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Appendix E1

Supplemental Methods

Head Motion Analyses

Measures of head motion were analyzed to verify image quality. For each participant, the cerebrospinal fluid signal-to-noise ratio (SNR) of the zero shift images (MFC echo time = 40 msec; T2 echo time = 15 msec; T2* echo time = 7 msec) and ghosting signal in the phase-encoding direction outside of the head were measured as suggested by Kaufman et al (41). SNR was estimated from the mean signal for an ROI within the head divided by the mean signal for air. Cerebrospinal fluid was chosen as the within-participant ROI as this is less affected by T2 signal decay (Fig E1, *A*). To avoid ghosting artifacts, air signal was sampled with an ROI outside the head in the read-encoding direction (Fig E1, *B*). As another indicator of head motion, ghosting signal in the phase-encoding direction outside of the head was measured (Fig E1, *C*). To compare cerebrospinal fluid SNR and ghosting signal means between the control group and each ADHD subgroup, one-way analysis of variance was conducted.

Image Processing

Parametric maps for the microscopic (magnetic tissue structures: eg, iron-rich glial cells, capillaries) and macroscopic (larger structures: eg, sinus air cavity) contributions to the total MFC (MFC_{mic} and MFC_{mac}, respectively) were also estimated (17). Quantitative analyses were restricted to total MFC values, which are relatively more accurate and less affected by artifacts. However, MFC_{mic} and MFC_{mac} maps were used to define a mask for voxels that are dominated by microscopic contributions to the total MFC signal. This mask was generated by binarizing the ratio map of MFC_{mic}/MFC_{mac}, with the MFC_{mic}-dominant voxels defined as those with ratios exceeding 1, and applied to the total MFC parametric map.

Serum Iron Measures

Two standard food surveys (ie, 24-Hour Food Recall, Quick Iron Questionnaire) were administered to approximate dietary iron intake and to screen for exclusionary factors that affect serum measures: non-omnivore diets, contraceptives, pregnancy or late menstruation, blood donation in the past 6 months, iron supplements, liver function abnormalities, and erythropoiesis-stimulating agents. Heme and non-heme iron intake (in milligrams per day) were calculated by using the U.S. Department of Agriculture National Nutrient Database for Standard Reference (*http://ndb.nal.usda.gov*). Multivitamin intake was also recorded.

On the day of MR imaging, blood samples were collected after a fasting period (4 hour minimum) from each participant to obtain serum ferritin level, iron level, total iron binding capacity, transferrin level, and complete blood count. Standard commercial kits were used to analyze the serum levels of ferritin with the electrochemiluminescence immunoassay method (Roche, Basel, Switzerland), iron levels and total iron binding capacity with the ferrozine colorimetric method (Ortho-Clinical, Rochester, NY), and transferrin levels with the quantitative immunoturbidimetry method (ARUP Laboratories, Salt Lake City, Utah). Normal ranges for each measure were provided by the New York University Hospital Center Clinical Laboratories

(Table E1). For measures with different normal ranges due to age and/or sex, secondary normalized measures were calculated as the ratio within the normal range (ie, negative: below normal, 0–1: within normal range, larger than 1: higher than normal). Normal ferritin cut-off values reported in the literature were also referenced (42–45). A total of two participants were excluded due to a blood draw exclusion criteria ($n = 1$) and abnormally high blood iron levels ($n = 1$).

Supplemental Results

Clinical Demographics

There were no significant differences in parent-identified ethnicity between the control and ADHD groups (12 of the 27 control subjects [44%] and 10 of the 22 ADHD patients [45%] were Caucasian, 11 control subjects [41%] and eight ADHD patients [36%] were African American, two control subjects [7%] and four ADHD patients [18%] were Latino, and two control subjects [7%] and no ADHD patients were other, including Asian or mixed; Pearson χ^2 [$df = 3$, $n = 49$] = 2.8 [two-sided], exact $P = .51$).

Half of the ADHD-nonmedicated subgroup was free of comorbidities, whereas the other half had the following comorbid disorders: anxiety not otherwise specified ($n = 2$), nocturnal enuresis ($n = 1$), reading disorder ($n = 1$), obsessive-compulsive disorder ($n = 1$), and oppositional defiant disorder ($n = 1$). Similarly, half of the ADHD-medicated subgroup consisted of comorbidity-free patients and the other half had the following comorbid disorders: separation anxiety disorder ($n = 1$), dysthymic disorder ($n = 1$), reading disorder ($n = 1$), expressive language disorder ($n = 1$), and oppositional defiant disorder plus bulimia plus sleep disorder ($n = 1$).

Brain Iron Indexes

Shapiro-Wilk tests of normality showed that the MFC data for all regions except the thalamus were normally distributed for the control and ADHD groups (globus pallidus: $W_{(27)} = 0.94$, $P = .095$ for control group and $W_{(22)} = 0.99$, $P = .978$ for ADHD group; putamen: $W_{(27)} = 0.93$, $P = .054$ for control group and $W_{(22)} = 0.95$, $P = .308$ for ADHD group; caudate nucleus: $W_{(27)} = 0.96$, $P = .365$ for control group and $W_{(22)} = 0.96$, $P = .575$ for ADHD group; thalamus: $W_{(27)} = 0.86$, $P = .002$ for control group and $W_{(22)} = 0.87$, $P = .007$ for ADHD group).

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