



# Brain iron levels in attention-deficit/hyperactivity disorder normalize as a function of psychostimulant treatment duration

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## ABSTRACT

Brain iron homeostasis is a dopamine-related mechanism that may be modified with long-term psychostimulant treatment in attention-deficit/hyperactivity disorder (ADHD). We previously reported that while medication-naïve youth with ADHD have reduced brain iron compared to controls and psychostimulant-medicated patients, no differences were detected between the latter groups. In this follow-up study, we examined whether the duration of psychostimulant treatment correlates with the degree of iron normalization.

Brain iron was indexed with MRI using an advanced method called magnetic field correlation (MFC) imaging and the conventional R2\* proton transverse relaxation rate method. MFC was acquired in 30 psychostimulant-medicated youth with comorbid-free ADHD and 29 age-matched controls (all males). R2\* was acquired in a subset of these individuals. Region-of-interest analyses for MFC and R2\* group differences and within-group correlations with age and years of psychostimulant treatment were conducted in the globus pallidus (GP), putamen (PUT), caudate nucleus (CN), thalamus (THL) and red nucleus.

No significant MFC and R2\* group differences were detected. However, while all regional MFC and R2\* significantly increased with age in the control group, MFC and R2\* increased in the GP, PUT, CN and THL with psychostimulant treatment duration in the ADHD group to a greater degree than with age. Our findings suggest that while youth with ADHD may have less prominent age-related brain iron increases than that seen in typical development, long-term use of psychostimulant medications may compensate through a normalizing effect on basal ganglia iron. Longitudinal studies following ADHD patients before and after long-term psychostimulant treatment are needed to confirm these findings.

## 1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is among the most prevalent neurodevelopmental disorders, with diagnosis rates capturing 11% of the US pediatric population (Anderson, 2018; Visser et al., 2014). Despite ongoing concerns of ADHD overdiagnosis (Adams, 2015; Bax et al., 2019; Layton et al., 2018; Storebø et al., 2018), psychostimulant medications remain the first-line treatment for ADHD and are increasingly prescribed in youth (6.6%) and adults (0.7%) (Anderson, 2018; Burcu et al., 2016; Visser et al., 2014). Psychostimulant medications act primarily on the striatal dopaminergic system and on the dopaminergic and noradrenergic systems in the frontal cortex (Faraone, 2018; Spencer et al., 2015; Volkow et al., 2005). A single-dose of the drug increases synaptic dopamine and noradrenaline levels by blocking catecholamine transporters. In ADHD, this acute increase in

catecholamines corresponds to reduced symptoms (Wilens, 2008) and transient normalization of brain function as demonstrated by reduced resting-state functional connectivity (Silk et al., 2017), enhanced activation in cognitive control brain circuits during executive function tasks (Rubia et al., 2014) and reduced activation in reward circuits during reward response tasks (Rubia et al., 2009). As such, dopamine deficiency within frontostriatal circuits is suspected to play a central role in the pathophysiology of ADHD (Swanson et al., 2007).

Consistent with the lifetime chronicity of ADHD (40–65% persist into adulthood) (Barbaresi et al., 2013; Faraone et al., 2006; Geissler and Lesch, 2011), patients typically remain on psychostimulant medications for several years (Barbaresi, 2014), yet the effects of long-term psychostimulant treatment on the brain remain unclear. While limited and somewhat inconsistent (Castellanos et al., 2002; Hoogman et al., 2017; Norman et al., 2016; Sethi et al., 2017; Shaw et al., 2014), the

*Abbreviations:* MFC, magnetic field correlation; GP, globus pallidus; PUT, putamen; CN, caudate nucleus; THL, thalamus; RN, red nucleus

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literature implicates alterations in the dopaminergic system, brain structure and brain function, including increased striatal dopamine transporters (Fusar-Poli et al., 2012), basal ganglia volumes (Sobel et al., 2010; Nakao et al., 2011; Greven et al., 2015; Pretus et al., 2017), white matter microstructure (de Luis-García et al., 2015; Schweren et al., 2016), brain activation during inhibitory control tasks (Norman et al., 2016) and resting-state functional connectivity between the putamen and the default mode network (Battel et al., 2016). In a previous study, we detected the first account of a concordant psychostimulant medication effect on brain iron levels in ADHD (Adisetiyo et al., 2014).

Brain iron homeostasis is a critical dopamine-related mechanism, and its dysregulation has been implicated in ADHD (Adisetiyo et al., 2014; Cortese et al., 2012; Lozoff, 2011; Tseng et al. 2018; Wang et al., 2017; Yazici et al., 2019). Along with its many essential roles in the brain (Anderson and Frazer, 2017; Morris et al., 2018), iron is required for the synthesis and metabolism of dopamine, noradrenaline and myelin (Beard, 2003; Beard and Connor, 2003; Daubner et al., 2011; Ramsey et al., 1996). Indeed, age-related brain iron accumulation has been well documented in healthy brain development and is associated with cognitive and motor functioning (Hallgren and Sourander, 1958; Lozoff, 2011). Animal studies have shown that reduced iron in basal ganglia regions not only alters catecholamine systems within these regions but also modifies the effects of psychostimulants (Jenney et al., 2016; Jones et al., 2002; Mohamed et al., 2011; Morse et al., 1999). Using non-invasive magnetic resonance imaging (MRI) methods (Dusek et al., 2013), we along with others have demonstrated that striatal and thalamic iron levels are significantly reduced in medication-naïve children and adolescents with ADHD compared to age-matched typically developing controls (Adisetiyo et al., 2014; Cortese et al., 2012). Conversely, psychostimulant-medicated youth with ADHD have comparable brain iron levels as controls (Adisetiyo et al., 2014). However, because the details of medication history were not accounted for in these previous studies, we could not infer whether long-term psychostimulant use was correlated with the degree of iron normalization. Nonetheless, these findings are consistent with studies that demonstrate increased brain iron levels with methamphetamine and cocaine use (Adisetiyo et al., 2018; Ersche et al., 2017; Melega et al., 2007).

As an extension of our prior work (Adisetiyo et al., 2014), the goal of this follow-up study was to replicate the normalized brain iron findings observed in psychostimulant-medicated children and adolescents with ADHD compared to age-matched controls and examine whether brain iron levels increase with the duration of psychostimulant treatment. We utilized an advanced MRI method called magnetic field correlation (MFC) imaging (Jensen et al., 2009, 2006; Jensen and Helpert, 2014) along with the conventional R2\* proton transverse relaxation rate method (Dusek et al., 2013) to index iron levels within dopamine and iron-rich basal ganglia regions that are known targets of psychostimulant medications (Faraone, 2018; Mink, 1996; Volkow et al., 2005). Multimodal imaging of brain iron was conducted to bolster the sensitivity and specificity for iron detection as the relationship between MRI metrics and brain tissue iron is complex and depends on numerous factors including the microscopic spatial distribution of iron and its molecular form (e.g., ferritin, hemosiderin) (Dusek et al., 2013). Because iron is strongly magnetic and has non-uniform microscopic spatial distributions in the brain (Connor et al., 1990; Iancu, 1992; Morris et al., 1992; Schenck and Zimmerman, 2004), it generates microscopic magnetic field inhomogeneities (MFIs) that significantly increase R2\* (Brown et al., 2014). While R2\* is highly sensitive to iron (Langkammer et al., 2010), it has limited specificity because it is affected by other dipolar interactions generated by tissue constituents unrelated to iron (Dusek et al., 2013). Compared to R2\*, MFC has a more direct relationship to MFIs because MFC is independent of dipolar relaxation mechanisms (Jensen et al., 2009, 2006; Jensen and Helpert, 2014). Capitalizing on the strengths of both metrics to index iron, we hypothesized that while age will correlate with brain iron accumulation in control individuals (Adisetiyo et al., 2012; Hallgren and Sourander,

1958), psychostimulant treatment duration will be the driver of iron increases in youth with ADHD, particularly in the striatum and its primary output structure, the globus pallidus (Everitt and Robbins, 2005; Mink, 1996).

## 2. Material and methods

### 2.1. Participants

Individuals with ADHD and typically developing controls (males, 8–18 years old) were recruited through our medical center and the local community. Parental informed consent and child's assent were obtained as approved by the Institutional Review Board. Inclusion required an estimated full-scale IQ  $\geq 79$  on the Kaufman Brief Intelligence Test, Second Edition (Kaufman and Kaufman, 1990), right-handedness on the Edinburgh Handedness Inventory (Oldfield, 1971) and absence of neurological, cognitive and chronic medical disorders. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition/Text Revision (DSM-IV-TR) diagnoses (American Psychiatric Association, 2000) were assessed by licensed clinicians or supervised trainees using the Schedule of Affective Disorders and Schizophrenia for Children – Present and Lifetime Version (K-SADS-PL) (Kaufman et al., 1997) administered to the parent and child; for a subset of participants recruited from a related study ( $n = 19$ ), the Mini-International Neuropsychiatric Interview for Children and Adolescents (Sheehan et al., 1998) was used in lieu of the K-SADS-PL to reduce assessment burden. Diagnosis of psychotic, major depressive, conduct, tic, anxiety, pervasive developmental disorders, history of traumatic head injury or contraindications to MRI were exclusionary. Medical and medication histories were reviewed and executive function (off all medications) was assessed using the Behavioral Rating Inventory of Executive Function – Parent and Teacher Versions (Gioia et al., 2000). For individuals with ADHD, inclusion required a current diagnosis of ADHD (with childhood diagnosis of ADHD combined subtype at 12 years old or younger) and a history of taking psychostimulant medications (i.e., methylphenidate, dextroamphetamine, dextroamphetamine/amphetamine, *L*-lysine-D-amphetamine). Prior to the study visit, parents were instructed to complete a medication form using medical records to document the start and end dates for each psychostimulant medication taken by their child; the form was reviewed during the study visit and, if necessary, follow-up calls were conducted to ensure discrepancies were resolved. The cumulative years of psychostimulant treatment duration were derived by summing the durations of each non-overlapping psychostimulant medication taken by the participant (Harstad et al., 2014); non-psychostimulant medications were not examined. For controls, regular medications and DSM-IV-TR diagnoses (current/past) were exclusionary. All participants were instructed to be off all medications on the day of the MRI scan.

### 2.2. Image acquisition and processing

An MRI brain scan was conducted using a 3T Siemens TIM Trio scanner (Siemens Healthineers, Erlangen, Germany). Whole-brain structural T1-weighted magnetization-prepared rapid acquisition gradient-echo (MPRAGE) images were acquired for tissue segmentation with the following parameters: repetition time (TR) = 1900 ms, echo time (TE) = 2.26 ms, voxel size =  $1.0 \times 1.0 \times 1.0 \text{ mm}^3$ , matrix =  $256 \times 256 \times 192$ , field of view (FOV) =  $256 \times 256 \times 192 \text{ mm}^3$ , flip angle =  $9^\circ$  and acquisition time = 4 min 26 s. For MFC estimation, asymmetric spin-echo (ASE) images were acquired with the segmented echo-planar imaging (EPI) approach, which minimizes EPI distortion (Porter and Heidemann, 2009): TR = 5550 ms, TE = 40 ms, voxel size =  $1.7 \times 1.7 \times 1.7 \text{ mm}^3$ , matrix =  $128 \times 128$ , FOV =  $220 \times 220 \text{ mm}^2$ , number of slices = 40, flip angle =  $90^\circ$ , bandwidth = 1346 Hz/pixel, EPI factor = 33, refocusing pulse time shifts = 0, -4, and -16 ms, no gaps, averages = 4, acquisition time = 6 min 40 s. Prior to MFC calculation, the ASE images were corrected

for Gibbs-ringing artifacts (Kellner et al., 2016) and motion-corrected. To correct for motion, the ASE 0-shift images (time shift = 0 ms) from each average were linearly co-registered to the first average's 0-shift image using SPM8 (Statistical Parametric Mapping, University College London, UK); the rigid-body transformation obtained was applied to all the ASE images corresponding to each average. The co-registered ASE images were averaged, and the MFC parametric map was then calculated on a voxel-by-voxel basis (Jensen et al., 2009, 2006). For  $R2^*$  estimation,  $T2^*$  gradient echo images were acquired with TR = 4380 ms, TEs = 4.92, 9.84, 14.76, 19.68, 24.60, 29.52, 34.44, 39.36, 44.28, 49.20 ms, voxel =  $1.7 \times 1.7 \times 1.5 \text{ mm}^3$ , matrix =  $128 \times 128$ , FOV =  $220 \times 220 \text{ mm}^2$ , number of slices = 82, flip angle =  $20^\circ$ , bandwidth = 260 Hz/pixel, no gaps, average = 1, and acquisition time = 5 min 34 s.  $R2^*$  parametric maps were generated as previously described (Adisetiyo et al., 2014). Structural scans were screened by two experienced raters for gross abnormalities and referred to a neuroradiologist when warranted. Images with incidental findings of clinical significance or severe artifacts (i.e., blurred images, signal loss) were excluded.

### 2.3. Region-of-interest analyses

Region-of-interest (ROI) analyses were conducted on the globus pallidus (GP), putamen (PUT), caudate nucleus (CN), and thalamus (THL) because these brain regions have the highest concentrations of dopamine and iron (Beard and Connor, 2003; Hallgren and Sourander, 1958), are targets of psychostimulants (Faraone, 2018; Volkow et al., 2005) and have been previously studied in ADHD (Adisetiyo et al., 2014; Cortese et al., 2012). Additionally, MFC and  $R2^*$  in these brain regions have been shown to significantly correlate with putative post-mortem iron concentrations in normal aging (Adisetiyo et al., 2012; Langkammer et al., 2010). The red nucleus (RN) was examined as a control ROI because it has high iron content but is not a target of psychostimulants (Faraone, 2018; Hallgren and Sourander, 1958; Volkow et al., 2005). To optimize anatomical accuracy, automated ROI and cerebrospinal fluid (CSF) segmentation was conducted on each participant's high-resolution MPRAGE using the Freesurfer software (<http://surfer.nmr.mgh.harvard.edu>). Every participant's ROIs, CSF mask, 0-shift ASE image and MFC map were normalized (resliced and resampled) to the MNI152 standard space ( $1 \text{ mm}^3$ ) using the non-linear registration algorithm from the Automatic Registration Toolbox software (Ardekani et al., 2005).

To exclude voxels with partial volume effects, CSF was removed from all parametric maps and ROIs were constrained with a consensus mask. The consensus masks for the GP, PUT, CN and THL were defined as ROI voxels with 100% overlap among all participants (i.e., 59 participants for MFC; 46 participants for  $R2^*$ ). Due to the unavailability of automatic RN segmentation, the RN consensus ROI was drawn on a normalized group average 0-shift ASE image with its boundary defined well within the clearly distinguishable anatomical boundary. For each participant, anatomical accuracy of the consensus ROIs was visually verified (Fig. 1) and applied to the corresponding parametric map to extract ROI means.

### 2.4. Statistical analyses

Statistical analyses were performed with the SPSS software package (v24.0; IBM, Armonk, NY). Data distributions were tested for normality by using the Shapiro-Wilk test. Group comparisons were conducted with two-tailed Student's  $t$ -test for normally distributed measures (Cohen's  $d$  for effect size), the Mann-Whitney  $U$  test for non-normally distributed measures (rank biserial correlation [ $r_{rb}$ ] for effect size) and the Fisher's exact test for nominal measures. A sequential Bonferroni-type false discovery rate (FDR) correction method was conducted to correct for multiple comparisons wherein an FDR corrected  $p$ -value significance threshold was calculated (Benjamini and Hochberg, 1995). The FDR approach was applied over the entire set of  $p$ -values from each analysis type (i.e., between group comparison, within group correlations).

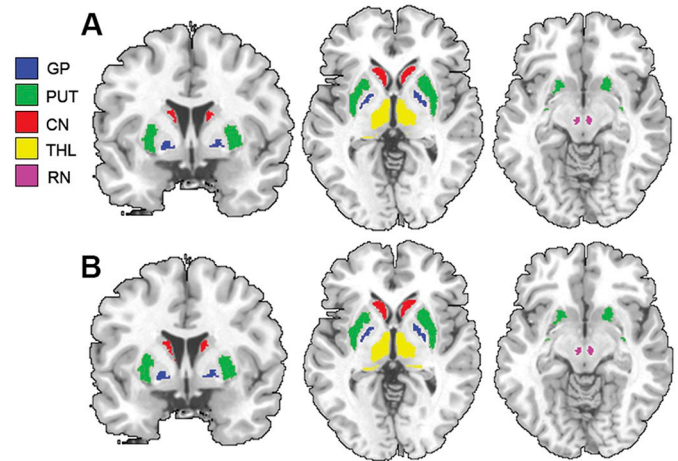


Fig. 1. Brain iron indices in deep gray matter regions.

Region-of-interest (ROI) analyses of magnetic field correlation (MFC) and the  $R2^*$  relaxation rate brain iron indices were conducted on the globus pallidus (GP), putamen (PUT), caudate nucleus (CN), thalamus (THL) and red nucleus (RN). A. Consensus ROIs from the cohort with MFC data ( $n = 59$ ). B. Consensus ROIs from the cohort with MFC and  $R2^*$  data ( $n = 46$ ).

To test whether brain iron indices increased with age (Hallgren and Sourander, 1958), ROI MFC and  $R2^*$  correlations with age were conducted within each group using Pearson's correlation ( $r$ ) for normally distributed measures and Spearman's correlation ( $r_s$ ) for non-normally distributed measures; all one-tailed. To test whether brain iron indices increased with the years taking psychostimulant medication in the ADHD group (Adisetiyo et al., 2014), ROI MFC and  $R2^*$  correlations with psychostimulant medication duration were similarly conducted. Additionally, as age and medication duration were collinearly related in the ADHD group, ROI MFC and  $R2^*$  partial correlations with years of psychostimulant duration (controlling for age), and vice versa, were conducted.

## 3. Results

### 3.1. Demographics

MFC maps were successfully acquired from 59 participants (controls  $n = 29$ , ADHD  $n = 30$ );  $R2^*$  maps were successfully acquired from a subset of these same participants (controls  $n = 23$ ; ADHD  $n = 23$ ). For the participants with MFC data (Table 1) and those with  $R2^*$  data (Supplementary Table S1), the control and ADHD groups did not significantly differ in age, ethnicity distribution and IQ; the ADHD group had significantly higher parent and teacher ratings of executive function deficits (all parent ratings reflected behavior off medication while teacher ratings reflected behaviors off or on medication).

All participants with ADHD were comorbid-free and had a history of taking psychostimulant medication; 22 of these participants (73.3%) were prescribed  $> 1$  type of psychostimulant medication over time and 1 individual had limited psychostimulant exposure ( $< 1$  week). For participants with MFC data (Table 1), the ADHD group consisted of 30 individuals that averaged 5.9 years (SD = 2.8, range = 0–12.6 years) on psychostimulant medication, with the average age of first treatment being 7.8 years old (SD = 2.8); 10 of these individuals also had a history of taking non-psychostimulant medication. For participants with  $R2^*$  data (Supplementary Table S1), the ADHD group consisted of 23 individuals that averaged 5.1 years (SD = 2.6, range = 0–10.1 years) on psychostimulant medication, with a similar age of treatment onset as the MFC cohort; 8 of these individuals also had a history of taking non-psychostimulant medication.

**Table 1**  
Demographics (MFC data).

	Control group	ADHD group	Group comparison	
	(n = 29)	(n = 30)	t	p-Value
Age (years, mean ± SD)	13.9 ± 3.5	14.0 ± 2.5	<sup>a</sup> 414	0.8
Age range (years)	8.2–18.6	8.5–18.8	–	–
Ethnicity (C:AA:O)	23:5:1	23:7:0	<sup>b</sup> 1.3	0.7
KBIT-2				
Verbal IQ	111.0 ± 10.9	106.5 ± 13.1	–1.4	0.2
Nonverbal IQ	105.9 ± 11.8	107.5 ± 14.1	0.5	0.6
Composite IQ	110.1 ± 11.1	108.5 ± 13.7	–0.5	0.6
ADHD subtype				
Combined	–	22	–	–
Inattentive	–	7	–	–
Hyperactive	–	1	–	–
BRIEF-Parent				
Behavioral regulation index	46.9 ± 7.7	61.5 ± 9.9	<sup>a</sup> 97	< 0.001
Metacognition index	47.8 ± 9.3	69.4 ± 6.7	10.3	< 0.001
Global executive composite	48.0 ± 9.3	68.3 ± 7.2	9.4	< 0.001
BRIEF-Teacher				
Behavioral regulation index	48.4 ± 4.5	61.7 ± 15.1	<sup>a</sup> 125	< 0.001
Metacognition index	50.7 ± 8.5	69.9 ± 16.4	5.1	< 0.001
Global executive composite	49.8 ± 6.6	68.5 ± 16.8	5.1	< 0.001

All participants, male, right-handed, comorbid-free, positive history of psychostimulant medication; MFC: magnetic field correlation; C: Caucasian; AA: African American; O: other; KBIT-2: Kaufman Brief Intelligence Test, second edition; BRIEF-Parent: Behavioral Rating Inventory of Executive Function-Parent version (T-scores); BRIEF-Teacher: Teacher version (T-scores, Controls = 25, ADHD = 24); t: Student's *t*-test (two-tailed); SD: standard deviation; df: degrees of freedom; – not applicable.

<sup>a</sup> Mann-Whitney *U* test (Exact Sig. two-tailed).

<sup>b</sup> Fisher's Exact Test (Exact Sig. two-sided).

### 3.2. Brain iron indices as a function of age and psychostimulant medication duration

There were no significant group differences in MFC and R2\* brain iron indices between the control and ADHD groups (Table 2), even when age was controlled as a covariate (Supplementary Table S2A–B). These results were further demonstrated by supplementary multiple regression analysis wherein group effects and group by age interaction effects on MFC and R2\* were not significant (Supplementary Table S3).

**Table 2**  
Group comparisons.

A. MFC (s <sup>-2</sup> )	Control group (n = 29)	ADHD group (n = 30)	Group comparison		
Region	Mean (SD)	Mean (SD)	Statistic	p-Value	Effect size
GP	579.4 (204.6)	546.7 (159.2)	<i>t</i> = 0.7	0.495	<i>d</i> = 0.2; small
PUT	242.9 (92.7)	244.3 (74.8)	<i>t</i> = –0.1	0.950	<i>d</i> = 0.0; small
CN	284.1 (88.8)	285.1 (88.2)	<i>U</i> = 422	0.851	<i>r</i> <sub>rb</sub> = 0.0; small
THL	143.5 (36.8)	153.6 (36.4)	<i>U</i> = 342	0.162	<i>r</i> <sub>rb</sub> = 0.2; small
RN	230.9 (77.6)	243.1 (121.1)	<i>U</i> = 423	0.863	<i>r</i> <sub>rb</sub> = 0.0; small
B. R2* (s <sup>-1</sup> )	Control group (n = 23)	ADHD group (n = 23)	Group comparison		
Region	Mean (SD)	Mean (SD)	Statistic	p-Value	Effect size
GP	31.0 (3.6)	31.0 (3.3)	<i>t</i> = 0.1	0.957	<i>d</i> = 0.0; small
PUT	19.5 (2.3)	19.9 (1.7)	<i>t</i> = –0.6	0.550	<i>d</i> = 0.2; small
CN	18.4 (1.6)	19.4 (2.7)	<i>U</i> = 214	0.275	<i>r</i> <sub>rb</sub> = 0.2; small
THL	18.7 (1.3)	18.7 (1.3)	<i>U</i> = 241	0.617	<i>r</i> <sub>rb</sub> = 0.1; small
RN	23.8 (4.0)	23.5(3.3)	<i>U</i> = 248	0.728	<i>r</i> <sub>rb</sub> = 0.1; small

GP: globus pallidus; PUT: putamen; CN: caudate nucleus; THL: thalamus; RN: red nucleus; *t*: Student's *t*-test (two-tailed); *U*: Mann-Whitney *U* test (Exact Sig. two-tailed); SD: standard deviation; *d*: Cohen's *d*; *r*<sub>rb</sub>: rank biserial correlation.

In the control group, MFC and R2\* indices significantly increased with age in the GP, PUT, CN, THL and RN with large effect sizes (Table 3; Fig. 2). In the ADHD group, simple correlation analysis with age revealed significant positive correlations with MFC (moderate effect sizes) in the GP, PUT and CN, with trends for increased R2\* with age in these regions. Alternatively, simple correlation analysis with psychostimulant treatment duration detected significant positive correlations with MFC and R2\* (large effect sizes) in the GP, PUT and CN and with MFC in the THL (Table 3; Supplementary Fig. S1). All significant findings survived FDR correction.

In the ADHD group, age and psychostimulant treatment duration were significantly correlated (*n* = 30 with MFC: *r* = 0.67, *p* < .001; *n* = 23 with R2\*: *r* = 0.58, *p* = .004). Accounting for the collinearity between age and treatment duration, partial correlation analyses of MFC and R2\* with medication duration (controlling for age) revealed significant positive correlations in the GP and PUT for both metrics, in the CN for R2\* and in the THL for MFC. Conversely, partial correlation analyses of MFC and R2\* with age (controlling for medication duration) found no significant correlations in any of the ROIs (Table 3; Fig. 2). All significant findings had moderate to large effect sizes and survived FDR correction. Similar findings were replicated in supplementary MFC analyses of the cohort with R2\* data (Supplementary Tables S2C, S3C, S4 and S5) and in supplementary analyses excluding individuals with additional non-psychostimulant medication history (Supplementary Tables S6 and S7).

## 4. Discussion

Psychostimulant medications are the most common treatment for ADHD and reduce inattentive and hyperactive/impulsive symptoms in approximately 70% of the population (Wilens, 2008). The acute therapeutic effects of psychostimulants have a rapid onset (30 min) and last up to 12 h. Consequently, management of ADHD symptoms involves long-term use of psychostimulant medications, often lasting years (Barbarese, 2014). Although limited, ADHD studies examining the effects of long-term psychostimulant treatment on the brain report alterations in the dopaminergic system (Fusar-Poli et al., 2012), GABAergic system (Solleveld et al., 2017), brain function (Battel et al., 2016), brain structure (Sobel et al., 2010; Nakao et al., 2011; Greven et al., 2015; Pretus et al., 2017; de Luis-García et al., 2015; Schwenen et al., 2016) and brain metabolites (Benamor, 2014). Likewise, we previously reported the first account of a medication effect on brain iron levels in children and adolescents with ADHD (Adisetiyo et al.,

**Table 3**  
Correlations with age and psychostimulant medication duration.

Iron index	Control group		ADHD group		
	vs. Age	vs. Rx Duration	vs. Age	vs. Rx Duration (control for Age) <sup>a</sup>	vs. Age (control for Rx Duration) <sup>a</sup>
<b>A. MFC (s<sup>-2</sup>)</b>	(n = 29)	(n = 30)			
Region	r <sub>s</sub> (p-value)	r (p-value)	r (p-value)	r (p-value)	r (p-value)
GP	0.76 (< 0.001)*	0.60 (< 0.001)*	0.44 (0.007)*	0.45 (0.007)*	0.07 (0.362)
PUT	0.72 (< 0.001)*	0.55 (0.001)*	0.45 (0.006)*	0.38 (0.021)*	0.13 (0.257)
CN	0.80 (< 0.001)*	0.45 (0.006)*	0.43 (0.009)*	0.25 (0.098)	0.19 (0.167)
THL	0.77 (< 0.001)*	r <sub>s</sub> = 0.33 (0.040)*	r <sub>s</sub> = 0.05 (0.398)	r <sub>s</sub> = 0.37 (0.025)*	r <sub>s</sub> = -0.19 (0.163)
RN	0.59 (< 0.001)*	r <sub>s</sub> = 0.26 (0.083)	r <sub>s</sub> = 0.24 (0.103)	r <sub>s</sub> = 0.15 (0.216)	r <sub>s</sub> = 0.11 (0.290)
<b>B. R2* (s<sup>-1</sup>)</b>	(n = 23)	(n = 23)			
Region	r <sub>s</sub> (p-value)	r (p-value)	r (p-value)	r (p-value)	r (p-value)
GP	0.80 (< 0.001)*	0.54 (0.004)*	0.36 (0.048)* <sup>#</sup>	0.44 (0.018)*	0.07 (0.385)
PUT	0.60 (0.001)*	0.66 (< 0.001)*	0.43 (0.021)* <sup>#</sup>	0.56 (0.003)*	0.08 (0.365)
CN	0.54 (0.004)*	r <sub>s</sub> = 0.54 (0.004)*	r <sub>s</sub> = 0.32 (0.071)	r <sub>s</sub> = 0.46 (0.015)*	r <sub>s</sub> = 0.02 (0.473)
THL	0.58 (0.002)*	r <sub>s</sub> = -0.18 (0.203)	r <sub>s</sub> = -0.08 (0.360)	r <sub>s</sub> = -0.17 (0.229)	r <sub>s</sub> = 0.03 (0.448)
RN	0.47 (0.012)*	r <sub>s</sub> = -0.13 (0.283)	r <sub>s</sub> = 0.18 (0.201)	r <sub>s</sub> = -0.29 (0.100)	r <sub>s</sub> = 0.31 (0.078)

GP: globus pallidus; PUT: putamen; CN: caudate nucleus; THL: thalamus; RN: red nucleus; Rx: psychostimulant medication; r: Pearson's correlation; r<sub>s</sub>: Spearman's correlation.

<sup>a</sup> Partial correlations.

\* p < .05, one-tailed (false discovery rate corrected).

<sup>#</sup> p < .05, one-tailed.

2014). Whereas medication-naïve individuals with ADHD had significantly reduced MFC brain iron indices in the PUT, CN and THL, individuals with a history of psychostimulant treatment had comparable MFC in basal ganglia regions as age-matched control individuals. In this follow-up study, we replicated our findings in another independent cohort, once again demonstrating that psychostimulant-medicated children and adolescents with ADHD have comparable MFC and R2\* indices of iron in basal ganglia regions as control individuals. Moreover, we found that the duration of psychostimulant treatment had a stronger statistical relationship to increased MFC and R2\* in the ADHD group than age. These findings suggest that while youth with ADHD appear to have less prominent age-related brain iron increases than that seen in typical development, normalization of brain iron levels may result from long-term psychostimulant treatment of ADHD.

#### 4.1. Disrupted iron homeostasis in ADHD

Iron homeostasis is a critical biological mechanism that is tightly regulated under physiological conditions (Anderson and Frazer, 2017; Duck and Connor, 2016). While required for virtually all basic cellular processes, iron is lethal to cells when unbound, especially in excess (Morris et al., 2018). Accordingly, intricate regulatory mechanisms for iron uptake, transport, usage and storage exist to ensure that iron supply meets the body's vast demands while preventing cell death (Morris et al., 2018). In the brain, iron is required for important neural processes implicated in ADHD including dopamine, noradrenaline and myelin synthesis and regulation (Beard and Connor, 2003). Maintaining iron homeostasis is essential for healthy brain development as low brain iron impairs catecholamine and myelin metabolism (Beard, 2003; Earley et al., 2014; Lozoff, 2011; Unger et al., 2007) and has been associated with developmental delay and cognitive deficits consistent with ADHD symptoms (McCann and Ames, 2007; Sidrak et al., 2014).

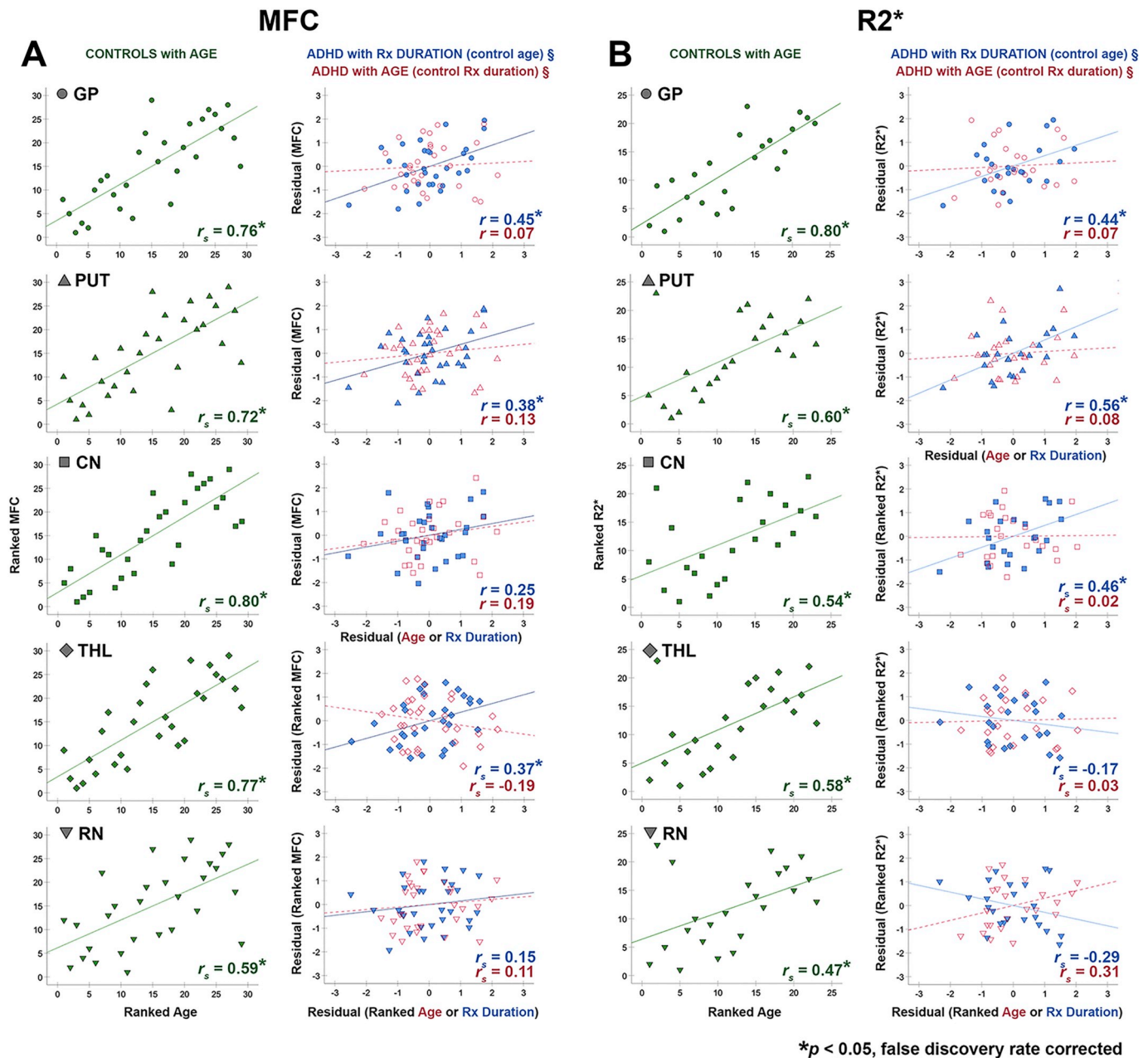
Indeed, growing evidence suggests that iron homeostasis may be disrupted in ADHD. Although the underlying mechanism are not understood, increased serum hepcidin (a master regulator of iron metabolism) (Yazici et al., 2019) and reduced brain iron levels (Adisetiyo et al., 2014; Cortese et al., 2012) have been detected in medication-naïve children with ADHD; variations in the hemochromatosis (HFE) gene (encodes for the iron uptake regulating HFE protein) (Nigg et al., 2016) and reduced serum ferritin (iron storage protein) (Tseng et al., 2018; Wang et al., 2017) have also been detected in studies of predominantly psychostimulant-medicated individuals with ADHD.

Moreover, iron status in toddlerhood has been shown to predict sensitivity to psychostimulants in children with ADHD, with severe iron deficiency linked to higher medication dosage within the first year of treatment (Turner et al., 2012). Worthy of note is the inconsistency of peripheral iron markers in ADHD (Donfrancesco et al., 2013; Tseng et al., 2018; Wang et al., 2017). Discrepant findings may reflect the indirect and complex relationship between iron levels in the periphery and brain (Duck and Connor, 2016), the sensitivity of serum measures to confounds (e.g., inflammation, appetite suppression) (Dignass et al., 2018) as well as genetic differences influencing brain iron uptake which have been observed in animal studies (Morse et al., 1999).

#### 4.2. Psychostimulants increase brain iron levels

Concordant with the literature on normal brain development (Beard and Connor, 2003; Hallgren and Sourander, 1958), we demonstrated that MFC and R2\* brain iron indices significantly increased with age in all regions examined in the control group. In the ADHD group, simple age correlations detected a similar positive correlation with MFC and R2\* in the GP, PUT and CN but to a lesser extent than simple correlations with psychostimulant medication duration, which detected positive correlations with MFC and R2\* in more regions (GP, PUT, CN and THL) and with greater statistical significance. However, as all the participants with ADHD had a history of psychostimulant treatment, interpretation of these simple correlation findings is limited due to the significant collinearity between age and psychostimulant treatment duration. After accounting for this collinearity, we confirmed that MFC and R2\* significantly increased with the duration of psychostimulant treatment in the GP, PUT, CN and THL even after controlling for age effects, whereas age no longer correlated with MFC or R2\* when controlling for treatment duration. While we cannot definitively conclude that brain iron indices do not vary with age in the ADHD group, these results support a stronger relationship between psychostimulant treatment duration and increased MFC and R2\*. Moreover, this psychostimulant medication effect was not detected in the RN control region, supporting the regional specificity of these results.

Along with reported changes in brain volume (Sobel et al., 2010; Nakao et al., 2011; Greven et al., 2015; Pretus et al., 2017), our findings add brain iron homeostasis to the list of neural processes likely altered by long-term psychostimulant treatment in ADHD. However, our supplementary volumetric analysis suggests that the long-term psychostimulant treatment effects on brain iron levels in basal ganglia regions



**Fig. 2.** Brain iron indices increased with age in controls but increased with medication duration in individuals with ADHD.

A. In the control group (green), magnetic field correlation (MFC) indices of brain iron significantly increased with age in the globus pallidus (GP), putamen (PUT), caudate nucleus (CN), thalamus (THL) and red nucleus (RN); Conversely, in the ADHD group, MFC significantly increased with psychostimulant medication (Rx) duration when controlling for age in the GP, PUT and THL (blue). This correlation was not observed with age when controlling for Rx duration (red). B. Similar results were found with R2\* relaxation rate indices of brain iron.  $r$ : Pearson's correlation,  $r_s$ : Spearman's correlation (ranked values plotted), §: partial correlations (residual values plotted). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

are unrelated to regional brain volume. Specifically, we found no significant correlations between regional brain volume with MFC or R2\* in the GP, PUT, CN and THL in either group (Supplementary Table S8). Additionally, regional volumes did not mediate the significant relationship between age and brain iron indices (MFC, R2\*) within these regions in controls (Supplementary Table S9). These supplementary findings, along with those from (Ersche et al., 2017), implicate potentially distinct mechanisms underlying changes in brain iron and volume during typical development and from long-term psychostimulant use.

Our findings are consistent with the growing literature that implicates elevated brain iron within basal ganglia regions with prolonged psychostimulant exposure to cocaine (Ersche et al., 2017; Adisetiyo et al., 2018) and methamphetamines (Melega et al., 2007). Given that

psychostimulants primarily modulate the dopaminergic system (Faraone, 2018; Spencer et al., 2015; Volkow et al., 2005), we speculate their long-term use may alter brain iron metabolism in basal ganglia regions via mechanisms linked to iron's role as a required cofactor for catecholamine synthesis and regulation (Daubner et al., 2011; Ramsey et al., 1996). Validation studies in animals and humans examining iron homeostasis and the dopaminergic system before and after long-term psychostimulant exposure are needed to elucidate the underlying mechanisms. Nonetheless, our findings suggest that long-term psychostimulant treatment may compensate for diminished age-related brain iron accumulation in ADHD that is observed in healthy brain development (Beard and Connor, 2003; Hallgren and Sourander, 1958).

#### 4.3. Strengths, limitations and future studies

To our knowledge, this is the first study to examine the effects of psychostimulant treatment duration on brain iron levels in children and adolescents with ADHD. There is one R2\* study of iron in the left ventral striatum of adults with ADHD (18–65 years old) that reported increased R2\* correlating with age but not medication duration (Sethi et al., 2017). However, this study was limited to a brain region with low MRI reliability for iron detection (proximity to nasal-tissue interface can introduce artifacts) and its focus on an adult ADHD population may capture a distinct window of disease trajectory, wherein brain iron accumulation are at the highest levels and reaches a plateau (Hallgren and Sourander, 1958). Reduced specificity of the R2\* metric for iron could also contribute to discrepant findings (Dusek et al., 2013). A strength of our study is that we conducted a multimodal brain iron assessment using the iron-sensitive R2\* metric and the more iron-specific MFC metric that is independent of some of the non-iron mechanisms affecting R2\* (Jensen et al., 2009, 2006; Jensen and Helpert, 2014). While both metrics correlate with putative postmortem iron concentration in healthy brain (Adisetiyo et al., 2012; Langkammer et al., 2010), variance in their sensitivity and specificity for iron in different brain regions have been demonstrated (Adisetiyo et al., 2018) and are reflected in our findings (Table 3). Confounds such as tissue microstructure, calcification and water and protein content affect R2\* and MFC differently (Brown et al., 2014; Dusek et al., 2013; Jensen and Helpert, 2014) which underscores the advantage of multimodal assessment of brain iron in that this approach capitalizes on the strengths of each modality for improved brain iron detection.

Study limitations are noted. Despite the moderate to large effect sizes of our findings, the limited sample size may have reduced statistical power. This may be reflected in our supplementary analysis of parental BRIEF ratings in the ADHD group, wherein correlations between high Behavioral Regulation Index scores and low MFC in the PUT and CN did not survive FDR correction (Supplementary Table S10). While this trend suggests a potential link between greater inhibitory control deficits and low striatal brain iron, future studies with larger cohorts and extensive measures of symptom severity are needed to confirm if brain iron and behavioral relationships exist. Also, as medication dose and non-psychostimulant medications were not systematically accounted for, their effects remain unclear. Nonetheless, potential non-psychostimulant medications effects were not confounding the results as similar findings were reproduced when ADHD patients with additional non-psychostimulant medication history were excluded in the supplementary analyses (Supplementary Tables S6 and S7). Additionally, while our study controlled for gender and comorbidity effects in the inclusion criteria, our findings are restricted to comorbidity-free males with ADHD and thus have limited generalizability to the broader ADHD population. Lastly, the cross-sectional design of this study prevents causal inferences about normalized brain iron in the ADHD group and given that all participants with ADHD had a history of psychostimulant treatment, the extent of aberrant age-related iron accumulation trajectories in ADHD and the impact of baseline brain iron levels prior to taking medication remain unknown.

#### 4.4. Conclusion

In conclusion, our results demonstrate that brain iron levels increase with the duration of psychostimulant treatment more prominently than with age in ADHD. While the results of this study are preliminary, they are consistent with the ADHD literature on long-term psychostimulant treatment effects on the brain that report alterations in the dopaminergic system (Fusar-Poli et al., 2012), GABAergic system (Solleveld et al., 2017), brain function (Battel et al., 2016), brain structure (Sobel et al., 2010; Nakao et al., 2011; Greven et al., 2015; Pretus et al., 2017; de Luis-García et al., 2015; Schwenen et al., 2016) and brain metabolites (Benamor, 2014). Together, these studies demonstrate that long-

term use of psychostimulant medications alter the brain in lasting ways beyond the acute single-dose effects. Elucidating the clinical significance of these long-term brain changes is imperative. If the degree of brain iron normalization corresponds with improved treatment outcomes, such evidence would inform clinicians to which patients are responding to treatment or could be used to monitor treatment adherence (Adler and Nierenberg, 2010; Rashid et al., 2018). Future longitudinal studies of medication-naïve individuals with ADHD before and after long-term psychostimulant treatment are needed to confirm this speculation, with consideration to medication onset (i.e., childhood versus adulthood), heterogeneity of ADHD profiles and treatment response. More critically, future studies are needed not only to clarify whether alterations in brain iron from long-term psychostimulant treatment are associated with improved outcomes but also whether such changes are detrimental in cases of misdiagnosis and improper prescription of psychostimulants.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.nicl.2019.101993>.

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