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Striatal volume changes in a rat model of childhood attention-deficit/hyperactivity disorder

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ABSTRACT

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common childhood neuropsychiatric disorders. Based on neuroimaging studies, the striatum is reported to be abnormal in size, but it is still not clear how they change during developmental stages. Spontaneously hypertensive rats (SHRs) are the commonly used animal model for ADHD. We investigated volume differences of the striatum at various ages before puberty in SHRs versus a control strain, Wistar–Kyoto rats (WKYs). Volumes of the bilateral striatum were measured using micrographs of Nissl-stained serial sections in both strains of rats at the ages of 4, 5, 6, 7, 8, 9, and 10 weeks ($n=4$, each strain at each age). The results demonstrated that the age of a significant striatal volume difference between SHRs and WKYs was 5 weeks; however, there was no significant difference for the corresponding total brain volume at each matched age. It suggested that the timing for striatal abnormalities in ADHD occurs during an early stage of childhood.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common childhood neurobehavioral disorders. It affects 8–12% of children, predominantly boys (Biederman and Faraone, 2005). The neurobiology of ADHD remains unknown. Numerous volumetric imaging studies have emphasized the important role of basal ganglia in the pathophysiology of ADHD (Hynd et al., 1993; Castellanos et al., 1994, 1996, 2002; Filipek et al., 1997; Mataro et al., 1997; Pineda et al., 2002). There are several nuclei comprising the basal ganglia; the globus pallidus internal segment and substantia nigra pars reticulata are the two output nuclei, while the main input nucleus is the striatum (the caudate and putamen collectively) (Uttera and Basso, 2008). The basal ganglia receive input from the neocortex via the striatum and send processed information to the prefrontal cortex, which is involved in motor planning, learning, and execution (Haber, 2003). Lou et al. (1984) noted decreased metabolism in the striatal region, particularly the caudate nuclei region, in patients with attention-deficit disorder. A monozygotic twin study showed significantly smaller-sized caudate nuclei in patients with ADHD compared to the other unaffected twin (Castellanos et al., 2003). An imaging study in girls with ADHD (with an age range of 5.3–16.0 years) found that the pallidum and caudate volumes were significantly correlated with symptom severity and cognitive performance (Castellanos et al., 2001). Castellanos et al. (2002) proposed that volumetric differences in the caudate nucleus may be transient and possibly related to an improvement in hyperactivity/impulsivity with increasing age in ADHD children.

Imaging studies, like those previously mentioned, enable the tracing of gross anatomical changes in brain regions of ADHD children; however, it is still difficult to explore microscopic changes in humans while avoiding interference from possible treatment effects, especially when studying age-dependent volume abnormalities of the brain. Animal models provide a good opportunity to detect microscopic developmental changes in the brain due to the availability of brain tissues and the shorter life cycle of animals. They also provide relatively simpler experimental conditions, including homogeneous subjects, and a lack of previous drug treatment, family interactions, and other social factors encountered in human ADHD patients. A validated animal model of ADHD is the spontaneously hypertensive rat (SHR) strain, which was derived from Wistar–Kyoto (WKY) rats. The SHR strain manifests almost all of the behavioral characteristics of ADHD, including sustained attention deficits, impulsivity, and hyperactivity that occur in novel situations (Sagvolden, 2000).

SHRs further exhibit brain pathologies similar to ADHD. For example, the striatum may be an important brain region for the pathology of ADHD in SHRs. There is a strong possibility that the dopamine transporter gene is overexpressed in the striatum of ADHD subjects (Russell

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et al., 2005), consistent with the finding that the dopamine transporter is increased in the prehypertensive SHR striatum (Russell, 2003), and extracellular dopamine levels are lower in the caudate nucleus (Linthorst et al., 1991; De Jong et al., 1995). Some volumetric comparisons of brain regions between SHRs and WKY rats were done. SHR brain volumes, specifically the prefrontal cortex, occipital cortex, and hippocampus, are smaller than those of WKY rats (Tajima et al., 1993; Sabbatini et al., 1999, 2000), whereas the volume of the neostriatum was unchanged (Sabbatini et al., 1999). MRI also revealed significantly increased ventricular volume in SHRs compared to WKY rats at 3 months of age (Bendel and Eilam, 1992). However, the above volumetric studies were conducted on adult rats. Hypertension would be a confounding factor in the SHR model of ADHD, since hypertension develops at 10–12 weeks of age (considered an adult) in SHRs, but hyperactivity is observed at 3–4 weeks of age before rats enter puberty (Russell et al., 2005).

The aim of this study was to trace age-series changes in striatal volume in the SHR model of ADHD. While hypertension was not present in young hyperactive SHRs, we compared volume differences of the striatum between male SHRs and WKY rats aged 4–10 weeks, which covers the growth stages from weaning to puberty. Findings from this study can advance our understanding of the timing of volume changes in the striatum with ADHD.

2. Methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee, National Ilan University. Male rats (SHR and WKY) aged 4–10 weeks were used ($n=4$, for each strain at each age) and there was no significant difference in mean body weights between SHRs and WKY rats at each matched age (Fig. 1A). The rats were anesthetized with pentobarbital (80 mg/kg, i.p.) and perfused transcardially with saline, followed by a 4% formalin solution. The brains were carefully dissected out and placed in 30% sucrose in a 10% formalin solution for 3 days. Serial frozen sections of the brain at a thickness of 40 μm were cut, and Nissl staining was performed. The rostral and caudal limits of the caudate–putamen (striatum) nucleus were equivalent to bregma 2.52 to −3.72 mm according to the rat brain atlas demonstrated in Paxinos and Watson (2005). According to the suggestion of Oorschot (1996), approximately 10 sections is an adequate sample for volume estimation of the striatum by the method of the Cavalieri estimator (Gundersen and Jensen, 1987), and every 10th coronal section from the caudal to the rostral ends was selected. This resulted in about 11–14 sections being selected per striatum.

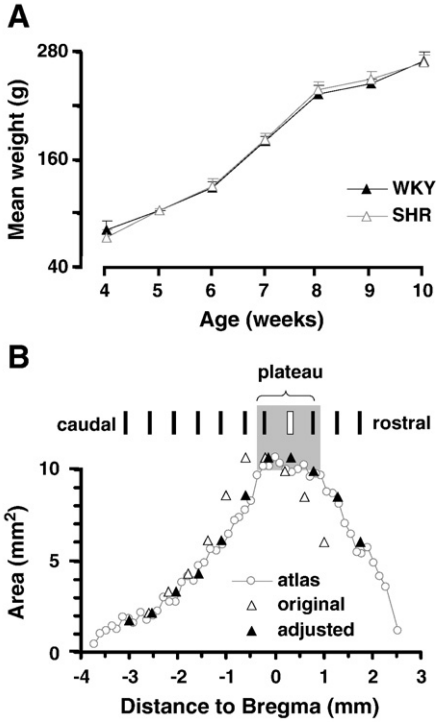

Fig. 1. (A) Mean body weights of SHRs and WKY rats at the age of 4–10 weeks ($n=4$ for each strain at each age). (B) The distribution of selected sections to the bregma (upper panel) and the adjusted striatal areas from the atlas and mean striatal areas from WKY rats (lower panel). The gray area indicates the plateau of the distribution curve, which covered approximately three selected coronal sections. The white rectangle indicates the middle section of the plateau, and the black ones are the three sections rostrally and seven sections caudally from this middle section. Distances between two adjacent selected sections were adjusted from 0.4 mm (original) to 0.476 mm (adjusted), and then the area distributions of the 11 sections matched the standard curve (atlas).

The boundaries of the striatal area were delineated also according to the criteria suggested by Oorschot (1996), and the method of point counting (West, 1993; Oorschot, 1996) based on the Cavalieri estimator was used to calculate the cross-sectional area of the striatum in each section and the volume of the striatum of each rat. Briefly, a lattice of regularly arranged points with interpoint distances of 0.2 mm was randomly superimposed over the section. The number of points falling on the striatum of interest (P) was counted, and these were multiplied by the area extending from each point (denoted as A, in this case was 0.2×0.2 mm^2). The striatal area in a single section was calculated as $P\times A$; the volume of the striatum was then calculated by $\Sigma P\times A\times t$, where t is the distance between sampled sections, basically, which was equal to the decuple thickness of a slice in this study.

Although the microtome was set at 40 μm, the thickness of a slice also needed to be calibrated for possible errors caused by the operator or slicing machine. Furthermore, there were about 11–14 sections selected per striatum in each rat, so the corresponding sites to the bregma of each section had to be calibrated for more-detailed comparisons between SHRs and WKY rats. The process on 10-week-old WKY rats is demonstrated as an example to illustrate the calibrating procedures. We used the striatal areas in the atlas of Paxinos and Watson (2005) as the standard for calibration, in which serial sections of the brain were 40 μm thick. We counted the striatal area of each section in the atlas by the same point counting method, which applies a lattice with 0.2×0.2-mm squares onto the region of interest. These area values were then plotted by distances to the bregma to form a distribution curve. On this curve, a plateau with a length of about 1.2 mm was observed; it covered approximately three selected coronal sections of WKY rats or SHRs in our preparation (as shown in Fig. 1B). Therefore, the mean areas of three successively selected coronal sections of WKY rats and SHRs were calculated, and the three sections with the maximum mean area were considered as the plateau of the distribution curve. Slices of WKY rats and SHRs were then realigned with the middle section of the plateau, and three sections rostrally and seven sections caudally from this middle section were selected for further analysis (Fig. 1B).

The mean values of the right striatal area of selected sections at the same distance to the bregma of the four WKY rats were calculated, and then plotted on a coordinate plane by distances to the bregma which were preliminary determined by assuming the most caudal section was located at −3 mm and the others progressively increased by 0.4 mm (which was the decuple thickness of a slice). Striatal area values of the atlas were also plotted on the same coordinate plane, but in order to eliminate brain size differences between the atlas and WKY rats in our study, all striatal area values of the atlas were adjusted by multiplying a constant, which was the ratio of the respective maximum right striatal area of WKY rats to the atlas (in this case, 10.65/12.48), to make the plateau of the atlas approximately match the corresponding distribution of striatal areas from WKY rats. Finally, all distances between two adjacent selected sections were further equally adjusted to make the striatal area distributions of the 11 sections match the standard curve. As shown in Fig. 1B, distances between two adjacent selected sections were adjusted from 0.4 to 0.476 mm, and then the distributions of the 11 sections all matched the standard curve. As a result, these sections included regions from sites of −3 to 1.76 mm (to the bregma), which covered more than 90% of the striatal volume. Actually, these parameters obtained from 10-week-old WKY rats were also applicable to 4–9-week-old rats. Therefore, according to Cavalieri's principle, the total volume of the striatum was then calculated by [$\sum$(each caudate–putamen area$\times$476 μm)] of the 11 selected coronal sections. The area of the entire section was also calculated by the method of point counting as used in the striatum, and the volume of the total brain (corresponding to the brain region containing the striatum) was calculated by [$\sum$(each whole brain area$\times$476 μm)] of the 11 selected coronal sections.

Data were analyzed for statistical significance by a two-tailed t-test. A P value of <0.05 was considered statistically significant. Right and left striatal volumes in a rat were compared using the paired t-test. Striatal volumes between WKY rats and SHRs were compared using the unpaired t-test. All data are expressed as the mean ± SE.

3. Results

Differences in striatal volumes at each age for both strains are shown in Fig. 2A. SHRs tended to have smaller total striatal volumes than WKY rats at 5–10 weeks of age but only those of rats 5 weeks of age were significantly smaller than those of WKY rats ($P<0.01$, Fig. 2A upper panel), even for individual comparisons of right or left striatal volumes (data not shown). However, there was no significant difference in corresponding total brain volumes between SHRs and WKY rats at each matched age (Fig. 2A lower panel). Bilateral volumes