippocampal region of dup(15q) subjects compared to ASD alone and subjects with both dup(15q) and ASD have a much higher incidence of epilepsy/seizures compared to controls (Battaglia et al., 1997; Schinzel and Niedrist, 2001; Dennis et al., 2006; Wegiel et al., 2012b). Based on these observations, it has been proposed that subjects with dup(15q) syndrome can be distinguished from subjects with ASD based on neuropathological observations (i.e. the number of developmental abnormalities and early onset Aβ plaques; Wegiel et al., 2012a,b; Frackowiak et al., 2013). In conjunction with our previous studies of the auditory brainstem in ASD (Kulesza and Mangunay, 2008; Kulesza et al., 2011), we hypothesize that the SOC nuclei are differentially affected in these two neurodevelopmental disorders [ASD and dup(15q)]. Herein, we investigate this hypothesis through a quantitative analysis of neuronal number and cell body morphology in the auditory brainstem of subjects with dup(15q) and compared these observations to ASD and control subjects.

EXPERIMENTAL PROCEDURES

Subjects

The tissue used in this study was obtained through the Autism Tissue Program (http://www.autismtissueprogram.org). All brain specimens were donated to the program by the subjects' family and all clinical diagnoses were obtained from the subjects' medical records and/or family surveys. All identifying information was removed from the records and LECOM's IRB granted exempt status for all procedures. All brainstems used in this study were preserved in 10% buffered formalin for at least 6 weeks, sectioned into 30-mm blocks, dehydrated in a series of ascending alcohols, embedded in polyethylene glycol (PEG), cut at a thickness of 50 μm, mounted onto glass slides and stained for Nissl substance with Cresyl Violet (for further details see Wegiel et al., 2012a,b). The distribution of amyloid-β was previously described in the forebrain and cerebellum of nine of the dup(15q) subjects (Wegiel et al., 2012a). Nine of the 12 dup(15q) subjects used in this study were characterized by genotyping as previously described (Wegiel et al., 2012a; Frackowiak et al., 2013).

Data collection

In all subjects studied, the nuclei of the SOC were identified within the tegmentum of the rostral medulla and caudal pons, anterior and medial to the facial nucleus (FN), lateral to axon bundles of the abducens nerve and medial lemniscus and posterior to the pontine nuclei (Kulesza, 2007, 2008; Kulesza et al., 2011). In subjects with ASD or dup(15q) syndrome, neurons of the SOC were arranged in topographically similar patterns as previously described for control subjects (Kulesza, 2007, 2008, 2014; Kulesza et al., 2011). Thus, each nucleus was identified based on location relative to brainstem nuclei, axon bundles (facial nerve, trapezoid body (tz)) and other SOC nuclei. Because the nuclei of the trapezoid body are nearly contiguous and subjects with ASD or dup(15q) syndrome exhibited dysmorphology (Kulesza and Mangunay, 2008; Kulesza et al., 2011), it is possible that a very small percentage (~1%) of these neurons were misclassified. Occasionally, large pools of neurons were found within or near the SOC, but outside of observed nuclear boundaries – such neurons were considered ectopic (see later).

All tissue sections were examined with an Olympus BX45 microscope and photographed with an Olympus DP12 digital camera. Data on cell body morphology were collected from all specimens used in this study (Table 2). For analysis of cell body morphology, tissue sections were randomly selected throughout the rostrocaudal extent of each nucleus. Cell bodies that were completely within the thickness of the tissue section and had a visible nucleolus were traced with the aid of a camera lucida attachment (Olympus; using a 40× objective with a final on paper magnification of 675×). Tracings were digitized into jpeg format using a flatbed scanner and these digitized tracings were imported into ImageJ (calibrated to a standard scale bar; available at http://rsb.info.nih.gov/ij/). Using the "Analyze" feature, measurements were made of cell body area, perimeter, long and short axes, circularity and orientation of the long axis ("angle"). For each soma, an index of circularity was calculated as follows:

$$\text{Circularity} = [4\pi * \text{Area}/\text{Perimeter}^2]$$

The products of this equation range from 0 to 1 and provide an estimate of cell body shape independent of size (Yin et al., 1990). A perfectly circular contour will have a value of "1" while a triangular contour will have a value ~0.5. Angle measurements for neurons in the medial superior olive (MSO) were made relative to the anatomical midline (i.e. the raphe). Neurons with a long axis parallel to the midline (i.e. vertical) will have an angle