Quantitative Regional Cerebral Blood Flow Differences in Normal and Attention-Deficit/Hyperactivity Disorder Rats

J. Danker[1], Z. M. Liu[1], T. Q. Duong[2]

[1]Ctr for Comparative NeuroImaging, Psychiatry, Univ Massachusetts Med Sch, Worcester, MA, United States, [2]Ctr for Comparative Neuroimaging, Psychiatry, Univ Massachussett Med Sch, Worcester, MA, United States

Introduction Attention-deficit/hyperactivity disorder (ADHD) is an early-onset developmental disorder that affects approximately 3-7% of school-age children and mainly occurs in males. It is marked by symptoms of inattention, hyperactivity, and impulsivity, and is among the most prevalent of childhood disorders. Children with ADHD also suffer from low self-image, interpersonal and scholastic problem, and are predisposed to develop other psychiatric problems later in life. Decreased prefrontal activation was observed in ADHD adolescents and was correlated with poorer performance on executive tasks [2]. A lower cerebral blood flow (CBF) in the prefrontal cortices and the cerebellum in humans with ADHD has been reported using single photon emission computerized tomography (SPECT) [1].

The goal of this study was to investigate the quantitative regional CBF differences between the ADHD (SHR) and normal (WKY) rats under basal conditions. The most widely used animal model for ADHD is the spontaneously hypertensive rat (SHR) because it shows many of the salient symptoms of ADHD, including hyperactivity and motor and cognitive impulsiveness [3]. SHR are bred from parental Wistar Kyoto rats (WKY) [4] which do not show the quasi-ADHD symptoms, making the WKY strain an ideal control. Since cerebral blood flow is intricately coupled to neural activity [1], we hypothesized that CBF measurements could offer insights into the regional neural activity differences between the control and ADHD animals under basal conditions. Multislice high-resolution (180x180x1500 μm) quantitative perfusion imaging was performed using magnetic resonance imaging. The accuracy of repeated CBF measurements within and across different animals was carefully evaluated. CBF differences between ADHD and control rats were analyzed for 10 brain sub-structures.

Methods Two groups of male rats (356 ± 48 grams) were imaged. *Group I* consisted of Wistor Kyoto rats (WKY, n = 9, control). *Group II* consisted of spontaneously hypertensive rats (SHR, n = 7, experimental). Typically, each rat was imaged twice on different days. Animals were secured in a MR-compatible rat stereotaxic headset with custom-designed ear- and tooth-bars with a built-in radiofrequency neck coil for arterial spin labeling. All imaging studies were performed under 2% isoflurane, in which animals respired spontaneously without mechanical ventilation.

CBF measurements were made on a 4.7T using the continuous arterial spin-labeling technique with four-shot, gradient-echo, echo-planar-imaging (EPI) acquisition. Paired images were acquired alternately – one with arterial spin labeling and the other without spin labeling (control). The MR parameters were: data matrix = 128 x 128, FOV = 2.56 x 2.56 cm^2, the same eight 1.5-mm slices as anatomy, TE = 15 ms, and TR = 2 s (90^o flip angle). Multiple CBF measurements were made over ~45 mins and averaged. Anatomy was acquired with double the spatial resolution using fast spin-echo (RARE) sequence.

Regions of interests (ROIs) were analyzed for: I. frontal cortex - dark blue; II. sensory cortex - light blue; III. hippocampus - red; IV. thalamus - green; V. globus pallidus - purple; VI. caudoputamen - orange; VII. corpus callosum - pink VIII. medial prefrontal cortex - white; IX. motor frontal cortex – yellow. Accuracy of repeated CBF measurement within and across different animals were evaluated for each subsructures. Differences in basal CBF values in different brain structures between SHR and WKY rats were compared. In addition, relative differences in regional CBF with respect to the whole brain average were also analyzed by dividing the regional CBF values by the average CBF values of the whole brain for each animal. Statistical tests between SHR and WKY rats were performed by using the unpaired student's t-test assuming unequal variances. All reported values and error bars on plots were in mean ± SD.

Results & Discussions Representative anatomic images from a WKY rat are shown in **Fig. 1A**. Overlaid on the anatomy are representative regions of interest (ROIs) of nine structures: Representative high-resolution echo planar images (EPI) and quantitative CBF images from the same animal are shown in **Fig. 1B** and **1C**, respectively. CBF was heterogeneous across the entire brain.

Deviation of CBF in different brain regions obtained from 7 repeated CBF measurements in the same animals ranged from 3 to 10%; the deviation of CBF in different brain regions obtained from 7 different animals ranged from 15 to 18%, indicating highly reproducible CBF measurements. Regional CBF of the SHR were statistically different (higher) from those of the WKY rats in all structures analyzed ($P<0.05$) except for the caudate putamen ($P=0.09$) and the globus pallidus ($P=0.12$) (**Fig. 2A**). Whole brain CBF of the SHR (1.5±0.2ml/g/min, mean±SD) was ~25% higher than that of the WKY rats (1.2±0.2ml/g/min), likely due to the hypertensive nature of the ADHD rat model. Following normalization to eliminate global CBF differences (**Fig. 2A**), CBF in the medial prefrontal cortex (pfc), a structure thought to be the equivalent of the human dorsolateral prefrontal cortex and widely implicated in ADHD, was found to be higher in SHR compared to WKY rats ($P<0.05$). The only other structure that was statistically different after normalization is the corpus callosum ($P<0.05$), although there could be partial volume effect due to the small structure of the corpus callosum. Since basal cerebral blood flow is intricately coupled to basal neural activity, these results suggest that there was abnormal basal neural activity in the corpus callosum and the medial prefrontal cortex between the control and AD/HD animals, consistent with the hyperactivity, impulsivity, inattention, and other ADHD-like behaviors in this animal model. Similar studies under awake and restraint conditions are under investigation.

Conclusions Highly accurate and reproducible quantitative CBF measurements were obtained. ADHD rats showed markedly higher regional and global blood flow when compared to the control WKY rats. After normalization to eliminate the global differences, regional CBF in the ADHD rats showed differentially higher CBF in the corpus callosum and the medial prefrontal cortex; the latter has been widely implicated in ADHD.

References [1] Kim et al. *Eur Arch Psych Clin Neurosci* 2002; 252:219. [2] Rubia et al. *Am J Psychiatry* 1999; 156:891. [3] Sagvolden et al. *Neurosci & Biobeh Rev* 2000; 24:31. [4] Okamoto et al. *Jpn Circ J* 1963; 27:282.