The effects of acute Fluoxetine administration on temporal discounting in youth with ADHD

Christina O Carlisi[1], Kaylita Chantiluke, PhD [1], Luke Norman[1], Anastasia Christakou, PhD[1], Nadia Barrett, MRCPsych[2], Vincent Giampietro, PhD[3], Michael Brammer, PhD[3], Andrew Simmons, PhD[3,4], Katya Rubia, PhD[1]

[1]Dept of Child & Adolescent Psychiatry, Institute of Psychiatry, Psychology and Neuroscience, King's College London, [2]South London and Maudsley NHS Foundation Trust, [3]Dept of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience, King's College London, [4]NIHR Biomedical Research Centre for Mental Health at South London and Maudsley NHS Foundation Trust,

Correspondence to:

Prof Katya Rubia

Department of Child Psychiatry/MRC Center for Social, Genetic and Developmental Psychiatry (SGDP), PO46, Institute of Psychiatry, 16 De Crespigny Park, London, SE5 8AF, UK

Tel.: 0044-207-8480463

Fax: 0044-207-2085800

Email: katya.rubia@kcl.ac.uk

Word count: 4500

Abstract: 245

Tables: 3

Figures: 2

Supplementary Material: 1

Acknowledgements: CC was supported by an NIHR BRC PhD studentship, and LN was supported by an MRC-IOP excellence PhD studentship.

Financial Support: This work was supported by the UK Department of Health via the National Institute for Health Research (NIHR) Biomedical Research Centre (BRC) for Mental Health at South London and the Maudsley NHS Foundation Trust and Institute of Psychiatry, Psychology and Neuroscience, King's College London.

Declaration of Interest: K.R. has received funding from Lilly for another project and consultancy fees from Lilly, Shire, and Novartis. MB is a consultant for P1 Vital, Ltd, Oxford, UK. The other authors have no conflict of interests to declare.

The authors assert that all procedures contributing to this work comply with the ethics standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

ABSTRACT

Background

Serotonin is under-researched in Attention-Deficit/Hyperactivity Disorder (ADHD), despite accumulating evidence for its involvement in impulsiveness and the disorder. Serotonin furthermore modulates temporal discounting (TD), which is typically abnormal in ADHD relative to healthy subjects, underpinned by reduced fronto-striato-limbic activation. This study tested whether a single acute dose of the selective serotonin reuptake inhibitor (SSRI) fluoxetine upregulates and normalises reduced fronto-striato-limbic neurofunctional activation in ADHD during TD.

Methods

Twelve boys with ADHD were scanned twice in a placebo-controlled randomised design under either fluoxetine (between 8-15mg, titrated to weight) or placebo while performing an individually adjusted functional magnetic resonance imaging (fMRI) TD task. Twenty healthy controls were scanned once. Brain activation was compared within patients under either drug condition and compared to controls to test for normalisation effects.

Results

Repeated-measures whole-brain analysis within patients revealed significant upregulation with fluoxetine in a large cluster comprising right inferior frontal cortex, insula, premotor cortex and basal ganglia, which furthermore correlated trend-wise with TD performance, which was impaired relative to controls under placebo, but normalised under fluoxetine. Fluoxetine furthermore downregulated default mode areas of posterior cingulate and precuneus. Comparisons between controls and patients under either drug condition revealed normalization with fluoxetine in right premotor-insular-parietal activation, which was reduced in patients under placebo.

Conclusions

The findings show that a serotonin agonist upregulates activation in typical ADHD dysfunctional areas in right inferior frontal cortex, insula and striatum as well as downregulating default mode network regions in the context of impulsivity and TD.

INTRODUCTION

Attention-Deficit/Hyperactivity Disorder (ADHD) is defined as age-inappropriate inattention and/or hyperactivity/impulsiveness (APA, 2013). It is one of the most common neurodevelopmental disorders with around 5% prevalence worldwide (Polanczyk *et al.*, 2014). ADHD patients have deficits in executive functions (EF) such as inhibition, attention and working memory (Willcutt *et al.*, 2008), underpinned by abnormalities in fronto-striatal, fronto-temporo-parietal and fronto-cerebellar networks (Hart *et al.*, 2012, Hart *et al.*, 2013, Rubia *et al.*, 2014a). Furthermore, they have deficits in timing functions (Noreika *et al*., 2013) and in "hot" EF, referring to EF involving motivation and affect such as reward-related decision-making (Kerr and Zelazo, 2004), as measured by temporal discounting (TD) and gambling tasks (Noreika *et al.*, 2013, Rubia *et al.*, 2009). Nonetheless, there is heterogeneity in cognitive impairments, with some patients not showing impairments or only in some cognitive domains which are likely underpinned by different pathophysiological pathways (Nigg *et al.*, 2005, Sonuga-Barke, 2003, Sonuga-Barke *et al.*, 2010).

TD tasks require choices between small immediate and larger delayed rewards and measure the extent to which a reward is subjectively discounted when delayed in time, i.e., the sensitivity to temporal delays measured in units of reward (Rubia *et al.*, 2009). The ability to inhibit immediate rewards and wait for larger future rewards depends on well-developed frontal lobe-mediated motivation control and temporal foresight and is key for mature decision making. TD matures with age (Christakou *et al.*, 2011) and varies between individuals, with steeper TD, i.e., more rapidly decaying rates of reward discounting with increasing time (Richards *et al.*, 1999), in more impulsive subjects (Noreika *et al.*, 2013, Rubia *et al.*, 2009). In individually-adjusted TD paradigms (Christakou *et al.*, 2011, Richards *et al.*, 1997), the immediate reward is adjusted using an algorithm based on previous choices for different delays, converging towards the value of the participant's subjective equivalent of the fixed delayed reward (Richards *et al.*, 1999). From this, a typically hyperbolic delay discounting function is calculated , the steepness of which indicates the individual TD rate, which is associated with impulsivity (Critchfield and Kollins, 2001, Richards *et al.*, 1999).

ADHD patients are impaired in TD tasks (Noreika *et al.*, 2013), with, however, some negative findings, mostly in non-individually-adjusted task versions (Scheres *et al.*, 2006, Scheres *et al.*, 2010, Sonuga-Barke *et al.*, 1992). FMRI studies of TD in healthy adults implicate ventromedial-fronto-limbic networks of reward-based decision-making and dorsolateral and inferior-fronto-insula-striato-parietal networks of temporal foresight (Christakou *et al.*, 2011, Wesley and Bickel, 2014). Despite documented TD deficits in ADHD, few fMRI studies have investigated its neurofunctional correlates. ADHD adolescents showed underactivation relative to controls during delayed choices in an adjusted

fMRI TD task in inferior frontal cortex (IFC), insula, striatal and cerebellar regions (Rubia *et al.*, 2009) and significantly weaker correlations between better TD and activation during delayed choices in IFC, superior temporal lobes, insula, supplementary motor area and cerebellum (Chantiluke *et al.*, 2014d). In adult ADHD, abnormal striato-limbic activation has been observed (Plichta *et al.*, 2009).

Neurotransmitters such as serotonin (5-HT) are implicated in ADHD (Oades, 2007, 2008), potentially via modulation of these neural circuits. Converging evidence across methodologies shows that serotonergic systems may be dysfunctional in ADHD (Oades, 2007), with evidence for chemical alterations of 5-HT systems, decreased 5-HT platelet levels (Spivak *et al.*, 1999), and increased ADHD-related behaviour after 5-HT depletion in ADHD patients (Zepf *et al.*, 2010). Second, there is evidence for an association between 5-HT-related polymorphisms and ADHD (Gizer *et al.*, 2009, Rommelse *et al.*, 2010) and methylphenidate treatment response (McGough *et al.*, 2009). Also, the selective serotonin reuptake inhibitor (SSRI) fluoxetine has been shown to be effective in reducing ADHD-related symptoms in children (Barrickman *et al.*, 1991, Quintana *et al.*, 2007) and to improve the efficacy of stimulants in human and animal studies (Findling, 1996, Gainetdinov *et al.*, 1999, Gammon and Brown, 1993). Furthermore, the concurrent administration of 5-HT and dopamine amino-acid precursors can improve ADHD symptoms (Hinz *et al.*, 2011). However, replication is needed as these studies are limited by comorbid samples (Quintana *et al.*, 2007) and non-randomised trials in small samples (Barrickman *et al.*, 1991). Last, in healthy adults, tryptophan depletion, which reduces brain 5-HT by up to 60%, downregulates activation in key ADHD deficit areas of IFC and basal ganglia (Lamar *et al.*, 2014, Lamar *et al.*, 2009, Rubia *et al.*, 2005), which are upregulated with serotonin agonists (Del-Ben *et al.*, 2005). Also, 5-HT has been implicated in reward-based decision-making in healthy adults (Cools *et al.*, 2011, Robinson *et al.*, 2012, Rogers, 2011), where striatal 5-HT levels have been shown to modulate choices of longer, delayed rewards (Doya, 2008, Schweighofer *et al.*, 2007, Tanaka *et al.*, 2007).

In conclusion, there is evidence that 5-HT is associated with ADHD, with impulsivity, in particular TD performance, and that it modulates IFC-striatal activation, a key ADHD deficit. Despite this, hardly any fMRI studies have tested the effects of serotonin agonists on brain function in ADHD. We have previously shown that in ADHD children, the SSRI fluoxetine versus placebo upregulated and normalised IFC-striatal and parietal underactivation during inhibition (Chantiluke *et al.*, 2014a, Chantiluke *et al.*, 2014c), and enhanced the deactivation in default mode network (DMN) regions during working memory (Chantiluke *et al.*, 2014b).

This study therefore aimed to investigate the effect of a single dose of fluoxetine relative to placebo on brain activation in ADHD adolescents during a TD task. Furthermore, to test for potential

normalisation effects of fluoxetine on abnormal brain activation in ADHD patients under placebo, we also compared brain activation during both drug conditions to that of healthy adolescents.

Based on previous findings, we hypothesized that ADHD adolescents under placebo would show steeper TD rates (Noreika *et al.*, 2013) and reduced IFC-insular-striatal activation during TD (Chantiluke *et al.*, 2014d, Plichta *et al.*, 2009, Rubia *et al.*, 2009). Furthermore, based on our fMRI studies showing upregulation and normalisation with fluoxetine in task-relevant regions during related tasks of cognitive control in ADHD (Chantiluke *et al.*, 2014a, Chantiluke *et al.*, 2014b, Chantiluke *et al.*, 2014c), and evidence for 5-HT modulation of IFC-striatal regions in healthy adults (Lamar *et al.*, 2009, Rubia *et al.*, 2005), we hypothesized that fluoxetine would upregulate IFC-insula-striato-parietal activation within patients and normalise regional underactivation relative to controls.

METHODS

Participants

Thirty-two right-handed (Oldfield, 1971) boys with (N=12) and without ADHD (N=20) were recruited from local clinics and support groups, aged 11-17 years, with IQ≥70 measured by the Wechsler Abbreviated Scale of Intelligence-Revised (WASI-R) short form (Wechsler, 1999). ADHD boys had a clinical DSM-IV diagnosis of non-comorbid ADHD, inattentive/hyperactive-impulsive combined subtype, assessed using the standardized Maudsley diagnostic interview (Goldberg and Murray, 2006). Patients scored above clinical threshold for ADHD symptoms on the Strengths and Difficulties Questionnaire (SDQ; (Goodman and Scott, 1999)) and the Conner's Parent Rating Scale-Revised (CPRS-R; (Conners *et al.*, 1998)). They also scored below clinical threshold for ASD on the Social Communication Questionnaire (SCQ; (Rutter *et al.*, 2003)). Nine ADHD boys were on psychostimulants but withheld medication for 48 hours prior to scanning.

Patients were scanned twice in a randomised, double-blind, placebo-controlled design. Due to the half-life of fluoxetine (1-3 days) and its metabolite norfluoxetine (5-16 days) (Wong *et al.*, 1995), scans were conducted 3-4 weeks apart. To ensure the fluoxetine dose had reached peak plasma levels (after 5-8 hours (Wong *et al.*, 1995)), patients were scanned 5 hours post-administration. Liquid fluoxetine was titrated to age and weight: boys 10-13 years and <30kg received 8mg, those >30kg received 10mg. Boys 14-17 years and <30kg received 10mg, and those >30kg received 15mg. Placebo was equivalent volumes of peppermint water, similar in taste and appearance to fluoxetine.

Twenty healthy age and handedness-matched control boys were recruited locally by advertisement and scanned once. Controls scored below clinical threshold on the SDQ, SCQ and CPRS-R and did not have any psychiatric condition.

Exclusion criteria for all participants were neurological disorders, drug/alcohol dependency and MRI contraindications.

The study was conducted according to the latest version of the Declaration of Helsinki. Ethical approval was obtained from the local Research Ethics Committee. Study details were explained to both child and guardian, and written informed consent was obtained for all participants.

Temporal Discounting fMRI paradigm

Prior to the first scan, subjects practiced the 12 min task once in a 'mock' scanner. Subjects choose by pressing a left or right button between receiving a small amount of money immediately (£0-£100) or £100 in one week, month or year. Delay choices (20 trials of each delay length) were randomised, but the delayed option was consistently displayed on the right side, and the variable immediate choices on the left side of the screen to minimize potential sensorimotor mapping effects. Choices were displayed for 4s, followed by a blank screen of at least 8s (inter-trial-interval: 12s). The amount of immediate reward was adjusted through an algorithm based on previous choices which was calculated separately for each of the three delays. This narrows the range of values, converging into an indifference point where the immediate reward is considered by the subject to be equivalent to the delayed amount for the given delay (Christakou *et al.*, 2011, Rubia *et al.*, 2009). This algorithm ensures equal numbers of immediate and delayed choices to be contrasted in the fMRI analysis.

Analysis of Performance Data

To estimate the steepness of TD for each subject, the indifference value between the immediate amount and the delayed £100 for each delay was calculated, equivalent to the subject's subjective value of £100 after each delay, and defined as the midpoint between the lowest immediate reward chosen by the subject and the next lowest immediate reward available (i.e. the value of the immediate reward offered at which point the subject began to instead consistently choose the delayed reward) (Christakou *et al.*, 2011, Rubia *et al.*, 2009).

Reward is typically discounted as a hyperbolic decay function depending on amount, delay and a free impulsiveness indicator *"k"*, calculated by fitting a hyperbolic function to the indifference values for each delay (see Supplement).

However, the limitations of the fMRI task adaption, i.e. relatively few trials and only three delay points, limit the goodness-of-fit of the data to a non-linear curve function. In addition, the distribution of *k*-values was not normal, skewed by low-frequency and high-value outliers. Thus, TD was measured using the area under the curve (AUC) which is more appropriate for investigations with quantitative, inferential statistics (Myerson *et al.*, 2001). The normalized subjective values of the delayed £100 for each delay were plotted against the normalized delays and AUC of these plots were calculated for each participant, using this obtained value as the main dependent variable. AUC correlated inversely with *k*-values ($r=-0.898$, $p<0.001$) whereby smaller AUC values denote steeper discounting rates, indicating increased choice impulsivity.

A repeated-measures within-group analysis of variance (ANOVA) was conducted within patients with medication condition (placebo, fluoxetine) as within-subjects variable to test for medication effects on TD. Two ANOVAs were conducted with group as independent variable and area under the curve (AUC) as dependent measure to test for differences in TD performance between controls and ADHD patients on either placebo or fluoxetine. To test for potential main effects of drug administration order and of an interaction between order and drug condition, order was included as a between-subjects factor in the repeated-measures ANOVA.

fMRI Image Acquisition

Gradient-echo echo-planar MR imaging (EPI) data were acquired at King's College London, Institute of Psychiatry's Centre for Neuroimaging Sciences on a 3T General Electric SIGNA HDx MRI scanner (GE Healthcare, UK). For details of scan acquisition, see Supplement.

fMRI Image Analysis

Event-related activation data were acquired in randomized trial presentation and analysed using the non-parametric XBAM software package developed at the Institute of Psychiatry, King's College London (www.brainmap.co.uk; (Brammer *et al.*, 1997)). The individual and group-level analyses methods are described in detail elsewhere (Brammer *et al.*, 1997, Bullmore *et al.*, 1999a, Bullmore *et al.*, 2001, Bullmore *et al.*, 1999b, Cubillo *et al.*, 2014b) and in the online Supplementary Material.

ANCOVA of within-patient medication effects

To investigate medication effects on brain activation within the ADHD group, a within-group repeated-measures analysis of covariance (ANCOVA) with motion as covariate and medication condition as within-subjects factor was conducted using randomization-based testing for voxel or cluster-wise differences, as described elsewhere (Bullmore *et al.*, 2001, Bullmore *et al.*, 1999b) and in the supplementary material. Voxel- and cluster- level statistical thresholds were set so as to obtain less than one false positive 3D cluster per map ($p<0.05$ was used for voxel and $p<0.005$ for cluster comparisons). The standardised BOLD response values for each participant were extracted for each of the significant clusters of the ANCOVA analyses and plotted to determine the direction of effects. Repeated-measures ANOVAs on the extracted BOLD response measures were conducted within patients to test for potential effects of scan-order and interactions between scan order and drug condition.

ANCOVA of between-group effects

One-way ANCOVAs with group as main factor and motion as covariate were conducted using randomization-based testing to test for case-control differences under placebo or fluoxetine (Bullmore *et al.*, 2001, Bullmore *et al.*, 1999b). For these comparisons, $p<0.05$ (voxel-level) and $p<0.05$ (cluster-level) were used. Standardized BOLD responses were then extracted from significant clusters for each participant and plotted to determine direction of effects.

Correlations with behaviour and IQ

To examine whether clusters which showed group effects in case-control comparisons were related to IQ or TD, the BOLD response in these clusters was extracted for each participant and Pearson correlations were performed with IQ and AUC within each group (ADHD placebo, ADHD fluoxetine, controls).

RESULTS

Participant characteristics

Univariate ANOVA revealed no significant group differences in age, but IQ, which was lower in ADHD (Table 1). However, since low IQ is associated with ADHD (Bridgett and Walker, 2006), IQ was not covaried as covarying for differences between groups that were not randomly selected violates ANCOVA assumptions (Miller and Chapman, 2001). Nonetheless, to assess potential effects of IQ on case-control comparisons, BOLD responses were correlated with IQ and analyses were

repeated covarying for IQ. As expected, patients had significantly lower CPRS-R *t*-scores, SDQ and SCQ scores than controls (Table 1).

Insert Table 1

Performance data

For repeated-measures ANOVA within-patients, no significant drug effects were found on AUC [F(df=1,11)=0.08, p=n.s.], reaction time (RT) [F(df=1,11)=0.08, p=n.s.] or omission errors (OM)[F(df=1,7=0.44), p=n.s.] (see Table 1). Case-control ANOVAs showed no differences in RT or OM, but controls had larger AUC than patients under placebo [F(df=1,30)=4, p<0.05] but no longer differed from patients under fluoxetine [F(df=1,30)=2, p=n.s.], suggesting that fluoxetine normalized case-control performance differences (see Table 1).

Drug administration order had no main effect on the primary behavioral outcome of AUC in the ADHD group [F(df=1,10)=.07, p=n.s.] and there was no interaction between scan order and drug condition [F(df=1,10)=1.31, p=n.s.].

fMRI data

Motion

No differences were found for largest head displacement in 3-dimensional space within the ADHD group under each drug condition [F(df=2,10)=0.51, p=n.s.]. Also, no group-by-displacement interaction was found between controls and ADHD under placebo [F(df=2,29)=2.63, p=n.s.] or fluoxetine [F(df=2,29)=2.54, p=n.s]. Nevertheless, to exclude potential effects of non-significant motion, motion parameters in 3D-Euclidian space were included as covariates in the fMRI analyses.

Group brain activation maps for delayed–immediate choices

For the contrast of delayed minus immediate choices, controls showed activation in dorsomedial PFC (dmPFC) and anterior cingulate cortex (ACC), insula, pre- and post-central gyri and parieto-occipital and cerebellar regions. ADHD patients on placebo showed activation in ACC, pre- and post-central gyrus, posterior cingulate (PCC), and occipito-cerebellar regions, while under fluoxetine they showed activation in right dorsolateral and inferior PFC (DLPFC/IFC)/ insula extending into basal ganglia (BG), ACC, temporo-parietal and occipital cortices and cerebellum (see Supplementary Material and Figure S1).

Within-group differences between ADHD patients on placebo versus fluoxetine

Repeated-measures ANCOVA revealed a significant drug effect in a large cluster comprising right IFC, insula, precentral and superior temporal cortices extending into BG, which was enhanced under fluoxetine relative to placebo (Figure 1, Table 2A). Post-hoc calculations in SPSS indicated an observed power of 89% (partial η^2=0.53). Activation in IFC was significantly negatively correlated in the placebo group with AUC (r=-0.676, p<0.016). Under Fluoxetine, however, the correlation was at a trend-level positive (r=0.563, p=0.057).

Under placebo relative to fluoxetine, patients had enhanced activation during delayed-immediate choices in two clusters, one comprising bilateral cerebellar hemispheres and vermis, PCC, precuneus and occipital lobe, and the other in left pre- and postcentral gyrus, extending into middle frontal gyrus and inferior parietal lobe (IPL) (Figure 1, Table 2B). Furthermore, activation in the cerebellum/PCC/precuneus cluster was negatively correlated with the IFC cluster that was upregulated under fluoxetine (r=-0.859, p<0.001).

Drug administration order had no effect on within-group differences in BOLD response [right IFC: *F*(df=1,10)=2.8, *p*=n.s.; cerebellum/occipital:*F*(df=1,10)=.40, *p*=n.s.; left pre/postcentral gyrus:*F*(df=1,10)=.88, *p*=n.s.], and there was no interaction between drug administration order and condition [IFC:*F*(df=1,10)=0.07, *p*=n.s.; cerebellum/occipital:*F*(df=1,10)=0.15, *p*=n.s.; left pre/postcentral gyrus:*F*(df=1,10)=.75, *p*=n.s.].

Insert Figure 1 + Legend and Table 2

Between-group differences

Controls versus ADHD patients on placebo

Between-group ANCOVA showed significantly increased activation in controls relative to ADHD under placebo for delayed–immediate choices in 3 right-hemispheric clusters comprising right pre- and postcentral gyri, extending into IPL and insula. Patients on placebo showed increased activation relative to controls in left anterior cerebellum/occipital lobe (Table 3A, Fig 2A). No significant correlations were observed between extracted BOLD response from these clusters and IQ. Furthermore, ANCOVA with IQ as covariate showed that all significant clusters remained with the exception of right insula. No correlations were observed between AUC and extracted BOLD response in any clusters.

Controls vs. ADHD patients on fluoxetine

Controls relative to ADHD patients on fluoxetine showed enhanced activation in left pre- and postcentral gyri reaching into IPL. No clusters were increased in ADHD relative to controls (Table 3B, Fig 2B). Thus, the right hemispheric clusters which were enhanced in activation in controls relative to ADHD under placebo were no longer observed, suggesting that fluoxetine normalized these activation differences. No significant correlations were observed between extracted BOLD response and IQ. ANCOVA with IQ as covariate showed that all significant clusters remained. No correlations were found between AUC and extracted BOLD response in significant clusters.

Insert Figure 2 + Legend and Table 3

DISCUSSION

Behaviourally, an acute dose of fluoxetine normalised TD abnormalities in ADHD relative to controls. At the brain level, within patients, fluoxetine relative to placebo significantly upregulated activation in a large right-hemispheric IFC-premotor-insular-striatal cluster, which correlated trend-wise with better TD. Fluoxetine additionally down-regulated activation in presumably default mode network activations in PCC/precuneus and in pre and postcentral gyrus/IPL as well as cerebellum. Relative to controls, patients on placebo showed underactivation in right insula, pre/postcentral gyrus and IPL but enhanced activation in left anterior cerebellum/PCC. Fluoxetine normalized all case-control differences, due to upregulation/downregulation of these regions within patients, but lead to underactivation in left-hemispheric pre/postcentral gyrus/IPL in ADHD relative to controls, due to downregulation of this activation within patients.

Fluoxetine relative to placebo upregulated a large right-lateralized cluster in ADHD comprising IFC, premotor cortex, insula and BG, which was associated trend-wise with better TD. This right IFC-insular-striatal network comprises key regions for TD important for integrating external information with internal value representations to support goal-directed EF (Christakou *et al.*, 2009, Christakou *et al.*, 2013a, Rubia *et al.*, 2009, Wesley and Bickel, 2014, Wittmann *et al.*, 2007). Right IFC is a key cognitive control hub region, crucial for inhibiting immediate reward choices as well as for inter-temporal bridging and future reward representation (Radua *et al.*, 2014, Wiener *et al.*, 2010). The BG are linked to a reward-valuation network that mediates reinforcement learning, reward-processing and inter-temporal bridging (Koch *et al.*, 2009, Peters and Büchel, 2011,

Wittmann *et al.*, 2007) while the insula plays a role in future reward-value representation as well as timing functions including temporal foresight (Carter *et al.*, 2010, Radua *et al.*, 2014, Wesley and Bickel, 2014, Wiener *et al.*, 2010). In particular right hemispheric IFC, insula and BG have been shown to be consistently hypoactivated in ADHD in meta-analyses of EF tasks (Cortese *et al.*, 2012, Hart *et al.*, 2013), including TD (Chantiluke *et al.*, 2014d, Rubia *et al.*, 2009). Right IFC underactivation has furthermore been shown to be disorder-specific relative to other childhood disorders such as conduct and obsessive-compulsive disorder (Norman *et al.*, 2015, Rubia, 2011, Rubia *et al.*, 2014a). In this study, we only observed underactivation in ADHD relative to healthy adolescents in insula and premotor regions, rather than in IFC and basal ganglia, presumably due to low power.

Given that our recent meta-analysis findings of a consistent upregulation with stimulants of right IFC, insula and BG activation in ADHD (Rubia *et al.*, 2014b), the findings suggest that serotonin agonists may have comparable upregulatory effects to stimulants. In fact, the upregulated region in VLPFC reaching into anterior insula, putamen and superior temporal lobe is in a very similar location to the cluster observed in our meta-analysis of methylphenidate effects on ADHD brain function (Talairach coordinates: 42,20,-12), with a sizeable effect size of 1 relative to the meta-analytic effect size of 1.5 (Rubia *et al.*, 2014b). Furthermore, it is strikingly similar to the upregulated IFC location in our fMRI studies of methylphenidate effects on inhibition and timing, with effect sizes of .7 and .2 respectively (Cubillo *et al.*, 2014b, Rubia *et al.*, 2014b, Smith *et al.*, 2013). The finding of right IFC-striatal upregulation together with normalisation of behavioural TD deficits extends previous evidence for modulation of behavioural TD rates with 5-HT (Schweighofer *et al.*, 2008, Schweighofer *et al.*, 2007) and of IFC-striatal activation with 5-HT modulators such as tryptophan depletion and SSRIs in healthy adults (Del-Ben *et al.*, 2005, Lamar *et al.*, 2009, Rubia *et al.*, 2005) to the ADHD population. It also extends our previous findings in ADHD that fluoxetine enhances and normalises frontal activation during other impulsiveness-related functions such as IFC-striatal regions during inhibition (Chantiluke *et al.*, 2014c) and DLPFC during working memory (Chantiluke *et al.*, 2014b). The findings of right IFC modulation suggest that indoleamine agonists have similar effects to catecholamine agonists on ADHD brain function, given that not only methylphenidate but also atomoxetine upregulated right IFC activation during inhibition and timing (Cubillo *et al.*, 2014a, Cubillo *et al.*, 2014b, Smith *et al.*, 2013).

The fact that fluoxetine normalised both, underactivation in right pre/postcentral gyri, insula and IPL *and* behavioural TD deficits in ADHD is in line with the role of lateral fronto-insular-striato-parietal circuitry in intertemporal choice (Bickel *et al.*, 2009, McClure *et al.*, 2004, Xu *et al.*, 2009) and for the modulation of these regions by 5-HT (Cools *et al.*, 2011, Long *et al.*, 2009, Robinson *et al.*,

2012, Rogers, 2011). Apart from fronto-insular-striatal regions, IPL are also consistently underactivated in ADHD during EF (Cortese *et al.*, 2012, Hart *et al.*, 2012, Hart *et al.*, 2013). We have found left IPL underactivation to be normalised in ADHD with fluoxetine during inhibition (Chantiluke *et al.*, 2014c). The upregulation and normalization with fluoxetine of insula, pre/postcentral and IPL deficits in ADHD thus provides promising novel evidence for modulatory effects of serotonin agonists on typically dysfunctional fronto-insular-parietal systems in ADHD.

The downregulation of PCC/precuneus under fluoxetine versus placebo likely reflects deactivation of the DMN, comprised of ACC, precuneus and PCC, thought to represent mind-wandering, and which is typically anti-correlated with task-positive regions as it needs to be switched off during cognitive effort (Northoff *et al.*, 2010). This is reinforced by the negative correlation under placebo of this cluster with the IFC activation. There is accumulating evidence that the DMN is insufficiently deactivated and anticorrelated with task-positive activation in ADHD (Christakou *et al.*, 2013b), leading to enhanced mind-wandering, poor attention, EF and timing functions. We have found a similar effect of fluoxetine enhancing the deactivation of PCC during working memory in ADHD patients (Chantiluke *et al.*, 2014b), which we also observed with methylphenidate and atomoxetine (Cubillo *et al.*, 2014a). The finding suggests that fluoxetine, like catecholamine agonists (Rubia *et al.*, 2014b), can strengthen the weak deactivation of the DMN in ADHD, presumably improving mind wandering. Given that the key functional deficits in ADHD are both reduced activation in key fronto-striato-parietal networks mediating EF as well as a reduced deactivation of the DMN (Rubia *et al.*, 2014a), the findings suggest that a 5-HT agonist positively modulates both “task-positive” as well as “task-negative” activation deficits of not switching off the DMN.

5-HT is relatively ubiquitous in the brain. However, 5-HT modulates specifically ADHD-relevant impulsivity-related functions mediated by ventrolateral-prefrontal regions which are dependent on 5-HT input such as inhibitory control and reward-related decision making (Dalley and Roiser, 2012). The upregulation with a 5-HT agonist of key right-hemispheric IFC-striatal activation that is typically abnormal in ADHD suggests that abnormal 5-HT may be underlying abnormal activation in these networks and not just catecholamine systems, in line with accumulating evidence of a role of 5-HT in ADHD (Barrickman *et al.*, 1991, Gizer *et al.*, 2009, Hinz *et al.*, 2011, McGough *et al.*, 2009, Oades, 2008, Quintana *et al.*, 2007, Rommelse *et al.*, 2010, Spivak *et al.*, 1999, Zepf *et al.*, 2010). However, it cannot be ruled out that fluoxetine had no indirect effects on other neurotransmitter systems which are known to be influenced by 5-HT such as dopamine, acetylcholine and other monoamines (Bymaster *et al.*, 2002, Mongeau *et al.*, 1997, Oades, 2008). All

3 main monoamine systems are likely to interact in a concerted manner to mediate impulsiveness-relevant functions (Dalley and Roiser, 2012).

The strength of this study is a carefully selected non-comorbid ADHD group. Limitations are a relatively small patient sample size and the fact that for ethical and financial considerations, the control group was scanned only once while patients were scanned twice. However, the randomisation accounted for potential training effects, and order did not affect the results. The significantly lower IQ in the ADHD group, typical for the population, is a limitation, in particular because IQ impacts upon decision making (Toplak *et al.*, 2010).However, covariance and correlation findings did not suggest that IQ confounded group differences. Finally, long-term stimulant use affects brain function and structure, so deficit findings may have been mitigated by the majority of patients taking stimulant medication (Hart *et al.*, 2012, Rubia *et al.*, 2014a).

Conclusions

A single fluoxetine dose in ADHD upregulated activation in key right IFC-premotor-insular-striatal circuitry that mediates TD and which correlated trend-wise with better TD, and enhanced the deactivation of posterior DMN regions. Moreover, fluoxetine, via upregulation of these right hemispheric regions, normalized underactivation in ADHD under placebo relative to controls in right premotor-insular-parietal areas and behavioural TD abnormalities. The findings show for the first time that a serotonin agonist can modulate right IFC -insular-striato-parietal neural mechanisms underlying poor temporal foresight in ADHD. While the study aim was to clarify the mechanism of action of an acute dose of fluoxetine, which has the advantage of revealing true drug effects not confounded by indirect symptom improvements after chronic administration, future studies need to assess longer-term effects, as clinical behavioural changes are typically observed after weeks of administration. Longer-term SSRI administration has been shown to lead to downregulation of 5-HT1a receptors and serotonin transporters (Lesch *et al.*, 1991) and may well have different effects on brain function than acute doses, which are clinically more informative.

REFERENCES

APA (2013). *Diagnostic and statistical manual of mental disorders*. American Psychiatric Publishing: Arlington, VA.

Barrickman, L., Noyes, R., Kuperman, S., Schumacher, E. & Verda, M. (1991). Treatment of ADHD with fluoxetine: A preliminary trial. *Journal of the American Academy of Child & Adolescent Psychiatry* **30**, 762-767.

Bickel, W. K., Pitcock, J. A., Yi, R. & Angtuaco, E. J. C. (2009). Congruence of BOLD Response across Intertemporal Choice Conditions: Fictive and Real Money Gains and Losses. *The Journal of Neuroscience* **29**, 8839-8846.

Brammer, M. J., Bullmore, E. T., Simmons, A., Williams, S. C. R., Grasby, P. M., Howard, R. J., Woodruff, P. W. R. & Rabe-Hesketh, S. (1997). Generic brain activation mapping in functional magnetic resonance imaging: A nonparametric approach. *Magnetic Resonance Imaging* **15**, 763-770.

Bridgett, D. J. & Walker, M. E. (2006). Intellectual functioning in adults with ADHD: a meta-analytic examination of full scale IQ differences between adults with and without ADHD. *Psychological Assessment* **18**, 1-14.

Bullmore, E., Brammer, M., Rabe-Hesketh, S., Curtis, V., Morris, R., Williams, S., Sharma, T. & McGuire, P. (1999a). Methods for diagnosis and treatment of stimulus-correlated motion in generic brain activation studies using fMRI. *Human Brain Mapping* **7**, 38-48.

Bullmore, E., Long, C., Suckling, J., Fadili, J., Calvert, G., Zelaya, F., Carpenter, T. A. & Brammer, M. (2001). Colored noise and computational inference in neurophysiological (fMRI) time series analysis: resampling methods in time and wavelet domains. *Human Brain Mapping* **12**, 61-78.

Bullmore, E. T., Suckling, J., Overmeyer, S., Rabe-Hesketh, S., Taylor, E. & Brammer, M. J. (1999b). Global, voxel, and cluster tests, by theory and permutation, for a difference between two groups of structural MR images of the brain. *Medical Imaging, IEEE Transactions on Medical Imaging* **18**, 32-42.

Bymaster, F. P., Zhang, W., Carter, P. A., Shaw, J., Chernet, E., Phebus, L., Wong, D. T. & Perry, K. W. (2002). Fluoxetine, but not other selective serotonin uptake inhibitors, increases norepinephrine and dopamine extracellular levels in prefrontal cortex. *Psychopharmacology* **160**, 353-361.

Carter, R. M., Meyer, J. R. & Huettel, S. A. (2010). Functional neuroimaging of intertemporal choice models. *Journal of Neuroscience, Psychology, and Economics* **3**, 27-45.

Chantiluke, K., Barrett, N., Giampietro, V., Brammer, M., Simmons, A., Murphy, D. G. & Rubia, K. (2014a). Inverse Effect of Fluoxetine on Medial Prefrontal Cortex Activation During Reward Reversal in ADHD and Autism. *Cerebral Cortex*.

Chantiluke, K., Barrett, N., Giampietro, V., Brammer, M., Simmons, A. & Rubia, K. (2014b). Disorder-dissociated effects of fluoxetine on brain function of working memory in attention deficit hyperactivity disorder and autism spectrum disorder. *Psychological Medicine* **45**, 1195-1205.

Chantiluke, K., Barrett, N., Giampietro, V., Santosh, P., Brammer, M., Simmons, A., Murphy, D. & Rubia, K. (2014c). Inverse fluoxetine effects on inhibitory brain activation in non-comorbid boys with ADHD and with ASD. *Psychopharmacology*, 1-12.

Chantiluke, K., Christakou, A., Murphy, C. M., Giampietro, V., Daly, E. M., Brammer, M., Murphy, D. G. & Rubia, K. (2014d). Disorder-specific functional abnormalities during temporal discounting in youth with Attention Deficit Hyperactivity Disorder (ADHD), Autism and comorbid ADHD and Autism. *Psychiatry Research: Neuroimaging* **223**, 113-120.

Christakou, A., Brammer, M., Giampietro, V. & Rubia, K. (2009). Right Ventromedial and Dorsolateral Prefrontal Cortices Mediate Adaptive Decisions under Ambiguity by Integrating Choice Utility and Outcome Evaluation. *The Journal of Neuroscience* **29**, 11020-11028.

Christakou, A., Brammer, M. & Rubia, K. (2011). Maturation of limbic corticostriatal activation and connectivity associated with developmental changes in temporal discounting. *NeuroImage* **54**, 1344-1354.

Christakou, A., Gershman, S. J., Niv, Y., Simmons, A., Brammer, M. & Rubia, K. (2013a). Neural and psychological maturation of decision-making in adolescence and young adulthood. *Journal of Cognitive Neuroscience* **25**, 1807-1823.

Christakou, A., Murphy, C., Chantiluke, K., Cubillo, A., Smith, A., Giampietro, V., Daly, E., Ecker, C., Robertson, D. & Murphy, D. (2013b). Disorder-specific functional abnormalities during sustained attention in youth with Attention Deficit Hyperactivity Disorder (ADHD) and with Autism. *Molecular Psychiatry* **18**, 236-244.

Conners, C. K., Sitarenios, G., Parker, J. A. & Epstein, J. (1998). The Revised Conners' Parent Rating Scale (CPRS-R): Factor Structure, Reliability, and Criterion Validity. *Journal of Abnormal Child Psychology* **26**, 257-268.

Cools, R., Nakamura, K. & Daw, N. D. (2011). Serotonin and Dopamine: Unifying Affective, Activational, and Decision Functions. *Neuropsychopharmacology* **36**, 98-113.

Cortese, S., Kelly, C., Chabernaud, C., Proal, E., Di Martino, A., Milham, M. P. & Castellanos, F. X. (2012). Toward systems neuroscience of ADHD: a meta-analysis of 55 fMRI studies. *American Journal of Psychiatry* **169**, 1038-1055.

Critchfield, T. S. & Kollins, S. H. (2001). Temporal discounting: Basic research and the analysis of socially important behavior. *Journal of Applied Behavior Analysis* **34**, 101-122.

Cubillo, A., Smith, A. B., Barrett, N., Giampietro, V., Brammer, M., Simmons, A. & Rubia, K. (2014a). Drug-specific laterality effects on frontal lobe activation of atomoxetine and methylphenidate in attention deficit hyperactivity disorder boys during working memory. *Psychological Medicine* **44**, 633-646.

Cubillo, A., Smith, A. B., Barrett, N., Giampietro, V., Brammer, M. J., Simmons, A. & Rubia, K. (2014b). Shared and Drug-Specific Effects of Atomoxetine and Methylphenidate on Inhibitory Brain Dysfunction in Medication-Naive ADHD Boys. *Cerebral Cortex* **24**, 174-185.

Dalley, J. W. & Roiser, J. P. (2012). Dopamine, serotonin and impulsivity. *Neuroscience* **215**, 42-58.

Del-Ben, C. M., Deakin, J. F. W., McKie, S., Delvai, N. A., Williams, S. R., Elliott, R., Dolan, M. & Anderson, I. M. (2005). The Effect of Citalopram Pretreatment on Neuronal Responses to Neuropsychological Tasks in Normal Volunteers: An fMRI Study. *Neuropsychopharmacology* **30**, 1724-1734.

Doya, K. (2008). Modulators of decision making. *Nature Neuroscience* **11**, 410-416.

Findling, R. L. (1996). Open-Label Treatment of Comorbid Depression and Attentional Disorders with Co-administration of Serotonin Reuptake Inhibitors and Psychostimulants in Children, Adolescents, and Adults: A Case Series. *Journal of Child and Adolescent Psychopharmacology* **6**, 165-175.

Gainetdinov, R. R., Wetsel, W. C., Jones, S. R., Levin, E. D., Jaber, M. & Caron, M. G. (1999). Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. *Science* **283**, 397-401.

Gammon, G. D. & Brown, T. E. (1993). Fluoxetine and methylphenidate in combination for treatment of attention deficit disorder and comorbid depressive disorder. *Journal of Child and Adolescent Psychopharmacology* **3**, 1-10.

Gizer, I. R., Ficks, C. & Waldman, I. D. (2009). Candidate gene studies of ADHD: a meta-analytic review. *Human Genetics* **126**, 51-90.

Goldberg, D. P. & Murray, R. (2006). *The Maudsley handbook of practical psychiatry*. Oxford University Press.

Goodman, R. & Scott, S. (1999). Comparing the Strengths and Difficulties Questionnaire and the Child Behavior Checklist: Is Small Beautiful? *Journal of Abnormal Child Psychology* **27**, 17-24.

Hart, H., Radua, J., Mataix-Cols, D. & Rubia, K. (2012). Meta-analysis of fMRI studies of timing in attention-deficit hyperactivity disorder (ADHD). *Neuroscience & Biobehavioral Reviews* **36**, 2248-2256.

Hart, H., Radua, J., Nakao, T., Mataix-Cols, D. & Rubia, K. (2013). Meta-analysis of functional magnetic resonance imaging studies of inhibition and attention in attention-deficit/hyperactivity disorder: Exploring task-specific, stimulant medication, and age effects. *JAMA Psychiatry* **70**, 185-198.

Hinz, M., Stein, A., Neff, R., Weinberg, R. & Uncini, T. (2011). Treatment of attention deficit hyperactivity disorder with monoamine amino acid precursors and organic cation transporter assay interpretation. *Neuropsychiatric Disease and Treatment* **7**, 31-38.

Kerr, A. & Zelazo, P. D. (2004). Development of “hot” executive function: The children’s gambling task. *Brain and cognition* **55**, 148-157.

Koch, G., Oliveri, M. & Caltagirone, C. (2009). Neural networks engaged in milliseconds and seconds time processing: evidence from transcranial magnetic stimulation and patients with cortical or subcortical dysfunction. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **364**, 1907-1918.

Lamar, M., Craig, M., Daly, E. M., Cutter, W. J., Tang, C., Brammer, M., Rubia, K. & Murphy, D. G. M. (2014). Acute tryptophan depletion promotes an anterior-to-posterior fMRI activation shift during task switching in older adults. *Human Brain Mapping* **35**, 712-722.

Lamar, M., Cutter, W. J., Rubia, K., Brammer, M., Daly, E. M., Craig, M. C., Cleare, A. J. & Murphy, D. G. M. (2009). 5-HT, prefrontal function and aging: fMRI of inhibition and acute tryptophan depletion. *Neurobiology of Aging* **30**, 1135-1146.

Lesch, K. P., Hoh, A., Schulte, H. M., Osterheider, M. & Müller, T. (1991). Long-term fluoxetine treatment decreases 5-HT1A receptor responsivity in obsessive-compulsive disorder. *Psychopharmacology* **105**, 415-420.

Long, A. B., Kuhn, C. M. & Platt, M. L. (2009). Serotonin shapes risky decision making in monkeys. *Social Cognitive and Affective Neuroscience* **4**, 346-356.

McClure, S. M., Laibson, D. I., Loewenstein, G. & Cohen, J. D. (2004). Separate neural systems value immediate and delayed monetary rewards. *Science* **306**, 503-507.

McGough, J. J., McCracken, J. T., Loo, S. K., Manganiello, M., Leung, M. C., Tietjens, J. R., Trinh, T., Baweja, S., Suddath, R., Smalley, S. L., Hellemann, G. & Sugar, C. A. (2009). A Candidate Gene Analysis of Methylphenidate Response in Attention-Deficit/Hyperactivity Disorder. *Journal of the American Academy of Child & Adolescent Psychiatry* **48**, 1155-1164.

Miller, G. A. & Chapman, J. P. (2001). Misunderstanding analysis of covariance. *Journal of Abnormal Psychology* **110**, 40-48.

Mongeau, R., Blier, P. & De Montigny, C. (1997). The serotonergic and noradrenergic systems of the hippocampus: their interactions and the effects of antidepressant treatments. *Brain Research Reviews* **23**, 145-195.

Myerson, J., Green, L. & Warusawitharana, M. (2001). Area under the curve as a measure of discounting. *Journal of the Experimental Analysis of Behavior* **76**, 235-243.

Nigg, J. T., Willcutt, E. G., Doyle, A. E. & Sonuga-Barke, E. J. (2005). Causal heterogeneity in attention-deficit/hyperactivity disorder: do we need neuropsychologically impaired subtypes? *Biological Psychiatry* **57**, 1224-1230.

Noreika, V., Falter, C. M. & Rubia, K. (2013). Timing deficits in attention-deficit/hyperactivity disorder (ADHD): Evidence from neurocognitive and neuroimaging studies. *Neuropsychologia* **51**, 235-266.

Norman, L., Carlisi, C. O., Lukito, S., Hart, H., Mataix-Cols, D., Radua, J. & Rubia, K. (2015). Comparative meta-analysis of functional and structural deficits in ADHD and OCD. In *World Congress on ADHD*: Glasgow.

Northoff, G., Qin, P. & Nakao, T. (2010). Rest-stimulus interaction in the brain: a review. *Trends in Neurosciences* **33**, 277-284.

Oades, R. (2007). The role of the serotonin system in ADHD: treatment implications. *Expert Reviews in Neurotherapeutics* **7**, 1357 - 1374.

Oades, R. (2008). Dopamine-serotonin interactions in attention-deficit hyperactivity disorder (ADHD). *Progress in Brain Research* **172**, 543 - 565.

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* **9**, 97-113.

Peters, J. & Büchel, C. (2011). The neural mechanisms of inter-temporal decision-making: understanding variability. *Trends in Cognitive Sciences* **15**, 227-239.

Plichta, M. M., Vasic, N., Wolf, R. C., Lesch, K.-P., Brummer, D., Jacob, C., Fallgatter, A. J. & Grön, G. (2009). Neural hyporesponsiveness and hyperresponsiveness during immediate and delayed reward processing in adult attention-deficit/hyperactivity disorder. *Biological Psychiatry* **65**, 7-14.

Polanczyk, G. V., Willcutt, E. G., Salum, G. A., Kieling, C. & Rohde, L. A. (2014). ADHD prevalence estimates across three decades: an updated systematic review and meta-regression analysis. *International Journal of Epidemiology* **43**, 434-442.

Quintana, H., Butterbaugh, G., Purnell, W. & Layman, A. (2007). Fluoxetine Monotherapy in Attention-Deficit/Hyperactivity Disorder and Comorbid Non-Bipolar Mood Disorders in Children and Adolescents. *Child Psychiatry and Human Development* **37**, 241-253.

Radua, J., Pozo, N. O. d., Gómez, J., Guillen-Grima, F. & Ortuño, F. (2014). Meta-analysis of functional neuroimaging studies indicates that an increase of cognitive difficulty during executive tasks engages brain regions associated with time perception. *Neuropsychologia* **58**, 14-22.

Richards, J. B., Mitchell, S. H., de Wit, H. & Seiden, L. S. (1997). Determination of discount functions in rats with an adjusting-amount procedure. *Journal of the Experimental Analysis of Behavior* **67**, 353-366.

Richards, J. B., Zhang, L., Mitchell, S. H. & Wit, H. (1999). Delay or probability discounting in a model of impulsive behavior: effect of alcohol. *Journal of the Experimental Analysis of Behavior* **71**, 121-143.

Robinson, O., Cools, R. & Sahakian, B. (2012). Tryptophan depletion disinhibits punishment but not reward prediction: implications for resilience. *Psychopharmacology* **219**, 599-605.

Rogers, R. D. (2011). The roles of dopamine and serotonin in decision making: evidence from pharmacological experiments in humans. *Neuropsychopharmacology* **36**, 114-132.

Rommelse, N. J., Franke, B., Geurts, H., Hartman, C. & Buitelaar, J. (2010). Shared heritability of attention-deficit/hyperactivity disorder and autism spectrum disorder. *European Child & Adolescent Psychiatry* **19**, 281-295.

Rubia, K. (2011). "Cool" Inferior Frontostriatal Dysfunction in Attention-Deficit/Hyperactivity Disorder Versus "Hot" Ventromedial Orbitofrontal-Limbic Dysfunction in Conduct Disorder: A Review. *Biological Psychiatry* **69**, e69-e87.

Rubia, K., Alegria, A. & Brinson, H. (2014a). Imaging the ADHD brain: disorder-specificity, medication effects and clinical translation. *Expert Review of Neurotherapeutics* **14**, 519-538.

Rubia, K., Alegria, A. A., Cubillo, A. I., Smith, A. B., Brammer, M. J. & Radua, J. (2014b). Effects of Stimulants on Brain Function in Attention-Deficit/Hyperactivity Disorder: A Systematic Review and Meta-Analysis. *Biological Psychiatry* **76**, 616-628.

Rubia, K., Halari, R., Christakou, A. & Taylor, E. (2009). Impulsiveness as a timing disturbance: neurocognitive abnormalities in attention-deficit hyperactivity disorder during temporal processes and normalization with methylphenidate. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**, 1919-1931.

Rubia, K., Lee, F., Cleare, A. J., Tunstall, N., Fu, C. H., Brammer, M. & McGuire, P. (2005). Tryptophan depletion reduces right inferior prefrontal activation during response inhibition in fast, event-related fMRI. *Psychopharmacology* **179**, 791-803.

Rutter, M., Bailey, A. & Lord, C. (2003). *The social communication questionnaire: Manual*. Western Psychological Services.

Scheres, A., Dijkstra, M., Ainslie, E., Balkan, J., Reynolds, B., Sonuga-Barke, E. & Castellanos, F. X. (2006). Temporal and probabilistic discounting of rewards in children and adolescents: Effects of age and ADHD symptoms. *Neuropsychologia* **44**, 2092-2103.

Scheres, A., Tontsch, C., Thoeny, A. L. & Kaczkurkin, A. (2010). Temporal Reward Discounting in Attention-Deficit/Hyperactivity Disorder: The Contribution of Symptom Domains, Reward Magnitude, and Session Length. *Biological Psychiatry* **67**, 641-648.

Schweighofer, N., Bertin, M., Shishida, K., Okamoto, Y., Tanaka, S. C., Yamawaki, S. & Doya, K. (2008). Low-Serotonin Levels Increase Delayed Reward Discounting in Humans. *The Journal of Neuroscience* **28**, 4528-4532.

Schweighofer, N., Tanaka, S. C. & Doya, K. (2007). Serotonin and the Evaluation of Future Rewards. *Annals of the New York Academy of Sciences* **1104**, 289-300.

Smith, A., Cubillo, A., Barrett, N., Giampietro, V., Simmons, A., Brammer, M. & Rubia, K. (2013). Neurofunctional Effects of Methylphenidate and Atomoxetine in Boys with Attention-Deficit/Hyperactivity Disorder During Time Discrimination. *Biological Psychiatry* **74**, 615-622.

Sonuga-Barke, E. (2003). The dual pathway model of AD/HD: an elaboration of neuro-developmental characteristics. *Neuroscience and Biobehavioral Reviews* **27**, 593 - 604.

Sonuga-Barke, E., Bitsakou, P. & Thompson, M. (2010). Beyond the Dual Pathway Model: Evidence for the Dissociation of Timing, Inhibitory, and Delay-Related Impairments in Attention-Deficit/Hyperactivity Disorder. *Journal of the American Academy of Child & Adolescent Psychiatry* **49**, 345-355.

Sonuga-Barke, E., Taylor, E., Sembi, S. & Smith, J. (1992). Hyperactivity and delay aversion—I. The effect of delay on choice. *Journal of Child Psychology and Psychiatry* **33**, 387-398.

Spivak, B., Vered, Y., Yoran-Hegesh, R., Averbuch, E., Mester, R., Graf, E. & Weizman, A. (1999). Circulatory levels of catecholamines, serotonin and lipids in attention deficit hyperactivity diiorder. *Acta Psychiatrica Scandinavica* **99**, 300-304.

Tanaka, S. C., Schweighofer, N., Asahi, S., Shishida, K., Okamoto, Y., Yamawaki, S. & Doya, K. (2007). Serotonin Differentially Regulates Short- and Long-Term Prediction of Rewards in the Ventral and Dorsal Striatum. *PLoS ONE* **2**, e1333.

Toplak, M. E., Sorge, G. B., Benoit, A., West, R. F. & Stanovich, K. E. (2010). Decision-making and cognitive abilities: A review of associations between Iowa Gambling Task performance, executive functions, and intelligence. *Clinical Psychology Review* **30**, 562-581.

Wechsler, D. (1999). *Wechsler abbreviated scale of intelligence*. Psychological Corporation: San Antonio, TX.

Wesley, M. J. & Bickel, W. K. (2014). Remember the Future II: Meta-analyses and Functional Overlap of Working Memory and Delay Discounting. *Biological Psychiatry* **75**, 435-448.

Wiener, M., Turkeltaub, P. & Coslett, H. B. (2010). The image of time: A voxel-wise meta-analysis. *NeuroImage* **49**, 1728-1740.

Willcutt, E., Sonuga-Barke, E., Nigg, J. & Sergeant, J. (2008). Recent developments in neuropsychological models of childhood psychiatric disorders. *Biological Child Psychiatry* **24**, 195-226.

Wittmann, M., Leland, D. & Paulus, M. (2007). Time and decision making: differential contribution of the posterior insular cortex and the striatum during a delay discounting task. *Experimental Brain Research* **179**, 643-653.

Wong, D. T., Bymaster, F. P. & Engleman, E. A. (1995). Prozac (fluoxetine, lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug: Twenty years since its first publication. *Life Sciences* **57**, 411-441.

Xu, L., Liang, Z.-Y., Wang, K., Li, S. & Jiang, T. (2009). Neural mechanism of intertemporal choice: from discounting future gains to future losses. *Brain Research* **1261**, 65-74.

Zepf, F. D., Gaber, T. J., Baurmann, D., Bubenzer, S., Konrad, K., Herpertz-Dahlmann, B., Stadler, C., Poustka, F. & Wöckel, L. (2010). Serotonergic neurotransmission and lapses of attention in children and adolescents with attention deficit hyperactivity disorder: availability of tryptophan influences attentional performance. *The International Journal of Neuropsychopharmacology* **13**, 933-941.

Table 1. Participant characteristics

Variables	Controls (N=20) Mean (SD)	ADHD (N=12) Mean (SD)	*F* test (DF)	*p* value
Demographic Data				
Age (years)	15.29 (1.78)	14.86 (1.71)	0.43 (1,30)	0.52
Handedness	88.4 (16.37)	92.92 (11.48)	0.70 (1,30)	0.41
IQ	118.9 (11.91)	94.5 (7.35)	40.71 (1,30)	< 0.001
CPRS-R total *T*-score *	48.63 (8.82)	82.83 (7.79)	113.79 (1,26)	< 0.001
SCQ total score †	2.24 (2.51)	6.58 (3.29)	16.32 (1,27)	< 0.001
SDQ total score ‡	4.89 (3.69)	20.75 (4.31)	116.32 (1,28)	< 0.001
Performance Data		**Plac/Fluox**		
AUC	0.557 (0.13)	0.440 (0.20)/0.458 (0.23)	-	-
RT (ms)	2141 (591.47)	2354 (578.6)/2306 (381.5)	-	-
OM	0.75 (1.83)	1.92 (2.4)/1.58 (2.0)	-	-

Abbreviations: SD=standard deviations; DF=degrees of freedom; Plac=placebo; Fluox=fluoxetine; RT=reaction time; ms=milliseconds; OM=omissions

* CPRS-R total *T*-score could not be obtained for 4 control participants

† SCQ scores could not be obtained from 3 control participants

‡ SDQ scores could not be obtained from 2 control participants

Table 2. Within-patient comparisons of activation differences for delayed-immediate choices

Brain regions of activation difference	Brodmann Area (BA)	MNI coordinates (x,y,z)	Voxels	Cluster *p* value	Partial eta^2
(A) ADHD Fluoxetine > ADHD Placebo					
R IFC/DLPFC/insula/precentral gyrus/STL/putamen/caudate/ globus pallidus	47/10/46/45/6/22	52,0,-20	107	0.003	0.53
(B) ADHD Placebo > ADHD Fluoxetine					
L + R lateral cerebellum & vermis/occipital lobe/ PCC/precuneus	30/31/23/19/18	-11,-75,-12	384	0.0002	0.38
L postcentral/precentral gyri/IPL/middle frontal gyrus	2/3/1/6/40/5	-30,-22,48	116	0.0007	0.38

Abbreviations: R=right; L=left; IFC=inferior frontal cortex; DLPFC=dorsolateral prefrontal cortex; STL=superior temporal lobe; PCC=posterior cingulate cortex; IPL=inferior parietal lobe. Partial eta^2 refers to the effect size of the differences in activation between groups.

Table 3. Case-control comparisons of activation differences for delayed-immediate choices

Subject contrast	Brain regions of activation difference	Brodmann Area (BA)	MNI coordinates (x,y,z)	Voxels	Cluster *p* value	Partial eta^2
(A) CONTROLS versus ADHD PLACEBO						
C > ADHD	R pre/postcentral gyrus/insula	6/4/3/2	56,-5,5	14	0.04	0.10
	R precentral/postcentral gyrus	6/4/3/2/1/40	41,-11,37	25	0.04	0.19
	R postcentral gyrus/IPL	4/1/2/40	-34,-22,45	14	0.04	0.24
	L postcentral gyrus/IPL	2/3/40	-34,-22,45	4	0.03	0.08
ADHD > C	L cerebellum (anterior)/occipital lobe/PCC	19/30	-14,-71,-12	16	0.02	0.12
(B) CONTROLS versus ADHD FLUOXETINE						
C > ADHD	L precentral/postcentral gyri/IPL	6/4/3/2/1/40	-34,-22,45	77	0.001	0.27
ADHD > C	No observed clusters	-	-	-	-	-

Abbreviations: R=right; L=left; IPL=inferior parietal lobe. Partial eta^2 refers to the effect size of differences in activation between groups.

Figure Legends

Fig. 1. Within-patient comparisons. Axial sections show medication effects within the ADHD group. RED=fluoxetine > placebo, BLUE=placebo > fluoxetine. Also shown are the statistical measures of the blood oxygen level-dependent (BOLD) response for each of the brain regions that showed a significant effect of medication within patients. R=right, L=left; IFC=inferior frontal cortex; STL=superior temporal lobe; PCC=posterior cingulate cortex; IPL=inferior parietal lobe; MFG=middle frontal gyrus. Talairach z-coordinates are indicated for slice distance (in mm) from the intercommissural line. The right side of the image corresponds to the right side of the brain.

Fig. 2. Case-control comparisons. Axial sections show the between-group ANCOVA findings between controls and patients under (A) placebo and (B) fluoxetine. RED=controls > ADHD, BLUE=ADHD > controls. Also shown are the statistical measures of the blood oxygen level-dependent (BOLD) response for each of the brain regions that showed a significant group effect. R=right, L=left, IPL=inferior parietal lobe, CB=cerebellum. Talairach z-coordinates are indicated for slice distance (in mm) from the intercommissural line. The right side of the image corresponds to the right side of the brain.