Challenging the brain

Insights from comprehensive structural & functional MRI studies

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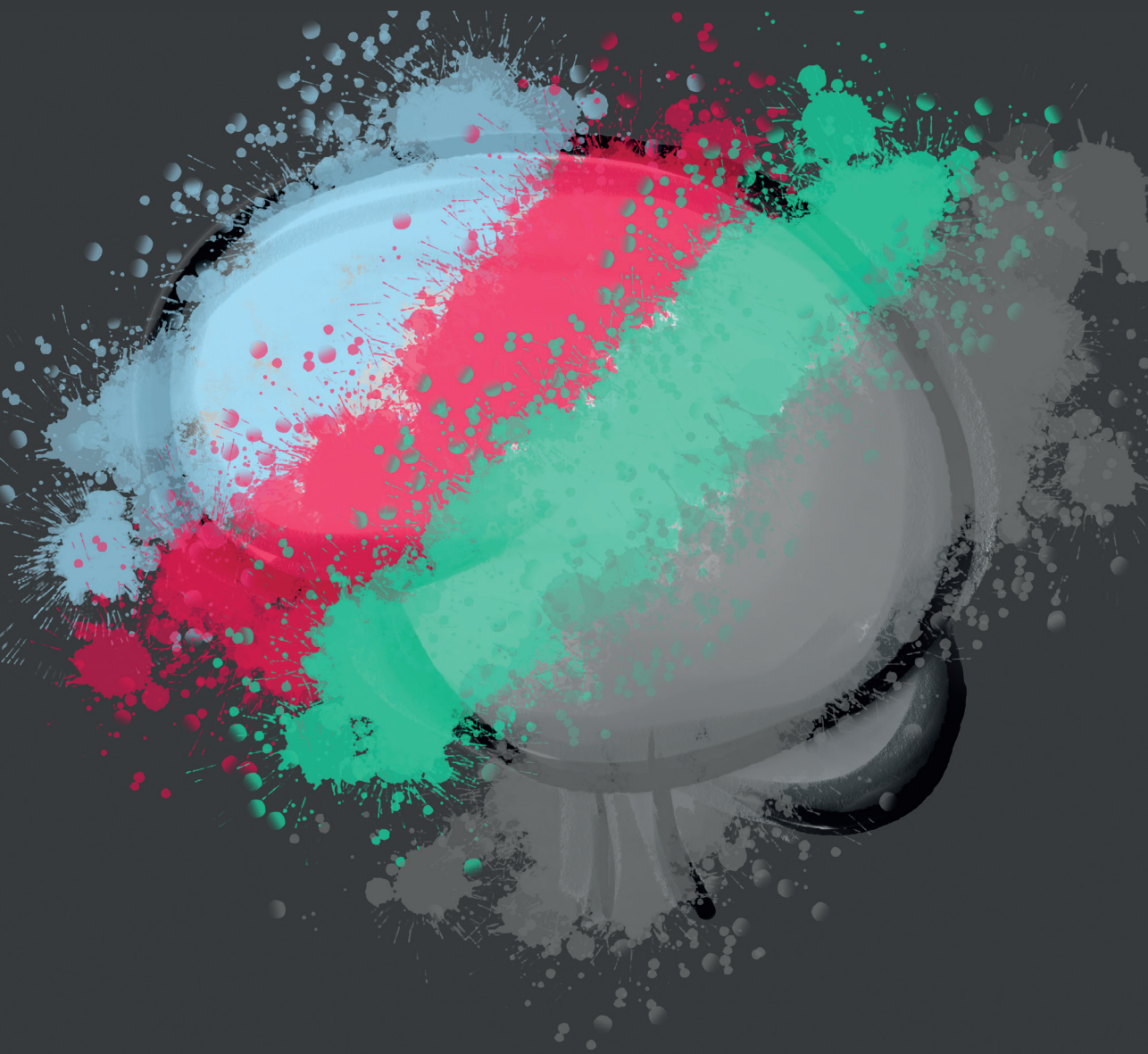

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Magic happens when you do not give up, even though you want to.

The universe always falls in love with a stubborn heart.

- JM Storm

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Chapter 1

General Introduction

General Introduction

Preface

13% of people worldwide are affected by mental health issues, which cause substantial reductions in quality of life and economic productivity. As such, mental health issues are a big burden for the individual and their social surroundings, as well as for society [World Health Organization, 2020]. Mental disorders are a diverse group of mental health issues that can be characterized by anxiety, emotional dysregulation (ED) or altered behavior of the individual [American Psychiatric Association, 2014]. Over the past decades, significant amounts of research efforts were invested to improve our understanding of the neurobiological mechanisms that contribute to, and may underlie, mental disorders.

It is also important to consider that the brain is a highly adaptable and malleable organ that can and needs to respond to many external factors and challenges [Kolb and Teskey, 2012]. In a way, failure to adapt can also be considered a mechanism that might underlie specific aspects of mental disorders. In fact, we can utilize the 'adaptability' of the brain to study the underlying mechanisms of specific brain disorders, and potentially also to advance the development of novel treatments. For example, by stimulating the brain in a specific, spatio-temporally well-controlled manner, and consequently considering the dynamic changes that occur in the brain as it adapts to those stimuli, we might be able to better understand the mechanisms involved in (mis-)processing of challenges to the brain that can modulate behavior, cognition or emotional regulation [Wojtalik et al., 2018].

The overall **aim of this thesis** is, by using comprehensive MRI studies, to investigate the influence of specific cognitive, pharmacological, and physical interventions on selective behavioural, cognitive and neuronal changes of the brain. We here; 1) investigated cognitive **task-based interventions** on specific brain functions, and next examined **medication effects** on certain symptom clusters and brain functions of individuals with ADHD, and 2) investigated the effects of a longitudinal **exercise intervention** on various measures of brain plasticity in young healthy adults.

General Introduction

Treatments and interventions for mental disorders

Over the past decades, researchers and medical professionals have worked together to develop treatments and interventions for a variety of mental disorders [Wojtalik et al., 2018]. Currently, one of the most common and most direct ways of treating a mental disorder is with medication. Unfortunately, most medications for mental disorders suffer from two major problems: 1) a strong heterogeneity in their efficacy between individuals, and sometimes even treatment resistance to the available treatments; and 2) adverse side effects are relatively common [Howes et al., 2022]. Therefore, efforts are being made to develop biomarkers and better measurement techniques that can identify disorder-specific neurobiological changes, which could aid patient stratification, improve the efficacy and reliability of medications, or help to develop better ones [Lozupone et al., 2017].

In addition to pharmacological treatment, behavioural therapy, cognitive interventions, or trainings are commonly being applied in psychiatry. In behavioural therapy, the focus lies on changing specific habits or introducing new ones [Antony et al., 2020]. In cognitive therapy, certain cognitive abilities, like working memory (WM), attention, or emotion regulation, are trained through specific cognitive tasks. A common therapeutic approach that has been shown to work well in many mental disorders is a combination of the two, namely cognitive-behavioural therapy [Leichsenring et al., 2006]. Most of these treatments are based on the premise that the brain is highly malleable. Neuroplasticity, also known as brain plasticity, refers to the brains ability to change and adapt in response to internal and external challenges, e.g. by reorganizing its structure, connections or function. Neuroplasticity is, for example, essential for brain development and learning, but also to recover from deleterious disturbances and changes occurring throughout life. Generally, two main types of neuroplasticity can be distinguished [Mateos-Aparicio and Rodríguez-Moreno, 2019]: functional and structural neuroplasticity. Structural plasticity refers to the formation of new synapses, new dendrites or even new nerve cells, and the growth of physiological support structures, like arteries that are essential for oxygen delivery, waste removal, and scaffold functions. Also, via processes like axonal sprouting, axons can grow new synapses that can establish contact or (re-)connect with other neurons, thereby making new links and neural pathways that allow to accomplish new functionalities. Functional plasticity, on the other hand, refers to changes in output, responsivity or function of a given brain network or structure that is typically induced by environmental factors or events [von Bernhardi et al., 2017]. Examples are the brain changes underlying learning and memory, or the induction of fear behavior after adverse experiences. Functional plasticity can be mediated via plastic changes at the level of the synapse, where the transmission of information can be weakened, or rather

strengthened after e.g. repeated experiences, like in long-term potentiation, which is the persistent increase in synaptic strength following high-frequency stimulation of a chemical synapse [Galván, 2010].

While plasticity is prominent during the period of early brain development, scientists nowadays propose that malleability of the brain, even though to a lower extent and only in specific brain regions (i.e. mainly in the hippocampus), is in fact maintained at many levels and can e.g. take place throughout all life stages [Kempermann et al., 2018; Nieto-Sampedro and Nieto-Díaz, 2004]. Recently, modern clinical experimental approaches such as magnetic resonance imaging (MRI) have made it possible to gather more detailed information on plasticity changes also in the in-vivo human brain.

MRI as a tool in intervention studies

MRI is a powerful imaging technique that allows to estimate a wide range of functional, structural, and biochemical features of the brain. MRI is non-invasive and accordingly, can be applied dynamically and longitudinally, and can be repeated in the same individual. Therefore, MRI can be used to evaluate the effects of specific challenges, perturbations or trainings on brain structure and physiology. Furthermore, while we can investigate effects of exposure to specific challenges, measures of cognition, physiology and other participant characteristics can be acquired in parallel. Having information about the structural and functional malleability of the brain, measured by MRI, and about the changes on behavioral or physiological levels, enables us to link these together and find correlates to eventually unravel neurobiological mechanisms underlying brain health and disease [Kalin, 2021]. In the following paragraphs a short overview of the MRI techniques used in this thesis will be given:

1) **T1-weighted MRI** is an example of an anatomical MRI technique used to assess regional volumetric differences in specific brain regions, such as gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF), but also smaller regions, including the hippocampus. It can be used to assess differences between groups or longitudinally within one participant. For that, the regions' outline is segmented and its volume or thickness can consequently be estimated using sophisticated algorithms. Volumetric measures provide information about in- or decreases of the structure of a region, but lack information on the exact underlying mechanisms that might cause these (adaptive) changes [Keller and Roberts, 2009].

2) **Functional MRI (fMRI)** is a tool to investigate stimulus-induced patterns of brain activity. FMRI is based on the observation that brain activation is accompanied by a local vascular response. This causes a change of the relative levels of oxygenated hemoglobin and deoxygenated hemoglobin that can be detected on the basis of

their differential magnetic susceptibility, which results in the blood-oxygen-level dependent (BOLD) signal. The size of the BOLD-signal can be related to the magnitude of neuronal activation in specific brain regions. FMRI has played an essential role e.g. in determining the functional organization of the human brain, also in response to specific tasks or trainings. **Resting-state fMRI (rs-fMRI)** is a derivative of fMRI that measures spontaneous BOLD fluctuations at rest, without any stimulus or activity [Chen and Glover, 2015].

3) **Arterial spin labelling (ASL)** is a technique with which we can estimate the amount of blood flowing to a region. This approach magnetically labels arterial blood water protons as an endogenous tracer, allowing for the measurement of tissue perfusion (cerebral blood flow; CBF), often expressed as mL of blood per 100g of tissue per minute. This can be compared between groups and also within an individual before and after a challenge to get information on perfusion changes. Brain perfusion is crucial for neuronal growth and synapse formation as a greater blood supply is essential to provide adequate nutrients to support neuronal plasticity and functional adaptation [Alsop et al., 2015].

4) Cerebral blood volume (CBV), is defined as the volume of blood present in a certain quantity of brain tissue, often expressed as milliliters of blood per 100g of brain tissue. Concentration-time curves may be constructed from signal intensity-time curves, and the area under these curves can then be used to determine CBV. CBV can be measured with so called **T1-mapping strategies**, for example through measuring the tissue T1 relaxation time before and after the arrival of a contrast agent. In this thesis, we used gadolinium for that purpose. The calculated CBV gives information about the hemodynamic correlate of oxygen metabolism, reflects brain activity and function and we can assume the density and architecture of vascularization of a region [Lindgren et al., 2014].

5) **MR Spectroscopy (MRS)** offers a noninvasive way of measuring biochemical changes in the brain. In contrast to the methods described above, it does not provide an image of the brain, but (typically) a one-dimensional spectrum. It makes use of the so-called "chemical shift" of neurometabolites to determine the location of the frequency axis of the spectrum, which is determined by the chemical environment protons reside. The frequency axis is often in parts per million (ppm), to provide a field-strength independent scale of the resonance frequency. The area under the curve of the neurometabolites in the spectrum can then be used to estimate the concentrations of specific metabolites in a given voxel of the brain [Kreis et al., 2020].

Part 1: cognitive and pharmacological interventions in Attention-Deficit/Hyperactivity Disorder (ADHD)

ADHD

In this thesis, we studied Attention-Deficit/Hyperactivity Disorder (ADHD), one of the most common neurodevelopmental disorders. The DSM-5 states a prevalence of 11.4% in primary school children [American Psychiatric Association, 2013]. Even though it is most commonly diagnosed in childhood, the disorder can persist into adulthood with a prevalence of 5% in adults [Caye et al., 2016].

ADHD is defined by the following symptoms: 1) the presence of developmentally inappropriate levels of hyperactive-impulsive and/or inattentive symptoms for 6 months or more; 2) symptoms occurring in different life settings (e.g., home and school); 3) symptoms that cause destructions in daily life; 4) some of the symptoms and impairments began in early to mid-childhood; and 5) no other disorder explains the symptoms [Faraone et al., 2015; World Health Organization, 2004]. Depending on the composition of their symptoms, ADHD can be classified as mostly inattentive, primarily hyperactive-impulsive, or both. ADHD is frequently associated with comorbidities, including mood and anxiety disorders, autistic spectrum disorders, oppositional defiant disorder, conduct disorder, eating disorders, and substance use disorders [Faraone et al., 2021]. Emotion dysregulation (ED), the inability to control and minimize the disruptive effects of irrelevant emotional stimuli on goal-oriented processes, has recently been suggested as an additional core symptom in especially adult ADHD [Hirsch et al., 2019]. Emotion regulation issues in adult ADHD include impaired emotional recognition, emotional responsivity, and emotional lability, further complicating the spectrum of classic symptoms [Beheshti et al., 2020]. Importantly, ED is associated with the persistence of ADHD into adulthood and predicts lower quality of life in young adults [Groenewold et al., 2013].

Even though the methodological capabilities in the field of neuroimaging research have been improving and the research efforts have been steadily growing, the underlying pathophysiological mechanisms of ADHD still remain only partially understood [Ghimire et al., 2020]. Nevertheless, neuroimaging has contributed significantly to our understanding of the alterations in the structure, function, and neurochemistry in ADHD. Structurally, for instance, in children with ADHD, the dorsolateral prefrontal cortex (DLPFC), caudate, pallidum, corpus callosum, and cerebellum are most commonly found to be smaller in size. The results from specific region of interest (ROI) approaches [Xavier Castellanos et al., 2002] and automated procedures [Sowell et al., 2003] suggest lower total brain sizes and substantial cortical modifications, indicating that the brain may be impacted more broadly.

On a functional level, ADHD has been increasingly proposed to be a condition of brain-wide network dysconnectivity rather than one that presents with region-specific deviances [Castellanos and Proal, 2012]. The use of rs-fMRI advanced this hypothesis significantly and a growing body of diverse research into rs-fMRI connectivity has consistently reported reduced connectivity within the default mode network (DMN; [Castellanos and Aoki, 2016; Gao et al., 2019b; Posner et al., 2014]), disrupted connectivity between the executive control network (ECN), the DMN [Gao et al., 2019a; Posner et al., 2014; Sutcubasi et al., 2020] and salience network (SN), and involvement of affective, motivational [Gao et al., 2019b; Posner et al., 2014] and somatosensory networks [Gao et al., 2019b].

Neurochemically, positron emission tomography (PET) and single-photon emission computed tomography (SPECT) research have frequently focused on changes in dopamine [Volkow et al., 2007]. This focus on striatal dopamine has been supported by structural and functional imaging findings indicating striatal abnormalities [Hoogman et al., 2017; Lei et al., 2015].

Interventions and treatments in ADHD

Methylphenidate

The first-line treatment for ADHD is pharmacotherapy with stimulants. MPH is a psychostimulant and the most commonly prescribed medication in ADHD. The pharmacological mechanisms of MPH result primarily from its direct inhibition of the dopamine and noradrenaline transporters [Cortese et al., 2017]. By increasing dopaminergic activity, MPH induces a number of significant behavioral and cognitive effects, such as changes in executive and attentional function [Jaeschke et al., 2021]. Studies utilizing MPH as an acute pharmacological challenge have been done mainly in children and adolescents with ADHD, and have reported decreased functional connectivity, measured with rs-fMRI, in e.g. the striatum and thalamus, and frontal regions. In typically-developing (TD) adults, an increase in connectivity between the thalamus and attention networks, and subcortical regions was found. FMRI assessments have shown several functional effects of MPH [Faraone, 2018; Schlösser et al., 2009]. For example, MPH was shown to increase activation of the dorsal attention network (DAN) whereas it alters deactivation of the default mode network (DMN) [Tomasi et al., 2011]. Another investigation discovered that MPH induced activity in the putamen only when a response inhibition error ('commission error') occurred, but not when response suppression was successful [Costa et al., 2013]. Several outcome measures of the Stroop color-word task were found to be improved after MPH administration [Moeller et al., 2014; Langleben et al., 2006], and this effect was likewise correlated with decreased activity in frontal

regions. This provides evidence that the apparent effects of MPH are dependent on the circumstances under which they are observed. MPH has also been shown to enhance regional cerebral blood flow (CBF) in more subcortical regions, while lowering CBF in the prefrontal cortex [Faraone, 2018; Schrantee et al, 2017]. Long-term MPH exposure was also shown to enhance activity in hippocampal areas and cerebellar regions, as well as change the strength of connections in a number of neural circuits [Schlösser et al., 2009; Faraone, 2018]. Additionally, long-term administration of psychostimulants (including MPH) was found to diminish structural and functional abnormalities in individuals with ADHD in a number of MRI investigations [Mueller et al., 2014; Schlösser et al., 2009; Schweitzer et al., 2004].

Dopamine transporter density in the striatum of individuals with ADHD has been reported to be negatively correlated with the length of time since the last exposure to psychostimulants and was positively correlated with the length of time since the last treatment, demonstrating that higher dopamine transporter density might be a side effect of prior stimulant medication and not a characteristic of the ADHD population as a whole [Fusar-Poli et al., 2012]. Interestingly, the theory of neural imprinting suggests that exposure to stimulants during stages of maturation may impact later development [Andersen, 2005]. In fact, research on animals suggests that the effects of MPH on development vary greatly depending on the age at which treatment begins [Canese et al., 2009]. In line, we found in a clinical trial comparing boys and men with ADHD that the effects of ADHD medication are modulated by age [Schrantee et al., 2016; Solleveld et al., 2017; Bouziane et al., 2018]. Specifically, we found that acute MPH decreased thalamic CBF only in children, but not in adults [Schrantee et al., 2017] and observed that prolonged MPH-treatment followed by an acute challenge with MPH significantly influenced CBF in the striatum and thalamus in children, but not adults, nor in the placebo conditions [Schrantee et al., 2016]. Accordingly, we also showed that acute MPH administration modulates one of the functional neural mechanisms underlying emotional processing, i.e. amygdala reactivity, in an age-dependent manner [Bottelier et al., 2017].

So far, studies still show mixed results and no consensus has been reached on the exact mechanisms and potential beneficial effects of MPH on the developing vs. the non-developing brain. Such heterogeneity results mainly from previous studies only including adults or children, making it difficult to compare them directly, and including individuals with different medication histories, making it impossible to rule out the influence of that on the results. We here compared the effects of acute and long-term MPH on both medication-naive children and adults with ADHD, to investigate the age-dependent neural mechanisms involved in the first exposure to MPH. In this thesis, we focused on the effects of prolonged MPH on neural mechanisms underlying emotional tasks, and acute MPH during rest and correlated them to several symptom scores.

Behavioral and cognitive Interventions

In addition to pharmacotherapy, the efficacy of behavioral interventions for ADHD have been frequently studied [Fullen et al., 2020; Waxmonsky et al., 2019; Young et al., 2020]. For instance, these interventions include cognitive trainings, such as WM training. Using a 'neurobiological systems' approach to analyze ADHD therapeutic outcomes is likely to aid in explaining how these therapies can affect brain changes and functionality and elucidate the biological mechanisms and cascades that drive the symptoms over time. Specifically, by attempting to change behaviors, researchers might be able to obtain more knowledge about the etiology and persistence of the behavior. Likewise, by finding mechanisms for treatments, critical components, or boundary conditions, the field might be able to help individuals with ADHD better. For example, top-down cognitive control processes have been shown to influence emotional regulation. For instance, in TD control (TDC) participants, the prefrontal cortex (PFC) was activated more during cognitive control tasks in the presence of emotional stimuli [Hung et al., 2018; Song et al., 2017], indicating the potential top-down suppression of emotional reactivity. Furthermore, taxing WM processes before or during exposure to emotionally salient stimuli was shown to reduce emotional reactivity in e.g. substance use disorders [Kaag et al., 2018]. Therefore, in this thesis, we investigated the potential of a WM training on emotional reactivity and the underlying neural mechanisms in adults with ADHD.

Part 2: A longitudinal exercise intervention

Exercise

Neuroimaging findings in exercise studies

It is generally known that engaging in regular physical activity is associated with several health benefits, including a lower chance of developing cardiovascular disease, stroke, and obesity. Furthermore, exercise interventions have recently been suggested to have a positive influence on the brain and e.g. benefit mental health outcomes and several brain disorders, including depression [Daley, 2008], anxiety [Aylett et al., 2018], and ADHD [Christiansen et al., 2019].

Regular physical exercise is thought to introduce both structural and functional changes in the brain. Indeed, the simple act of exercising/physical activity has been shown to increase hippocampal neurogenesis, a specific form of structural plasticity that refers to stem cells giving rise to the birth of new neurons in the adult brain [Czéh and Lucassen, 2007; van Praag et al., 1999]. Exercise also was found to present antidepressant effects [McKercher et al., 2009; Olson et al., 2006], and improved learning abilities

[Anderson et al., 2000; Van Praag et al., 1999; van Praag et al., 2005]. The early investigations on the neural correlates of exercise in humans focused first on possible structural brain changes. Multiple cross-sectional and prospective longitudinal MRI investigations in humans have revealed that high-intensity and chronic aerobic exercise increased and/or restored age-related declines in brain volume, particularly in the hippocampus [Erickson et al., 2011]. Firth et al. [2018] described in their meta-analysis that the most significant exercise effects in were found in older people. Nevertheless, one study also revealed rapid hippocampus volume increases in younger adults [Thomas et al., 2012]. While important, the studies on volume changes are lacking information on the biological substrates of the exercise-related changes. Several possible underlying mechanisms have been proposed in both animal and human studies [Lucas et al., 2015; Voss et al., 2011], including changes in perfusion, vascularization, synaptic plasticity, neurogenesis, and other molecular and cellular changes. All in all, physical activity appears to beneficially modulate several different pathways that may subsequently modify brain anatomy and function in both animals and humans [Kandola et al., 2016; Voss et al., 2013].

While exercise meets the criteria for a low-risk, socially acceptable and stigma-free therapeutic option, it so far has been mostly studied in middle-aged and older individuals. Relatively little was known about the mechanistic changes induced by exercise in brain structure and function, especially of young adults. Moreover, a study, investigating exercise in a multi-modal way, including a variety of neuroimaging-based measurements of volume, vasculature, and microstructure and also measures of peripheral health like blood markers, that would provide more insight into the underlying mechanisms, was lacking in the field. Therefore, we here used comprehensive MRI measures combined with several physiological, and peripheral outcome measures to investigated underlying neural mechanisms of exercise. We compared a low- and high-intensity regime in young, healthy, but otherwise non-trained, adults in a longitudinal, randomized trial.

Experimental setup and contents of this thesis

In this thesis, comprehensive MRI techniques were used to study functional and structural changes in the brain induced by different stimuli. These included: taxing WM, emotional tasks, stimulant-medication and an exercise intervention. Both 3T and 7T MRI techniques were applied to measure the influences on functional reactivity and connectivity, on brain metabolite concentrations, and on volumetric measures, CBF and CBV of the brain. Additionally, measures of behaviour, cognition and peripheral physiology were applied to correlate them to the observed MRI-based neurobiological changes. The content of the experimental chapters is as follows:

Chapter 2) Functional reactivity of the amygdala during an emotional task in male adult ADHD participants, in response to a WM task.

In this chapter we investigated the underlying neurobiological mechanisms of ED in male adults with ADHD. Previous studies in e.g. healthy controls and individuals with substance use disorders had suggested that a WM training was able to decrease ED [Andrade et al., 2012; van den Hout et al., 2014; Kaag et al., 2018; Markus et al., 2016; McClelland et al., 2006]. We therefore hypothesized that targeting WM processes could also benefit individuals with ADHD.

To that end, we developed a novel fMRI paradigm in which we interleaved emotionally negative and neutral stimuli with a low and high load WM task, and investigated whether a high load WM task could attenuate potential hyperreactivity to emotional stimuli of the amygdala in adults with ADHD. In contrast to previous reports however, we did not find a hyperreactivity of the amygdala to the emotional stimuli in the adults with ADHD compared to the TDC participants. Probably consequently, a significant effect of the load of the WM task on amygdala reactivity to the emotional stimuli was not found either.

Chapter 3) Functional reactivity of the amygdala during an emotional face-matching task in medication-naive male children and adults with ADHD; modulation by prolonged MPH treatment.

In this chapter, we also studied emotion regulation processes in ADHD. We investigated whether prolonged MPH treatment would have an influence on functional measures that may underlie emotional processing in medication-naive children and adults with ADHD. For that purpose, we used data of the "effects of Psychotropic drugs On Developing brain-MPH" ("ePOD-MPH") randomized controlled trial, which was a 16-week double-blind, randomized, placebo-controlled, multi-centre trial with MPH, and a blinded endpoint evaluation in stimulant treatment-naive participants with ADHD [Bottelier et al., 2014].

We did not find any effect of prolonged MPH on amygdala reactivity to an emotional face-matching fMRI task in children or adults with ADHD. Interestingly, the MPH treatment did have a positive effect on the behavioural symptoms of ADHD in both children and adults with ADHD. Additionally, we found indications that internalizing symptoms at baseline (i.e. symptoms of anxiety and depression) could be used to predict the change in ADHD symptoms from baseline to during and post-trial in only the MPH-treated adults. This indicated that adults with high internalizing problems may benefit most from a prolonged MPH treatment.

Chapter 4) Functional connectivity of the thalamus, striatum, dorsal anterior cingulate cortex and prefrontal cortex during rest in medication-naive male children and adults with ADHD and TDC participants; modulation by acute MPH.

This chapter reports specifically on the baseline data from the ePOD-MPH trial (i.e. before randomization). We investigated the effects of an acute MPH challenge on resting-state functional connectivity, and studied whether these effects differed between children and adults. In addition, we investigated whether MPH normalized potentially aberrant patterns of connectivity compared to age-matched TDC participants.

Interestingly, we found the opposite effects of MPH in children and adults on dopamine-sensitive regions (striatum, thalamus), but not on the dorsal anterior cingulate cortex (dACC) or prefrontal cortex (PFC). This might be due to possible changes in the dopamine and noradrenergic systems during maturation.

Chapter 5) Structural and functional plasticity of the hippocampus in young, healthy adult participants, modulated by 12 weeks of low- or high-intensity physical activity.

In this comprehensive, multi-modal 3T and 7T MRI, randomized controlled trial, we randomized young adult, non-athletic volunteers to a 12-week low- or high-intensity exercise program. We used a series of state-of-the-art methods to investigate changes in hippocampal volume, and explore potential underlying changes in vasculature, perfusion, neuro-metabolites, and peripheral growth factors.

Surprisingly, both exercise groups showed increases in cardiovascular fitness. Maybe as a consequence, we could not find differential effects between the high-intensity (aerobic) and low-intensity (toning) exercise groups regarding some of the structural and functional measures in the hippocampus of these young adults. However, we showed small but significant effects of exercise in general on hippocampal volume, neurometabolism and vasculature across exercise conditions, highlighting the importance of a multi-modal, whole-brain approach to assess macroscopic and microscopic changes underlying exercise-induced brain changes.

Chapter 6) General Discussion

The content of the described publications is summarized and, while not claiming to be comprehensive or complete, subsequently discussed in relation to recent literature.

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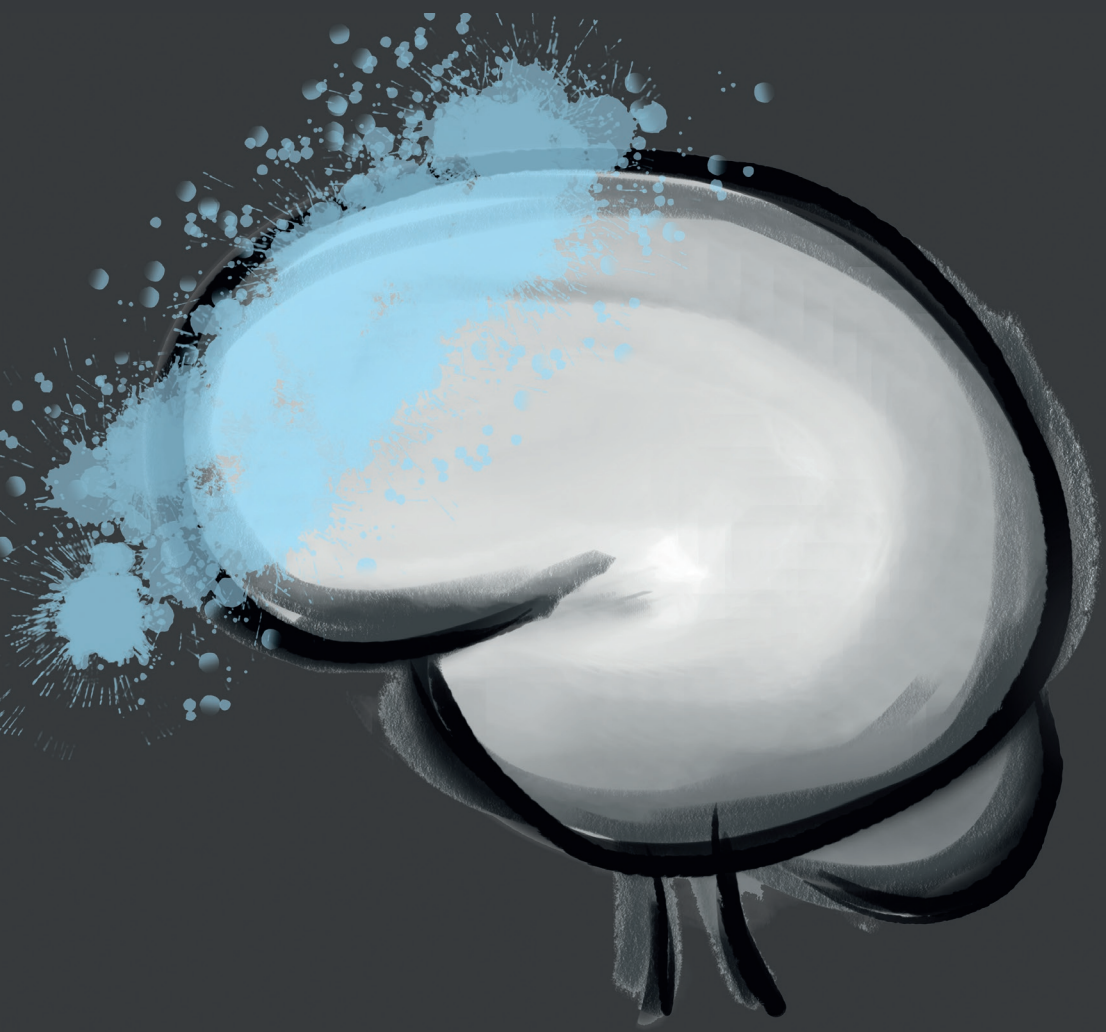
Chapter 2

Targeting amygdala hyperreactivity in ADHD



Targeting working memory to modify emotional reactivity in adult attention deficit hyperactivity disorder: a functional magnetic resonance imaging study

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Abstract

Understanding the neural mechanisms of emotional reactivity in Attention-Deficit/ Hyperactivity Disorder (ADHD) may help develop more effective treatments that target emotion dysregulation. In adult ADHD, emotion regulation problems cover a range of dimensions, including emotional reactivity (ER). One important process that could underlie an impaired ER in ADHD might be impaired working memory (WM) processing. We recently demonstrated that taxing WM prior to the exposure of emotionally salient stimuli reduced physiological and subjective reactivity to such cues in heavy drinkers, suggesting lasting effects of WM activation on ER. Here, we investigated neural mechanisms that could underlie the interaction between WM and ER in adult ADHD participants. We included 30 male ADHD participants and 30 matched controls. Participants performed a novel functional magnetic resonance imaging paradigm in which active WM-blocks were alternated with passive blocks of negative and neutral images. We demonstrated group-independent significant main effects of negative emotional images on amygdala activation, and WM-load on paracingulate gyrus and dorsolateral prefrontal cortex activation. Contrary to earlier reports in adolescent ADHD, no impairments were found in neural correlates of WM or ER. Moreover, taxing WM did not alter the neural correlates of ER in either ADHD or control participants. While we did find effects on the amygdala, paracingulate gyrus, and dorsal lateral prefrontal cortex activation, we did not find interactions between WM and ER, possibly due to the relatively unimpaired ADHD population and a well-matched control group. Whether targeting WM might be effective in participants with ADHD with severe ER impairments remains to be investigated.

Introduction

In addition to deficits in attention, hyperactivity, and impulsivity [American Psychiatric Association, 2013], emotional dysregulation (ED) is considered a core symptom in adults with Attention-Deficit/Hyperactivity Disorder (ADHD) [Hirsch et al., 2019]. ED is defined as the inability to control and minimize the disrupting effects of irrelevant emotional stimuli on goal-oriented processes [Barkley and Fischer, 2010; Wehmeier et al., 2010]. In adult ADHD, problems with emotion regulation include emotional recognition, emotional responsivity, and emotional lability, adding to the complexity of the spectrum of classic symptoms [Beheshti et al., 2020]. Importantly, ED predicts lower quality of life in young adults [Groenewold et al., 2013] and is associated with the persistence of ADHD into adulthood [Barkley and Fischer, 2010]. A recent meta-analysis demonstrated that pharmacological treatments have limited efficacy for ED in adults [Lenzi et al., 2018], yielding therapeutic challenges. As such, better insights into underlying (neural) mechanisms of ED in adult ADHD could help develop more effective treatments.

A critical aspect of ED in ADHD is impaired emotional reactivity (ER): the threshold, intensity, or duration of affective arousal, which can be measured through the processing of emotionally salient stimuli [Graziano and Garcia, 2015]. Adult participants with ADHD do not appear to have deficits in the explicit regulation of emotions, but display emotional hyper-responsivity [Materna et al., 2019]. For example, higher emotional lability has been associated with a hyper-connectivity of the cortico-amygdalar network, including the anterior cingulate cortex, both in children and adolescents [Hafeman et al., 2017; Hulvershorn et al., 2014]. In children with ADHD, the processing of negative emotional faces stimuli has been associated with amygdala hyper-connectivity and hyperreactivity [Brotman et al., 2010; Posner et al., 2011a; Posner et al., 2011b; Quinlan et al., 2017] and was notably also linked to ED [Herrmann et al., 2010]. In adults with ADHD, amygdala hyperactivity has further been demonstrated in response to salient stimuli [Maier et al., 2014; Plichta et al., 2009; Tajima-Pozo et al., 2018], but divergent findings have been reported as well [Hägele et al., 2016; Tajima-Pozo et al., 2015] and the exact neural mechanisms that underlie ER in adult ADHD thus remain still unclear.

Emotional regulation has been shown to be influenced by top-down cognitive control processes. For example, in controls, the prefrontal cortex (PFC) was activated stronger in the presence of emotional stimuli during cognitive control tasks [Hung et al., 2018; Song et al., 2017] and cognitively demanding tasks could tune down amygdala reactivity to emotional stimuli, suggesting top-down suppression of ER [van Dillen et al., 2009; Erk et al., 2007]. Furthermore, taxing WM during or prior to the exposure of emotionally salient stimuli reduced ER in both anxiety and substance use disorders [Andrade et al., 2012; van den Hout et al., 2014; Kaag et al., 2018; Markus et al., 2016; May et al., 2010; McClelland et al., 2006]. Additionally, WM training has been shown to

improve ER outcomes in healthy individuals as well as in individuals with psychiatric disorders other than ADHD [Barkus, 2020; Schweizer et al., 2013]. Whether this affects (the neural mechanisms underlying) emotional processing remains to be determined. Indeed, an underdeveloped working memory (WM) system may underlie impaired ER in ADHD [Groves et al., 2020]. More specifically, ADHD participants perform worse [Marx et al., 2011] and show reduced WM-related PFC activation in WM-tasks [Burgess et al., 2010; Cortese et al., 2012; Ko et al., 2013]. Altogether, this indicates that while WM and ER are strongly related, the underlying neural mechanisms of how emotional and WM processes interact in ADHD are still unclear [Tsai et al., 2020].

Therefore, this study aims to test whether targeting WM processes can reduce ER-related neural activity in adult ADHD, through top-down PFC suppression of amygdala hyperactivity. In order to disentangle neural mechanisms of emotional and WM processes, we used a novel functional magnetic resonance imaging (fMRI) paradigm, interleaving emotional stimuli with WM-load blocks. We expected that ADHD participants, compared to controls, would show higher levels of amygdala activation in response to negative emotional, relative to neutral images. Moreover, participants with ADHD were expected to show decreased dorsolateral PFC (dlPFC) and paracingulate gyrus (paCG) responses to high versus low WM-load tasks. We furthermore expected amygdala reactivity in participants with ADHD to be reduced in response to negative emotional blocks following high WM-load more so than in controls.

Methods

Thirty adults with ADHD and 30 controls (19–35 years of age) were included in the study. Inclusion criterion for the ADHD group was prior clinical ADHD diagnosis according to the DSM-IV [American Psychiatric Association, 2013]; controls were excluded with a score > 4 on the ADHD Rating Scale (ADHD-RS) [Kooij et al., 2008]. Controls were matched to the participants with ADHD, based on age educational-level, tobacco use (Fagerström Test for Nicotine Dependence)[Heatherton et al., 1991], alcohol use [Alcohol Use Disorders Identification Test (AUDIT)] [Saunders et al., 1993], cannabis use [Cannabis Use Disorders Identification Test (CUDIT)] [Adamson and Sellman, 2003], and the use of additional substances [Drug Use Disorders Identification Test (DUDIT)] [Berman et al., 2005]. Medicated participants with ADHD (N = 12) were instructed to refrain from ADHD medication use for seven days before the MRI scan. Exclusion criteria were: history of brain trauma, neurological disease, excessive consumption of alcohol (AUDIT > 12), cannabis (CUDIT > 12) or other drugs (DUDIT > 12), and MRI contra-indications. For control participants: psychiatric disorders for which they had ever received treatment; for participants with ADHD: psychiatric disorders other than ADHD for which they received

treatment at the moment of the experiment. Anxiety, depression, and impulsivity were assessed using the State and Trait Anxiety Inventory (STAI) [Marteau and Bekker, 1992], Beck's Depression Inventory (BDI) [Beck et al., 1961], and Barratt's Impulsiveness scale (BIS) [Patton et al., 1995], respectively. Written informed consent was obtained from all participants. The study was approved by the Ethics Review Board of the University of Amsterdam.

Statistical analyses

Data points more than three standard deviations from the mean were removed as outliers (results before and after outlier removal did not differ). Analyses of task and fMRI data used linear mixed-effects models (lme4 Rv.3.5.3) [Bates et al., 2015; R Development Core Team, 2011]. In the case of non-normal distributions, transformations were applied. The main and interaction effects of emotional load, WM load, and group were assessed as fixed effects. We used an adjusted top-down model selection process using the Bayesian information criterion (BIC) for model comparison [Fabozzi et al., 2014; Schwarz, 1978]. The model best capturing the data was reported and compared using χ^2 tests and BICs. $P < 0.05$ was considered statistically significant (Supplementary Materials 1.5).

MRI acquisition

Participants were scanned on a 3T whole-body MR system (Philips, Best, The Netherlands) using a 32-channel receive-only head-coil. T1-weighted (T1w) scans were obtained using a 3D-TFE sequence (resolution = 0.8mm^3, FOV = 240 × 256 × 200mm, TR/TE = 9.8ms/4.5ms). Functional scans were acquired using a 2D-GE-EPI sequence (resolution = 2.5 × 2.5 × 2.2mm, FOV = 240 × 240 × 131.8mm, TR/TE = 1500ms/30ms, FA = 70°, MB-factor = 3, SENSE = 1.5). A scan with opposite phase-encoding-direction was used for distortion correction.

fMRI paradigm

The experimental fMRI paradigm (Figure 1) comprised a blocked design wherein active blocks with either the zeroback or the two-back task ('WM-block') were interleaved with passive blocks consisting of either emotionally neutral or emotionally negative pictures ('EMO-block'). This resulted in four conditions: two-back followed by negative pictures (2E), two-back followed by neutral pictures (2N), zero-back followed by negative pictures (0E), and zeroback followed by neutral pictures (0N). The order of the blocks was randomized per participant, under the conditions that 1) the experiment started with a WM-block and 2) blocks were never immediately followed by the same type. The WM-blocks consisted of a standard 0-back and 2-back task ([Cousijn et al., 2014]; Supplementary Methods 1.2). During the emotional block, 64 pictures from the

International Affective Picture System (IAPS; Supplementary Materials 1.2) were shown (32 emotionally negative and 32 emotionally neutral; Figure 1) [Lang et al., 2005]. The percentage of correct responses in the n-back task were compared between conditions and groups. All participants performed a recognition task after the fMRI experiment to determine whether both groups paid equal attention to the images during the fMRI-task. Subsequently, participants performed a validation task, in which they rated the valence of all images using the Self-Assessment Manikin (SAM) rating from one ('negative') to nine ('positive') [Lang, 1980] (Supplementary Materials 1.2–1.3).

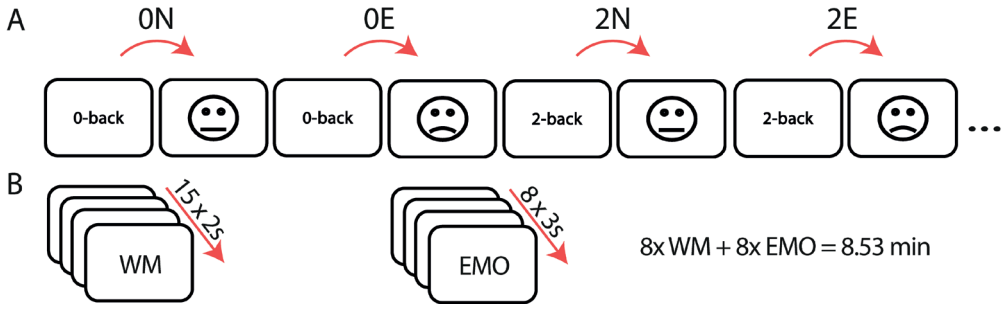

Figure 1. Study design: A) Interleaved active n-back blocks and passive emotional stimuli blocks. The effect of WM-blocks preceding emotional stimuli was assessed by subdividing the emotional stimuli into four conditions: neutral-after-0-back (0N), emotional-after-0-back (0E), neutral-after-2-back (2N), emotional-after-2-back (2E). B) The working memory (WM) blocks consisted of 15 trials lasting 2s each. The emotional images blocks (EMO) consisted of 8 trials lasting 3s each. Every condition was shown twice, resulting in 8 WM and 8 EMO-blocks randomly interleaved, which results in a total task duration of 8:53min

fMRI processing

Preprocessing was performed using FMRIPREP v1.2.3 [Esteban et al., 2019; Esteban et al., 2020] (RRID: SCR_016216). Each T1w scan was normalized to MNI space. Functional data preprocessing included motion correction (FLIRT), distortion correction (3dQwarp), followed by co-registration to the T1w. Independent component analysis (ICA) based Automatic Removal Of Motion Artifacts (AROMA) was used to generate data that was non-aggressively denoised. Subsequently, data were spatially smoothed (6mm FWHM) and high pass-filtered (342s) using FSL (Supplementary Methods 1.5). FMRI data were entered into the first-level analysis (FSL/FEAT v.6.00; RRID: SCR_002823) [Jenkinson et al., 2012]. The model was designed to estimate the effect of WM-load on the neural correlates of emotional processing (Supplementary Materials 1.5). To explore whole-brain activity in the main task contrasts (two-back vs. zero-back; emotional vs. neutral), the first-level contrast-of-parameter-estimates (COPE) maps were analyzed using non-parametric permutation testing (5000 permutations), using FSL Randomise. Thresholds for all analyses were initially set at $p < 0.05$ with family-wise error corrections using

threshold-free cluster enhancement [Winkler et al., 2014]. Mean framewise displacement per participant was added as a confound regressor. ROI) analyses used predefined ROIs: Amygdala, dlPFC, and paCG (Supplementary Methods 1.4). The amygdala is a key region in emotional processing [Sergerie et al., 2008], the dlPFC and paCG play an important role in executive functioning, especially during WM-back tasks [Miró-Padilla et al., 2018], whereas the paCG shows overlapping activation during negative affect and cognitive control paradigms [Lin et al., 2015; Shackman et al., 2011]. Hemisphere differences were tested using paired t-tests and found to be significant for the dlPFC, but not the amygdala. The left and right dlPFC were therefore analyzed separately, whereas parameters were averaged across hemispheres for the amygdala. Featquery (FSL) was used to extract the COPEs, which were converted to percentage change.

Results

Participants

Participants with ADHD and controls were well-matched (Table 1). Two participants were removed from the analysis, due to extreme motion during the fMRI-task (framewise displacement > 1.5 * 95% CI).

Table 1 Participants characteristics

		ADHD		controls		
		M (SD)	N	M (SD)	N	Statistics*
Age (y)		25.18 (4.06)	28	24.40 (3.86)	30	t (56) = −0.75, p = 0.46
Education		4.07 (1.63)	28	4.13 (1.63)	30	U = 416.00, z = −0.06, p = 0.95
Medication use			12/28			
ADHD-RS						
	Inattention (child)	6.71 (2.12)	28	1.33 (1.56)	30	U = 815.50, z = 6.22, p < 0.001
	Hyperactivity (child)	4.57 (2.74)	28	0.47 (0.78)	30	U = 783.00, z = 5.83, p < 0.001
	Inattention (adult)	5.36 (2.78)	28	1.23 (1.19)	30	U = 764.50, z = 5.42, p < 0.001
	Hyperactivity (adult)	3.71 (1.90)	28	1.07 (0.91)	30	U = 752.00, z = 5.26, p < 0.001
Comorbid psychiatric disorders						
	MDD		5		NA	
STAI (trait)		42.19 (10.81)	27	34.10 (5.27)	30	U = 601.50, z = 3.15, p = 0.002
STAI (state)		38.25 (9.58)	28	30.60 (5.51)	30	U = 621.00, z = 3.13, p = 0.002
BDI		6.71 (6.26)	28	3.63 (2.99)	30	U = 546.00, z = 1.97, p = 0.049
BIS		70.00 (9.49)	28	56.43 (6.95)	30	t (56) = −6.24, p < 0.001
AUDIT		5.82 (3.74)	28	5.97 (3.36)	30	U = 395.00, z = -0.39, p = 0.67
DUDIT		2.11 (2.63)	28	1.83 (2.38)	30	U = 441.50, z = 0.30, p = 0.72
CUDIT		2.50 (4.38)	28	2.40 (3.50)	30	U = 378.00, z = -0.72, p = 0.47
Tobacco use			11/28		10/30	χ^2 (1, n = 58) = 0.006, p = 0.94
Motion (FD, mm)		0.15 (0.07)	28	0.12 (0.03)	30	U = 348.00, z = -1.12, p = 0.26

*Normally distributed data were tested using independent samples t-tests, otherwise, Mann–Whitney tests, or χ^2-test were used

Education (Dutch system): 1 = VMBO/VMBO-T; 2 = MBO; 3 = HAVO; 4 = HBO; 5 = VWO; 6 = WO; *ADHD-RS* ADHD-Rating Scale; *STAI* State-Trait Anxiety Inventory; *BDI* Beck Depression Inventory; *BIS* Barrat's Impulsivity Scale; *AUDIT* Alcohol Use Disorders Identification Test; *DUDIT* Drug Use Disorders Identification Test; *CUDIT* Cannabis Use Disorders Identification Test

Behavioral measures

We found no interaction between WM-load and group on n-back accuracy ($\chi2(1) = 1.68$, $p = 0.19$, $\Delta BIC = -3.07$), but a main effect of WM-load was found ($\chi2(1) = 40.29$, $p < 0.001$, $\Delta BIC = 34.87$) (Figure 2A). The d-prime of the recognition task suggests that more attention was paid towards negative emotional images ($\chi2(1) = 6.78$, $p < 0.01$, $\Delta BIC = 1.36$), and that attention reduced after high-load WM-blocks ($\chi2(1) = 34.87$, $p < 0.001$, $\Delta BIC = 1.36$) (Figure 2B). Additionally, we found a trend towards an interaction effect of group and emotional image type ($\chi2(1) = 3.68$, $p = 0.05$, $\Delta BIC = -1.74$), suggesting that participants with ADHD paid more attention to the negative emotional images compared to controls. The validation task showed that negative images were perceived as more negative than the neutral images by both groups ($\chi2(1) = 143.29$, $p < 0.001$, $\Delta BIC = 138.55$) (Figure 2C).

fMRI

Exploratory whole-brain analysis assessed the effects of WM-load, emotional image type, and group. Permutation tests revealed the expected main effects of the WM-load and a main effect of the emotional image type in the executive and salience network, respectively (Figure 3, Supplementary Results 2.2). In contrast to our hypothesis, we found no main effect of group nor any two or three-way interactions of WM-load, emotional image type, and group.

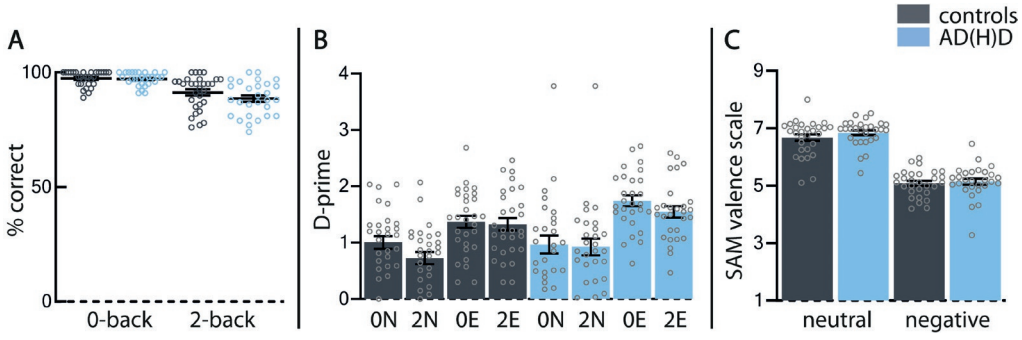

Figure 2 Behavioral data: A) Task performance; No significant differences between participants with ADHD (blue) and controls (gray). The performance of the 2-back task was lower than the 0-back task. B) Recognition task; Neutral images were recognized less correctly than negative images. There is a trend towards participants with ADHD recognizing negative images better than controls. C) Validation task; Both groups rated negative images as more negative than neutral images. Error bars represent the standard error

In line with the whole brain results, we found no significant interaction between WM-load and group on PaCG activation and left dlPFC (PaCG: $\chi2(1) = 2.01$, $p = 0.15$, $\Delta BIC = -2.73$; all other $\Delta BIC < -4.74$; left dlPFC: $\chi2(1) = 2.62$, $p = 0.11$, $\Delta BIC = -2.13$), but a main effect of WM-load was found (PaCG: $\chi2(1) = 41.12$, $p < 0.001$, $\Delta BIC = 36.38$; left dlPFC:

$\chi2(1) = 56.35$, $p < 0.001$, $\Delta BIC = 51.605$)(Figure 4A). Thus, high load WM-blocks (two-back) elicited more activity in the PaCG and left dlPFC than zero-back blocks, regardless of group. In the right dlPFC, we found a trend-significant interaction between group and WM-load ($\chi2(1) = 4.10$, $p = 0.04$, $\Delta BIC = -0.65$; all other $\Delta BIC < -3.73$), indicating that there was less WM-related right dlPFC activity in participants with ADHD compared to controls, which suggests that WM processes are only limitedly impaired in the investigated ADHD population.

We assessed the effects of the emotional image type and group on amygdala activity during emotional processing (Figure 4B). Model comparisons revealed evidence for a main effect of emotional images ($\chi2(1) = 37.32$, $p < 0.001$, $\Delta BIC = 45.65$), showing higher amygdala activity for negative than neutral images, but this was not moderated by group. We furthermore found no significant three-way interactions between emotional image type, preceding WM-block, and group ($\chi2(1) = 1.40$, $p = 0.84$, $\Delta BIC = -20.37$; all other $\Delta BIC < -5.19$), which suggests that taxing WM did not influence ER-related amygdala activation. Additionally, we assessed the effects of emotional images, the preceding WM-load and group on paCG activity during emotional processing, but found no three or two-way interactions ($\chi2(1) = 3.66$, $p = 0.45$, $\Delta BIC = -18.10$; all other $\Delta BIC < -4$); neither did we find a main effect of emotional images ($\chi2(1) = 0.97$, $p = 0.32$, $\Delta BIC = -4.47$), which suggests, in contrast to our hypothesis, paCG activity did not react to emotional salient stimuli, in either the ADHD or control group.

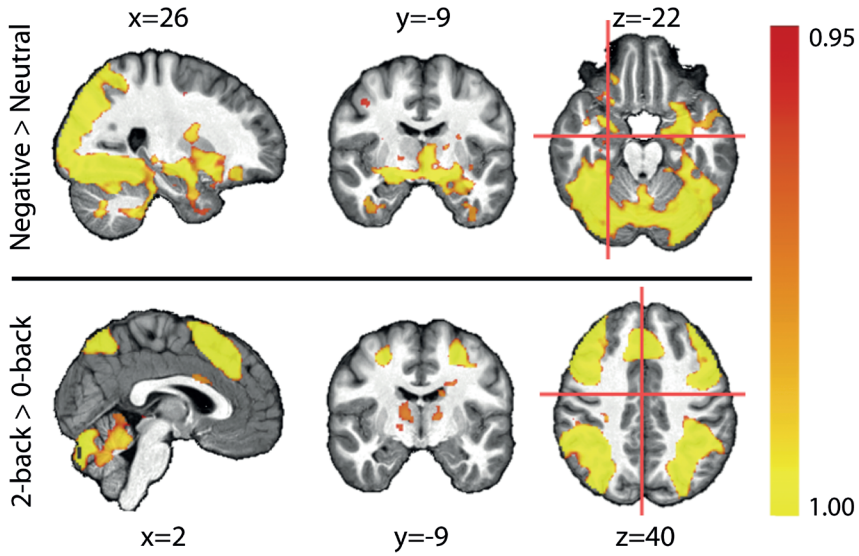

Figure 3 Whole brain activation maps calculated with permutation tests: BOLD signal for negative vs. neutral images (top row) and the 2-back vs. 0-back task (bottom row). Values shown are corrected and therefore correspond to the statistically significant regions. Colors correspond to 1-p results; with red = 0.95 (corresponding to p = 0.05) and yellow = 1.00

Discussion

The primary aim of this study was to investigate whether taxing WM could ameliorate ER in adults with ADHD, and how WM and emotional reactivity (ER) would interact on a neural level. We demonstrated a significant main effect of negative emotional images on amygdala activation and a significant main effect of WM-load on activation of the paCG and dlPFC across both groups but did not find strong evidence for group differences. These findings were in line with the WM-task performance, which also did not reveal any group differences. Contrary to our hypothesis, neither amygdala nor paCG activity was reduced in response to negative vs. neutral images after inducing a high WM-load in participants with ADHD or controls.

Emotional dysregulation in ADHD

ED in ADHD consist of a complex combination of dimensions, including emotion recognition, ER, and emotional lability. In the current study, however, we did not find amygdala hyperactivity in response to negative emotional stimuli in adults with ADHD. This contrasts earlier findings of amygdala hyperactivity in response to negative emotional faces [Brotman et al., 2010; Posner et al., 2011a; Posner et al., 2011b; Quinlan et al., 2017] and related to delay aversion [van Dessel et al., 2018; van Dessel et al., 2019; Lemiere et al., 2012] in children and adolescents with ADHD and in response to loss of anticipation [Tanaka et al., 2018; Wilbertz et al., 2017] and delayed rewards [Plichta et al., 2009] in adults with ADHD. Interestingly, the only two other studies that investigated ER in adult ADHD using stimuli similar to ours (i.e., IAPS images), also failed to find differences between participants with ADHD and controls [Hägele et al., 2016; Tajima-Pozo et al., 2018]. In our study, valence ratings of the IAPS images by participants with ADHD were comparable to that of controls, implying that the lack of amygdala hyperactivity was not due to the lack of experiencing the images as negative. As such, amygdala hyperactivity in participants with ADHD might be related to deficits in the processing of specific negative stimuli (e.g., loss anticipation), instead of general deficits in emotion regulation. This is in line with an earlier notion that ER, as an aspect of ED in participants with ADHD, is influenced by many factors including age and is much more complex than originally thought [Graziano and Garcia, 2015].

Working memory dysfunction in ADHD

In line with previous literature, we found that paCG and dlPFC were activated during the WM-task (high vs. low WM-load) [Müller and Knight, 2006; Roth and Courtney, 2007], but we did not find group differences in WM-related left dlPFC and paCG activation or behavior. In the right dlPFC, however, we found a trend-significant interaction between group and WM-load, suggesting blunted WM-related right dlPFC activation in participants

with ADHD, which is consistent with other studies in adults [Burgess et al., 2010; Cortese et al., 2012; Ko et al., 2013], and adolescents with ADHD [Fassbender et al., 2011; Massat et al., 2012; Mattfeld et al., 2016; Prehn-Kristensen et al., 2011]. In general, studies in adults with ADHD found less differences in neural recruitment between participants and controls than those in children with ADHD [Cortese et al., 2012]. This suggests compensatory effects with age that has been proposed to involve parietal, occipital, and subcortical structures (i.e., cerebellum and basal ganglia) that may overcome deficits presented earlier in life [Cortese et al., 2012; Frazier et al., 2007]. These compensatory mechanisms could explain why we did not find strong evidence for WM-dysfunction and associated frontal hypoactivity in adults with ADHD [Konrad and Eickhoff, 2010; Schweitzer et al., 2000; Schweitzer et al., 2004]. Nevertheless, previous studies have been inconsistent in their operationalization of WM tasks (e.g., n-back, recognition), assessing multiple aspects of WM. As a possible contributor, differences in task complexity and difficulty of different tasks may have affected the extent of activation, and may even have activated different brain regions [Müller and Knight, 2006; Roth and Courtney, 2007]. As this makes it challenging to compare results, we suggest that future studies take the complexity of WM tasks into account by including WM tasks that place greater demand on executive components of WM.

Effect of working memory activation on emotional reactivity

Behaviorally, we found that participants recognized negative emotional images better but had worse recognition of images that were preceded by high-load WM-blocks, indicating an influence of WM-load on emotional memory processes. In the neuroimaging data, however, the amygdala and paCG activation were not reduced in response to negative emotional images when they were preceded by high WM-load tasks, suggesting no influence of WM-load on the neural correlates of ER. Previous studies that aimed to identify an interaction between WM and ER in controls and other patient populations used emotional interference tasks in which WM-tasks were performed during the presentation of neutral and negative emotional stimuli. Using such paradigms, high WM-load reduced amygdala activation to negative emotional stimuli in participants with an increased risk of ED [Richter et al., 2013]. Also, negative emotional stimuli enhanced WM-related dlPFC activity in participants with major depressive disorder [Kerestes et al., 2012], adolescents with ADHD [Passarotti et al., 2010], and control adolescents, but not in control adults [Mueller et al., 2017]. These effects were supported by impaired WM performance in presence of negative emotional stimuli in adolescents [López-Martín et al., 2013; Marx et al., 2011; Villemonteix et al., 2017] and adults with ADHD [Marx et al., 2014]. Together, these studies suggest interaction effects between WM-load and ER-related amygdala (hyper-) activity. We were, however, unable to replicate these findings using a paradigm in which high WM-load was induced prior to emotional stimulation,

instead of during the presentation of emotional stimuli. While our negative findings may, at least in part, be due to the limitations of our paradigm, an additional explanation is that improving ER by inducing high WM-loads will only be effective when severe impairments of ER are present. This may not always have been the case in our ADHD sample, as no group differences were found in amygdala reactivity to emotional images and only weak evidence was found for right dlPFC hypoactivity. Divergent emotional processing in ADHD has been linked to a variety of features in individuals with ADHD [Beheshti et al., 2020], such as externalizing symptoms, including conduct problems [Gillberg et al., 2004] and internalizing symptoms, such as depression and anxiety symptoms [Jarrett and Ollendick, 2008]. The participants with ADHD we included here showed relatively

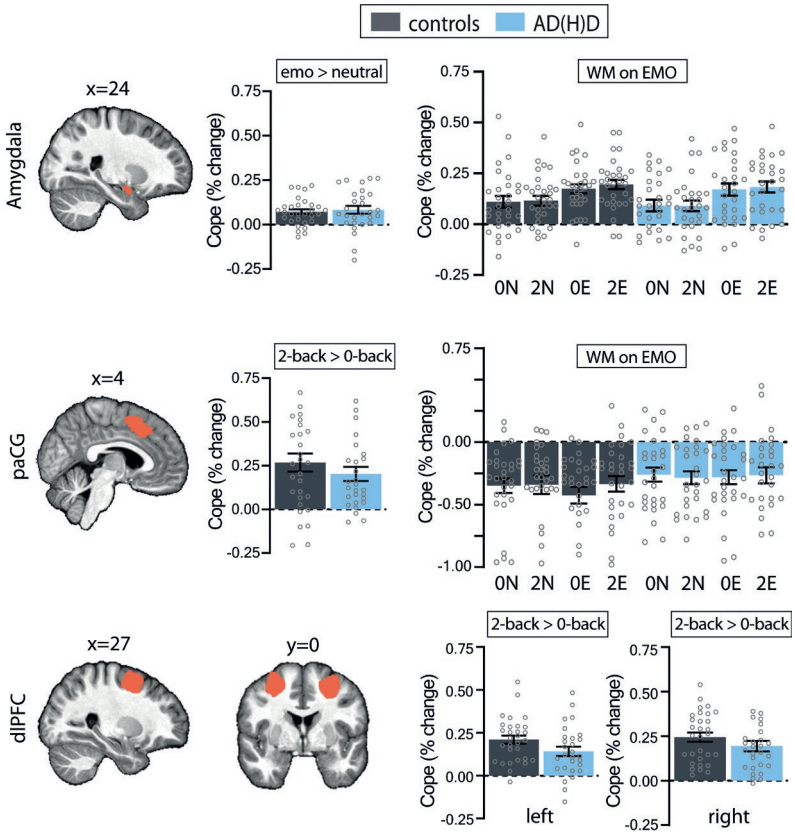

Figure 4. Region of Interest analysis: A) Amygdala activity during negative vs. neutral images (left) and emotional images preceded by working memory blocks (right) divided into the four conditions (0N, 2N, 0E, 2E). B) PaCG activity during 2-back vs. 0-back blocks (left) and emotional images preceded by working memory blocks (right) divided into the four conditions (0N, 2N, 0E, 2E). C) Left (left) and right (right) dlPFC activity during 2-back vs. 0-back blocks. paCG = paracingulate gyrus; dlPFC = dorsolateral prefrontal cortex; Error bars represent the standard error.

low scores on symptoms of anxiety and depression (although higher than the controls), which provides an indication that ER capacities may not have been strongly affected in the studied population. Indeed, other studies that found amygdala hyperactivity in individuals with ADHD also reported higher symptoms of depression and anxiety in their study populations [Maier et al., 2014; Wilbertz et al., 2013]. Interestingly, recent studies demonstrated that different subtypes of ED exist and that not all individuals with ADHD experience ED in all its complexities [Hirsch et al., 2019]. This thus yields the possibility that our current sample of participants with ADHD may have only relatively mild impairments in ER.

As the dimensions of ED in adult ADHD are complex and multifaceted [Beheshti et al., 2020], it is essential to develop paradigms that can disentangle the effects of executive and emotional processes in adult ADHD. Our novel fMRI-task design allows us to investigate the interactions of WM and emotional processes, like ER, which may help to understand the underlying neural mechanisms and develop novel training for ADHD.

Conclusion

We assessed possible neural correlates of the effects of targeting WM taxing on ER in adults with ADHD. We found no group differences in response to the emotional blocks on amygdala activation nor of WM-load on paCG and dlPFC activation. Although studies suggested an interaction between WM-load and emotional stimuli on the amygdala (hyper-) activity, we could not replicate these findings using our paradigm. These results might be due to compensatory effects in the adult participants with ADHD. Furthermore, targeting WM might still be effective in individuals with severe impairments in emotion regulation. These findings contribute to the understanding of the neural mechanisms of ER in controls and participants with ADHD.

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Supplementary Materials

Supplementary methods

Pilot study

Seventeen healthy male control participants between the ages of 20 and 29 years old were included in a pilot study before the main study, with the same in- and exclusion criteria as for the controls of the main study. Based on that study, it was concluded that a sample of 30 male participants with ADHD and 30 matched healthy male controls would be sufficient to demonstrate a relevant effect of the task.

fMRI task

The following images from the IAPS database were used for the fMRI task.

Table 1. Images used from the IAPS database.

Negative images	old	3053,3102,3080,3131,3000,3170,3064,3120,3015,3001,3100,9040,3130,3060,3069,3110,9410,3261,3530,3350,3071,3225, 9405,9940,9810,9220,9910,9340,9635.1,9295,9901,9921
	new	7380,9520,9902,9920,9830,9904,9903,9301,6230,6021,9900,9000,9911,9031,3010,3150
Neutral images	old	2396,4559,7365,3302,7043,2122,2377,2397,2038,2690,8466,2351,8001,2411,2488,2309,2770,2191,2032,2514,2749,8032, 2273,7255,7161,7012,7034,7081,7092,7207,7233,7020
	new	7059,7001,7632,7021,5740,7036,7044,7179,5533,7014,7017,7033,7493,7513,2092,8160

The experimental fMRI paradigm comprised a blocked design wherein active blocks with either the zero-back or the two-back task ('WM block') were interleaved with passive blocks consisting of either emotionally neutral or emotionally negative pictures ('EMO block'). This resulted in four conditions: a two-back block followed by negative pictures (2E), a two-back block followed by neutral pictures (2N), a zero-back block followed by negative pictures (0E), and a zero-back block followed by neutral pictures (0N). The order of the blocks was randomized per participant, under the conditions that 1) the experiment started with a WM block and 2) blocks were never immediately followed by a block of the same type.

A WM block consisted of 15 letters that were shown on the screen one by one. During the zero-back condition, participants were instructed to use their index finger to press a button on the left response box when they saw an 'x' and to press a button on the right response box when they saw any letter other than 'x'. During the two-back condition, participants were instructed to press the left button when the letter on the screen was the same as two letters before and the right button when the letter on the screen was different from two letters before. A total of 64 pictures shown during the experiment (32 emotionally negative and 32 emotionally neutral) were retrieved from the International Affective Picture System (IAPS). Every condition (2E, 2N, 0E, 0N) was shown twice, resulting in 16 blocks. The WM blocks lasted 30s each, the EMO blocks

24s each (8 stimuli with a duration of 2.5s and 0.5s inter-stimulus interval). Instructions before and in between the blocks were 5s long. In total, the task was 8:53min long. The percentages of correct responses in the n-back task were extracted and compared between conditions and groups.

Recognition task

All participants performed a recognition task after the fMRI experiment to determine whether both groups paid equal attention to the images while conducting the fMRI task. Images were shown on a computer screen one-by-one and participants were requested to indicate on a scale from 0 to 10 whether they recognized the pictures from the fMRI-task. A rating of 0 indicated they were confident they did not recognize the picture, a rating of 10 indicated they were confident they recognized the picture, and a rating of 5 indicated they were uncertain whether they recognized the picture or not. The recognition task included the 64 pictures that were shown during the fMRI experiment ('old' images) plus 32 'new' images (16 emotionally neutral and 16 emotionally negative) also retrieved from the IAPS and meeting the same criteria as the other images. The recognition data were analyzed to obtain d-prime (d') as a measure of discriminability, which is unaffected by response bias [Macmillan, 1993]. The rating scale was first divided into recognized as new (0-4), unsure (5), and recognized as old (6-10) per participant. Trials with a rating of unsure were discarded for the analysis. To calculate d', the z score that corresponds to the false-alarm rate was subtracted from the z-score that corresponds to the hit-rates [Macmillan, 1993]. The recognition task data were binned into the task conditions of the fMRI task, resulting in d' values per participant for 0N, 2N, 0E, and 2E. To correct for possible ceiling effects, the log-linear approach was used [Hautus, 1995].

Validation task

After the recognition task, the participants were requested to assess their subjective arousal in response to the images to measure the validity of the stimuli in our sample. During this validation task, participants rated the valence of all the images shown to them during the recognition task using the Self-Assessment Manikin (SAM) rating from 1 to 9, with one being 'negative' and nine being 'positive' (SAM; [Lang, 1980]). Ratings of all neutral and negative images were averaged per participant, respectively.

fMRI analysis

Preprocessing with fMRIprep:

Preprocessing was performed using FMRIPREP v1.2.3. Each T1w scan was bias-corrected, skull-stripped, and subsequently normalized to MNI space using non-linear registration. Functional data preprocessing included motion correction using FLIRT and distortion

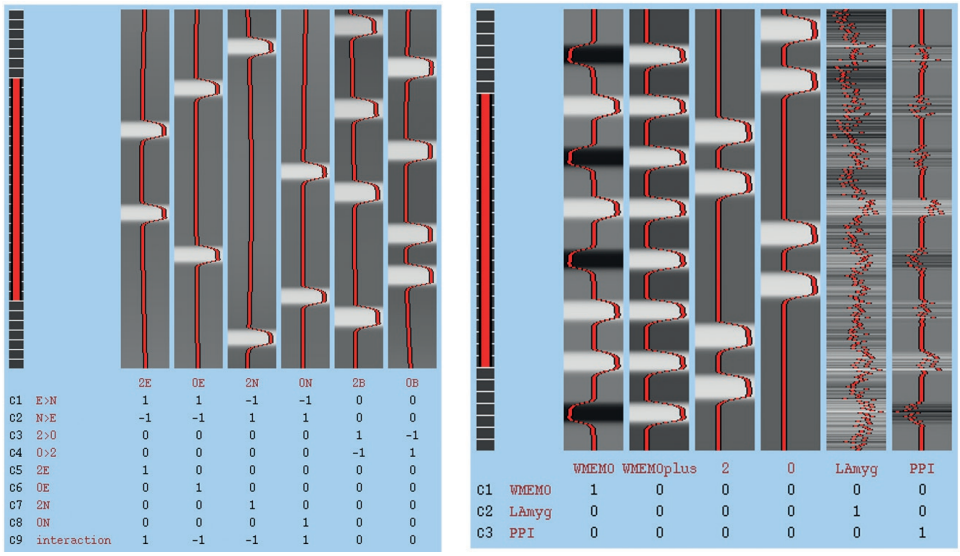
correction using an implementation of the TOPUP technique using 3dQwarp. This was followed by co-registration to the corresponding T1w using boundary-based registration with 9 degrees of freedom. Motion correcting transformations, field distortion correcting warp, BOLD-to-T1w transformation, and T1w-to-template (MNI) warp were concatenated and applied in a single step using antsApplyTransforms (ANTs v2.1.0) with Lanczos interpolation. Independent component analysis (ICA) based on Automatic Removal Of Motion Artifacts (AROMA) was used to generate data that was non-aggressively denoised. Subsequently, data were spatially smoothed (6mm FWHM), and a high pass-filter (342s) was applied using FSL. Since amygdala fMRI signals may be particularly affected by susceptibility artifacts induced by the magnetic field inhomogeneities in the ventral part of the brain, we calculated temporal signal-to-noise-ratio (tSNR) maps for the whole brain (Supplementary Results 2.1). The design matrices in Supplementary Figure 1 are examples of the ones used in this study.

ROI analysis

To assess the effect of WM-load on emotional processing, ROI analyses were conducted using predefined ROIs. The left and right amygdala were chosen as ROIs because they are key regions in emotional processing and were extracted from the Harvard-Oxford Subcortical Structural Atlas with a threshold of 90%. The dlPFC and the paCG were chosen as ROIs as they play a key role in executive functioning, especially during n-back tasks. Additionally, overlapping activation in studies using negative affect and cognitive control paradigms was found in the paCG. The dlPFC and paCG masks were based on the most robust activated clusters from a meta-analysis across 1091 WM fMRI studies (Neurosynth). For the amygdala, hemisphere differences were tested using a paired t-test and found not to be significant. Therefore, all parameters were averaged across hemispheres. For the dlPFC, hemisphere differences were tested using a paired t-test and found to be significant. The left and right dlPFC were, therefore, analyzed separately. Featquery (FSL) was used to extract the COPEs, which were converted to a percentage change.

Statistical Analysis

To assess all data for normality, histograms, and QQplots were inspected, and a Shapiro-Wilk test was performed. In the case of a non-normal distribution, a transformation was applied. Data points that were more than three standard deviations from the mean were removed as outliers. Demographics were analyzed with SPSS v.23 (IBM, Chicago, USA). Normally distributed data were tested using independent t-tests; otherwise, a Whitney-U test was used. To account for more than one source of random variability within-participant and across-participants, analyses of the task and fMRI data used linear mixed-effects models in Rv.3.5.3 [R Development Core Team, 2011] using the lme4 package [Bates et al., 2015]. The main and interaction effects of emotional load (EMO: emotional/



Supplementary Figure 1. A) Design Matrix of one representative participant for the task-based analysis. The model was designed to estimate the effect of working memory load on the neural correlates of emotional processing. B) Design Matrix of one representative participant for the PPI analysis. The model included the timeseries of the ROI as well as the task regressors and the PPI regressor.

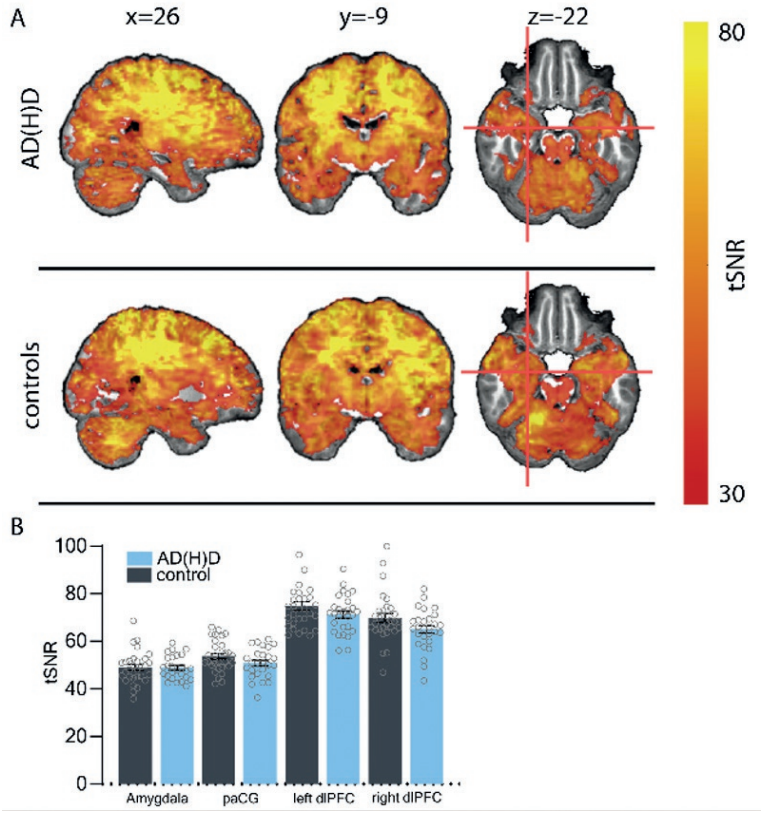
neutral), working memory (WM: 2-back/0-back), and group (GRP: ADHD/control) were assessed as fixed effects. The model selection process was based on an adjusted top-down procedure, where the full model (three-way interaction) was tested based on the Akaike information criterion (AIC) using the step function, which resulted in the most complex fixed effects structure that would then further be tested.

A random effect per subject was always included in the model (SUBJECT|1), controlling for individual variation among participants. The simplest model was then increased in complexity, adding fixed effects until all fixed effects that resulted from step one were included and compared using the Bayesian information criterion (BIC) [Fabozzi et al., 2014; Schwarz, 1978]. Random slopes were then added and tested based on BICs and χ^2 tests. If these were found to add in explaining the variance, they were added as random effects and all fixed effects were tested again based on the described semi top-down strategy. The model found best capturing the data was consequently reported and compared to other models using χ^2 tests and BICs. All statistical tests were conducted using a significance level of $p < 0.05$. ΔBIC is calculated by subtracting the BIC value of the tested model from the model it is being compared to. A negative ΔBIC, therefore, indicates that the tested model did not explain the variance better, whereas

a positive ΔBIC indicates that the tested model did explain the variance better. A ΔBIC between 0 and 2 can be interpreted as weak evidence, 2-6 as positive evidence, 6-10 as strong evidence, and ΔBIC>10 as very strong evidence against the model with the higher BIC value [Raftery, 1995].

Psychophysiological Interaction (PPI) analysis

To investigate the effect of WM-load during emotional processing on the connectivity of the ROIs (left and right amygdala, dlPFC, and paCG) to the rest of the brain, a PPI analysis was conducted. ICA-AROMA denoised functional data were entered into the first-level analysis (FSL/FEAT v.6.00), which included the time-series per ROI as well as the task regressors and the PPI regressor (Supplementary Figure 1). Whole-brain analyses were performed as described above, using the first level data of the PPI analysis.



Supplementary Figure 2. Temporal signal-to-noise analysis. A) Whole brain tSNR maps for ADHD patients (up) and controls (down). B) Average tSNR per participant (circles) and group (bars) per ROI and for both ADHD patients (blue) and controls (gray). paCG = paracingulate Gyrus, dlPFC = dorsolateral prefrontal cortex.

Supplementary results

Temporal Signal-to-Noise (tSNR)

Since amygdala fMRI signals may be particularly affected by susceptibility artifacts induced by the magnetic field inhomogeneities in the ventral part of the brain [Merboldt et al., 2001], we calculated tSNR maps for the whole brain [Welvaert and Rosseel, 2013].

The average tSNR in the amygdala for participants with ADHD was 48.88(5.05), and for controls was 48.96(6.83). In the paCG, the average tSNR for participants with ADHD was 50.8(6.29), and for controls was 53.71(6.57). In the left dlPFC, the average tSNR for participants with ADHD was 71.21(8.12), and for the controls was 74.74(10.34), in the right dlPFC, it was 64.95(8.41) for participants with ADHD and 69.79(10.43) for controls. As previously reported, a tSNR of around 50 requires the acquisition of around 350 volumes to detect a 2% signal change [Murphy et al., 2007]. We acquired 370 volumes. We could, therefore, conclude that we had sufficient tSNR to measure activation in our regions of interest.

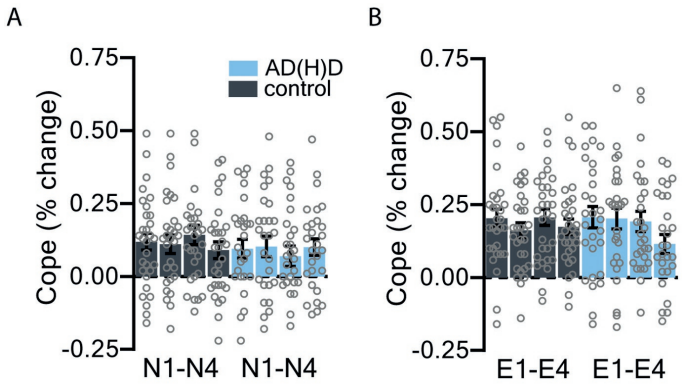
Whole Brain Analysis – Cluster Table

Supplementary Table 2. Clusters resulting from the whole brain analysis, assessing the effects of working memory load and emotional stimulus type.

Contrast	Region	Cluster Index	Voxels	MAX	MAX X (vox)	MAX Y (vox)	MAX Z (vox)	COG X (vox)	COG Y (vox)	COG Z (vox)
Negative images > neutral images										
	Occipital Cortex, Amygdala, Left Thalamus	5	39784	1	27	42	22	44.5	37.2	33.4
	Superior Frontal Gyrus	4	1210	0.994	48	90	47	45.3	91.7	47.2
	Right Precentral Gyrus	3	308	0.959	19	64	47	19.8	66.6	48.4
	Right Precentral Gyrus	2	162	0.957	25	59	58	22.8	61.1	59.5
	Paracingulate Gyrus	1	10	0.951	44	89	34	44.2	88.5	34.8
2-back > 0-back										
	Frontal Pole, Inferior Frontal Gyrus, Middle Frontal Gyrus, Paracingulate Gyrus	5	34443	1	27	91	28	44.2	59.2	54.8
	Cerebellum	4	8119	1	29	27	7	44.9	30.2	18.9
	Left Inferior Temporal Gyrus	3	287	0.986	71	34	31	70.1	34.9	32
	Right Inferior Temporal Gyrus	2	141	0.982	18	38	31	17.4	37.9	
	Brain Stem	1	15	0.955	44	46	35	43.5	47.4	

Habituation Analysis

A habituation analysis of the emotional response within the amygdala was performed to exclude the possibility of a difference in adaptive reduction in response to the emotional stimuli between participants with ADHD and controls. Blocks of emotional stimuli were analyzed depending on when they were presented (N1-N4/E1-E4), with the numbers indicating the position of the block in time. For the negative stimulus type there was no interaction effect of group and time found ($\chi^2(3) = 4.85$, $p = 0.18$, $\Delta BIC = -11.5$). Also, no main effects of group or time were found (all other $\Delta BIC < -8.35$). For the neutral stimulus type there was no interaction effect of group and time found ($\chi^2(3) = 1.13$, $p = 0.77$, $\Delta BIC = -15.01$). Also, no main effects of group or time were found (all other $\Delta BIC < -5.24$). We can, therefore, conclude that there was no habituation effect to negative or neutral emotional stimuli in the amygdala, for neither participants with ADHD nor controls.

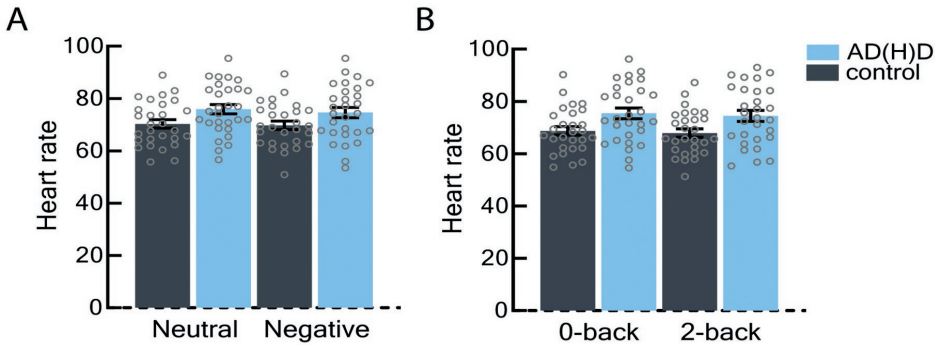


Supplementary Figure 3. Habituation analysis. A) Amygdala activity in response to the first (N1), second (N2), third (N3) and fourth (N4) block of neutral images, for ADHD patients (blue) and controls (gray). B) Amygdala activity in response to the first (E1), second (E2), third (E3) and fourth (E4) block of negative images, for ADHD patients (blue) and controls (gray).

Heart rate analysis

Heart rate was measured during scanning using a PPU to obtain plethysmographs. The average heart rate per WM load and type of emotional stimulus was calculated using MATLAB scripts. For the heart rate during the working memory task, no interaction effect of group and working memory load could be found ($\Delta BIC < -2.76$). A main effect of group was found ($HR \sim GRP + (1|SUBJECT)$; $\chi^2(1) = 5.70$, $p < 0.02$, $\Delta BIC = 1.02$). For heart rate during emotional stimuli, no interaction effect of group and type of emotional stimulus were found ($\Delta BIC < -0.16$). Also, no main effects of group or emotional stimuli were found, even though there was a trend towards a group effect. This indicates that participants with ADHD have a higher heart rate, independent of which task they were doing. We

can conclude that the task had no different effect on participants with ADHD than on controls. participants with ADHD demonstrated a higher heart rate in general, which could be an effect of ADHD medication.



Supplementary Figure 4. Heart rate analysis. A) Heart rate of participants with ADHD(blue) and controls (gray) during the presentation of neutral and negative emotional stimuli. B) Heart rate of participants with ADHD(blue) and controls (gray) during the low-load (0-back) and high-load (2-back) working memory task.

PPI

Whole Brain

For the whole brain PPI analysis, we assessed the effects of WM-load, emotional stimulus type, and group. Permutation tests did not show any connectivity of the left and right amygdala, nor paCG or left or right dlPFC with the rest of the brain. When contrasting emotionally negative and neutral images and high versus low WM-load, we found no differences in connectivity of the paCG or left or right dlPFC with the rest of the brain. Furthermore, we found no interaction effect of WM-load and emotional stimulus type on the connectivity of the left and right amygdala nor paCG or left or right dlPFC with the rest of the brain. Neither did we find any group differences.

In line with the presented task effects, no group differences were found, which could be explained by a low impairment of ER in our current ADHD patients. Positive structural and functional amygdala-frontal coupling as a mechanism that provides top-down control of emotions has been shown, e.g., in controls [Banks et al., 2007; Ochsner et al., 2009], during emotion regulation tasks [Stein et al., 2007] and more specifically as a top-down inhibitory effect of the PFC on the amygdala [Banks et al., 2007]. Moreover, studies using PPI-based analyses showed increased co-activation between the amygdala and the medial PFC [Erk et al., 2006] and between the amygdala and the dlPFC in depression [Siegle et al., 2007] during cognitive-emotional tasks. In contrast, Hägele et al., who also used IAPS stimuli, did not find any PPI effects using a seed in the left and

right amygdala, which they interpreted as due to high inter-individual differences in patients [Hägele et al., 2016]. These differences, including, e.g., differences in perception of stimuli or different symptom severities, make it difficult to reach the necessary power to calculate a PPI in patient populations, and indicate that effect sizes in these kinds of analyses are very small [O'Reilly et al., 2012].

Methodological considerations

To make sure our results were not due to methodological limitation, we assessed temporal signal-to-noise-ratio (tSNR) maps per participant, investigated habituation effects in the amygdala, and the influence by heart rate. Amygdala fMRI signals can be especially affected by susceptibility-artifacts induced by inhomogeneities in the subcortex [Merboldt et al., 2001]. Therefore, we assessed temporal signal-to-noise-ratio (tSNR) maps per participant ([Welvaert and Rosseel, 2013]; Supplementary Results 2.1), which were sufficiently high in all ROIs for all participants. Additionally, amygdala activation to emotional stimuli has been suggested to show a different adaptive reduction between ADHD patients and controls [Breiter et al., 1996; Garrett et al., 2012]. However, we did not find any evidence for such habituation effects (Supplementary materials and results 2.3). As BOLD contrast is based on hemodynamic changes, heart rate (HR) might cause fluctuations in the signal [Shmueli et al., 2007]. Task effects were not found to be influenced by HR (supplementary materials and results 2.4). Therefore, it is unlikely that our results were affected by these methodological challenges.

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Chapter 3

Prolonged MPH & amygdala reactivity in ADHD

Effects of prolonged methylphenidate treatment on amygdala reactivity and connectivity: a randomized controlled trial in stimulant treatment-naive, male participants with ADHD

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Abstract

Background: Problems with emotional processing are widely reported in individuals with attention deficit/hyperactivity disorder (ADHD). Although methylphenidate (MPH) effectively alleviates inattention and hyperactivity symptoms in ADHD, its effects on emotional processing and internalizing symptoms have remained elusive. While we previously found that acute MPH administration modulated neural mechanisms underlying emotional processing in an age-dependent manner, the effects of prolonged administration remained unknown.

Objectives: Therefore, we investigated: (i) Whether prolonged MPH treatment influences neural substrates (amygdala reactivity and connectivity) of emotional processing, and (ii) whether these effects are modulated by age.

Methods: The "effects of Psychotropic drugs On Developing brain-MPH" ("ePOD-MPH") randomized controlled trial was a 16-week double-blind, placebo-controlled, multi-center trial with MPH in 50 boys (10–12 years of age) and 49 men (23–40 years of age), all stimulant treatment-naive and diagnosed with ADHD. Participants performed an emotional face-matching task during functional magnetic resonance imaging. We assessed their symptoms of ADHD and internalizing symptoms at baseline, during the trial (8 weeks), and 1 week after the trial end (17 weeks).

Results and Conclusions: We did not find effects of prolonged MPH treatment on emotional processing, as measured by amygdala reactivity and connectivity and internalizing symptoms in this trial with stimulant treatment-naive participants. This differs from our findings on emotional processing following acute MPH administration and the effects of prolonged MPH treatment on the dopamine system, which were both modulated by age. Interestingly, prolonged MPH treatment did improve ADHD symptoms, although depressive and anxiety symptoms showed a medication-independent decrease. Furthermore, our data indicate that baseline internalizing symptoms may be used to predict MPH treatment effects on ADHD symptoms, particularly in (male) adults with ADHD.

Introduction

Methylphenidate (MPH), the primary pharmacological treatment for attention-deficit/hyperactivity disorder (ADHD), effectively alleviates symptoms of inattention and hyperactivity in individuals with ADHD. However, individuals with ADHD also present difficulties in emotion processing, independent of other comorbidities [Lenzi et al., 2018]. Divergent emotional processing in ADHD has been linked to both externalizing symptoms, such as conduct problems [Gillberg et al., 2004], and to internalizing symptoms associated with symptoms of anxiety and depression [Jarrett and Ollendick, 2008; Sciberras et al., 2014]. Additionally, it has been found to impact the quality of life of individuals with ADHD seriously and was in fact associated with poorer daily life functioning [Kuhne et al., 1997; Riley et al., 2006; Schei et al., 2016; Sciberras et al., 2014]. Clinical experience suggests that MPH may positively affect emotion regulation, as supported by a recent meta-analysis [Lenzi et al., 2018].

One possible pathophysiological substrate underlying emotional processing in ADHD may involve a dysfunctional striato-amygdalo-medial prefrontal cortical network [Shaw et al., 2014]. Likewise, in ADHD, specific brain regions related to emotion processing have shown altered connectivity to the rest of the brain [Icer et al., 2018]. For example, more emotional problems, particularly externalizing symptoms, were associated with a hyperconnectivity of the cortico-amygdalar network, including the anterior cingulate cortex, both in children and adolescents [Damiani et al., 2020; Hafeman et al., 2017; Hulvershorn et al., 2014]. Furthermore, different self-regulation problem dimensions were associated with stronger negative whole-brain functional connectivity patterns in children [Rohr et al., 2020]. Additionally, in adolescents with ADHD (aged 11–16 years), hyperreactivity and -connectivity of the amygdala were reported in response to fearful faces, which was notably increased further after MPH abstinence [Posner et al., 2011a]. Also, an acute MPH challenge has been found to normalize altered resting-state circuits in children and adults with ADHD [Pereira-Sanchez et al., 2021]. However, even though a few studies have shown these positive effects of stimulants on internalizing emotional symptoms [Biederman et al., 2009; Coughlin et al., 2015], the exact neural mechanisms underlying changes in emotional processing in ADHD remained unclear, especially following more prolonged durations of stimulant treatment.

Increasing preclinical evidence suggests that the effects of ADHD medication are modulated by age [Andersen, 2003; Andersen, 2005; Urban et al., 2012], which we also found to be the case in a clinical trial comparing boys and adults with ADHD [Schrantee et al., 2016; Solleveld et al., 2017]. Accordingly, we had previously shown that acute MPH administration modulates one of the functional neural mechanisms underlying emotional processing, i.e., amygdala reactivity, in an age-dependent manner [Bottelier et al., 2017]. Additionally, preclinical studies have shown that prolonged treatment

during adolescence induced anxiety and depressive-like behavior [Bolaños et al., 2003] and increased impulsivity during adulthood [Somkuwar et al., 2016]. The most comprehensive study on long-term effects of ADHD medication to date, i.e., the multi-modal treatment study of ADHD (MTA), found that children treated with ADHD medication had higher rates of anxiety and depression (19.1%) than children receiving behavioral therapy only (4.3%), as measured 6 years after treatment onset. However, this effect had disappeared after 8 years [Molina et al., 2009].

Therefore, in the current study, we set out to: (i) investigate whether prolonged treatment with MPH influences internalizing symptoms and the neural substrates underlying emotional processing in stimulant-naive participants with ADHD, and (ii) assess whether these effects are modulated by age. Based on the literature, we expected that MPH would increase amygdala reactivity and the connectivity to the prefrontal cortex during an emotional face-matching, functional magnetic resonance imaging (fMRI) paradigm in children but not, or less so, in adults.

Methods

The present study is part of the "effects of Psychotropic drugs On Developing brain-MPH" ("ePOD-MPH") randomized controlled trial (RCT), which was a 16-week double-blind, randomized, placebo-controlled, multi-center trial with MPH, and a blinded endpoint evaluation in stimulant treatment-naive participants with ADHD [Bottelier et al., 2014]. The primary objective of the ePOD-MPH RCT was to report on the age-dependent effects of MPH on the outgrowth of the dopaminergic system, as published elsewhere [Schrantee et al., 2016]. The current study investigated the secondary outcome measures, namely functional measures underlying these changes, including emotional processing. The study protocol applied the code of medical ethics and was registered by the Central Committee on Research Involving Human Subjects (an independent registry) on March 24, 2011 (identifier NL34509.000.10) and subsequently at the Netherlands National Trial Register (identifier NL2955/NTR3103). The enrollment started with the first patient on October 13, 2011, ended on June 15, 2015, and was monitored by the Clinical Research Unit of the Amsterdam University Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

Participants

We included 50 stimulant treatment-naive boys (10–12 years of age) and 49 stimulant-treatment naive men (23– 40 years of age) in the ePOD-MPH RCT. They were diagnosed with ADHD and recruited through clinical programs at the Child and Adolescent Psychiatry Center Triversum (Alkmaar), the Department of Child and Adolescent

Psychiatry at the Bascule/AMC (Amsterdam), and the PsyQMental Health Facility (The Hague). An experienced psychiatrist (MAB) diagnosed all children and adults. They met criteria for ADHD according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, 4th edition), as confirmed by a structured interview, i.e., the Diagnostic Interview Schedule for Children [NIMH-DISC-IV: authorized Dutch translation [Ferdinand and van der Ende, 1998]] and the Diagnostic Interview for ADHD (DIVA 2.0) for adults [Kooij, 2012]. The DSM-IV requirement of at least six inattention or hyperactivity/impulsivity symptoms was applied to both children and adults. Exclusion criteria were: comorbid axis I psychiatric disorders requiring treatment with medication at study entry; and a history of major neurological or medical illness or clinical treatment with drugs influencing the dopaminergic system (for adults before 23 years of age), such as stimulants, neuroleptics, antipsychotics, and/or D2/D3 agonists. More detailed inclusion and exclusion criteria are listed in the Supplementary Methods. All participants and parents or legal representatives of the children provided written informed consent after receiving a complete description of the study.

Intervention, randomization, and blinding

After baseline (BL) assessments, we stratified participants by age and randomized them to either placebo or MPH treatment (1:1), using a permuted block randomization scheme generated by the local Clinical Research Unit. The treating physician prescribed the study medication under double-blind clinical guidance (reduction of ADHD symptoms) following Dutch treatment guidelines. Participants received oral dosages of short-acting MPH starting with 1–2 doses of 0.3 mg/kg daily. Dosages were increased weekly with 5–10 mg/day to a maximum of 50 mg/day until the target clinical dosage was reached, in line with clinical guidelines in the Netherlands. If, after decreasing the dosage, serious side-effects occurred, the participant returned to the previous dosage and dosage modifications were more gradual thereafter. Decisions about dosage modifications were always and only done by the treating psychiatrist (mean dosage per person in Supplementary Results). Participants, care providers, and research personnel were blinded to the treatment condition (Supplementary Methods for further details). The Medical Center Alkmaar hospital pharmacy assigned participants to a specific allocation, using sequentially numbered containers. The appearance of the placebo tablet was identical to the MPH tablet and was manufactured and labeled according to GMP guidelines (2003/94/EG). We obtained data at three timepoints: at BL, at 8 weeks into treatment (during treatment = DT), and 1 week after the treatment had ended (post treatment=PT) (Figure 1). Short-acting MPH has a half-life ($t_{1/2}$) of approximately 2 hours; therefore, MPH is cleared approximately 10 hours after the last MPH administration. We used a wash-out period of 1 week to ensure that no acute effects of MPH influenced the PT assessment. Adherence to the study medication was monitored at each of the control

visits and was expressed as a percentage, based on the number of tablets remaining divided by the number of tablets that should have remained (based on the daily dose, adjusted at each of the control visits). Adult participants received coaching sessions, and parents of children received psychoeducation.

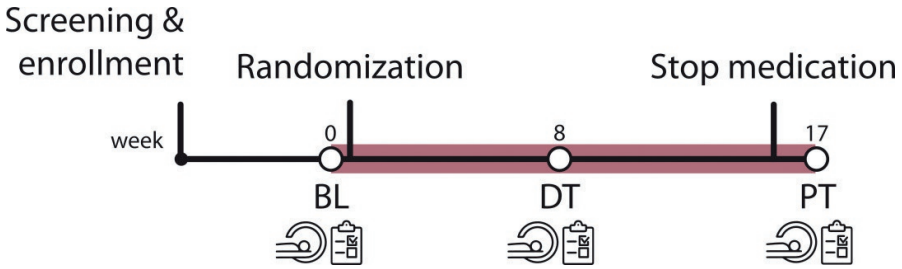

Figure 1. Timeline of the ePOD-MPH RCT. We measured fMRI activity and connectivity on an emotional face-matching task at three time points: at BL before randomization, 8 weeks during treatment (DT), and 1 week after the trial end (PT). Furthermore, we assessed clinical measures of ADHD, anxiety, and depression at these same timepoints.

Clinical and behavioral variables

In children, we assessed ADHD severity, anxiety, and depressive symptoms using the disruptive behavior disorder rating scale (DBD-RS) [Pelham Jr. et al., 1992], Child Depression Inventory (CDI) [Kovacs, 1985], and the child version of the Screen for Child Anxiety Related Disorders (SCARED) [Muris P, Bodden D, Hale W, 2007]. In adults, we used the Attention-Deficit Hyperactivity Disorder-Self Report (ADHD-SR) [Kooij, 2012], Beck's Depression Inventory (BDI) [Beck et al., 1961], and Beck's Anxiety Inventory (BAI) [Beck et al., 1988]. We assessed all clinical scales at BL, DT, and PT (Figure 1). Behavioral response data (accuracy and reaction times) of the fMRI task were extracted from E-Prime.

fMRI

Participants performed an emotional face-matching fMRI paradigm at BL, DT, and PT (Figure 1). We presented a practice run before the first MRI scan and used two versions of the task to minimize learning effects. The emotional face-matching paradigm consisted of a blocked design and was adapted from a task previously used to assess drug effects on amygdala reactivity [Bottelier et al., 2017; Hariri et al., 2002]. The emotional stimuli consisted of angry and fearful faces, and the neutral stimuli consisted of ellipses assembled from scrambled faces (Supplementary Figure S1). During the task, we recorded reaction-time to button press and accuracy.

The MRI study was performed on a 3T Philips scanner (Philips Healthcare, Best, Netherlands) using an eight-channel receive-only head coil. Eight children and one

adult were scanned on a 3T Phillips scanner at a different center (Philips Healthcare, Best, Netherlands). A 3D T1-weighted anatomical scan was acquired for registration purposes, and fMRI data were acquired using a single-shot echo-planar imaging sequence (parameters: TR/TE = 2300/30ms, resolution = 2.3 × 2.3 × 3mm, 39 sequential slices, FA = 80°, dynamics = 70). Preprocessing was performed using FMRIPREP v.1.2.3 [Esteban et al., 2019; Esteban et al., 2020](RRID: SCR 016 216). Each T1-weighted (T1w) scan was normalized to MNI space. Functional data preprocessing included motion correction (FLIRT), distortion correction (3dQwarp), followed by co-registration to the T1w. Independent component analysis-based Automatic Removal Of Motion Artifacts (ICA-AROMA) was used to generate non-aggressively denoised data. Subsequently, data were spatially smoothed (6mm FWHM) and high pass-filtered (100s) within FSL/FEAT (FSL/FEAT v.6.00; RRID: SCR 002 823) (Supplementary Methods for further detail).

FMRI data were entered into the first-level analysis in FSL/FEAT [Jenkinson et al., 2012]. For our regions of interest (ROI) analyses of the emotional face-matching task, mean signal intensity for the left and right amygdala [Posner et al., 2011b] was extracted from the first level contrasts using masks from the Harvard–Oxford atlas (thresholded at 50%). To explore whole-brain activity in the main task contrasts (faces vs shapes; shapes vs faces), the first-level contrast-of-parameter-estimates (COPE) maps were analyzed pairwise using nonparametric permutation testing (5000 permutations) in FSL Randomise. Thresholds for all analyses were initially set at $P < 0.05$ with family-wise error corrections using threshold-free cluster enhancement [Winkler et al., 2014]. From a total of 198 MRI scans, 34 scans could not be entered into the statistical analysis (17.2%). Exclusion criteria for MRI scans were: technical problems (0.6%), mean frame-wise displacement >0.5 mm (2%), scrubbing >15% (1.5%), drop-out (8.6%), or incomplete understanding of the task <70% accuracy (4.5%).

Psychophysiological interaction (PPI) analyses were conducted to assess connectivity during the emotional face-matching task. The left and right amygdala were chosen as seed regions and separately entered into two first-level models. Whole-brain analyses were performed as described before, using the first level data of the PPI analysis.

Statistical analysis

All statistical analyses were conducted using R v.3.5.3 [R Development Core Team, 2011]. Clinical and behavioral variables were analyzed intention-to-treat, and fMRI activity was analyzed per protocol with the significance level set at $P < 0.05$ (two-sided). All data were checked for normality and, in the case of nonnormality, transformed accordingly. To account for missing data points and the longitudinal nature of the RCT, linear mixed effects models were used to analyze clinical and behavioral variables and fMRI activity to investigate the main effect of scan session (BL, DT, PT), medication group (placebo, MPH), and age group (children, adults), and its corresponding interaction effects using

the lme4 package [Bates et al., 2015]. For the amygdala reactivity data, the average framewise displacement per participant was added to the model as a covariate. A variable representing the scanner that was used was tested as a possible covariate and found to not contribute significantly. Additionally, we tested whether leaving out the participants with comorbidities changed the results of our analyses. Model selection was based on an adjusted topdown procedure, in which the resulting models were compared using the Bayesian information criterion (BIC), and consequently, the model best capturing the data was reported using approximate F-tests based on the Kenward–Roger approach [Kenward and Roger, 1997]. Follow-up pairwise comparisons were corrected for multiple testing using a Sidak correction. Exploratory prediction analyses were done using linear models (lm); BL ADHD severity scores were included as a covariate.

Results

Clinical characteristics and randomization

A total of 99 participants with ADHD were randomized to either MPH or placebo. No serious adverse events were reported in any of the participants. Treatment groups did not differ in age, intelligence quotient (IQ), depressive or anxiety symptoms, and ADHD severity at BL (Table 1). One adult in the placebo group had a current panic disorder. Discarding the data from this participant did not change the results, and therefore we decided to include these data in the analyses.

Treatment assignment

In Supplementary Figure S2, treatment allocation and dropout rates are reported according to CONSORT standards. One adult was excluded from the analysis due to undisclosed previous stimulant treatment. Eight adults underwent the PT scan at 8 weeks instead of at 17 weeks of the trial due to significant technical changes (software upgrade) to the MRI scanner. The mean treatment duration did not differ between both treatment groups in adults ($t(42) = -0.02$, $p = 0.98$) or children ($t(45) = 0.15$, $p = 0.88$). Medication conditions did not differ in age, IQ, or ADHD, depression and anxiety symptoms, or motion parameters after exclusion of scans (Supplementary Table S1).

	Children MPH $n = 25$ mean ± SD	Placebo $n = 25$ mean ± SD	Statistics	Adults MPH $n = 24$ mean ± SD	Placebo $n = 24$ mean ± SD	Statistics
Age (y)	11.4 ± 0.8	11.3 ± 0.9	$t(48) = -0.42$, $P = 0.67$	28.6 ± 4.6	29.0 ± 4.9	$t(46) = -0.59$, $P = 0.55$
Estimated IQ[1]	104.8 ± 21.0	103.4 ± 15.1	$t(46) = 0.28$, $P = 0.77$	107.9 ± 8.8	107.9 ± 6.4	$t(42) < 0.01$, $P > 0.9$
ADHD subtype, no.						
Inattentive	14	14		11	5	
Hyperactive/impulsive	0	1		0	0	
Combined	11	10		13	19	
ADHD symptoms						
DBD-RS[2] inattention	21.7 ± 3.2	22.8 ± 3.4	$t(47) = -1.18$, $P = 0.24$	–	–	
DBD-RS[2] hyperactivity	15.0 ± 5.0	16.4 ± 6.3	$t(47) = -0.90$, $P = 0.37$	–	–	
ADHD-SR[3]	–	–		31.8 ± 9.9	31.1 ± 9.7	$t(46) = 0.24$, $P = 0.82$
Comorbid psychiatric disorders:						
Panic disorder	0	0		0	1	
Depressive symptoms[4]	8.1 ± 4.4	8.5 ± 4.6	$t(46) = 0.87$, $P = 0.79$	6.3 ± 5.5	8.1 ± 6.2	$t(44) = -1.05$, $P = 0.30$
Anxiety symptoms[4]	25.9 ± 17.4	29 ± 16.9	$t(47) = -0.63$, $P = 0.53$	9.1 ± 6.7	8.9 ± 8.2	$t(46) = 0.08$, $P = 0.94$
Adherence (%)	84 ± 15	80 ± 18	$t(44) = 0.88$, $P = 0.38$	90 ± 8	86 ± 8	$t(43) = 1.98$, $P = 0.06$
Average MPH dose (mg)	31.3 ± 7.3	34.4 ± 7.9	$t(45) = 0.80$, $P = 0.43$	51.1 ± 9.8	55.2 ± 8.7	$t(42) = -0.18$, $P = 0.86$

[1]For children: Wechsler Intelligence Scale for Children (WISC) (Kort *et al.*, 2002). For adults: National Adults Reading Test (NART) (Schmand *et al.*, 1992); [2]DBD-RS (Pelham Jr *et al.*, 1992); and [3]ADHD-SR (Kooij, 2012).
[4]Depressive symptoms and anxiety symptoms: children, CDI (Kovacs, 1985) and SCARED (Muris *et al.*, 1998); adults, BDI (Beck *et al.*, 1961) and BAI (Beck *et al.*, 1988).

Behavioral outcomes

Linear mixed-effects model analyses showed a significant medication x scan-session effect for ADHD symptoms in adults ($F(2,78) = 4.82$, $p = 0.01$). Posthoc tests revealed that ADHD symptoms in the MPH group decreased significantly more than in the placebo group from BL to 8 weeks during treatment (DT) and continued to be lower 1-week PT (ADHD-SR: DT: $t(101) = -2.21$, $p = 0.03$; PT: $t(95) = -2.33$, $p = 0.02$). In children, the inattention subscale showed a significant medication × scan-session effect (DBD-RS-A: $F(2,83) = 5.47$, $P < 0.01$), but not the hyperactivity subscale (DBD-RS-H: $F(3,90= 2.48$, $p = 0.07$). Post hoc tests revealed that inattention symptoms in the MPH group decreased significantly more than in the placebo group from BL to 8 weeks DT and continued to be lower 1 week PT (DBD-RS-A: DT: $t(116) = -3.62$, $p < 0.01$; PT: $t(111) = -3.77$, $p < 0.01$). For the hyperactivity subscale in children, we found a significantly larger decrease for the MPH group than for placebo at DT, but not at PT (DBD-RS-H: DT: $t(94) = -2.16$, $p = 0.03$; PT: $t(88) = -1.87$, $p = 0.07$) (Figure 2A; Supplementary Table S2).

For anxiety symptoms, a main effect of scan session was only found in the children (SCARED: $F(2,93) = 22.70$, $p < 0.01$; BAI: $F(2,85) = 2.01$, $p = 0.14$); both the MPH and placebo conditions in children showed improvement from BL to 1 week PT (MPH: $t(96) = 3.32$, $p < 0.01$; placebo: $t(94) = 5.17$, $p < 0.001$) (Figure 2B; Supplementary Table S2). For depressive symptoms, in both children and adults, a main effect of scan session was found (CDI: $F(2,91) = 38.17$, $p < 0.01$; BDI: $F(2,44) = 4.05$, $p = 0.02$). Post hoc tests showed that both the MPH and placebo conditions in children improved from BL to 1week PT (CDI: MPH: $t(93) = 5.36$, $p < 0.001$; placebo: $t(92) = 6.58$, $p < 0.001$); however, in adults, the effect was

driven by a small effect of the placebo group from BL to DT (BDI: t(90) = 2.77, p = 0.02). No treatment effects were found from BL to PT (BDI: MPH: t(91) = 1.45, p = 0.32; placebo: t(90) = 0.79, p = 0.71) (Figure 2B; Supplementary Table S2).

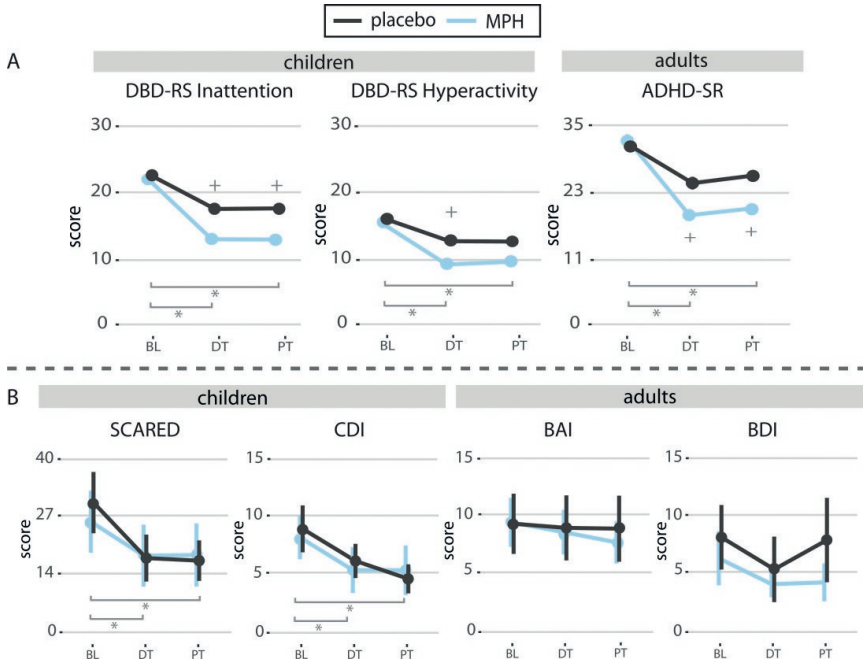

Figure 2. (A) ADHD symptoms. Line graphs show mean with 95% CI ADHD symptoms. A medication (MPH, placebo) × session (BL, DT, PT) effect was found for ADHD symptoms in adults and the inattention subscale in children but not the hyperactivity subscale. Post hoc tests revealed that ADHD symptoms in the MPH group decreased significantly more than in the placebo group from BL to DT and continued to be lower at PT in adults and the inattention subscale in children. We found a significantly larger decrease for the MPH group than placebo at DT for the hyperactivity subscale in children, but not at PT. (B) Clinical variables. Line graphs show the mean with 95% CI of anxiety, and depression scores. A main effect of session (BL, DT, PT) was found for anxiety and depressive symptoms in children, and depressive symptoms in adults. For depression and anxiety symptoms, both the MPH and placebo conditions in children showed improvements from BL to PT, but not adults. ∗= post hoc effect of session; += post hoc effect of medication condition.

Prediction analysis revealed a significant interaction effect of BL depressive and anxiety symptoms and medication condition on ADHD symptom change from BL to DT and BL to PT in adults, but not children (DT-BL: BDI: $F(4,31) = 8.93$, $p < 0.01$; BAI: $F(4,32) = 10.70$, $p < 0.01$; PT-BL: BDI: $F(4,34) = 13.26$, $p < 0.01$; BAI: $F(4,35) = 13.67$, $p < 0.01$). Post hoc tests showed that adults in the placebo condition did not show any relation between the

clinical variables and ADHD symptom change. In contrast, a negative association was found in the MPH condition, meaning higher BL depression and anxiety scores predicted a larger ADHD symptom severity decrease (Figure 3).

fMRI results

Emotional face-matching paradigm as expected, the emotional face-matching task elicited activity in the bilateral amygdala, bilateral and medial prefrontal cortex, and bilateral occipital and parietal areas, including the fusiform gyrus at BL. For activation maps, see Bottelier et al. [2017].

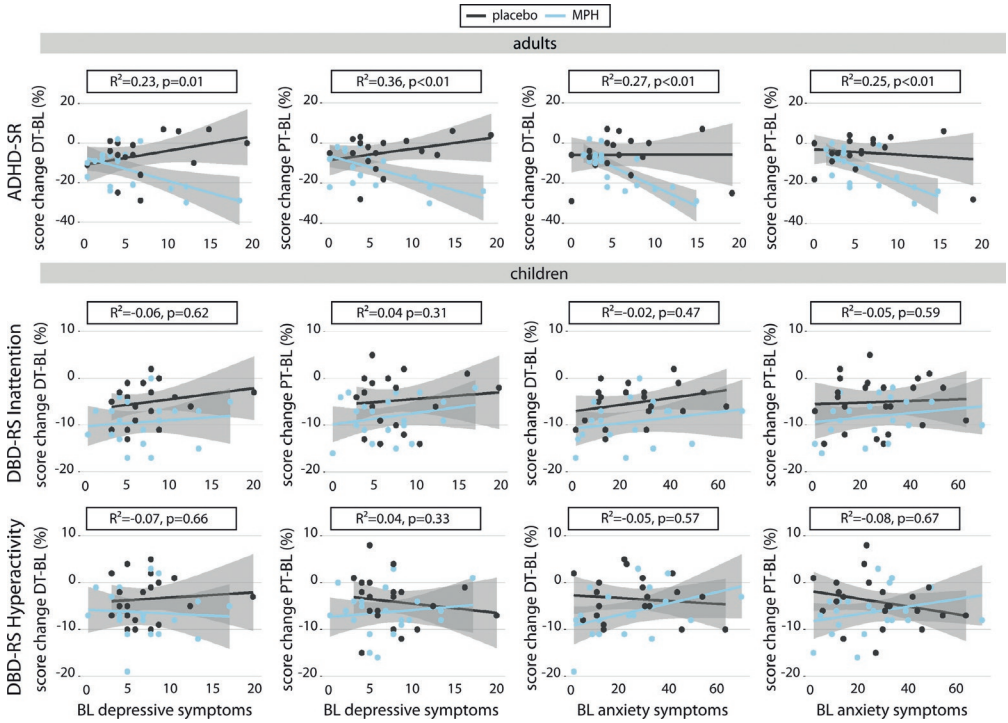

Figure 3. Prediction analysis. Scatterplots showing linear regressions between BL anxiety or depression symptoms and ADHD symptom severity change from BL to DT and BL to PT. Higher clinical scores at BL significantly predicted a higher decrease in ADHD symptom severity in adults treated with MPH (but not placebo) and not in children.

Linear mixed-effects model analyses did not show a significant age × medication × scan-session interaction on left or right amygdala reactivity (left: $F(11,181) = 0.91$, $p = 0.53$; right: $F(10,172) = 0.74$, $p = 0.69$), nor a significant scan-session × medication interaction in children (left: $F(5,70) = 1.23$, $p = 0.30$; right: $F(5,69) = 1.22$, $p = 0.31$) or adults (left: $F(5,93) =$

0.69, p = 0.63; right: F(5,93) = 1.17, p = 0.33), nor any main effects of scan session (children: left: F(2,63.61) = 0.68, p = 0.51; right: F(2,60.58) = 1.27, p = 0.29; adults: left: F(2,83.85) = 0.86, p = 0.43; right: F(2,82.64) = 0.85, p = 0.43), or medication (children: left: F(1,39.45) = 0.14, p = 0.70; right: F(1,40.54) = 0.85, p = 0.36; adults: left: F(1,42.50) = 1.12, p = 0.30; right: F(1,43.12) = 2.57, p = 0.12) (Figure 4A; Supplementary Table S2). Furthermore, none of the clinical questionnaires of ADHD, depression, or anxiety in either the children or adults correlated with the left or right amygdala reactivity in any of the sessions (Supplementary Table S4). Accuracy and reaction time (RT) data did not show any medication × scan-session interaction (children: accuracy faces: F(5,72.66) = 0.99, p = 0.50; accuracy shapes: F(3,68.83) = 1.24, p = 0.30; RT faces: F(5,66.93) = 1.06, p = 0.39; RT shapes: F(5,87.10) = 0.84, p = 0.52; adults: accuracy faces: F(5,85.32) = 0.09, p = 0.66; accuracy shapes: F(3,82.97) =

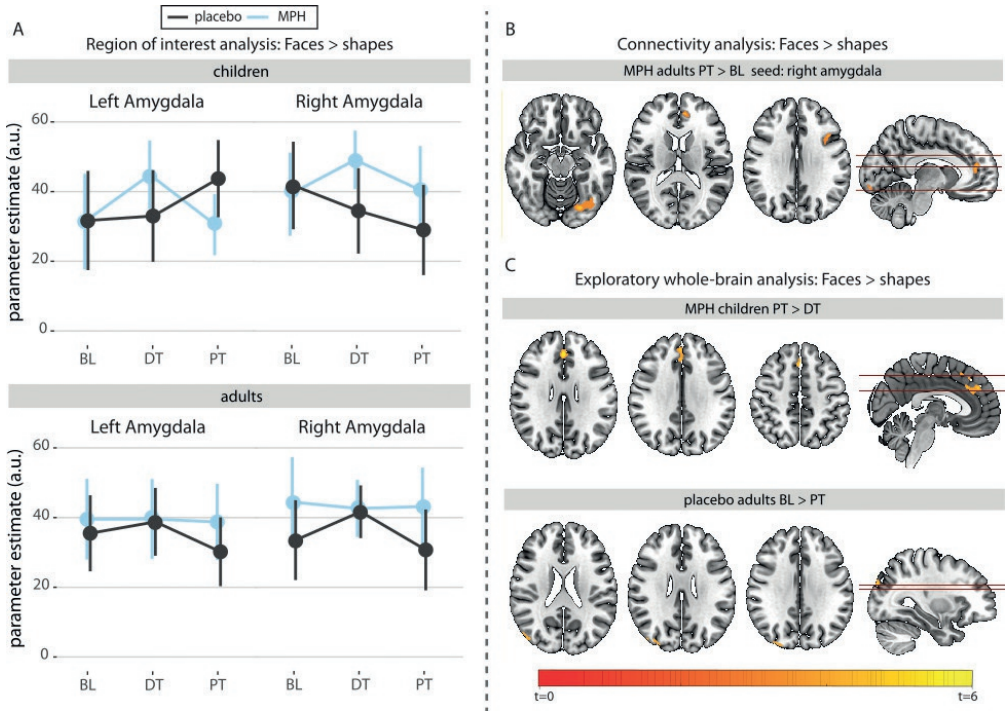

Figure 4. FMRI results of the emotional face-matching task. (A) ROI analysis. Line graphs display mean with 95% CI, showing no significant interaction or main effects of session (BL, DT, PT) and medication (MPH, placebo).
(B) PPI-analysis.Whole-brain maps per group showed increased connectivity between the right amygdala and the occipital fusiform gyrus, paracingulate gyrus, and inferior frontal gyrus in adults treated with MPH from BL to PT. (C) Exploratory whole-brain analysis. Whole-brain maps show increased reactivity in the superior frontal cyrus and paracingulate cortex in children treated with MPH from BL to DT and decreased reactivity in the lateral occipital cortex in adults treated with placebo from BL to PT.

0.04, p = 0.99; RT faces: F(3,81.50) = 1.36, p = 0.26; RT shapes: F(5,67.13) = 1.02, p = 0.42), nor medication main effects (Supplementary Table S2, Supplementary Figure S4).

Connectivity and exploratory whole-brain analyses PPI analysis per group indicated that MPH increased the connectivity between the right amygdala and the occipital fusiform gyrus, paracingulate gyrus, and inferior frontal gyrus in adults from BL to 1 week PT (Figure 4B; Supplementary Table S3). Exploratory whole-brain analyses revealed that MPH increased reactivity to the emotional face-matching task in the superior frontal gyrus and paracingulate cortex in children in the period of 8 weeks during treatment (DT) to 1 week after treatment. Additionally, a decrease in reactivity in the lateral occipital cortex was found in placebo-treated adults from BL to 1 week PT (Figure 4C).

Discussion

In this 4-month RCT in stimulant treatment-naive boys and men with ADHD, MPH did not influence internalizing symptoms or neural substrates underlying emotional processing, although MPH positively affected ADHD symptoms in both the children and adults, compared to placebo. Furthermore, we did not find that age modulated any of these effects. However, PPI analyses showed an increase of connectivity between the right amygdala and frontal regions only in the MPH-treated adults. In our exploratory whole-brain reactivity analyses we found small increases in cortico-limbic circuits in the MPH-treated children and in MPH-treated adults, we showed decreasing effects in the lateral occipital cortex. Interestingly, higher BL depressive and anxiety symptoms in adults predicted larger ADHD symptom reductions in the MPH but not the placebo condition. These results were independent of BL ADHD severity, providing further evidence for the important role of internalizing symptoms in obtaining clinical response and the role of ADHD medication herein.

Previous studies, including our research in the same sample of ADHD participants (BL only), showed that acute MPH administration normalized the heightened amygdala reactivity during emotional processing in individuals with ADHD [Bottelier et al., 2017; Posner et al., 2011a]. In contrast, in the current study on prolonged MPH treatment, we did not find evidence for altered amygdala reactivity, nor was this response age dependent. Previous studies comparing individuals with and without ADHD using emotional processing tasks have found mixed results, with some studies reporting increased left amygdala reactivity [Brotman et al., 2010; Posner et al., 2011b], whereas others only found significant results for either adults or children, or only in participants with certain comorbidities [Shaw et al., 2014]. Consequently, functional impairments of the amygdala, and therefore also the influence of MPH thereon, may be heterogeneous and highly dependent on the task. Furthermore, studies have suggested that ADHD-related deficits in the PFC

may be responsible for the deficient integration of information of regions responsible for perception and emotion recognition [Winston et al., 2003]. Therefore, medication effects should be assessed within the functional network associated with the task.

Indeed, in our exploratory whole-brain connectivity analyses we found small yet specific effects in the MPH treated children within cortico-limbic circuits. Although these findings require replication in larger samples, they indicate that MPH induces changes in top-down control processes, as MPH has been found before to primarily affect fronto-parietal circuits [Faraone et al., 2019]. In line with our results, the "dyscontrol hypothesis" postulates that externalizing symptoms in ADHD are not emerging from direct dysfunctional emotional processing itself, but rather from executive dysfunction, affecting top-down processes, such as the capacity to suppress responses evoked by emotional stimuli [Posner et al., 2014]. Research on internalizing symptoms and their neural correlates in ADHD participants is scarce. One study found that increased connectivity of the amygdala with prefrontal regions is associated with higher internalizing emotional regulation problems in children with ADHD [Uchida et al., 2015]. Although we did not find changes in amygdala-prefrontal connectivity in children, in MPH-treated adults, we showed a significant increase in connectivity between the right amygdala and frontal regions, including paracingulate gyrus and inferior frontal gyrus. Additionally, this group showed increased connectivity between the right amygdala and the fusiform gyrus during the task. This pathway is thought to be important for emotional feedback from the amygdala during visual processing in the fusiform gyrus [Vuilleumier et al., 2004]. While several studies have linked deficits in connectivity in this particular pathway to problems with emotional processing in various disorders [Herrington et al., 2011], future studies should consider investigating the influence of MPH on internalizing symptoms and its relation to the neural mechanisms of emotional processing in ADHD further as research on this topic is still scarce.

Children across both treatment conditions scored lower on scales of anxiety and depression during and after the trial. This finding points towards a general trial effect [Arkes and Harkness, 1980], including the consequences of a diagnosis and the subsequent support, rather than medication-specific improvements in these symptoms. It is important to note that the BL anxiety scores in this sample were in the clinical range for anxiety symptoms for most children (54% SCARED > 25), but their BL depression scores were identified as "none to mild" for 84% of the sample. However, in adults, the BL depression and anxiety scores were in the subclinical range for most (BDI: 83% less than mild depressive symptoms; BAI: 70% less than mild anxiety symptoms). Therefore, it is perhaps not surprising that anxiety symptoms did not change over the course of the trial.

Depressive symptoms in adults transiently decreased during the trial in the placebo condition. This effect was minimal and is likely due to the significant variance and individual differences in this measure. Despite the low prevalence of anxiety and

depressive symptomatology, our results are of clinical importance. Contrary to previous preclinical and some human studies [Bolaños et al., 2003; Somkuwar et al., 2016], we did not observe an increase in depressive and anxiety symptoms in the MPH condition during the 16 weeks in this well-controlled trial. This is in line with another long-term (3-year) study, which found a reduced risk for developing depressive symptoms associated with previous medication [Chang et al., 2016]. Moreover, the effect of MPH on internalizing symptoms is likely patient-specific and Coughlin et al. (2015) argued in their meta-analysis that the positive impact on anxiety symptoms outweighs the risk of psychostimulants inducing anxiety in children with ADHD, and so far, the causal link between stimulant treatment and internalizing symptoms thus remains debated.

Interestingly, higher depression and anxiety symptoms at BL predicted a larger ADHD symptom-severity reduction during the trial (week 8) and after the trial end (1 week PT) in the adult MPH condition, but not in the adult placebo group or in children. The current guidelines for treating adult ADHD state that comorbid depression and anxiety require treatment before starting stimulant medication, as stimulants could introduce internalizing symptoms as a side-effect [JJ et al., 2004; Kollins, 2008]. In contrast, we did not find an MPH induced increase in internalizing symptoms in adults or children with ADHD, and therefore do not confirm previous findings [Fredriksen et al., 2014; Molina et al., 2009]. As such, we could argue that worries about MPH introducing internalizing symptoms in children or adults might be unwarranted and MPH should be considered as a potential treatment for adults with ADHD and anxiety and/or depression comorbidities. However, close monitoring of side-effects should always be ensured and future studies in samples with more severe internalizing symptoms should replicate these findings. Notably, adults' depression and anxiety scores at BL in our study were in the subclinical range. Therefore, future studies should additionally consider investigating the interactions between MPH, depression and anxiety, and ADHD symptoms within an ADHD population with more severe internalizing symptoms.

These results are conflicting with the recent findings of Masi et al., who reported that higher ED at BL, as assessed by the CBCL dysregulation profile (including symptoms of anxiety/depression, aggression, and inattention), predicted higher ADHD symptoms at follow-up after 4 weeks of MPH treatment in children and adolescents [Masi et al., 2020]. In their trial, individuals were followed for 4 weeks of MPH treatment, whereas we assessed our participants (both children and adults) after 8 and 17 weeks. Additionally, the operationalization of emotion regulation problems differed between the two studies; while Masi et al. assessed emotion dysregulation defined as a combination of internalizing and externalizing symptoms (depression, anxiety, attention, and aggression) and considered absolute values of ADHD symptom severity at PT, we focused on internalizing symptoms of anxiety and depression in relation to changes in ADHD symptom severity, possibly explaining the differences in results.

A critical strength of our current study is its design. To rule out the influence of a history of medication use, we included only stimulant treatment-naive individuals. For ethical reasons, we could not extend the follow-up period to more than 4 months and did not include healthy control participants in our study; therefore, we cannot argue how amygdala reactivity changed compared to healthy control participants. Further limitations of our study are that its results cannot be extrapolated to all children and adults with ADHD, as we only studied male participants within a specific age range. We chose to include only male participants to limit participant variation. Females and males differ considerably in their patterns of brain growth [Giedd et al., 1999] and ADHD is most prevalent in male individuals [Polanczyk et al., 2007]. Additionally, the fact that patients in the MPH groups were prescribed short-acting MPH, and that DT scans were carried out throughout the day, may have resulted in increased variability in fMRI activity within these groups at that timepoint. However, this is not reflected in differences in variance between the groups. Even though we applied advanced and state-of-the-art motion correction methods, we had to exclude several scans due to motion in the MRI scanner; consequently, framewise displacement did not differ between groups included in the analysis (Supplementary Table S1). Furthermore, internalizing symptoms are known to change over development, and as such, its operationalization varies in children and adults, even though we assume similar underlying neural processes [Shaw et al., 2014]. This makes a comparison of the symptomatology between the age groups challenging, especially keeping in mind the complexity of the multiple domains that might span emotion regulation problems [Graziano and Garcia, 2015].

Future studies should clarify the effects of prolonged stimulant therapy on the differential effects on internalizing and externalizing comorbidities in ADHD, both separately and together, and their underlying neural mechanisms. In conclusion, we did not find evidence for the effects of prolonged MPH on internalizing symptoms nor neural substrates underlying emotional processing. Nevertheless, we did demonstrate that MPH improved ADHD symptoms the most in adults with the highest depressive and anxiety symptoms at BL, suggesting that adult ADHD patients with comorbidities could also benefit from treatment with MPH. Furthermore, we did not confirm that MPH treatment increased internalizing symptoms in either children or adults, suggesting that worries about (early) prescription of MPH might be unwarranted. Nevertheless, future studies in an ADHD population with more severe internalizing symptoms should confirm these findings to guide treatment in patients at risk for, or presenting with, comorbidities.

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Supplementary Materials

Supplementary Methods

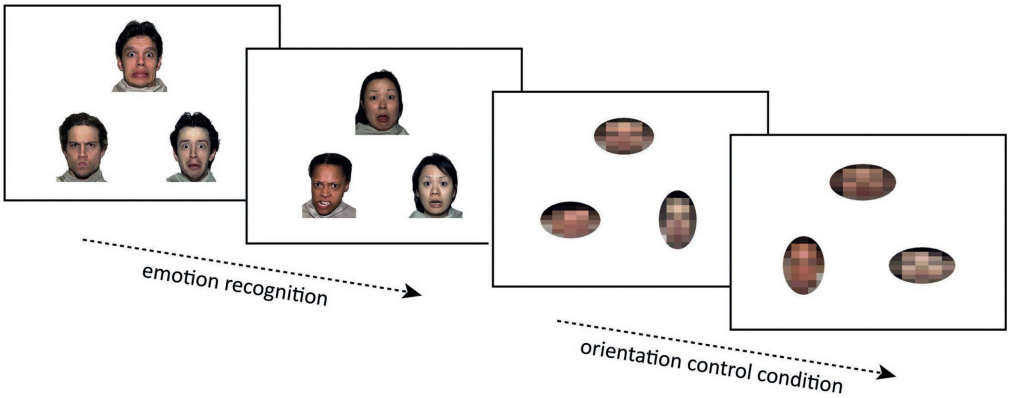
Participants

Boys aged 10-12 years and men aged 23-40 years were included. Inclusion criteria were meeting criteria for a diagnosis of and requiring treatment with medication for ADHD (Inattentive, Hyperactive/Impulsive or Combined subtype). The diagnosis was determined by an experienced clinician based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; [American Psychiatric Association, 1994]), which was confirmed with a (semi-)structured interview [Ferdinand and van der Ende, 1998] in children; Diagnostic Interview for Adult ADHD (DIVA; [Kooij, 2012]). The DSM-IV requirement of at least six inattention or hyperactivity/impulsivity symptoms was applied to both children and adults. Participants were not eligible when they had received clinical treatment influencing the DA system (for adults before age 23), such as stimulants, neuroleptics, antipsychotics, D2/D3 agonists, or when they had a current or previous dependency on drugs that influence the DA system (for adults before age 23). Other exclusion criteria were an estimated IQ < 80 (Block Design and Vocabulary subtests of the WISC-III-R [Kort et al., 2002], Dutch Adult Reading Test [Schmand et al., 1992], and/or a history of significant medical or neurological trauma or illness (see Figure 2 for a CONSORT flow diagram).

fMRI paradigm

Subjects performed an emotion recognition fMRI paradigm at three different time points during the trial. To further minimize learning effects, a practice run was presented before the first MRI scan. Two versions of the tasks were used to overcome learning effects.

The emotion recognition paradigm consisted of a blocked design and was adapted from a task previously used to assess drug effects on amygdala reactivity [Bottelier et al., 2017; Hariri et al., 2002]. The emotional stimuli consisted of angry and fearful faces, whereas the neutral stimuli consisted of ellipses assembled from scrambled faces (Supplementary Figure 1). Two blocks of emotional stimuli were interleaved with three neutral blocks, each block (30s) containing six trials (5s) (6 trials per block x 5 blocks = 30 trials (15min)). For each emotional trial, three stimuli were presented simultaneously, and subjects had to decide which one of the lower two stimuli expressed the same emotion as the target stimuli presented above. Similarly, for each neutral trial, three stimuli were presented, but subjects had to decide which of the bottom two ellipses was identically oriented to the target ellipse. During the task, reaction time to button press and accuracy were recorded.



Supplementary Figure 1.fMRI task paradigm.

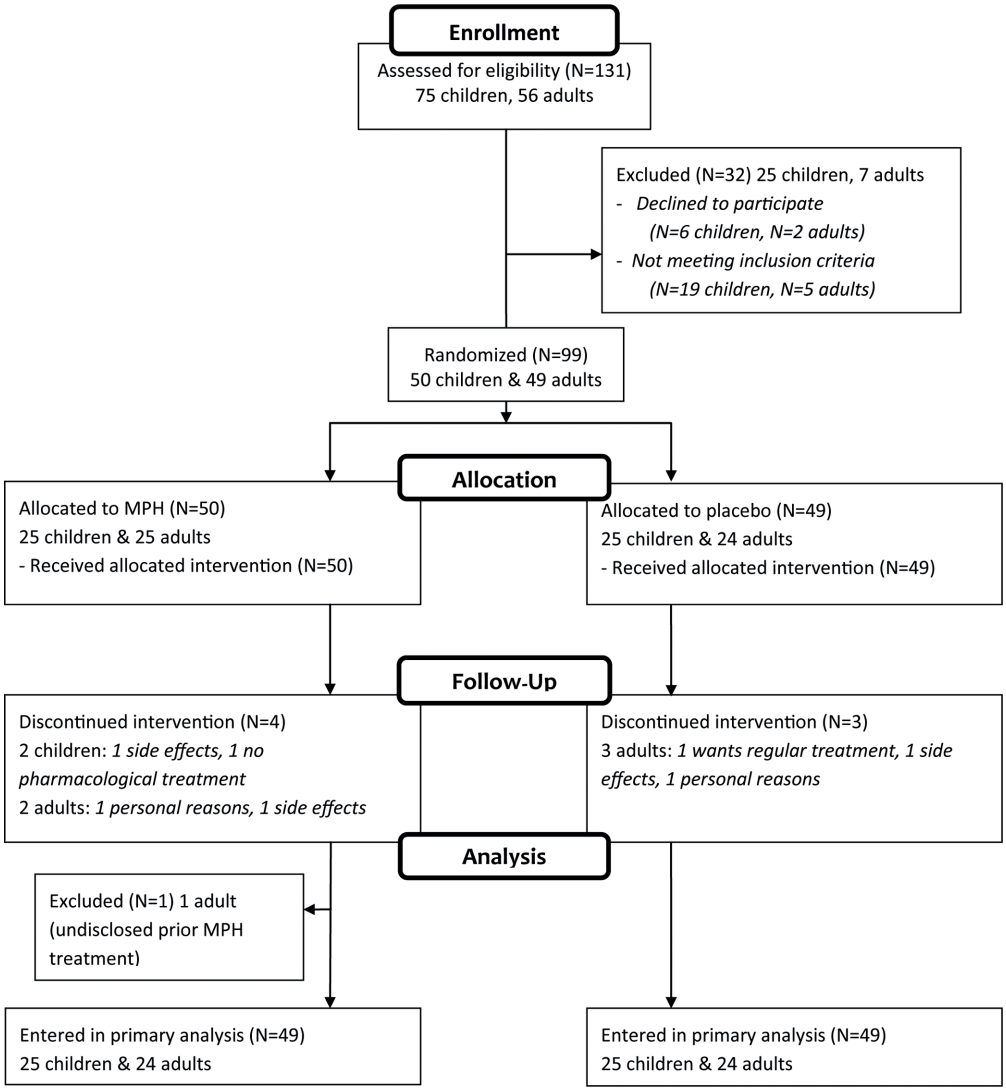
MRI acquisition

The MRI study was performed on a 3.0 T Philips scanner (Philips Healthcare, Best, The Netherlands) using an 8-channel receive-only head coil. A high-resolution 3D T1-weighted anatomical scan was acquired for registration purposes, and fMRI data were obtained using a single-shot echo-planar imaging sequence Parameters were: TR/TE=2300/30ms, resolution=2.3 × 2.3 × 3mm, 39 sequential slices, FOV=220x220x117mm, GE-EPI read-out, no gap, 80° flip angle, 70 dynamics were used.

MRI preprocessing

Preprocessing was performed using FMRIPREP v1.2.3. Each T1w scan was bias-corrected, skull-stripped, and subsequently normalized to MNI space using non-linear registration. Functional data preprocessing included motion correction using FLIRT and distortion correction using an implementation of the TOPUP technique using 3dQwarp. This was followed by co-registration to the corresponding T1w using boundary-based registration with 9 degrees of freedom. Motion correcting transformations, field distortion correcting warp, BOLD-to-T1w transformation, and T1w-to-template (MNI) warp were concatenated and applied in a single step using antsApplyTransforms (ANTs v2.1.0) with Lanczos interpolation. Independent component analysis (ICA) based on Automatic Removal Of Motion Artifacts (AROMA) was used to generate data that was non-aggressively denoised. Subsequently, data were spatially smoothed (6mm FWHM), and a high pass-filter (100s) was applied using FSL.

First-level analyses were performed by modeling the signal changes using the stimulation paradigm (faces vs. shapes), convolved with a canonical hemodynamic response function. Data from subjects with extreme motion (framewise displacement > 1mm) were removed from the analysis.



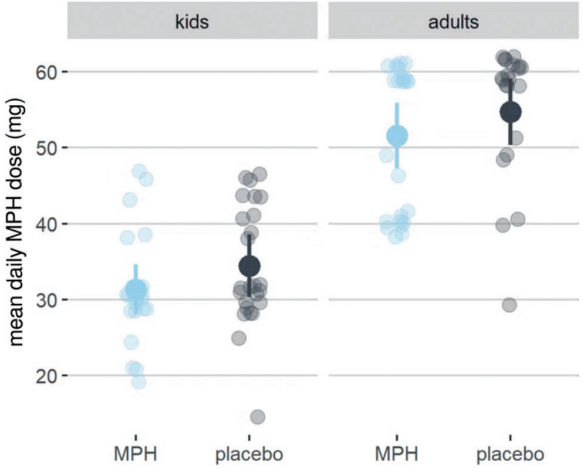
Supplementary Figure 2.Treatment assignment.

Supplementary Results

Supplementary Table 1. Characteristics of the participants included in the fMRI Analysis of the randomized controlled trial at Baseline**.**

	Children		Adults	
	MPH	placebo	MPH	placebo
	n=17	n=15	n=21	n=22
	mean±SD	mean±SD	mean±SD	mean±SD
Age (y)	11.4±0.9	11.3±1.1	28.04±4.5	28.8±5.1
Estimated IQ[1]	107.3±20.9	101.9±13.8	107.2±7.9	107.5±6.4
ADHD subtype (N)				
Inattentive	9	9	10	4
Hyperactive/impulsive	0	0	0	0
Combined	8	6	11	18
ADHD symptoms				
DBD-RS Inattention	21.9±3.6	23.5±2.6	-	-
DBD-RS Hyperactivity	14.3±5.5	15.1±7.4	-	-
ADHD-SR	-	-	33.9±10.1	31.5±10.1
Depressive symptoms[2]	7.8±4.8	9.7±5.3	6.5±5.5	7.7±6.1
Anxiety symptoms[2]	27.1±19.4	31.4±18.83	9.9±6.8	9.0±8.2
Adherence	83%±19	79%±20	90%±8	86%±9
Framewise displacement (mm)				
Baseline	0.37±0.24	0.46±0.24	0.15±0.10	0.12±0.05
During Treatment	0.23±0.08	0.35±0.20	0.10±0.05	0.15±0.10
Post-Treatment	0.34±0.29	0.39±0.28	0.16±0.12	0.17±0.09

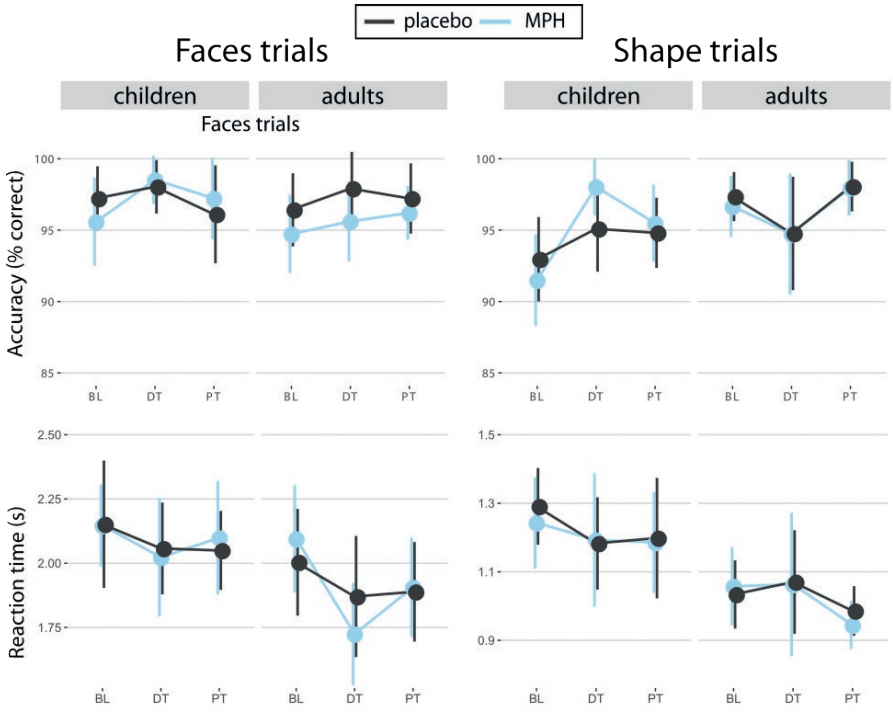
[1]for children: Wechsler Intelligence Scale for Children (WISC) (Kort et al. 2002); for adults: National Adults Reading Test (NART) (Schmand et al. 1992); DBD-RS=disruptive behavior disorder rating scale (Pelham Jr. et al. 1992); ADHD-SR=Attention Deficit Hyperactivity Disorder-Self Report (Kooij 2012);
[2]Depressive symptoms and anxiety symptoms: children: Child Depression Inventory (CDI) (Kovacs 1985); Screen for Child Anxiety Related Disorders (SCARED) (Muris et al. 1998); adults: Beck's Depression Inventory (BDI) (Beck et al. 1961); Beck's Anxiety Inventory (BAI) (Beck et al. 1988)



Supplementary Figure 3. Mean and confidence interval of the dose of MPH treatment or placebo over the whole trial, including data points per participant.

Supplementary Table 2. Statistics.

	session * MED (+FD)	Main effect session (BL/DT/PT) (+FD)	Main effect medication/placebo (+FD)
Children:			
left amygdala reactivity	F(5,70.14)=1.23, *p*=0.30, ΔBIC=18.09	F(2,63.61)=0.68, *p*=0.51, ΔBIC=9.49	F(1,39.45)=0.14, *p*=0.70, ΔBIC=6.19
right amygdala reactivity	F(5,68.46)=1.22, *p*=0.31, ΔBIC=16.45	F(2,60.58)=1.27, *p*=0.29, ΔBIC=6.5	F(1,40.54)=0.85, *p*=0.36, ΔBIC=3.65
Accuracy face trials	F(5,72.66)=0.99, *p*=0.50, ΔBIC=18.19	F(2,67.70)=1.00, *p*=0.22, ΔBIC=5.98	F(1,36.40)=1.00, *p*=0.96, ΔBIC=4.59
Accuracy shape trials	F(3,68.83)=1.24, *p*=0.30, ΔBIC=9.8	F(2,67.01)=6.15, ***p*<0.01**, ΔBIC=-3.2	F(1,37.12)=0.38, *p*=0.54, ΔBIC=4.2
Reaction time face trials	F(5,66.93)=1.06, *p*=0.39, ΔBIC=17.45	F(2,58.61)=2.51, *p*=0.09, ΔBIC=4.27	F(1,40.83)=0.01, *p*=0.93, ΔBIC=4.56
Reaction time shape trials	F(5,87.10)=0.84, *p*=0.52, ΔBIC=17.51	F(2,77.09)=1.78, *p*=0.16, ΔBIC=5.31	F(1,43.89)=0.02, *p*=0.89, ΔBIC=4.36
CDI	F(3,96.13)=0.63, *p*=0.60, ΔBIC=11.63	F(2,90.77)=38.17, ***p*<0.01**, ΔBIC=-4.69	F(1,46.88)=0.09, *p*=0.77, ΔBIC=49.91
SCARED	F(3,97.39)=0.51, *p*=0.67, ΔBIC=12.49	F(2,92.72)=22.70, ***p*<0.01**, ΔBIC=-29.09	F(1,46.99)=0.03, *p*=0.87, ΔBIC=5.10
DBD-RS Inattention	F(2,83.49)=5.47, ***p*<0.01**, ΔBIC=-69.10	F(2,85.25)=61.44, ***p*<0.01**, ΔBIC=-38.4	F(1,44.08)=12.69, ***p*<0.01**, ΔBIC=-4.1
DBD-RS Hyperactivity	F(3,90.33)=2.48, *p*=0.07, ΔBIC=7.26	F(2,83.87)=30.80, ***p*<0.01**, ΔBIC=-32.28	F(1,45.51)=3.09, *p*=0.09, ΔBIC=2.4
Adults:			
left amygdala reactivity	F(5,92.69)=0.69, *p*=0.63, ΔBIC=20.54	F(2,83.85)=0.86, *p*=0.43, ΔBIC=8.21	F(1,42.50)=1.12, *p*=0.30 ΔBIC=3.78
right amygdala reactivity	F(5,92.08)=1.17, *p*=0.33, ΔBIC=18.17	F(2,82.64)=0.85, *p*=0.43, ΔBIC=8.26	F(1,43.12)=2.57, *p*=0.12, ΔBIC=2.21
Accuracy face trials	F(5,85.32)=0.09, *p*=0.66, ΔBIC=20.33	F(2,76.39)=0.09, *p*=0.66, ΔBIC=8.63	F(1,43.15)=1.15, *p*=0.19, ΔBIC=2.93
Accuracy shape trials	F(3,82.97)=0.04, *p*=0.99, ΔBIC=17.06	F(2,81.06)=3.33, *p*=0.04, ΔBIC=2.88	F(1,41.92)=0.08, *p*=0.78, ΔBIC=4.70
Reaction time face trials	F(3,81.50)=1.36, *p*=0.26, ΔBIC=10.08	F(2,72.83)=5.97, ***p*<0.01**, ΔBIC=-2.07	F(1,44.81)=0.01, *p*=0.97, ΔBIC=5.02
Reaction time shapes trials	F(5,67.13)=1.02, *p*=0.42, ΔBIC=19.52	F(2,59.09)=1.87, *p*=0.17, ΔBIC=5.96	F(1,43.65)=0.19, *p*=0.65, ΔBIC=4.75
BAI	F(5,96.99)=1.02, *p*=0.41, ΔBIC=19.57	F(2,84.82)=2.01, *p*=0.14, ΔBIC=6.20	F(1,46.82)=0.06, *p*=0.81, ΔBIC=4.76
BDI	F(3,88.57)=1.18, *p*=0.32, ΔBIC=12.75	F(2,43.61)=4.05, ***p*=0.02**, ΔBIC=1.84	F(1,43.61)=1.04, *p*=0.31, ΔBIC=3.79
ADHD-SR	F(2,78.3)=4.82, ***p*=0.01**, ΔBIC=-36.59	F(2,80.48)=31.46, ***p*<0.01**, ΔBIC=-38.97	F(1,43.95)=3.18, *p*=0.08, ΔBIC=2.38



Supplementary Figure 4. Accuracy and reaction time measures from the fMRI task.

Supplementary Table 3. PPI whole-brain analysis and exploratory task reactivity whole-brain analysis of the emotion recognition taks (Faces > shapes).

	Seed	*Cluster-index*	*# of voxels*	*Max. T-value*	*MNI-coordinates*			*Brain areas*
					X	*Y*	*Z*	
Connectivity								
PT >BL								
Adults MPH	Right amygdala	1	38	4.27	-8	46	16	67% Paracingulate Gyrus, 13% Cingulate Gyrus, anterior division, 1% Superior Frontal Gyrus
		2	45	4.27	-42	18	32	45% Middle Frontal Gyrus, 7% Inferior Frontal Gyrus, pars opercularis, 2% Inferior Frontal Gyrus, pars triangularis
		3	196	5.45	-26	-78	-16	69% Occipital Fusiform Gyrus, 5% Lingual Gyrus, 4% Lateral Occipital Cortex, inferior division
Reactivity								
PT> BL								
Adults placebo	Whole-brain	1	23	4.68	52	-74	22	55% Lateral Occipital Cortex, superior division, 2% Lateral Occipital Cortex, inferior division
		2	42	5.38	30	-88	30	33% Lateral Occipital Cortex, superior division, 25% Occipital Pole
DT > PT								
MPH kids	Whole-brain	1	40	4.28	2	22	50	29% Superior Frontal Gyrus, 22% Paracingulate Gyrus
		2	122	4.97	0	36	28	66% Paracingulate Gyrus, 18% Cingulate Gyrus, anterior division

Supplementary Table 4. Correlations of amygdala reactivity and clinical measures per session (significance level alpha=0.008 after bonferroni correction per age group and session (number of tests=6)).

	BL *Placebo*	*MPH*	***DT*** *Placebo*	*MPH*	***PT*** *Placebo*	*MPH*
Left amygdala						
Children						
Depressive symptoms	t(12)=-0.41, *p*=0.69	t(15)=-0.66, *p*=0.52	t(16)=0.20, *p*=0.84	t(15)=-0.32, *p*=0.75	t(13)=0.09, *p*=0.93	t(14)=-1.13, *p*=0.28
Anxiety symptoms	t(13)=-1.23, *p*=0.24	t(15)=-0.24, *p*=0.81	t(16)=0.66, *p*=0.52	t(13)=-0.13, *p*=0.90	t(14)=-0.90, *p*=0.39	t(14)=-0.15 ,*p*=0.89
ADHD: inattention subscale	t(13)=1.38, *p*=0.19	t(14)=0.04, *p*=0.97	t(13)=0.31, *p*=0.17	t(13)=-0.79, *p*=0.44	t(11)=0.53, *p*=0.61	t(13)=0.36, *p*=0.72
ADHD: hyperactivity subscale	t(13)=1.42, *p*=0.18	t(14)=0.15, *p*=0.88	t(13)=0.31, *p*=0.76	t(13)=-0.56, *p*=0.58	t(11)=0.27, *p*=0.79	t(13)=-0.75, *p*=0.46
Adults						
Depressive symptoms	t(19)=0.96, *p*=0.35	t(19)=1.01, *p*=0.33	t(18)=-0.31, *p*=0.76	t(18)=0.71, *p*=0.49	t(18)=1.26, *p*=0.22	t(19)=0.69, *p*=0.49
Anxiety symptoms	t(20)=2.62, *p*=0.02	t(18)=0.62, *p*=0.54	t(16)=0.24, *p*=0.81	t(18)=-0.29, *p*=0.78	t(17)=0.23, *p*=0.82	t(19)=0.60, *p*=0.55
ADHD symptoms	t(19)=1.45, *p*=0.16	t(16)=1.47, *p*=0.16	t(15)=1.46, *p*=0.17	t(17)=-0.28, *p*=0.79	t(18)=-0.42, *p*=0.68	t(18)=0.37, *p*=0.72
Right amygdala						
Children						
Depressive symptoms	t(12)=0.03, *p*=0.98	t(15)=0.20, *p*=0.84	t(16)=-0.17, *p*=0.87	t(15)=0.57, *p*=0.58	t(13)=0.29, *p*=0.78	t(14)=0.68, *p*=0.50
Anxiety symptoms	t(13)=-0.68, *p*=0.51	t(15)=-0.40, *p*=0.70	t(16)=0.97, *p*=0.35	t(15)=0.57, *p*=0.58	t(13)=1.13, *p*=0.28	t(14)=-0.65, *p*=0.52
ADHD: inattention subscale	t(13)=1.05, p=0.31	t(14)=-1.06, *p*=0.31	t(13)=0.06, *p*=0.95	t(13)=1.21, *p*=0.74	t(11)=-0.44, *p*=0.67	t(13)=0.15, *p*=0.88
ADHD: hyperactivity subscale	t(13)=1.42, *p*=0.18	t(14)=-0.95, *p*=0.36	t(13)=-1.48, *p*=0.16	t(13)=0.34, *p*=0.25	t(11)=-1.29, *p*=0.22	t(13)=-0.62, *p*=0.55
Adults						
Depressive symptoms	t(19)=0.73, *p*=0.47	t(19)=0.69, *p*=0.50	t(18)=-1.19, *p*=0.25	t(18)=0.56, *p*=0.58	t(18)=0.55, *p*=0.59	t(19)=-0.45, *p*=0.66
Anxiety symptoms	t(20)=0.88, *p*=0.39	t(18)=0.73, *p*=0.47	t(16)=-0.22, *p*=0.83	t(18)=0.61, *p*=0.55	t(17)=-0.41, *p*=0.69	t(19)=-0.52, *p*=0.61
ADHD symptoms	t(19)=0.92, *p*=0.37	t(16)=1.36, *p*=0.19	t(15)=1.48, *p*=0.16	t(17)=-0.16, *p*=0.88	t(18)=-1.06, *p*=0.30	t(18)=-2.12, *p*=0.05

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Chapter 4

Acute MPH & rs-fMRI connectivity in ADHD

Effects of a single-dose methylphenidate challenge on resting-state functional connectivity in stimulant-treatment naive children and adults with ADHD

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Abstract

Prior studies suggest that methylphenidate, the primary pharmacological treatment for attention-deficit/hyperactivity disorder (ADHD), alters functional brain connectivity. As the neurotransmitter systems targeted by methylphenidate undergo significant alterations throughout development, the effects of methylphenidate on functional connectivity may also be modulated by age. Therefore, we assessed the effects of a single methylphenidate challenge on brain network connectivity in stimulant-treatment naïve children and adults with ADHD. We obtained resting-state functional MRI from 50 boys (10–12 years of age) and 49 men (23–40 years of age) with ADHD (DSM IV, all subtypes), before and after an oral challenge with 0.5 mg/kg methylphenidate; and from 11 boys and 12 men as typically developing controls. Connectivity strength (CS), eigenvector centrality (EC), and betweenness centrality (BC) were calculated for the striatum, thalamus, dorsal anterior cingulate cortex (dACC), and prefrontal cortex (PFC). In line with our hypotheses, we found that methylphenidate decreased measures of connectivity and centrality in the striatum and thalamus in children with ADHD, but increased the same metrics in adults with ADHD. Surprisingly, we found no major effects of methylphenidate in the dACC and PFC in either children or adults. Interestingly, pre-methylphenidate, participants with ADHD showed aberrant connectivity and centrality compared to controls predominantly in frontal regions. Our findings demonstrate that methylphenidate's effects on connectivity of subcortical regions are age-dependent in stimulant-treatment naïve participants with ADHD, likely due to ongoing maturation of dopamine and noradrenaline systems. These findings highlight the importance for future studies to take a developmental perspective when studying the effects of methylphenidate treatment.

Introduction

In recent years, attention-deficit/hyperactivity disorder (ADHD) has been increasingly considered a disorder of brain-wide network dysconnectivity rather than of region-specific deficits [Castellanos and Proal, 2012; Samea et al., 2019]. Methylphenidate, the primary pharmacological treatment for ADHD, has been proposed to alter functional connectivity in various brain-wide functional circuits affected by ADHD [Pereira-Sanchez et al., 2021]. For instance, normalized connectivity in fronto-parietal-cerebellar circuits has been observed in children with ADHD following acute methylphenidate. This was first observed by An et al., demonstrating that a single dose of methylphenidate compared to placebo, upregulated abnormally decreased local connectivity in bilateral ventral prefrontal cortices and the cerebellar vermis, and downregulated abnormally increased local connectivity in the right parietal and visual areas in children with ADHD [An et al., 2013]. Similarly, Silk et al. found that a single dose of methylphenidate compared to placebo normalized increased functional connectivity in occipital, temporal, and cerebellar regions and visual, executive, and default mode networks in adolescents with ADHD [Silk et al., 2017]. More recently, alterations in fronto-parietal-cerebellar circuits have also been observed following prolonged methylphenidate treatment in medication-naïve children with ADHD [Yoo et al., 2018]. Finally, preliminary evidence suggests that such a normalization might also occur in adults with ADHD [Cary et al., 2017; Picon et al., 2020]. However, due to methodological heterogeneity in previous studies, including prior use of stimulant medications, results remain inconclusive [Pereira-Sanchez et al., 2021].

Methylphenidate acts by inhibiting dopamine and noradrenaline reuptake in the brain [Cortese et al., 2017]. As the dopamine system undergoes significant alterations throughout development [Chen et al., 2010], methylphenidate-induced effects on functional connectivity may be modulated by age. For example, a recent longitudinal study demonstrated an age-dependent effect of prolonged stimulant treatment-response on cingulo-opercular network connectivity [Norman et al., 2021b]. Moreover, exposure to stimulants during sensitive stages of maturation might cause developmental alterations, a process called neuronal imprinting [Andersen, 2005]. Indeed, animal studies suggest that the age at initiation of methylphenidate treatment affects its influence on development in a highly specific manner [Canese et al., 2009]. In the same sample as described here, we also observed that the effects of methylphenidate may be modulated by age; we found that acute methylphenidate decreased thalamic cerebral blood flow only in children, but not in adults [Schrantee et al., 2017]. Moreover, we observed that prolonged methylphenidate-treatment followed by an acute challenge with methylphenidate significantly influenced cerebral blood flow in the striatum and thalamus in children, but not adults, nor in the placebo conditions [Schrantee et al., 2016].

Here, we aimed to assess the effects of a single-dose challenge of methylphenidate on resting-state functional MRI (rs-fMRI) network connectivity in stimulant-treatment naïve children and adults with ADHD using graph theoretical measures, to investigate potential age-dependent neural mechanisms involved in stimulant-induced changes in ADHD. Based on previous findings, we expected significant methylphenidate-induced alterations in connectivity of the striatum, thalamus, dorsal anterior cingulate cortex (dACC), and prefrontal cortex (PFC). We expected that these effects would differ between children and adults because of functional maturation of the dopamine and noradrenaline system [Chen et al., 2010]. In addition, based on studies reporting altered connectivity in individuals with ADHD compared to typically developing control participants, we hypothesized that an acute dose of methylphenidate would strengthen connectivity for these four brain regions in adults, whereas in children, we expected increased connectivity in frontal regions (PFC and dACC) and decreased connectivity in the thalamus and striatum.

Methods

We included 50 stimulant treatment-naive boys (10–12 years of age) and 49 stimulant-treatment naive men (23–40 years of age) that were part of the "effects of Psychotropic drugs On the Developing brain methylphenidate" (ePOD-MPH) trial (NTR3103 and NL34509.000.10; [Bottelier et al., 2014; Schrantee et al., 2016]). They were recruited through clinical programs at the Child and Adolescent Psychiatry Center Triversum (Alkmaar, The Netherlands), the Department of Child and Adolescent Psychiatry at the Bascule/AMC (Amsterdam, The Netherlands), and the PsyQ Mental Health Facility (The Hague, The Netherlands). All participants were diagnosed with ADHD (DSM-IV, all subtypes) by an experienced psychiatrist, using a structured interview, (Diagnostic Interview Schedule for Children (NIMH-DISC-IV): authorized Dutch translation [Ferdinand and van der Ende, 1998]) and the Diagnostic Interview for ADHD (DIVA 2.0) for adults [Kooij et al., 2008]. In addition, as a typically developing comparison group, we included 11 boys (aged 10–12 years) and 12 men (aged 23–40 years) as non-ADHD control participants, who received pre-methylphenidate scans only (Table 1).

Exclusion criteria were: comorbid axis I psychiatric disorders requiring treatment with medication at study entry, a history of major neurological or medical illness or clinical treatment with drugs influencing the dopamine system (for adults before 23 years of age), such as stimulants, neuroleptics, antipsychotics, and/or D2/D3 agonists (see Supplementary Material for more detail). The study was approved by the medical ethical committee and consequently monitored by the Clinical Research Unit of the Amsterdam University Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

All participants and parents or legal representatives of the children provided written informed consent.

The primary outcome measure of the ePOD-MPH trial was to report on the modification by age of methylphenidate treatment on the outgrowth of the dopamine system by using pharmacologic MRI [Schrantee et al., 2016]. Here, we report on acute effects of methylphenidate on the baseline rs-fMRI measurement of the trial, during which ADHD participants underwent two MRI scans, one before and one 90min after an oral challenge of short-acting methylphenidate (Sandoz B.V., Weesp, the Netherlands; 0.5mg/kg with a maximum of 20 mg in children and 40mg in adults). The dose was chosen so that 80% of dopamine transporters were occupied [Swanson and Volkow, 2003], and we chose 90min of waiting period for optimal occupation of these transporters. Typically developing control subjects did not receive a challenge of methylphenidate.

Table 1 Characteristics of participants included in the rs-(MRI) analysis. Significaant effects are indicated in bold (p < 0.05)

	Children ADHD	Controls		Adults ADHD	Controls	
	n = 33	*n* = 10	Statistics	*n* = 48	*n* = 11	Statistics
	mean ± SD	mean ± SD		mean ± SD	mean ± SD	
Age (y)	11.4 ± 0.9	11.5 ± 0.8	t(17) = −0.4, *p* = 0.67	28.5 ± 4.6	25.1 ± 1.9	**t(39) = 3.9, *p* < 0.01**
Estimated IQ[a]	106.1 ± 18.9	101.8 ± 7.9	t(36) = 1.0, *p* = 0.31	107.8 ± 7.5	108.0 ± 5.8	t(19) = −0.1, *p* = 0.92
ADHD subtype, no.						
Inattentive	16	–		16	–	
Hyperactive/impulsive	1	–		0	–	
Combined	16	–		33	–	
ADHD symptoms						
DBD-RS Inattention[b]	21.7 ± 3.5	3.8 ± 3.0	**t(17) = 15.9, *p* < 0.01**	–	–	
DBD-RS Hyperactivity[b]	15.9 ± 5.5	4.0 ± 2.6	**t(33) = 9.4, *p* < 0.01**	–	–	
ADHD-SR[c]	–	–		32.7 ± 9.7	11.5 ± 5.6	**t(26) = 9.5, *p* < 0.01**
Depressive symptoms[d]						
CDI	7.9 ± 4.2	3 ± 3.2	**t(16) = 3.8, *p* < 0.01**	–	–	
BDI	–	–	–	7.2 ± 5.8	2.7 ± 2.0	**t(53) = 5.0, *p* < 0.01**
Anxiety symptoms[d]						
SCARED	26.4 ± 16.3	11.1 ± 6.6	**t(29) = 4.2, *p* < 0.01**	–	–	
BAI	–	–	–	9.0 ± 7.4	2.2 ± 1.7	**t(55) = 5.1, *p* < 0.01**
Framewise Displacement						
pre-MPH	0.04 ± 0.02	0.03 ± 0.008	**F(1,57) = 7.53, *p* < 0.01**	0.01 ± 0.01	0.02 ± 0.004	F(1,58) = 1.47, *p* = 0.23
post-MPH	0.03 ± 0.01	–	F(1,52) = 0.05, *p* = 0.83	0.01 ± 0.005	–	F(1,58) = 0.70, *p* = 0.41
MPH challenge, mg	18.7 ± 2.2			38.3 ± 2.6		

[a]for children: Wechsler Intelligence Scale for Children (WISC) (Kort et al., 2002); for adults: National Adults Reading Test (NART) (Schmand et al., 1992).
[b]DBD-RS = disruptive behavior disorder rating scale (Pelham Jr. et al., 1992).
[c]ADHD-SR, Attention Deficit Hyperactivity Disorder-Self Report (Kooij, 2012).
[d]Depressive symptoms and anxiety symptoms: children: Child Depression Inventory (CDI) (Kovacs, 1985); Screen for Child Anxiety Related Disorders (SCARED) (Muris et al., 1998); adults: Beck's Depression Inventory (BDI) (Beck et al., 1961); Beck's Anxiety Inventory (BAI) (Beck et al., 1988).

Resting-state fMRI

Data were acquired on 3 T Philips scanners (Philips Healthcare, Best, The Netherlands) using an 8-channel receive-only head coil. A 3D T1-weighted anatomical scan was acquired for registration purposes, and rs-fMRI data were acquired using a single-shot echo-planar imaging sequence (TR/TE = 2300/30ms, resolution = 2.3 x 2.3 x 3mm, 39 sequential slices, FA = 80°,dynamics = 130).

Preprocessing was performed using FMRIPREP v1.2.3 ([Esteban et al., 2020]; RRID: SCR_016216), including ICA-AROMA. Subsequently, white matter (WM) and cerebral spinal fluid (CSF) signals (obtained before ICA-AROMA) were regressed out and high-pass-filtering (100 s) was applied using FSL. The Brainnetome atlas was used to define 246 parcels ([Fan et al., 2016]; Figure 1a,b) and fMRI signal time-series per participant were extracted and z-scored (Figure 1c). Framewise displacement (FD) values were calculated from low-pass filtered motion parameter time-series according to Gratton et al. [2020] to remove respiration artifacts (Supplementary Methods) and fMRI signal timepoints where FD >0.2mm were scrubbed. Participants were excluded from further analyses if mean FD >0.2 mm or if the number of volumes after scrubbing ≤104.

Cleaned fMRI time-series were then used to calculate connectivity matrices using Pearson correlations, resulting in a 246 x 246 connectivity matrix per participant, which was absolutized for further analyses (Figure 1c,d). Temporal signal-to-noise ratio (tSNR) maps were calculated per participant to remove low-tSNR nodes (Supplementary Methods).Graph theory measures were calculated for the whole brain from connectivity matrices using the Brain Connectivity Toolbox ([Rubinov and Sporns, 2010]; [RRID:SCR_004841]; Figure 1e). Quality control measures as defined by Ciric et al. [2017], as well as the number of negative correlations and average correlation coefficients, were calculated (Supplementary Figure 1; Supplementary Table 1). Connectivity strength (CS), betweenness centrality (BC), and eigenvector centrality (EC) were calculated and consequently averaged for four regions of interest (ROIs): striatum, thalamus, dorsal anterior cingulate cortex (dACC), and prefrontal cortex (PFC) (Brainnetome region numbers per ROI in Supplementary Table 2). The striatum was selected because it is rich in dopamine transporters and is the primary target of methylphenidate. The thalamus and dACC were selected because animal literature has demonstrated the largest age-dependent effects of methylphenidate in these two important projections from the striatum [Andersen, 2005]. Finally, the PFC was selected due to its hypothesized importance and its interconnection with other areas that are affected by ADHD [Mehta et al., 2019].

We decided not to take lateralization into account, mainly for statistical reasons. An additional division into left and right would have significantly decreased the statistical power of our study. Additionally, we did not have any a-priori hypotheses about lateralization of methylphenidate induced resting-state connectivity changes in either the striatum, thalamus, dACC or the PFC, as pre-registered with the ePOD-MPH randomized controlled trial. Correlations of all connectivity measures and FD can be found in Supplementary Table 3. Further details on the analysis methods can be found in the Supplementary Material.

Statistical analysis

Statistical analyses were conducted using R v.3.5.3 [R Development Core Team, 2011]. All data were checked for normality and, in case of non-normality, log-transformed. Linear mixed-effects models were used to analyze changes in fMRI connectivity per age group separately to investigate the main effect of methylphenidate (pre and post-challenge of acute methylphenidate) using the lme4 package [Bates et al., 2015]. Linear models were used to analyze the differences between the ADHD participants and controls at pre-methylphenidate. The average whole-brain CS per participant was added to the model as a covariate. FD and a variable representing the scanner that was used were tested as possible covariates, but not significant and thus not included in the models. Multiple comparison correction within modalities was performed using Sidak's correction: $\alpha^* = 1-(1-\alpha)1/m$, with $\alpha = 0.05$ and $m = 4$ (number of ROIs), which resulted in an $\alpha^* = 0.0127$.

Results

Participants

Of the 99 ADHD patients scanned, data from 81 participants with ADHD and 21 typically developing controls were analyzed (Table 1). One adult ADHD participant was excluded because of undisclosed prior stimulant treatment (more details about the trial are published elsewhere [Schrantee et al., 2016]. Seventeen children with ADHD were excluded due to excessive motion (whose characteristics did not differ from the included children (Supplementary Results)).

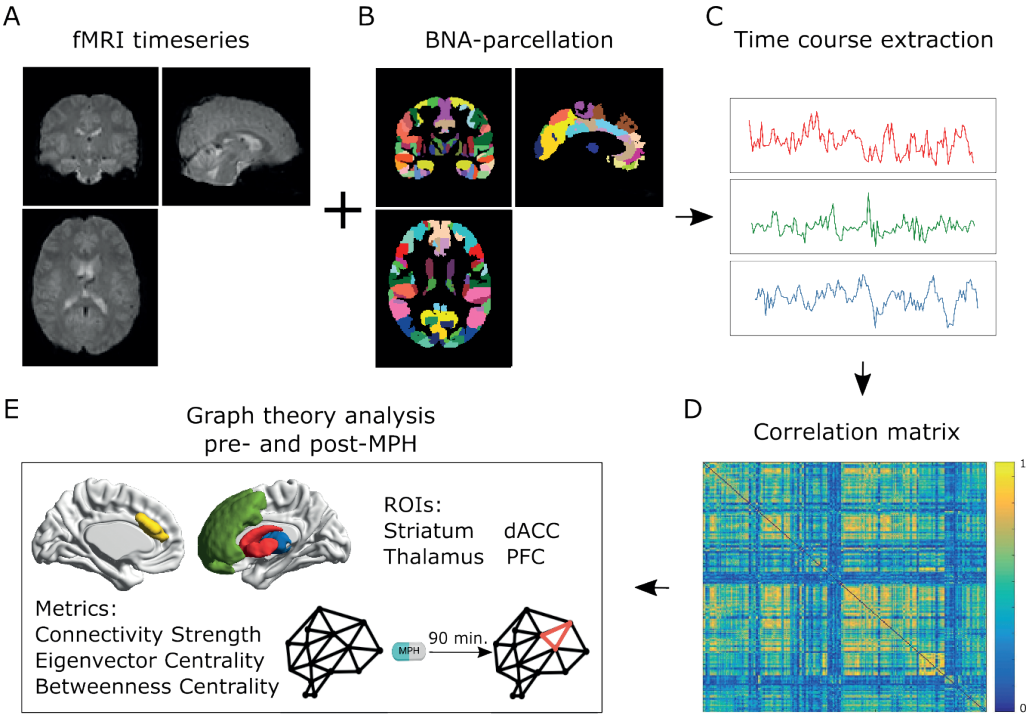

Figure 1. Analysis overview. (a and b) to construct functional brain networks per participant, the Brainnetome atlas (BNA) was used to define 246 parcels [Fan et al., 2016]. (c and d) the cleaned time series were then used to calculate connectivity matrices using Pearson correlations, resulting in a 246 x 246 connectivity matrix per participant, which was absolutized for further analyses. (e) Graph theory measures were calculated from the connectivity matrices using the brain connectivity toolbox [Rubinov and Sporns, 2010]. Connectivity strength (CS), betweenness centrality (BC), and eigenvector centrality (EC) were calculated for four regions of interest (ROIs): Striatum, thalamus, dorsal anterior cingulate cortex (dACC), and prefrontal cortex (PFC).

Rs-fMRI connectivity

All results of the statistical tests, as well as the estimated means and 95% confidence intervals, can be found in Table 2.

Striatum

Pre to post-methylphenidate, in children with ADHD, CS, and EC significantly decreased, but changes in BC did not survive multiple comparison corrections. Pre to post-methylphenidate, in adults with ADHD, the opposite effect was found; both CS and EC significantly increased, but BC did not change significantly.

Pre-methylphenidate, neither children nor adults with ADHD differed significantly from the respective controls in any of the connectivity metrics. Post-methylphenidate,

in children with ADHD, none of the connectivity metrics differed significantly from the respective young controls. Post-methylphenidate, in adults with ADHD, CS and EC differed significantly from the adult controls, but BC did not differ significantly. (Figure 2a; Table 2).

Table 2. Results of statistical tests.

Region of Interest		ADHD pre- vs. post-methylphenidate		ADHD (pre-methylphenidate) vs. controls		ADHD (post-methylphenidate) vs. controls	
		Estimated mean difference [95% CIs]	Statistics	Estimated mean difference [95% CIs]	Statistics	Estimated mean difference [95% CIs]	Statistics
Striatum	*Children*						
	Strength	−9.3 [−9.6 −9.1]	F(1,47) = 11.5, $p < 0.01$	−0.7 [−5.4 4.07]	F(1,40) = 1.0, $p = 0.32$	8.7 [4.2 13.2]	F(1,51) = 3.7, $p = 0.06$
	EC (×10²)	−0.8 [−0.8 −0.8]	F(1,47) = 6.8, $p = 0.01$	0.07 [−0.3 0.5]	F(1,40) < 0.1, $p = 0.97$	0.8 [0.5 1.3]	F(1,51) = 4.8, $p = 0.03$
	BC	−16.4 [−16.8 −16.0]	F(1,47) = 5.5, $p = 0.02$	−10.5 [−23.5 2.6]	F(1,40) = 0.2, $p = 0.64$	5.9 [−6.7 18.5]	F(1,51) < 0.1, $p = 0.93$
	Adults						
	Strength	8.2 [8.1 8.3]	F(1,45) = 9.0, $p < 0.01$	−6.6 [−10.5 −2.7]	F(1,52) = 1.8, $p = 0.19$	14.8 [−18.8 −10.8]	F(1,54) = 11.1, $p < 0.01$
	EC (×10²)	0.9 [0.8 0.9]	F(1,44) = 12.8, $p < 0.01$	−0.5 [−0.9 −0.2]	F(1,52) = 1.3, $p = 0.25$	−1.4 [−1.6 −1.0]	F(1,54) = 9.7, $p < 0.01$
	BC	12.8 [12.5 13.0]	F(1,45) = 1.4, $p = 0.24$	0.8 [−10.0 11.6]	F(1,52) < 0.1, $p = 0.78$	−12.0 [−23.0 −0.9]	F(1,54) = 0.9, $p = 0.36$
Thalamus	*Children*						
	Strength	−13.8 [−14.0 −13.6]	F(1,47) = 37.9, $p < 0.01$	−2.9 [−7.1 1.4]	F(1,40) = 4.6, $p = 0.04$	10.9 [6.90 14.97]	F(1,51) = 8.6, $p < 0.01$
	EC (×10²)	−1.2 [−1.3 −1.2]	F(1,46) = 32.3, $p < 0.01$	−0.2 [−0.6 0.1]	F(1,40) = 2.0, $p = 0.17$	0.9 [0.6 1.4]	F(1,51) = 4.6, $p = 0.03$
	BC	−20.9 [−21.2 −20.5]	F(1,47) = 13.8, $p < 0.01$	−10.6 [−1.4 19.8]	F(1,40) < 0.1, $p = 0.83$	10.3 [1.5 19.1]	F(1,51) < 0.1, $p = 0.93$
	Adults						
	Strength	9.4 [9.3 9.5]	F(1,45) = 12.4, $p < 0.01$	−3.1 [−6.6 0.4]	F(1,52) = 0.7, $p = 0.41$	−12.5 [−16.1 −9.0]	F(1,54) = 7.6, $p < 0.01$
	EC (×10²)	0.9 [0.9 0.9]	F(1,45) = 13.5, $p < 0.01$	−0.2 [−0.6 0.1]	F(1,52) = 0.5, $p = 0.50$	−1.2 [−1.5 −0.9]	F(1,54) = 7.0, $p = 0.01$
	BC	13.1 [13.0 13.3]	F(1,44) = 8.0, $p < 0.01$	20.7 [12.3 29.1]	F(1,52) = 10.7, $p < 0.01$	7.6 [−0.9 16.2]	F(1,54) = 4.3, $p = 0.04$
dACC	*Children*						
	Strength	4.6 [4.4 4.8]	F(1,47) = 5.6, $p = 0.02$	−3.2 [−7.0 0.72]	F(1,40) = 3.1, $p = 0.08$	−7.7 [−11.4 −4.0]	F(1,51) = 4.4, $p = 0.04$
	EC (×10²)	0.4 [0.4 0.4]	F(1,47) = 4.4, $p = 0.04$	0.1 [−0.2 0.5]	F(1,40) < 0.1, $p = 0.90$	−0.3 [−0.7 0.1]	F(1,51) = 0.2, $p = 0.68$
	BC	61.9 [60.0 63.8]	F(1,46) = 5.7, $p = 0.02$	67.4 [30.2 103.64]	F(1,40) = 2.6, $p = 0.11$	5.5 [−29.8 40.8]	F(1,51) < 0.1, $p = 0.96$
	Adults						
	Strength	1.6 [1.5 1.7]	F(1,45) = 0.4, $p = 0.55$	−12.9 [−16.1 −9.7]	F(1,52) = 8.3, $p < 0.01$	−14.5 [−11.2 17.8]	F(1,54) = 13.7, $p < 0.01$
	EC (×10²)	0.1 [0.1 0.1]	F(1,45) = 0.7, $p = 0.41$	−1.1 [−1.4 −0.7]	F(1,52) = 7.3, $p < 0.01$	−1.2 [−1.5 −0.8]	F(1,54) = 11.4, $p = 0.01$
	BC	−9.6 [−10.6 −8.6]	F(1,45) = 0.9, $p = 0.36$	50.6 [17.0 84.3]	F(1,52) = 3.1, $p = 0.08$	60.2 [25.6 94.9]	F(1,52) = 6.4, $p = 0.01$
PFC	*Children*						
	Strength	−6.6 [−6.9 −6.2]	F(1,46) = 2.0, $p = 0.16$	15.7 [8.0 23.3]	F(1,40) = 9.2, $p < 0.01$	22.2 [14.9 29.5]	F(1,51) = 16.7, $p < 0.01$
	EC (×10²)	−0.1 [−0.1 −0.1]	F(1,45) < 0.1, $p = 0.83$	0.03 [−0.2 0.3]	F(1,40) = 1.2, $p = 0.27$	0.1 [−0.1 0.3]	F(1,51) = 0.7, $p = 0.40$
	BC	13.5 [12.7 14.2]	F(1,46) = 3.2, $p = 0.08$	−15.3 [−30.4 −0.14]	F(1,40) = 5.7, $p = 0.02$	−28.8 [−43.3 −14.4]	F(1,51) = 8.9, $p < 0.01$
	Adults						
	Strength	−9.4 [−9.5 −9.3]	F(1,44) = 6.3, $p = 0.02$	−9.0 [−2.6 15.2]	F(1,52) = 1.1, $p = 0.30$	0.4 [−6.0 6.9]	F(1,54) < 0.1, $p = 0.78$
	EC (×10²)	−0.3 [−0.3 −0.3]	F(1,45) = 5.8, $p = 0.02$	−0.6 [−0.8 −0.4]	F(1,52) = 8.8, $p < 0.01$	−0.3 [−0.5 −0.1]	F(1,54) = 1.3, $p = 0.25$

Note: Linear mixed-effects models were used to analyze changes in fMRI connectivity per age group separately to investigate the main effect of methylphenidate (pre- and post-challenge of acute methylphenidate). Linear models were used to analyze the differences between the ADHD participants and controls at pre- and post-methylphenidate. The average whole-brain CS per participant was added to the model as a covariate. Multiple comparison correction within modalities was performed using Sidak's correction, which resulted in an $\alpha^* = 0.0127$. Significant effects are indicated in bold.
Strength = Connectivity Strength; EC = Eigenvector Centrality (shown in $\times 10^2$ for legibility); BC = Betweenness Centrality.

Thalamus

Pre to post-methylphenidate, in children with ADHD, CS, EC, and BC decreased significantly. Pre to post methylphenidate, in adults with ADHD, the opposite effect was found; CS, EC, and BC increase significantly. Pre-methylphenidate, children with ADHD did not differ significantly from the young controls in any of the connectivity metrics.

Pre-methylphenidate, adult ADHD participants showed lower BC than adult controls, but CS and EC did not differ significantly. Post methylphenidate, in children with ADHD, CS was significantly different from the respective controls, but EC and BC were not significantly different. Post-methylphenidate, in adults with ADHD, CS and EC were significantly different from the respective controls (Figure 2b; Table 2).

dACC

Pre to post-methylphenidate, in children with ADHD, CS, BC and EC changes did not survive multiple comparison corrections. Pre to post methylphenidate, in adults with ADHD, none of the connectivity metrics changed significantly. Pre-methylphenidate, children with ADHD did not differ significantly from the respective controls in any of the connectivity metrics.

Pre-methylphenidate, adult ADHD participants showed higher CS and EC values than the adult controls, but BC did not differ significantly. Post-methylphenidate, in children with ADHD, none of the connectivity metrics differed from the controls. Post-methylphenidate, in adults with ADHD, CS, EC and BC differed significantly from (Figure 2c; Table 2).

PFC

Pre to post-methylphenidate, in children with ADHD, none of the graph-theory metrics changed significantly. Pre to post-methylphenidate, in adults with ADHD, CS and EC did not survive multiple comparison corrections, BC was found to increase significantly.

Pre-methylphenidate, children with ADHD showed significantly higher CS values than controls. None of the other connectivity measures differed significantly. Adults with ADHD showed no differences to controls in CS, but significantly higher EC values and lower BC values than the control group. Post-methylphenidate, in children with ADHD, EC was not significantly different from the young controls, but CS and BC were found to be significantly different. Post-methylphenidate, in adults with ADHD, none of the connectivity metrics were significantly different from the adult controls (Figure 2d; Table 2).

Discussion

The goal of this study was to investigate the effects of acute methylphenidate on rs-fMRI connectivity in stimulant-treatment naïve children and adults with ADHD. In line with our hypotheses, we found that methylphenidate decreased measures of connectivity and centrality in subcortical ROIs in children with ADHD, but increased the same metrics in adults with ADHD, indicating an age-dependent acute effect of methylphenidate in dopamine-sensitive regions. Surprisingly, we found no major effects of methylphenidate in frontal ROIs in either children or adults. Interestingly, at pre-methylphenidate, participants with ADHD showed aberrant connectivity and centrality predominantly in frontal ROIs compared to controls.

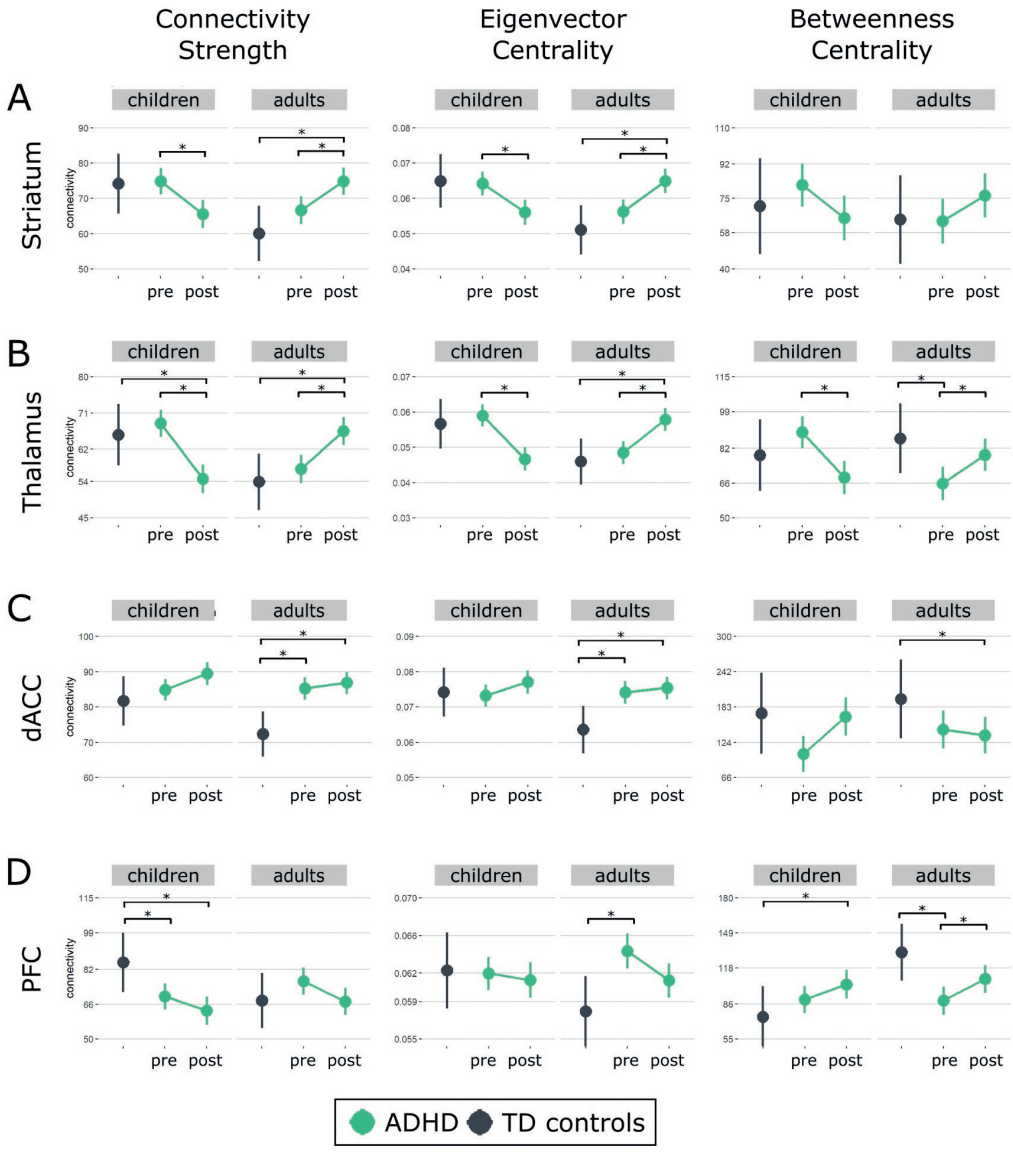

Figure 2. Functional connectivity within the ROIs. Connectivity strength, eigenvector centrality, and betweenness centrality are shown for the (a) striatum (b) thalamus (c) dorsal anterior cingulate cortex (dACC) and (d) prefrontal cortex (PFC). Estimated marginal means and 95% confidence intervals at pre-MPH (pre) and post-MPH (post) for children and adults are shown in green. Estimated means and 95% confidence intervals for TD controls are shown in black. Significant effects are indicated with an asterisk (*; $p < 0.0127$)

Effect of methylphenidate in children with ADHD

A recent review on the effects of stimulant medication on rs-fMRI connectivity in individuals with ADHD shows that methylphenidate appears to modulate several rs-fMRI networks, but the number of studies is small, and the results are heterogeneous [Pereira-Sanchez et al., 2021]. In line with findings from Silk et al. [2017], we observed that acute methylphenidate decreased connectivity in the striatum and thalamus, whereas in the dACC we found nonsignificant increases in connectivity after a single dose of methylphenidate. This is in agreement with a previous study reporting that acute methylphenidate increased connectivity in frontal regions [An et al., 2013]. Notwithstanding, our study has some methodological differences compared to previous studies. Firstly, all our participants were stimulant-treatment naïve, whereas in other studies medication status was inconsistent. Therefore, our study rules out the influence of prior medication on connectivity through prolonged effects of stimulants on the dopamine system. For example, prolonged MPH treatment has been shown to impact (proxy measures of) dopamine function in juvenile animals and children [Andersen, 2005; Moll et al., 2001; Schrantee et al., 2016]. Furthermore, long-term stimulant treatment normalized delayed structural maturation of the PFC in individuals with ADHD, which may reflect dopaminergic adaptive processes [Shaw et al., 2009; Xavier Castellanos et al., 2002].

Secondly, we assessed graph theory metrics, whereas Silk et al., 2017 used Network Based Statistics to identify connections that are affected by methylphenidate, and An et al., 2013 used regional homogeneity, reflecting local synchronized brain activity, considered to be a measure of functional segregation [Lv et al., 2018]. As such, our study extends prior literature from connectivity metrics to topology metrics, which allows us to not only assess individual nodes or global connectivity, but to assess the importance and integration of pre-specified nodes within the global network. In subcortical regions, methylphenidate affects average connectivity (CS) and nodal importance (EC), suggesting changes in the role of these regions in both local and global network topology. In frontal regions on the other hand, we observe marginal increases in global importance (BC) following methylphenidate, which might indicate a more important role for these regions regarding information flow in the network [Farahani et al., 2019; Wang et al., 2010]. Thirdly, both previous studies included placebo conditions, whereas we used a pre-post design. Finally, the dose that we used was slightly higher than Silk et al. ([2017]; 0.41 mg/kg) and substantially higher than An et al. (10mg), which may have affected functional connectivity differently [An et al., 2013], particularly considering the inverted-U relationship between dopamine levels and cognition [Arnsten and Rubia, 2012; Froudist-Walsh et al., 2020].

Although previous studies have reported that methylphenidate normalizes brain activity [Czerniak et al., 2013; Rubia, 2011] and connectivity [An et al., 2013], our results do

not support these findings. Instead, in accordance with a recent meta-analysis [Cortese et al., 2021], pre-methylphenidate, we show no group differences in connectivity in subcortical ROIs, and our findings suggest that methylphenidate-induced changes in connectivity deviate from the control-like state. We could speculate that these discrepancies are due to divergent brain development in ADHD, affecting local vs. global metrics differently. As such, methylphenidate could normalize local connectivity and activity, as demonstrated by previous studies, but compensate for altered network structure on a global level, as found here. Alternatively, the deviation from the control-like state ("normal" to "abnormal") may also represent potential "side effects" of the medication.

Future studies are needed to determine whether these different connectivity patterns reflect compensatory processes or unwanted side effects of medication. In the PFC on the other hand, we found higher CS compared to controls, one of the latest brain regions to mature [Mills et al., 2014]. This is partly in line with two recent meta-analyses proposing increased connectivity within the executive control network in children with ADHD [Gao et al., 2019; Sutcubasi et al., 2020], potentially reflecting greater mental effort to compensate for executive function in ADHD.

Effect of methylphenidate in adults with ADHD

The present study is, to our knowledge, the first to investigate the acute effects of methylphenidate in stimulant-treatment naive adults with ADHD. In agreement with our hypotheses, our findings indicate that methylphenidate increased overall connectivity and importance of striatal and thalamic nodes within the brain network. Our results show overlap with regions identified in a study investigating prolonged effects of methylphenidate in adults [Cary et al., 2017], and correspond to findings from typically-developing adults showing that acute methylphenidate increased connectivity between the thalamus and attention networks, and subcortical regions [Farr et al., 2014; Mueller et al., 2014]. These findings, together with the absence of major differences in connectivity when compared to controls, suggest that the mechanisms underlying the effects of methylphenidate on subcortical connectivity are largely comparable between adults with and without ADHD. However, this is in contrast with evidence from Positron Emission Tomography (PET) studies reporting significant differences in striatal dopamine release between adults with ADHD and controls following a stimulant challenge; albeit in different directions [Cherkasova et al., 2013; Volkow et al., 2007]. Together, this suggests that differential effects of methylphenidate on subcortical dopamine release may not directly translate into differential subcortical connectivity between individuals with ADHD and controls.

The pattern observed in cortical regions is more complex. In the dACC, methylphenidate did not induce changes in connectivity in participants with ADHD, despite higher pre-methylphenidate connectivity compared to controls. Such hyperconnectivity [Guo

et al., 2020] could be speculated to be a result of developed compensatory processes, in response to reduced network efficiency [Konrad and Eickhoff, 2010], particularly in adults who were never treated with ADHD medication. Interestingly, the absence of normalized dACC after methylphenidate could suggest that such processes are dopamine and noradrenaline-independent. Alternatively, individual differences may be too large to observe group differences, or such processes affect other network measures than those studied here. Conversely, in the PFC, we found that BC increased, whereas CS and EC decreased after methylphenidate. This would mean that methylphenidate increases the role of the PFC as a global communication hub (i.e., BC), but reduces connectivity of the PFC with other regions (i.e., CS and EC); meaning that the PFC connections become more specialized for network communication.

Age-dependent effects of methylphenidate in ADHD

The effects of methylphenidate on the brain have been proposed to be age-dependent [Andersen, 2005; Canese et al., 2009; Norman et al., 2021b]. Indeed, we previously showed that thalamic cerebral blood flow was reduced following acute methylphenidate in children, but not in adults with ADHD [Schrantee et al., 2016]. Accordingly, we here find an opposite effect of acute methylphenidate in thalamic and striatal connectivity in children compared to adults. Nevertheless, these age-effects may not be specific to ADHD, as functional connectivity changes over development, complicating intergenerational comparisons [Tooley et al., 2021; Váša et al., 2020]. Functional segregation appears predominant in children, whereas functional integration prevails in adults.

System neuroscience models suggest that increased segregation reflects efficient network functioning, and that excessive integration can be a correlate of brain dysfunction. If excessive cross-network functional integration were confirmed to be a consistent feature of ADHD, it could represent a therapeutic target [Pereira-Sanchez et al., 2021; Wig, 2017]. As such, typical development of functional connectivity is characterized by simultaneous reduction of local circuitry and strengthening of long-range connectivity [Grayson and Fair, 2017; Supekar et al., 2010]. Nevertheless, we can speculate that the difference in methylphenidate-induced connectivity changes between children and adults might result from maturation of dopaminergic and noradrenergic systems [Chen et al., 2010]. For instance, adults display a more segregated architecture in the frontoparietal network, including the dorsal basal ganglia (i.e., caudate nucleus) [Fair et al., 2009], possibly through changes in the dopamine system in the frontal cortex [DR and DA, 1994; MS et al., 1991; MS and P, 1992]. This network is, for example, important for the top-down regulation of emotion and attention [Zhou et al., 2007]. Indeed, a recent longitudinal study on the effects of stimulant treatment response and age found a significant influence on cingulo-opercular network connectivity [Norman et al., 2021b].

The age-dependent effects on striatal and thalamic connectivity reported here could therefore be due to compensatory mechanisms taking place in the adults, especially given that they were stimulant treatment naïve before the study. It has been argued that the neuropathology of childhood remittent cases could be attributed largely to a delayed frontal cortex maturation, whereas the neuropathology of persistent cases is linked more to pathology in extra-frontal and subcortical structures [Francx et al., 2015]. In summary, this suggests that the efficacy of stimulant therapy may not be based on normalization only, but rather depend on combinations of factors that return the network organization to typical topology for some systems while reorganizing others. In other words, it might be that altered networks in the brain do not need to return to the control state to function in the desired way, a restructuring of function could be sufficient. It is therefore important that future studies take age-dependent effects into account.

In addition, previous studies have suggested potential neural differences between persistent and remitted adults with ADHD [Mattfeld et al., 2014]. By definition, our adult ADHD sample had persistent ADHD, whereas this remains to be assessed for our pediatric sample [Caye et al., 2016; Kessler et al., 2005]. Longitudinal (f)MRI studies on ADHD persisters and remitters with childhood ADHD will be crucial to gain more insight into the differences in brain connectivity of persisting and remitting ADHD in childhood [Rubia, 2018]. Speculatively, in addition to developmental differences, our results may partially be explained by neuronal differences between these two ADHD phenotypes. Norman et al., indeed found reduced connectivity within the inferior frontal gyrus in children with ADHD to be indicative of longitudinal risk for ADHD inattention symptoms [Norman et al., 2021a]. Additionally, because we included stimulant treatment naïve individuals with ADHD, the adults might not represent a typical sample, as most adults with ADHD will receive medication before adulthood. For a long time, it has been debated if ADHD may also be developed in adulthood, with no previous symptoms in childhood ("adult-onset ADHD"; [Castellanos, 2015]). However, a recent review argues that symptoms in adults indeed exist but that their source would be either symptoms that were previously surpassed, were not properly assessed before, or not detected earlier [Taylor et al., 2021].

One of the main strengths of this study is that we included both stimulant-treatment naïve boys and men with ADHD and that, compared to previous studies on the acute effects of methylphenidate, we included a larger number of participants. However, limitations of our study are that the results cannot be extrapolated to all children and adults with ADHD, because we only studied participants with restricted age ranges. Furthermore, we included only male participants to reduce heterogeneity, but this limits the generalizability to female participants. Additional studies are needed in females,

since female sex hormones modulate dopamine transporter expression [Wagner et al., 2007]. Furthermore, the comparisons between participants with ADHD and control participants have to be interpreted with caution, due to the small control groups and because control participants did not receive a methylphenidate challenge. Ethical considerations did not permit us to administer methylphenidate to the young controls, therefore, the controls were assessed only once. Due to this limitation, we cannot fully exclude the possibility of a scan order effect causing differences, even though children and adults showed effects in opposite directions in this study, which makes that explanation unlikely. Moreover, we acquired only a relatively short scan of 5min, which might have made the intra-/intersession reliability lower.

Conclusion

Taken together, in line with our hypothesis, we found opposing effects of acute methylphenidate on connectivity strength and the relative importance of the nodes in subcortical regions, in children compared to adults. In contrast with what we expected, MPH induced changes in connectivity of frontal cortical regions were marginal. They did not indicate differences between age groups, and mainly global importance of these regions (i.e., their importance as a hub) within the network was increased. Therefore, we conclude that acute methylphenidate-effects on connectivity measures in dopamine-sensitive subcortical, but not cortical regions, are different in children and adults with ADHD, possibly due to changes of the dopamine and noradrenergic systems during maturation.

These findings highlight the importance for future studies to investigate the age-dependent effects of long-term methylphenidate treatment, ideally in previously medication-naive individuals, on graph-theoretical connectivity measures, with a focus on centrality measures of subcortical regions. Additionally, we did not find normalizing effects of acute methylphenidate in either of the age groups, indicating that the previously found normalization towards a control state might be present on the local connectivity level, whereas on the global network level methylphenidate may give rise to reorganization of function.

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Supplementary Materials

Supplementary Methods

Participants

Boys aged 10-12 years and men aged 23-40 years were included. Inclusion criteria were meeting criteria for a diagnosis of and requiring treatment with medication for ADHD (Inattentive, Hyperactive/Impulsive or Combined subtype). The diagnosis was determined by an experienced clinician based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; [American Psychiatric Association, 1994]), which was confirmed with a (semi-)structured interview [Ferdinand and van der Ende, 1998] in children; Diagnostic Interview for Adult ADHD (DIVA [Kooij, 2012]). The DSM-IV requirement of at least six inattention or hyperactivity/impulsivity symptoms was applied to both children and adults.

Participants were not eligible when they had received clinical treatment influencing the DA system (for adults before age 23), such as stimulants, neuroleptics, antipsychotics, D2/D3 agonists, or when they had a current or previous dependency on drugs that influence the DA system (for adults before age 23). Other exclusion criteria were an estimated IQ < 80 (Block Design and Vocabulary subtests of the WISC-III-R [Kort et al., 2002], Dutch Adult Reading Test [Schmand et al., 1992], and/or a history of significant medical or neurological trauma or illness.

MRI acquisition

The MRI study was performed on a 3.0 T Philips scanner (Philips Healthcare, Best, The Netherlands) using an 8-channel receive-only head coil. Eight children and 1 adult were scanned on a 3T Philips scanner at a different center than the rest of the participants, also using an 8-channel head coil. A high-resolution 3D T1-weighted anatomical scan was acquired for registration purposes, and fMRI data were obtained using a single-shot echo-planar imaging sequence Parameters were: TR/TE=2300/30ms, resolution=2.3×2.3×3mm, 39 sequential slices, FOV=220x220x117mm, GE-EPI read-out, no gap, 80° flip angle, 130 dynamics, total scan duration 4:98 minutes.

Participants were instructed to keep their eyes open and let their mind wander. In order to increase compliance, light blue blocks (disappearing one by one every minute) on a white background were shown, indicating the duration of the scan. The screen was placed directly behind the scanner and participants were able to see it through a mirror system attached to the head coil.

MRI preprocessing

Preprocessing was performed using FMRIPREP v1.2.3 ([Esteban et al., 2019], RRID: SCR_016216). Each T1w scan was bias-corrected, skull-stripped, and subsequently normalized to MNI space using non-linear registration. Functional data preprocessing included motion correction using FLIRT and distortion correction using an implementation of the TOPUP technique using 3dQwarp. This was followed by co-registration to the corresponding T1w using boundary-based registration with 9 degrees of freedom.

Motion correcting transformations, field distortion correcting warp, BOLD-to-T1w transformation, and T1w-to-template (MNI) warp were concatenated and applied in a single step using antsApplyTransforms (ANTs v2.1.0) with Lanczos interpolation. Independent component analysis (ICA) based on Automatic Removal Of Motion Artifacts (AROMA) was used to generate data that was non-aggressively denoised [Pruim et al., 2015]. Two brain volumes were removed from the start of each scan to ensure that the steady-state equilibrium was attained.

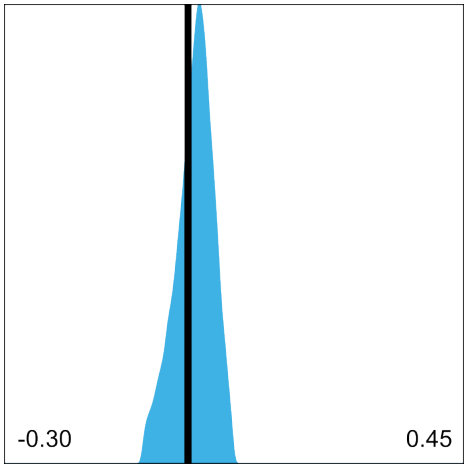
High frequency motion was quantified by determining the percent of relative power above 0.1 Hz within each motion direction, focusing on motion in the y-translation (phase-encoding), using scripts and protocols from [Gratton et al., 2020], using a low-pass Butterworth filter of 1st order, with normalized cutoff frequency 0.1/(0.5/TR) with TR=2.3.

Temporal signal-to-noise (tSNR) maps were calculated per participant to calculate the lowest-quartile mask per age group per session. Parcels overlapping with the lowest-quartile of the tSNR maps with more than 70% of voxels for more than 10% of the participants were excluded from the connectivity matrices [Meijer et al., 2017] (Table 1).

Firstly, connectivity strength was measured, a measure of the temporal correlations in the blood oxygenation level-dependent (BOLD) signals between brain regions at rest. Secondly, two centrality measures were assessed, which reflect the influence a brain region has on the whole-brain network. We measured eigenvector centrality (EC), a measure of the extent to which a given brain region is connected to other highly connected regions, and betweenness centrality (BC), indicating the number of shortest connections that pass through it.

Supplementary Table 1. Excluded regions based on low temporal signal-to-noise ratios.

Gyrus	Left and Right Hemisphere	Label ID.L	Label ID.R	Anatomical and modified Cyto-architectonic descriptions	lh.MNI(X,Y,Z)	rh.MNI(X,Y,Z)
OrG, Orbital Gyrus	OrG_L(R)_6_4	47	48	*A11m, medial area 11*	-6, 52, -19	6, 57, -16
	OrG_L(R)_6_5	49	50	*A13, area 13*	-10, 18, -19	9, 20, -19
STG, Superior Temporal Gyrus	STG_L(R)_6_1	69	70	*A38m, medial area 38*	-32, 14, -34	31, 15, -34
MTG, Middle Temporal Gyrus	MTG_R_4_1		82	*A21c, caudal area 21*	-65, -30, -12	65, -29, -13
ITG, Inferior Temporal Gyrus	ITG_L(R)_7_1	89	90	*A20iv, intermediate ventral area 20*	-45, -26, -27	46, -14, -33
	ITG_L(R)_7_3	93	94	*A20r, rostral area 20*	-43, -2, -41	40, 0, -43
	ITG_L(R)_7_4	95	96	*A20il, intermediate lateral area 20*	-56, -16, -28	55, -11, -32
	ITG_L(R)_7_7	101	102	*A20cv, caudoventral of area 20*	-55, -31, -27	54, -31, -26
PhG, Parahippocampal Gyrus	PhG_L(R)_6_1	109	110	*A35/36r, rostral area 35/36*	-27, -7, -34	28, -8, -33
	PhG_L(R)_6_2	111	112	*A35/36c, caudal area 35/36*	-25, -25, -26	26, -23, -27
	PhG_L(R)_6_4	115	116	*A28/34, area 28/34 (EC, entorhinal cortex)*	-19, -12, -30	19, -10, -30
	PhG_L(R)_6_5	117	118	*TI, area TI(temporal agranular insular cortex)*	-23, 2, -32	22, 1, -36
MVOcC, MedioVentral Occipital Cortex	MVOcC _L(R)_5_1	189	190	*cLinG, caudal lingual gyrus*	-11, -82, -11	10, -85, -9
	MVOcC _L(R)_5_2	191	192	*rCunG, rostral cuneus gyrus*	-5, -81, 10	7, -76, 11
	MVOcC _L(R)_5_3	193	194	*cCunG, caudal cuneus gyrus*	-6, -94, 1	8, -90, 12
LOcC, lateral Occipital Cortex	LOcC _L(R)_4_3	203	204	*OPC, occipital polar cortex*	-18, -99, 2	22, -97, 4



Supplementary Figure 1. Residual QC-FC correlations after denoising. The distribution of all edgewise QC-FC correlations after denoising shows a narrow distribution and a distribution center close to 0 [Ciric et al., 2017].

Supplementary Table 2. Adjacency matrices - quality control.

variable	Children	Adults
# negative correlations	7766.26 ± 4327.95	6638.26 ± 4386.57
% negative correlations	0.13 ± 0.07	0.11 ± 0.07
Average Pearson's R	0.31 ± 0.08	0.33 ± 0.10

Supplementary Table 3. BNA parcellation numbers per region of interest (ROI).

Image	ROI	BNA atlas parcellation numbers
	Striatum	219, 220, 225-230
	Thalamus	231-246
	dACC	179, 180
	PFC	3-6, 11-28, 31, 32, 35, 36, 41, 42, 47, 48, 51, 52, 179, 180, 187, 188

Supplementary Results

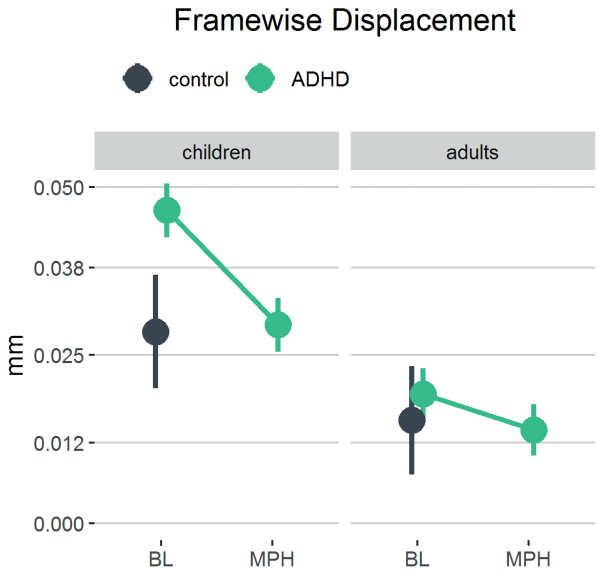
Participants

Included adult ADHD participants were significantly older than the TD adults (mean age ADHD= 28.5±4.6; mean age control 25.1±1.9; $t(39)=3.90$, $p<0.01$; but 80% of the adults with ADHD were under 30). TD controls did not differ from the ADHD participants in IQ ($p>0.05$). In addition, children and adults with ADHD differed from TD controls in ADHD symptom severity, as well as in anxiety and depressive symptoms.

Pre-methylphenidate, FD differed significantly between children and adults (ADHD: $t(45)=5.48$, $p<0.01$; controls: $t(13)=4.99$, $p<0.01$). Excluded children did not differ in age, IQ, ADHD symptom-severity, anxiety symptoms, or depressive symptoms from the included children ($p>0.05$). Children with ADHD had significantly higher FDs than controls at pre-methylphenidate, but FD significantly decreased post-methylphenidate ($F(1,87)=18.84$, $p<0.01$). Pre-methylphenidate, adult ADHD FD values did not differ from controls, but they decreased post-methylphenidate ($F(1,96)=10.08$, $p<0.01$)(Supplementary Figure 2). Post-methylphenidate, FD of ADHD participants did not differ from controls. Importantly, except in two cases, (CS and BC in the PFC) none of the connectivity measures were correlated with motion (Supplementary Table 4).

Supplementary Table 4. Correlation results of all connectivity measures with Framewise Displacement.

variable	Children BL	MPH	Adults BL	MPH
STRIATUM				
strength	r=-0.07,p=0.60	r=-0.24, p=0.12	r=-0.12, p=0.38	r=-0.20, p=0.18
Eigenvector centrality	r=-0.01, p=0.94	r=0.2, p=0.19	r=-0.12, p=0.37	r=-0.03, p=0.85
Betweenness Centrality	r=0.23, p=0.08	r<0.01, 0.98	r=0.02, p=0.91	r=-0.18, p=0.24
THALAMUS				
strength	r=0.01, p=0.92	r=0.01, p=0.96	r=-0.06, p=0.67	r=-0.25, p=0.10
Eigenvector centrality	r=0.17, p=0.21	r=0.10, p=0.53	r=-0.03, p=0.84	r=-0.14, p=0.36
Betweenness Centrality	r=0.18, p=0.17	r=0.24, p=0.12	r=0.23, p=0.09	r<-0.01, p>0.99
DACC				
strength	r=-0.11, p=0.43	r=-0.17, p=0.27	r=-0.06, p=0.67	r=-0.26, p=0.08
Eigenvector centrality	r=-0.01, p=0.93	r=-0.06, p=0.7	r=-0.07, p=0.59	r=-0.10, p=0.51
Betweenness Centrality	r=-0.19, p=0.14	r=-0.3, p=0.05	r=-0.10, p=0.47	r=-0.05, p=0.74
PFC				
strength	r=-0.10, p=0.46	r=0.17, p=0.26	r=0.52, p<0.01	r=0.3, p=0.04
Eigenvector centrality	r=-0.03, p=0.80	r=-0.01, p=0.95	r=-0.02, p=0.86	r=0.16, p=0.28
Betweenness Centrality	r=-0.02, p=0.86	r=-0.29, p=0.05	r=-0.58, p<0.01	r=-0.25, p=0.08



Supplementary Figure 2. Mean Framewise Displacement at pre and post MPH challenge for participants with ADHD (green) and control participants (gray). Showing estimated means with confidence intervals.

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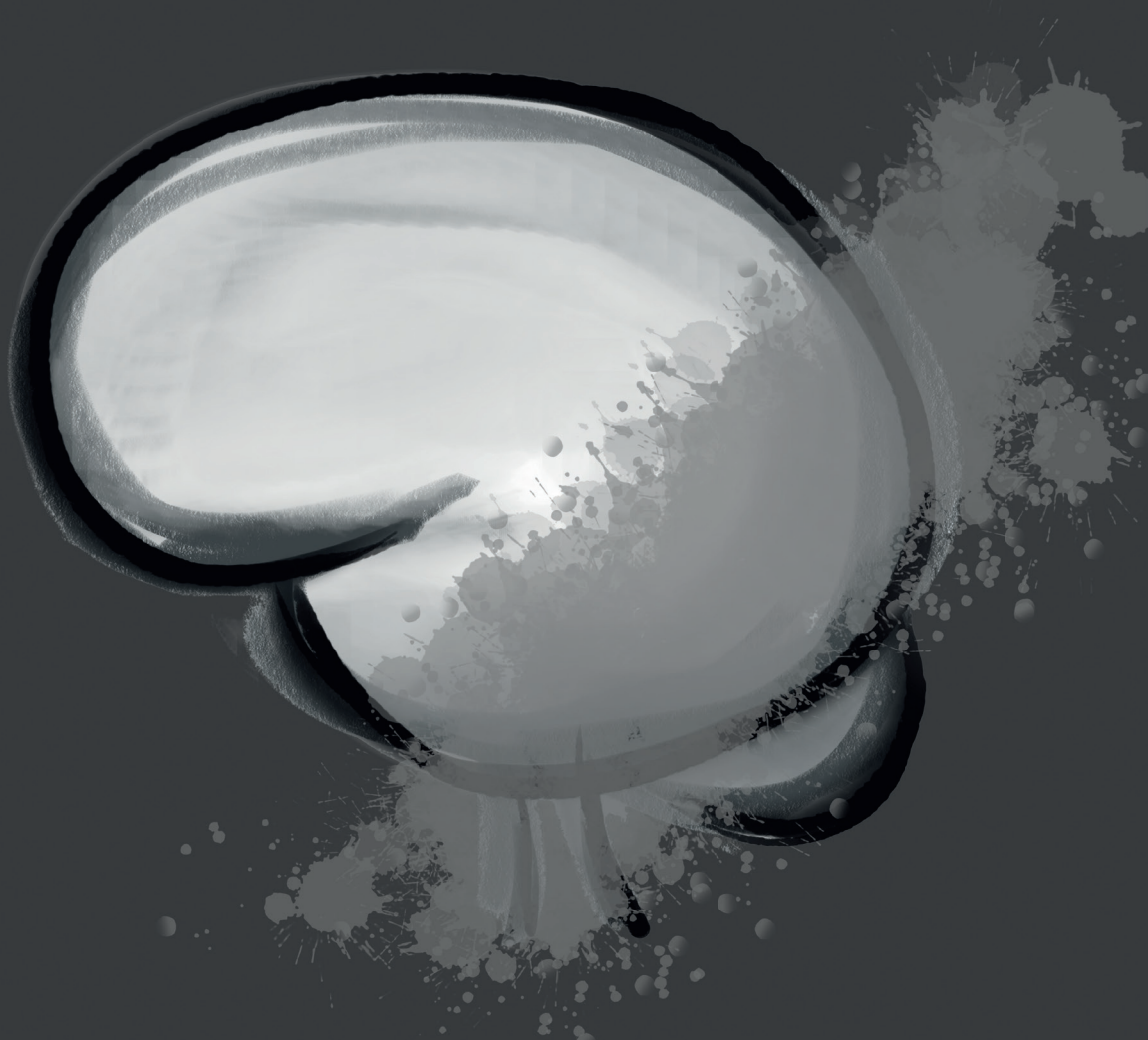
Chapter 5

Exercise Intervention & hippocampal structure and function



A Randomized Controlled Trial on the Effects of a 12-Week High vs. Low-Intensity Exercise Intervention on Hippocampal Structure and Function in Healthy, Young Adults

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Abstract

Physical exercise affects hippocampal structure and function, but the underlying neural mechanisms and the effects of exercise intensity remain incompletely understood. Therefore, we undertook a comprehensive, multi-modal 3T and 7T MRI randomized controlled trial (Netherlands Trial Register NL5847) in which we randomized 52 young, non-athletic volunteers to a 12-week low or high-intensity exercise program. Using state-of-the-art methods, we investigated changes in hippocampal volume, as well as changes in vasculature, neuro-metabolites, and peripheral growth factors as potential underpinnings. Cardiorespiratory fitness improved over time ($p < 0.001$), but no interaction with exercise intensity was found ($p = 0.48$). Accordingly, we did not observe significant interactions between exercise condition and time on MRI measures (all $p > 0.06$). However, we found a significant decrease in right hippocampal volume ($p < 0.01$), an increase in left hippocampal glutathione ($p < 0.01$), and a decrease of left hippocampal cerebral blood volume ($p = 0.01$) over time, regardless of exercise condition. Additional exploratory analyses showed that changes in brain-derived neurotrophic factor ($p = 0.01$), insulin-like growth-factor ($p = 0.03$), and dorsal anterior cingulate cortex N-acetyl-aspartate levels ($p = 0.01$) were positively associated with cardiorespiratory fitness changes. Furthermore, a trend toward a positive association of fitness and gray-matter cerebral blood flow ($p = 0.06$) was found. Our results do not provide evidence for differential effects between high-intensity (aerobic) and low-intensity (toning) exercise on hippocampal structure and function in young adults. However, we show small but significant effects of exercise on hippocampal volume, neurometabolism and vasculature across exercise conditions. Moreover, our exploratory results suggest that exercise might not specifically only benefit hippocampal structure and function, but rather has a more widespread effect. These findings suggest that, in agreement with previous MRI studies demonstrating moderate to strong effects in elderly and diseased populations, but none to only mild effects in young healthy cohorts, the benefits of exercise on the studied brain measures may be age-dependent and restorative rather than stimulatory. Our study highlights the importance of a multi-modal, whole-brain approach to assess macroscopic and microscopic changes underlying exercise-induced brain changes, to better understand the role of exercise as a potential non-pharmacological intervention.

Introduction

Physical exercise can have numerous positive effects on our body and brain; including reductions in the risk for cardiovascular disease, stroke, and obesity. Furthermore, it has been found to promote brain plasticity and positively affect brain structure and function in both rodents and humans [Demnitz et al., 2021; Dorsman et al., 2020; Haskell et al., 2007; Intlekofer and Cotman, 2013; Venkatraman et al., 2020; Voss et al., 2013]. Therefore, the possibility to use physical activity to improve brain health has received much attention lately as a low-cost and easy to apply, non-pharmacological intervention [Cole et al., 2019; van Praag et al., 2005]. So far, however, the exact underlying mechanisms by which exercise can benefit the brain, and what role exercise intensity plays, have remained incompletely understood.

The first studies investigating brain correlates of exercise-induced changes sought to determine structural brain alterations. Using magnetic resonance imaging (MRI), multiple cross sectional and prospective-longitudinal studies in humans have shown that high-intensity aerobic exercise increased or normalized age-related decreases in brain volume, particularly in the hippocampus [Erickson et al., 2011; Pajonk et al., 2010]. In their meta-analysis, Firth et al. found the most substantial exercise effects in older adults [Firth et al., 2017], even though some studies also reported rapid hippocampal volume increases in younger adults [Thomas et al., 2016].

Volume changes alone lack information on biological substrates of exercise-related changes [Czéh and Lucassen, 2007]. Both animal and human studies have proposed several underlying mechanisms [Lucas et al., 2015; Voss et al., 2013], such as changes in perfusion, as measured with cerebral blood flow (CBF), vascularization as measured with cerebral blood volume (CBV), synaptic plasticity and neurogenesis as estimated by neuro-metabolite concentrations, and other molecular and cellular changes [Bullitt et al., 2009; Burdette et al., 2010; Dorsman et al., 2020; Guiney et al., 2015; Pereira et al., 2006; Stillman et al., 2018; Suwabe et al., 2018]. For instance, rodent studies have shown exercise-induced increases in angiogenesis and neurogenesis [Bloor, 2005; Czéh and Lucassen, 2007; Kerr et al., 2010; Vivar and van Praag, 2017]. Physical exercise was further shown to alter specific neuro-metabolites; Biedermann et al. and Wagner et al. reported decreased right hippocampal glutamate (Glu) levels of mice and humans after prolonged exercise [Biedermann et al., 2012; Wagner et al., 2015], and similar results were found in the human occipital cortex [Dennis et al., 2015] and the rat striatum [Meeusen et al., 1997]. Cross-sectional studies have further associated higher fitness of endurance-trained, middle-aged adults with higher N-acetyl aspartate (NAA) levels in their frontal cortex [Erickson et al., 2012; Gonzales et al., 2013].

In summary, physical exercise in both animals and humans influences various mechanisms that may alter brain structure [Kandola et al., 2016; Voss et al., 2013]. In this respect, Thomas et al. were one of the first to investigate volume changes in young, healthy adults in a multimodal approach [Thomas et al., 2016]. They used several neuroimaging measures of volume, vasculature, and microstructure, and specifically found a temporary increase in volume and myelination, but no vascular changes. So far, the role of exercise intensity has received little attention [Lucas et al., 2015; Wilke, 2020], even though a recent meta-analysis stressed the importance of high-intensity training (heartrate (HR) > 80% of maximum HR) for improving fitness in younger adults [Bacon et al., 2013; Wen et al., 2019].

Therefore, we here undertook a comprehensive, multi-modal study to compare the effects of a 12-week high vs. low-intensity exercise paradigm in young, healthy, but otherwise non-athletic volunteers. We studied exercise-induced changes in hippocampal volume, and additionally, its potential underpinnings, like changes in angiogenesis, synaptic plasticity, neurogenesis, and peripheral growth factors. Because earlier studies had indicated that certain changes only occur in specific hippocampal subfields [Nuninga et al., 2019], we further explored changes in hippocampal subfield volume and relations of all measures to individual changes in cardiorespiratory fitness. We used 3T MRI to study outcomes related to vascular changes and 7T MRI to obtain high resolution anatomical delineation of hippocampal subfields and reliable quantification of various neuro-metabolites [Terpstra et al., 2016]. To study exercise intensity and control for baseline differences in fitness, we randomized participants, after stratification for age, sex, and baseline VO2max, to a high-intensity, aerobic exercise condition, or a low-intensity, stretching and toning (active control) exercise condition.

Based on earlier literature, we hypothesized that cardiorespiratory fitness and hippocampal volume increases would occur in the high-, but not low-intensity exercise condition. Based on an increase in hippocampal volume, we furthermore expected changes in vascularization, as measured with cerebral blood flow (CBF), and cerebral blood volume (CBV), along with vascular endothelial growth factor (VEGF) concentrations, as a marker for vascular maintenance and remodeling. Moreover, neuronal remodeling was expected, estimated through changes in neuro-metabolite concentrations of NAA, glutathione (GSH), glutamate (Glu), and glutamine (Gln), and brain-derived neurotrophic factor (BDNF) as well as peripheral insulin-like growth factor-1 (IGF-1), as markers for neuronal development. We chose the dorsal anterior cingulate cortex (dACC) as a control region and regarded whole-brain gray matter (GM) changes as evidence for non-specific effects.

Methods

Participants and Experimental Design Participants were recruited through posters and online advertisements. We included 52 healthy, non-athletic volunteers (30 women and 22 men, aged 18-30 years old, Table 1) in a 3month randomized, controlled trial (Netherlands Trial Register NL5847; Figure 1). After stratification for age, sex, and baseline cardiorespiratory fitness, participants were randomized to a 12-week intervention of high or low-intensity exercise training. Before and after the intervention, participants performed a maximal exercise test to measure cardiorespiratory fitness and underwent MRI measurements (Figure 2A).

Participants that were classified as athletic based on the definition by Haddad Herdy and Uhlendorf [Haddad Herdy and Uhlendorf, 2010], i.e., $VO2max_{males}$ > 55 ml/kg/min, $VO2max_{females}$ > 45 ml/kg/min, were excluded. Furthermore, participants who engaged in intensive sports (>3 times/week) were also excluded. In addition, we excluded participants based on the following criteria: BMI >30 kg/m^2 (>class 1 obesity), MRI contra-indications, a history of chronic renal insufficiency, allergy to gadolinium-containing compounds, a history of psychiatric disorders, excessive smoking (>1 pack/day), excessive alcohol consumption (>21 units/week), or other regular drug use. Additionally, females were only included if they were on hormonal contraceptives to control for the effects of the hormonal cycle. We obtained written informed consent from all participants, and the study was approved by the local Medical-Ethical Committee of the Amsterdam University Medical Centre, University of Amsterdam (NL55943.018.15).

Table 1. Participant characteristics and fitness measures.

	HIGH-INTENSITY		*LOW-INTENSITY*		*STATISTICS*3*	
	female (N=13) Mean ± SD	*male (N=11) Mean ± SD*	*female (N=13) Mean ± SD*	*male (N=10) Mean ± SD*	*female*	*male*
Participant characteristics:						
Age (y)	23.87 ± 2.59	22.09 ± 2.09	24 ± 2.58	24.64 ± 4.15	t(23.92)=-0.14, p=0.89	t(14.88)=1.81, p=0.09
Education	7.00 ± 1.11	6.70 ± 1.49	7.31 ± 1.03	6.36 ± 2.01	T(23.00)=-0.75, p=0.46	t(18.31)=0.44, p=0.67
Body Mass Index (kg/m²)	23.12 ± 3.19	23.55 ± 3.17	23.67 ± 2.51	23.46 ± 2.23	t(23.70)=0.52, p=0.60	t(17.95)=-0.08, p=0.94
IQ estimate (DART)	106.47 ± 5.40	107.60 ± 6.33	107.21 ± 7.20	105.55 ± 7.41	t(23.06)=-0.32, p=0.76	t(18.94)=0.69, p=0.50
Fitness measures:						
VO2max pre (kg/mL/min)	33.71 ± 5.18	41.98 ± 7.44	34.44 ± 4.75	41.78 ± 6.22	t(27.80)=-0.18, p=0.85	t(19.39)=-0.07, p=0.95
VO2max post (kg/mL/min)	38.79 ± 5.25	45.25 ± 6.83	36.15 ± 6.14	43.59 ± 6.93	t(23.44)=-1.18, p=0.25	t(18)=-0.54, p=0.60
Max. Heart Rate pre (beats/min)	182.92 ± 8.36	192.12 ± 4.85	190.64 ± 7.97	187.03 ± 6.68	t(27.94)=2.59, **p=0.01**	t(18.18)=-2.01, p=0.06
Max. Heart Rate post (beats/min)	185.08 ± 9.57	187.11 ± 6.68	190.10 ± 6.88	184.07 ± 6.68	t(21.79)=1.54, p=0.14	t(18.81)=-1.04, p=0.31
Resistance pre (watts)	200.33 ± 33.03	294.09 ± 49.84	221.00 ± 31.58	276.82 ± 42.56	t(27.94)=1.75, p=0.09	t(19.52)=-0.87, p=0.39
Resistance post (watts)	225.38 ± 30.38	330.45 ± 53.03	239.62 ± 29.04	291.50 ± 47.26	t(23.95)=1.22, p=0.23	t(18.99)-1.78, p=0.09
Intervention:						
Duration of exercise (h)	32.44 ± 14.81	26.46 ± 7.13	31.69 ± 9.51	26.78 ± 14.69	t(20.63)=-0.15, p=0.88	t(17.97)=-0.60, p=0.56
*Percent of hours with HR>80% of max. HR*1*	35.92 ± 17.11	35.84 ± 19.92	13.51 ± 9.39	4.53 ± 3.69	t(18.04)=-4.21, **p<0.01**	t(10.83)=-5.11, **p<0.01**

*Note: pre= pre-exercise intervention; post = post-exercise intervention; Education (Dutch System; Scheerens et al., 2012): 0= no education, 1= Elementary School, 2= VMBO, 3= VMBO-T, 4= MBO, 5=HAVO, 6= VWO, 7= HBO, 8= WO (University), Body Mass Index=weight (kg) / [height (m)]^2; NLV= Dutch reading test estimating intelligence quotient (IQ); max. Heart Rate= max. Heart Rate measured during VO2max test; Resistance= max. resistance of ergometer during VO2max test; Duration of exercise= Hours spent exercising over the 12-week exercise intervention; *1= Percentage of hours spent exercising with a HR above 80% of individuals max. HR (220-age); *3=Independent samples t-tests, in case of non-normal distribution, data were transformed.*

Exercise-Intervention

All participants were enrolled in an exercise program for 12 weeks, in which they were instructed to exercise three times a week for 45min [Astorino et al., 2017; Haskell et al., 2007] at the university sports center (USC). Their presence and active engagement were monitored by tracking their sports center visits using an automated fingerprint entrance system and by using weekly questionnaires on exercise duration and activities. Additionally, participants received a HR monitor (Polar, Finland) to measure HR during each training session (Table 1). Participants randomized to high-intensity exercise performed high-intensity interval training, targeting HR zones above 80% of their maximum HR. Participants randomized to low-intensity exercise performed stretching and toning exercises (active control condition), targeting HR zones under 60% of their maximum HR. In collaboration with the sports scientists of the USC, we provided a list of generic exercise group classes offered at the USC which were supervised by an experienced fitness instructor, that participants were allowed to choose from (Supplementary Material 1.5; Supplementary CERT Checklist [Slade et al., 2016]). Participants that were engaged in physical activity before the study were instructed to do the recommended sports classes on top of their usual activities. For motivation purposes, participants were contacted regularly to check in on their progress and one experimenter joined them at least once during the intervention period to train with them (more detail in Supplementary Material 1.5). The Dutch version of the International Physical Activity Questionnaire (IPAQ; [Vandelanotte et al., 2005]) was used to measure physical activity during walking, intermediate and vigorous intensities before and after the intervention.

Cardiorespiratory Fitness Participants underwent a cardiopulmonary exercise test on an ergometer before and after the exercise intervention to assess individual cardiorespiratory fitness. After 2min of rest (baseline measurement), an incremental bicycle protocol (which was dependent on weight and sex) was started with a 3-min warm-up period, followed by an increase in resistance (watts) every minute until maximal effort (maximum resistance) or exhaustion, which was immediately followed by a 2-min recovery period at 50 watts resistance. Breath-by-breath gas exchange measurement data were obtained to determine maximum oxygen uptake (VO2max (mL/kg/min)) [Astorino et al., 2017]. VO2max data were time-averaged using 10s intervals [Wagner et al., 2015]. For exercise tests to be considered maximal, participants had to reach both a plateau in VO2max and a respiratory exchange ratio of >1.1 CO_2/O_2. VO2max tests took place at least 24h before the MRI scans.

MRI Acquisition

Participants were scanned on a 7T whole-body MR system (Philips, Best, The Netherlands) using a dual-channel transmit coil and a 32-channel receive head-coil, and on a 3T whole body MR system (Philips, Best, The Netherlands) using a body transmit coil and a

32-channel receive head-coil. A 24h gap between the last workout and MRI scanning was ensured to minimize the potential influences of dehydration on brain volume and acute exercise effects [Maddock et al., 2011].

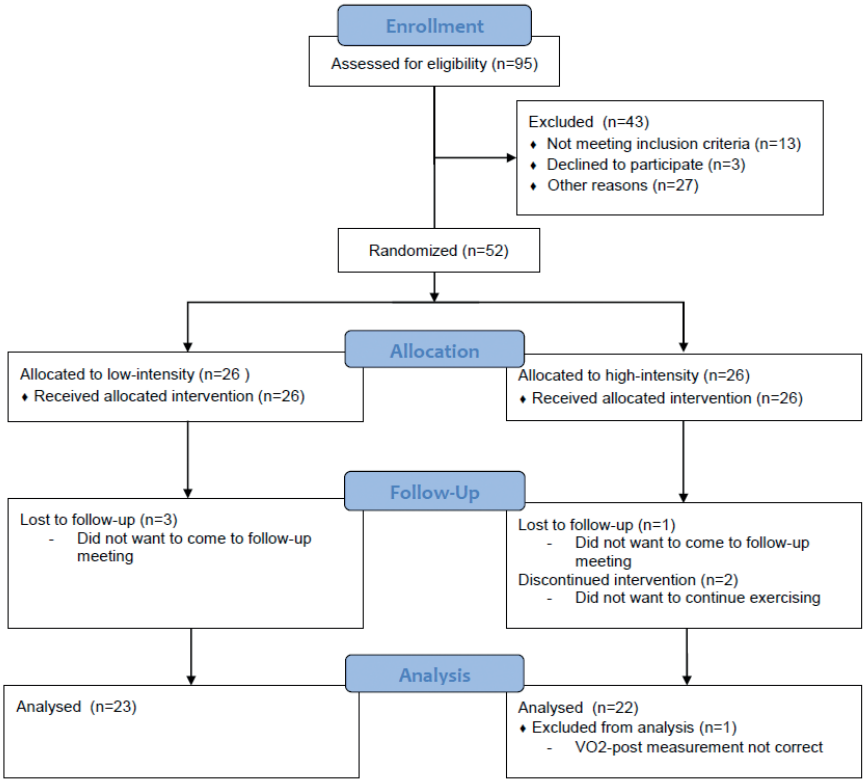

Figure 1. CONSORT flow chart.

7T MRI

Whole-brain T1-weighted data were obtained with a sagittal 3D magnetization-prepared rapid gradient echo (MPRAGE) sequence (TR/TE = 4.1/1.8ms; TI = 1300ms; 0.9 × 0.9 × 0.9mm^3 isotropic voxels; flip-angle = 7°). T2weighted data covering the hippocampus were obtained using a coronal multi-slice turbo spin-echo (TSE) sequence (TR/TE = 6000/80ms; voxel-size = 0.4 × 0.4 × 2mm; flip-angle = 110°) (Figure 2B). Single voxel 1H-MRS data were collected from the left hippocampus and dACC with a semi-localized adiabatic selective refocusing (sLASER) sequence (TR/TE = 5000/36ms; FOCI pulses [Arteaga de Castro et al., 2013]) to ensure correct adiabatic behavior of the FOCI pulses: B1 > 17μT; bandwidth = 4 kHz; 2048 data points; voxel-size = 30 × 15 × 15mm; NSA dACC = 64; NSA hippocampus = 128 (Figure 2C; Supplementary Figure 1). Non-water suppressed spectra were obtained for quantification and eddy-current correction.

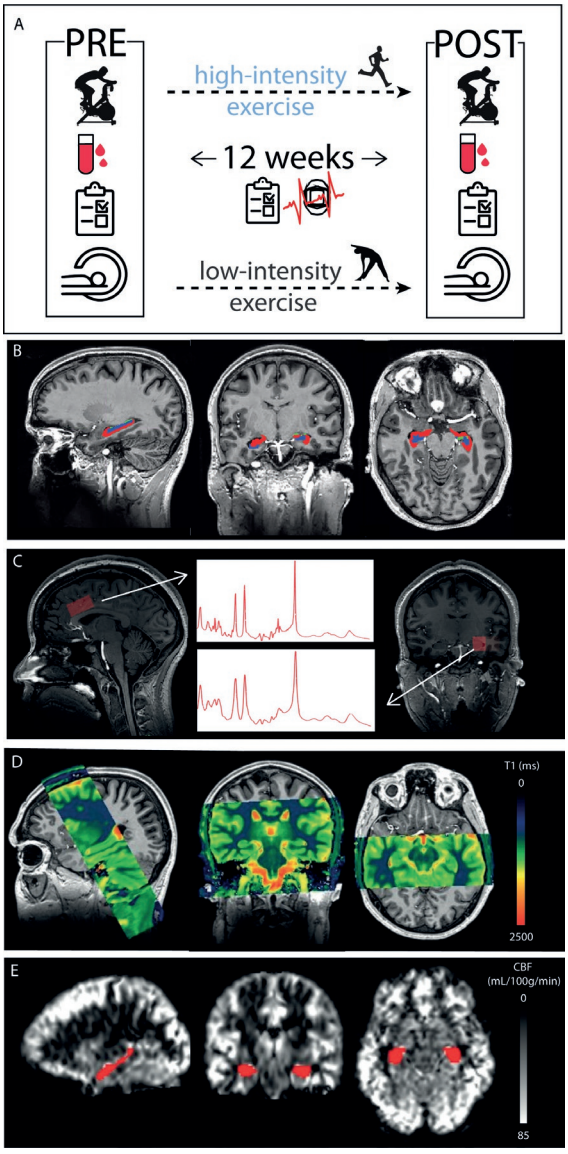

Figure 2. Study methods: (A) Participants were enrolled in a 12-week low- (active control) and high-intensity exercise intervention. Several measures, including a cardiorespiratory fitness test (VO2max), and peripheral growth factors (blood sampling) were conducted before (PRE) and after (POST) the exercise intervention. Additionally, HR, exercise frequency, and exercise questionnaires were collected during the intervention. Furthermore, several MRI measures were collected before and after the exercise regime: (B) T1- and T2-weighted scans were conducted at 7T for segmentation purposes. (C) Single voxel spectroscopy was conducted at 7T in the dACC (left) and left hippocampus (right). (D) T1-mapping using a steady-state contrast-enhanced method was conducted at 3T to derive CBV and R1. (E) A pCASL sequence was used at 3T to obtain CBF values.

3T MRI

To obtain CBV and myelination (R1 = (1/T1)), quantitative T1 measurements of the hippocampus and sagittal sinus blood were performed before and after gadolinium contrast administration (0.1mL/kg, 1–2mL/s followed by 20mL saline (0.9% NaCl)) (Gadovist, Bayer B.V., Mijdrecht, The Netherlands). For brain T1-mapping, a 3D Look-Locker sequence with 40 inversion times was performed as described by Lindgren et al. [2014], in a coronal slab covering the hippocampus (Figure 2D) with the following parameters: TR/TE = 10/4ms; flip-angle = 5°; TI = 110ms, inter-shot TR = 6s, resolution = 1.15 × 1.15 × 2mm, acquisition time = 6min. Blood T1 values were obtained using a single-slice multi-time-point inversion recovery sequence planned perpendicular to the posterior sagittal sinus with parameters: TR/TE=110/16ms, flip angle =95°; resolution = 1.5 × 1.5mm; slice-thickness = 2mm. To obtain whole brain CBF measures we used a gradient-echo single-shot EPI pseudo-continuous arterial spin labeling (pCASL) sequence with background suppression (TR/TE = 4091/16ms; label-duration = 1650ms, post-label delay = 1525ms; voxel-size = 3 × 3 × 5mm) (Figure 2E). For CBF quantification, an additional M0 scan was acquired using the same imaging parameters, except for the TR = 2000ms, and without labeling and background suppression.

MRI Data Analysis

Volume

Using both T1-weighted and T2-weighted scans, segmentations of the hippocampus were performed in native space using Automatic Segmentation of Hippocampal Subfields (ASHS) software [Wisse et al., 2016; Yushkevich et al., 2015]. This method automatically generates segmentations based on a segmentation atlas [Wisse et al., 2016] with a machine-learning algorithm using similarity-weighted voting and learning-based bias-correction techniques (Figure 2B). The following subfields were defined: whole hippocampus, consisting of CA1, CA2, CA3, CA4, DG, subiculum, head, tail, entorhinal cortex, and cysts. Segmentation of the dACC was performed with Freesurfer v.5.3.0 [Reuter et al., 2012]. Gray matter (GM) and white matter (WM) segmentations were performed with SPM12.

For the main analyses, all measures were calculated for the whole left and right hippocampus. The dACC was used as a control region, and whole-brain GM changes were regarded as region-unspecific effects. Further exploratory analyses involved volume measures of hippocampal subfields: CA1, CA3, and dentate gyrus, and hippocampal GM and WM.

1H-MRS

Pre-processing included optimized coil combination, eddy current correction, and spectral registration [Near et al., 2015]. Spectra were fitted using LCModel with a simulated basis set with a measured macromolecular baseline (Supplementary Methods 1.1). Metabolite concentrations for glutamate, glutamine, glutathione (GSH), and N-acetyl-aspartate (NAA) were calculated using water-scaling and were corrected for partial volume effects using the tissue volume fractions [Gasparovic et al., 2006]. T1-weighted scans were segmented using SPM12 to determine the contributions of GM, WM, and CSF to each voxel. Spectral quality measures calculated with LCModel, and signal-to-noise ratio (SNR > 30), linewidth (FWHM > 19Hz), and Cramér–Rao lower bounds (CRLB ≤ 40), were used to exclude lower-quality spectra [Kreis et al., 2020; Öz et al., 2021].

CBF

ASL post-processing was performed using Explore ASL [Mutsaerts et al., 2020]. Motion was estimated, spike frames > mean + 3 standard deviations (SD) were deleted, and motion estimation was repeated. ASL perfusion-weighted images were registered to GM-tissue probability maps of each participant using six degrees-of-freedom (DOF). Label and control images were pairwise subtracted (M), corrected for slice gradients, and averaged. CBF was calculated using the single-compartment model [Alsop et al., 2015], using a separate M0 image and individual hematocrit values that were derived from blood samples to calculate T1-blood values. Before and after quantification, voxel-based outlier rejection was applied. GM-tissue probability maps were normalized using Diffeomorphic Anatomical Registration analysis using Exponentiated Lie algebra (DARTEL), and T1-to-MNI transformation fields were applied to CBF maps [Ashburner, 2007]. Median ROI CBF was based on voxels remaining after excluding voxels with CBF values exceeding 2.5 times the mean CBF over the entire volume, assumed to originate from large vessels. Thresholded left and right hippocampal masks (Harvard-Oxford Subcortical Structural Atlas) were masked for GM, and median ROI CBF values were calculated per participant.

CBV and Myelination

ROI averaged Look-Locker signal time curves were generated from different ROIs. T1 values for each ROI were calculated using a 3-parameter fit of the Look-locker signal equation [Deichmann and Haase, 1992]. For determination of blood T1 values, 5 pixels that showed the highest average signal intensity, averaged over the last ten inversion times, within the sagittal sinus, were selected. Subsequently, T1 was determined by averaging signals from different combinations of pixels and choosing combinations that resulted in the lowest T1 fit-error based on a 3-parameter fit of the multi-timepoint inversion recovery curve. Finally, CBV was calculated using equations by Lindgren et al. with brain tissue density = 1.04g/ml, hematocrit levels in large vessels = 0.45, and hematocrit levels in small vessels =

0.25, and GM CBV was corrected for the expected fast-water exchange-effects [Lindgren et al., 2014; Shin et al., 2006]. Hippocampal values were consequently expressed relative to GM values. Furthermore, R1 values were obtained for left and right hippocampal WM by calculating 1/T1 to estimate myelination [Stüber et al., 2014].

Peripheral Neurotrophic Factors

Blood samples were collected before the MRI measurements, pre and post-intervention, to obtain: 1) brain-derived neurotrophic factor (BDNF) levels as a proxy for exercise effects on hippocampal neuronal health, plasticity, and possibly neurogenesis [Adlard et al., 2005; Aguiar et al., 2010; Marlatt et al., 2012]; 2) insulin-like growth factor 1 (IGF) as a proxy for cell proliferation and the inhibition of cell death [Åberg et al., 2003], 3) free vascular endothelial growth factor (VEGF) levels, as a prime regulator of angiogenesis [Fabel et al., 2003]; and 4) hematocrit levels. For BDNF and IGF, a total of 4mL serum was collected (15min centrifugation at 1000×g). For VEGF, 8mL serum was collected in PECT tubes [Niers et al., 2011b] through an open system, drop by drop, without using a tourniquet (60min centrifugation at 4°C at 1,700×g). All samples were aliquoted and stored at −80°C. Growth factors were quantified using enzyme linked immunosorbent assays (ELISAs) according to the manufacturer's instructions (R&D Systems; DVE00 for VEGF; DBD00 for BDNF; DG100 for IGF), and optical densities were converted into concentrations using an LP4 logistic fit (Graphpad Prism 5).

Statistical Analysis

Sample size calculations can be found in the Supplementary Methods (1.4). All data were checked for normality and, in the case of non-normality, transformed accordingly. To account for missing data and the longitudinal nature of the trial, linear mixed-effects models were used to investigate the condition (high vs. low-intensity exercise) × time (pre vs. post-intervention) interaction effects in Rv.3.5.3 [R Development Core Team, 2011] using the lme4 package [Bates et al., 2015]. Sex (female vs. male) was tested as a possible predictor but did not contribute to any of the models. Model selection was based on an adjusted top-down procedure, in which the resulting models were compared using the Bayesian information criterion (BIC), and subsequently, the model best capturing the data was reported using χ^2-tests and BICs [Schwarz, 1978]. Bayes factors (BF) were calculated, indicating the strength of evidence, using BIC approximation [Wagenmakers, 2007]. The evidence categories of Wetzels et al. were used [Wetzels et al., 2011] (Supplementary Table 1). We regarded changes in cardiovascular fitness and hippocampal and dACC volume as primary hypotheses, and changes in neuro-metabolites, CBV, myelination, CBF, and neurotrophic factors as secondary hypotheses.

Statistical tests regarding the interaction effects were corrected for multiple comparisons within modalities using Sidak's correction: $\alpha^* = 1 - (1 - \alpha)^{(1/m)}$, with $\alpha = 0.05$ and

being the number of interaction and main effects (m = 3), which resulted in an α* = 0.02. Additionally, Tukey corrected t-tests were used as post-hoc tests.

Furthermore, exploratory analyses testing associations of all variables with changes in VO2max were conducted using linear models in R, including the baseline measure of the explanatory variables and VO2max as covariates (α = 0.05). We additionally tested exercise-condition as a possible covariate, which did not contribute to the model. Mean and standard deviation per timepoint per variable are reported in Supplementary Table 4.

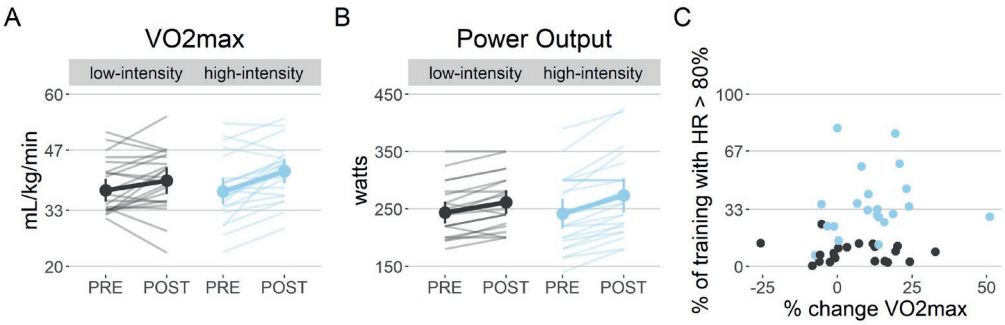

Figure 3. Cardiorespiratory fitness: (A) Cardiorespiratory fitness (VO2max) was found to increase over time irrespective of the exercise group ($p < 0.01$), even though post-hoc tests show only a significant increase in the high-intensity group ($p < 0.01$). (B) The ergometer power output during the VO2max test increased over time irrespective of the exercise group ($p < 0.01$), with the post-hoc test showing a significant increase in both groups (both $p < 0.01$). (C) Even though participants in the high-intensity group spent significantly more time in the target HR zone (80% of max. HR) than the low-intensity group ($p < 0.01$), the hours spent exercising was not associated with changes in fitness ($p = 0.19$).

Results

Six participants dropped out during the exercise program and one participant was removed from the analysis because of incomplete VO2max data. Therefore, the low-intensity condition consisted of 10 males and 13 females, the high-intensity condition of 11 males and 11 females. Conditions did not differ in age, sex, education, IQ-estimation, VO2max, or BMI at baseline (Table 1).

Cardiorespiratory Fitness

Hours spent exercising demonstrated high compliance with the exercise program in both exercise groups (Table 1). As expected, participants in the high-intensity condition spent significantly more time exercising in the intended higher HR regime than the low-intensity condition, which did not explain the change in VO2max ($t(40) = 1.34$, $p = 0.19$; Figure 3C). The low and high-intensity exercise groups did not show a significant change from pre to post-intervention on the total score or scores for walking and intermediate intensity activities as measured with the IPAQ questionnaire. However, a significant condition x time effect was found on vigorous intensity activities ($\chi^2(1) = 5.46$, $p = 0.02$), indicating an increase in the high-intensity group but not in the low-intensity group (Supplementary Results 2.3).

Nevertheless, contrary to our expectations, we found no condition x time effect on VO2max; instead, we found decisive evidence (BF > 100) for an effect of time ($\chi^2(1) = 15.43$, $p < 0.001$; low-intensity: 4.7%, high-intensity: 12.65% change) (Figure 3A). Nevertheless, post-hoc tests revealed only a significant increase in the high-intensity condition (low: $t(49) = 1.72$, $p = 0.09$; high: $t(49) = 4.20$, $p < 0.01$). In line with the results on VO2max, we found no interaction effect, but decisive evidence (BF > 100) for a main effect of time ($\chi^2(1) = 38.92$, $p < 0.001$) on the maximal resistance attained. Post-hoc tests revealed a significant increase in both conditions ($t(23) = 4.67$, $p < 0.01$; $t(24) = 7.02$, $p < 0.01$; Figure 3B). No group effects on HR during the VO2max test were found (Supplementary Table 2).

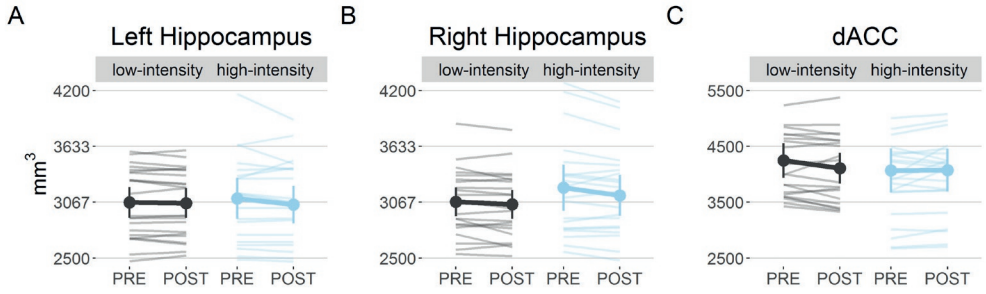

Figure 4. Volume measures: (A) Left hippocampal volume did not show any differences over time. (B) Right hippocampal volume decreased over time, irrespective of the exercise group ($p < 0.01$). Post-hoc tests revealed only a significant decrease in the high-intensity exercise group ($p = 0.03$). (C) The control region, the dorsal anterior cingulate cortex (dACC), did not show any significant changes over time.

MRI Volume

For left and right hippocampal volume, one baseline scan had to be removed from the analysis due to incomplete hippocampal coverage of the T2-weighted scan. Left and right hippocampal volumes were analyzed separately, based on previous literature reporting lateralized effects of exercise [Bracht et al., 2016; Nauer et al., 2020]. We found no interactions between condition and time for either left and right whole hippocampal volume. However, we found substantial evidence (BF = 4.48) for a negative main effect of time in the right hippocampus ($\chi^2(1) = 7.51$, $p < 0.01$). Post-hoc tests further revealed only a significant decrease in the high-intensity condition ($t(47) = 2.22$, $p = 0.03$; Figures 4A,B). Consequently, we sought to determine whether this change was specific to a certain hippocampal subfield but found no significant effects (Supplementary Table 2). We did not find changes in volume in our control region, the dACC (Figure 4C).1H-MRS

We were unable to obtain 1 baseline and 1 post spectrum in the left hippocampus and 7 baseline dACC spectra due to technical difficulties. Due to further exclusion based on stringent quality control measures (Supplementary Results 2.1) a total of 42 baseline and 43 post hippocampal spectra, and 40 baseline and 51 post dACC high-quality spectra were included in the analyses. No condition-by-time interactions were found for any neuro-metabolites investigated, i.e., glutamate, glutamine, glutathione, and NAA, in the hippocampus and the dACC (Supplementary Table 2). However, we found strong evidence (BF = 11.19) for increased GSH in the left hippocampus across conditions (main effect of time: $\chi^2(1) = 9.21$, $p < 0.01$)(Figure 5).

Vascularization and Myelination

No condition-by-time interactions were found for CBF and CBV, in the left and right hippocampus, and GM (Supplementary Table 2; Figure 6). However, anecdotal evidence (BF = 2.79) for a decrease of CBV in the left hippocampus was found (main effect of time: $\chi^2(1) = 5.97$, $p = 0.01$), with post-hoc tests showing a slight reduction in CBV in the low-intensity condition ($t(43) = 1.94$, $p = 0.05$).

No condition-by-time interactions were found for R1 in the left and right hippocampal WM (Supplementary Table 2).

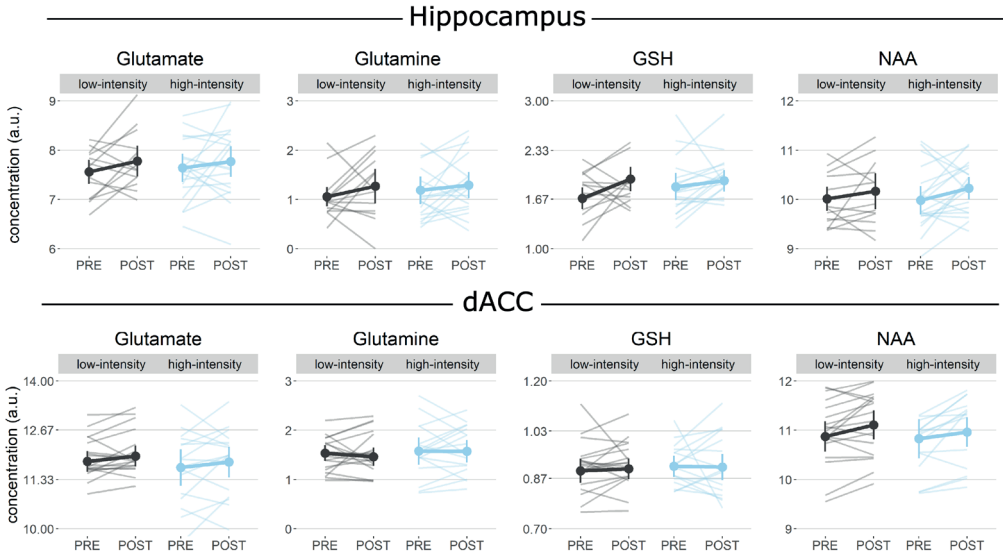

Figure 5. 1H-MRS: GSH in the hippocampus was found to increase over time, irrespective of the exercise group ($p < 0.01$). No other metabolites in the hippocampus or the control region, dorsal anterior cingulate cortex (dACC), were found to change over time.

Peripheral Neurotrophic Factors

We found no interaction effect of condition and time, and no main effects of time for BDNF, VEGF, or IGF levels ($BF < 100^{-1}$) (Supplementary Table 2). No significant interaction effect of condition and time was found for hematocrit ($\chi^2(2) = 0.03$, $p = 0.98$), but a main effect of time was found, indicating an increase in both exercise groups ($\chi^2(1) = 12.90$, $p < 0.01$; $BF = 8.4$).

Regression Analyses

MRI Regression analyses demonstrated no association between changes in hippocampal or dACC volume and change in VO2max (left: $F(1, 41) = 0.17$, $p = 0.95$; right: $F(1, 41) = 0.43$, $p = 0.65$; dACC: $F(1, 40) = 0.30$, $p = 0.59$). There was no association between changes in hippocampal Gln, Glu, GSH and NAA and VO2max change ($F(1, 26) = 0.38$, $p = 0.55$; $F(1, 26) = 0.42$, $p = 0.74$; $F(1, 25) = 0.77$, $p = 0.52$; $F(1, 25) = 0.54$, $p = 0.72$). In the dACC, Glu, Gln, and GSH changes ($F(1, 26) = 0.46$, $p = 0.64$; $F(1, 26) = 0.30$, $p = 0.83$; $F(1, 26) = 0.96$, $p = 0.43$) were not associated with VO2max change, but increased VO2max was associated with increased dACC NAA levels ($F(1, 26) = 7.14$, $p = 0.01$) (Supplementary Figure 2). While not significant, GM CBF showed a trend toward an association with VO2max change ($F(1, 45) = 3.06$, $p = 0.06$). No other associations with VO2max changes were found (Supplementary Table 3).

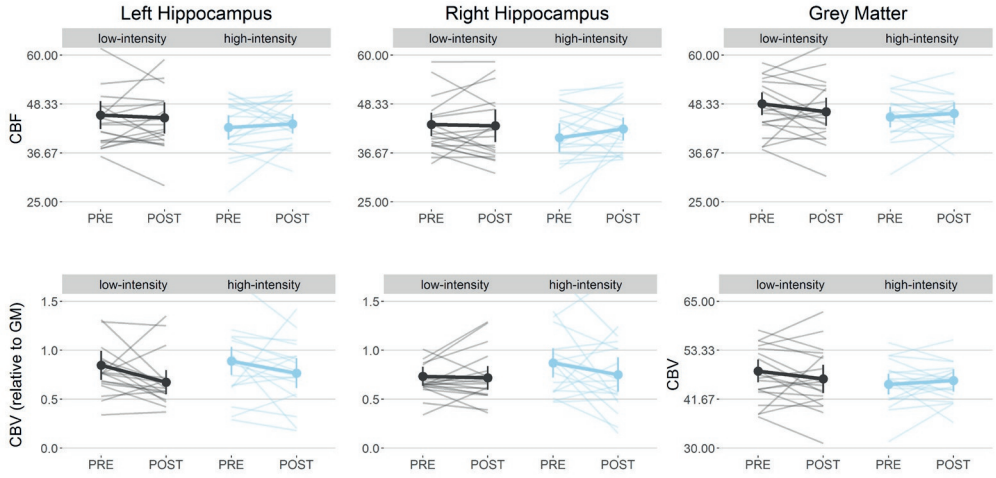

Figure 6. Vasculature: No vascular changes were found over time. CBV, Cerebral blood volume; CBF, Cerebral blood flow.

Peripheral Neurotrophic Factors

BDNF and IGF level changes were found to be positively associated with change in VO2max ($F(1,35) = 6.84$, $p = 0.01$; $F(1,35) = 6.27$, $p < 0.01$) (Supplementary Figure 2), whereas VEGF level changes were not ($F(1, 36) = 0.22$, $p = 0.63$, $F(1, 36) = 1.09$, $p = 0.30$).

Discussion

We investigated the effects of a 12-week high- vs. low-intensity exercise intervention paradigm on various structural and functional brain changes. Despite adherence to the intervention in both groups (Figure 3C), we found that cardiovascular fitness increased significantly independent of the exercise intensity. Nevertheless, post-hoc tests revealed that this effect was driven by significant increases in the high-intensity group. While we did not find differential effects of exercise intensity on changes in hippocampal volume, vasculature, or metabolite measures, we found a significant decrease in the right hippocampal volume, an increase in left hippocampus GSH levels, and a decrease in left hippocampal CBV across conditions over time. However, these specific changes were not associated with individual changes in cardiorespiratory fitness. Instead, BDNF and IGF, as well as dACC NAA levels (as a control region), were positively associated with cardiorespiratory fitness changes.

Hippocampal Volume and Its Relation to Exercise

We found decreased right hippocampal volume, particularly in the high-intensity exercise condition. This effect was not found to be driven by changes in specific hippocampal subfields as previously suggested [Nuninga et al., 2019]. Although most studies, particularly in older adults, have reported increases in hippocampal volume following exercise [Biedermann et al., 2016; Erickson et al., 2011; Thomas et al., 2016], Wagner et al. [2015] also demonstrated that young, healthy participants (age 21-28) who failed to benefit from an exercise program showed decreases in hippocampal volume.

These findings suggest that changes in hippocampal volume are highly variable between individuals, especially in young adults [Lupien et al., 2007]. Indeed, a meta-analysis concluded that exercise does not stimulate the hippocampal growth in young participants but instead prevents its volume decline as it occurs with increasing age [Firth et al., 2017]. Additionally, many studies that found hippocampal volume increases through exercise interventions in younger adults were conducted in patient populations instead of healthy individuals [van der Kleij et al., 2018; Krogh et al., 2014; Liu et al., 2019; McKercher et al., 2009; Szulc-Lerch et al., 2018].

Hence, exercise effects on hippocampal volume may be dependent on age and disease [Wilckens et al., 2021], and therefore exercise could be regarded as a restorative, rather than stimulatory intervention.

Neuro-Metabolites, BDNF, and IGF and Their Relation to Exercise

To further understand the potential underpinnings of the volume reductions of the right hippocampus, we investigated neuro-metabolite concentrations and peripheral BDNF and IGF concentrations as markers of neuronal remodeling. Utilizing the potential of ultra-high field MRI, we resolved numerous (low-concentration) neuro-metabolites, such as Glu, Gln, and GSH additionally to NAA. This was important as previous studies in young adults reported both increases [den Ouden et al., 2018] and decreases [Wagner et al., 2015] in Glx (Glu + Gln) and NAA after aerobic exercise.

We found increases in GSH levels in the left hippocampus over time, independent of exercise intensity, which were mainly driven by an increase in the low-intensity condition. GSH is known to be responsible for the survival and function of neural cells and for sustaining dendrite integrity and cognitive function [Dringen, 2000]. Reducing GSH levels in hippocampal neurons of mice resulted, e.g., in dendritic disruption, glial activation in CA1, and cognitive impairment [Fernandez-Fernandez et al., 2018]. GSH levels have previously been shown to increase in rats after long-term exercise [Somani et al., 1995], but not in the hippocampus; therefore, this finding was somewhat surprising, particularly given the absence of findings in other metabolites involved in neuronal integrity in the hippocampus. We can speculate that increases in GSH levels detected here could be indicative of cell proliferation, but replication of these findings is

needed, and associations with fitness changes will need to be confirmed. Furthermore, neuro-metabolite levels in this study were only measured in the left hippocampus, whereas volume decreases were found particularly in the right hippocampus. Previous studies have found metabolite changes due to exercise interventions in both the left [den Ouden et al., 2018] and right hippocampus [Wagner et al., 2015], and therefore future studies should consider acquiring bilateral hippocampal MRS data.

Although no associations between fitness and hippocampal NAA were found, increased fitness was associated with increased NAA levels in the dACC (the control region). NAA is a well-accepted marker of neuronal viability, and exercise-induced increases in NAA could potentially reflect improvements in neuronal health. In the rodent hippocampus, NAA changes have been linked to neurogenesis [Czéh and Lucassen, 2007; Fuchs et al., 2004; Park et al., 2014; Zhu et al., 2017], a form of structural hippocampal plasticity that was recently re-confirmed to take place also in the human brain [Boldrini et al., 2018; Kempermann et al., 2018; Lucassen et al., 2020; Moreno-Jiménez et al., 2019] and is thought to underlie associations between exercise and hippocampal volume [Czéh and Lucassen, 2007; Déry et al., 2013; Erickson et al., 2011; Marlatt et al., 2012; Nuninga et al., 2019; Vivar and van Praag, 2017]. In our exploratory analyses, we found a novel positive association between NAA in the dACC and fitness. A previous study [den Ouden et al., 2018] has found changes in NAA in the hippocampus that we could not replicate. This exploratory finding thus requires further replication, in order to investigate whether exercise induced alterations in neurometabolites are region-specific or global.

We further found individual BDNF and IGF changes to be associated with individual fitness changes. While BDNF has been suggested as a primary candidate, IGF also plays a vital role in stimulating neurogenesis in the hippocampus [Fabel et al., 2003; Voss et al., 2011]. Most rodent studies determined hippocampal levels of BDNF and IGF, whereas we determined neurotrophic factors in peripheral blood, which may not directly reflect changes in the hippocampus or its subregions. Nevertheless, BDNF and IGF associations with fitness indicate that individual changes in cardiorespiratory fitness were indeed associated with markers of neuronal health.

In summary, we found exercise-independent increases of GSH, possibly indicating a gain in cell proliferation over time. Additionally, dACC NAA levels, as well as BDNF and IGF levels, were positively associated with fitness, hinting toward a relationship of cardiovascular fitness and neuronal remodeling in young, healthy adults.

Changes in Vasculature and VEGF and Its Relation to Exercise

Also vascularization was investigated as a potential underlying mechanism of exercise-induced changes in hippocampal volume. Changes in vascularization can be estimated using CBF, CBV, as well as the peripheral neurotrophic factor VEGF.

One of the first studies on exercise-induced changes in vascularization suggested strong, positive effects of exercise on hippocampal angiogenesis, estimated with CBV, in both rodents and older adults [Pereira et al., 2006]. They argued that oxygen and neurotrophic factors can reach the brain more efficiently through formations of new blood vessels and could, therefore, positively affect cardio-pulmonary and cognitive functions. Subsequent studies have not yet replicated beneficial exercise-induced changes in blood volume but found a positive association of fitness changes and changes in hippocampal vasculature (CBF and CBV) in a population of older adults [Maass et al., 2015]. In contrast, Thomas et al. found no effects of exercise on CBV [2016] and we found a decrease in left hippocampal CBV over time in young adults which is suggestive of no or negative effects of exercise on hippocampal vasculature.

Furthermore, even though non-significantly, increases in individual GM CBF were associated with improved individual fitness, indicating global, whole-brain changes rather than specific fitness effects on hippocampal CBF, but these results need replication. CBF is a potential marker for neuronal activity, as blood supply needs to be guaranteed during higher energy demands [Venkat et al., 2016]. These findings are in line with several other studies that found widespread beneficial effects of exercise on CBF [Chapman et al., 2013; Dougherty et al., 2019], and no effects on CBF in the hippocampus [Chapman et al., 2016].

In line with the absence of exercise effects on hippocampal vasculature, we also did not find any exercise-induced changes in peripheral VEGF levels. VEGF is thought to play a pivotal role in the formation of new blood vessels. Its peripheral levels were not changed in our study, even though we used collection in PECT tubes, which should provide an accurate estimation of in-vivo circulating VEGF levels [Niers et al., 2011a].

In sum, independent of fitness, we found CBV in the left hippocampus to decrease over time, as a potential marker for angiogenesis. Interestingly, even though non-significant, we found indications that increased fitness is positively associated with global increases in CBF.

Important Factors in Exercise Research

Contrary to our hypothesis and to previous studies, mainly performed in middle-aged or older adults [Erickson et al., 2011; Maass et al., 2016], our high-intensity aerobic exercise intervention did not improve cardiorespiratory fitness significantly more than the low intensity stretching and toning intervention. Although related exceptions have been reported [Coen et al., 2011], this was an unexpected finding, as low-intensity training was previously found not to influence VO2max [Maass et al., 2016], and a meta-analysis stressed the importance of high-intensity training (HR > 80% of the maximum HR) for improving fitness in younger adults (18–45 years old) [Bacon et al., 2013]. Moreover, the two groups strictly adhered to the instructed intensities of the respective interventions,

as is further evidenced by the significantly higher percentage of maximum HR attained during the training sessions in the high-intensity condition (Figure 3C).

These findings, therefore, may suggest that engaging in stretching and toning activities might have caused sufficiently large changes in activity and/or lifestyle to increase fitness in young, otherwise non-athletic volunteers. Indeed, previous studies have argued that also low intensity exercise such as yoga and pilates might be beneficial for cardiovascular fitness in young individuals [Fernández-Rodríguez et al., 2019; Sreehari et al., 2013], even though sample sizes were small and effect sizes were medium. This would partially explain why we did not observe an interaction effect between the low and high-intensity conditions (despite the fact that only the high-intensity group showed a significant increase in VO2max). Nevertheless, our findings suggest that perhaps in some individuals, even minimal time of elevated HR might be enough to increase cardiorespiratory fitness, which is an important finding for those starting to exercise again, or in rehabilitation medicine. However, this suggestion needs to be interpreted with caution and requires further investigation, as time spent in these high HR regimes did not explain changes in VO2max. As an alternative explanation, it has been suggested that some forms of exercise (regardless of their intensity) are more "mindful" than others, presuming possible synergistic effects of physical and cognitive activity on brain structure and function; however, this remains to be confirmed in future studies [Diamond and Ling, 2016; Hillman et al., 2019].

On the other hand, however, other studies have found that moderately intense exercise benefits neuronal health most [Coelho et al., 2013; Knaepen et al., 2010; Kramer and Erickson, 2007]. Therefore, speculatively, our high-intensity intervention may have been too intense, which might have evoked an accompanying stress reaction, which in turn could have deteriorated, rather than improved, some of the measures studied here [Clark and Mach, 2016; Droste et al., 2003; Naylor et al., 2005]. Although we did not measure stress hormone levels, we found no association between cardiorespiratory fitness or volume measures and subjective stress scores (measured with the Depression Anxiety Stress Scale DASS-S), indicating that this explanation is less likely.

Taken together, our findings highlight the importance of a passive control condition that refrains from exercise entirely in future studies; in addition to an active control condition (as implemented in this study), and different levels of exercise intensities (e.g., to control for possible environmental "enrichment" effects, including changes in social interactions, and individual motivation), to investigate if lifestyle changes or exercise training change brain health in young, healthy adults. In our study, we did not directly supervise the exercise sessions, but chose a more naturalistic approach, in which partic-

ipants could choose from a predefined list of classes at the sports center. These classes were supervised by a certified fitness instructor. This approach has the advantage that it resembles better how exercise would be implemented in everyday life and introduces less stress and pressure. Nevertheless, it also meant that we had less control over the classes that participants chose to follow, which might explain some of the variance in the cardiovascular fitness measures in this study.

Notably, previous studies have been inconsistent in their operationalization of (maximal) cardiorespiratory fitness (e.g., VO2plateau, VO2peak, VO2vat), thereby making it challenging to compare results [Astorino et al., 2017; Bullock et al., 2017; Déry et al., 2013; Erickson et al., 2012; Gonzales et al., 2013; Maass et al., 2015; den Ouden et al., 2018; Thomas et al., 2016; Wagner et al., 2015]. Therefore, we warrant it essential to harmonize the analyses and detailed reporting of such outcome measures.

It has further been suggested that individuals differ in the extent to which they are susceptible to fitness-based interventions, which may subsequently also influence the relationship between exercise and brain-related changes [Déry et al., 2013; Silva-Batista et al., 2020; Wagner et al., 2015; Weatherwax et al., 2016]. Upon confirmation in more extensive studies (e.g., the IGNITE study [Erickson et al., 2019]), it would become essential to develop individualized exercise programs to confer neurobiological benefits [Firth et al., 2017]. So far, the most prominent advantages of exercise for the brain seem to apply mainly to middle-aged or older adults and diseased populations, and less so to younger, healthy adults, due to possible ceiling effects and an already optimal neuronal health. Nevertheless, it is important to mention that we studied a relatively homogenous group (comparable BMI, age, fitness), which might have made ceiling effects even more likely. Overall, our results point toward the hypothesis that exercise benefits on the human brain are restorative rather than stimulatory.

Interestingly, evidence is accumulating that exercise effects might be transient and change relatively rapidly in young adults. For instance, Van Der Borght et al. [2009] found that the vasculature in rodents changed rapidly 3 days after exercise training but also declined again after 24h of inactive behavior. Thomas et al. [2016] even observed temporary changes in young healthy adults, at least for the anterior hippocampus. Specifically, they found a temporary effect of exercise intensity on the volume and myelination of the anterior hippocampus. As these changes were temporary, consistency and regularity of training seem to be essential factors, which could have influenced our measures. In our study, we intentionally controlled for rapid exercise effects on perfusion by guaranteeing a 24h gap between the last exercise session and MRI measurements, as we were interested in the prolonged effects of exercise intensity.

Conclusion

In sum, we found that cardiorespiratory fitness improved independent of exercise intensity in these young, non-athletic volunteers, but observed no differential effects of exercise intensity over time for hippocampal volume, vasculature, or neuro-metabolite measures. We found a fitness-independent decrease in the right hippocampal volume, an increase in GSH, and a decrease in CBV in the left hippocampus over time. In exploratory analyses, changes in BDNF and IGF levels, as well as dACC NAA levels, were found to be associated with individual cardiorespiratory fitness changes, indicating a beneficial effect of exercise on neuronal health on an individual level, independent of the exercise intervention intensity.

All in all, the benefits of physical activity are likely not attributable to a single mechanism but probably involve multiple biological changes within the body and brain that could differ across individuals. In our study in a young population, exploratory analyses suggest that cardiovascular fitness shows positive associations with CBF and markers of neuronal viability, arguing that exercise does not seem to benefit the hippocampus specifically.

Our findings highlight the utility of a multimodal approach in assessing exercise induced neural integrity. Work of this kind will help to bridge the gap between animal and human studies by studying neuronal changes that occur on the macroscopic and microscopic level, as well as understand the role of exercise intensities to use physical activity as a potential future treatment for various disorders in humans.

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Supplementary Materials

CERT ✓ Consensus on Exercise Reporting Template

A Checklist for what to include when reporting exercise programs

Section/Topic	Item #	Checklist item	Location **	
			Primary paper (page, table, appendix)	† Other (paper or protocol, website (URL)
WHAT: materials	1	Detailed description of the type of exercise equipment (e.g. weights, exercise equipment such as machines, treadmill, bicycle ergometer etc)	Suppl. Mat. 1.5	*1)
WHO: provider	2	Detailed description of the qualifications, teaching/supervising expertise, and/or training undertaken by the exercise instructor	Main text p.9	*2)
HOW: delivery	3	Describe whether exercises are performed individually or in a group	Main text p.9	*2)
	4	Describe whether exercises are supervised or unsupervised and how they are delivered	Main text p.9	*2)
	5	Detailed description of how adherence to exercise is measured and reported	Main text p.9	*3)
	6	Detailed description of motivation strategies	Main text p.9/10	*4)
	7a	Detailed description of the decision rule(s) for determining exercise progression	Suppl. Mat. 1.5	*3)
	7b	Detailed description of how the exercise program was progressed	Suppl. Mat. 1.5	*3)
	8	Detailed description of each exercise to enable replication (e.g. photographs, illustrations , video etc)	Suppl. Mat. 1.5	*1)
	9	Detailed description of any home program component (e.g. other exercises, stretching etc)	Suppl. Mat. 1.5	*5)
	10	Describe whether there are any non-exercise components (e.g. education, cognitive behavioural therapy, massage etc)	Suppl. Mat. 1.5	*5)
	11	Describe the type and number of adverse events that occurred during exercise	Suppl. Mat. 1.5	*6)
WHERE: location	12	Describe the setting in which the exercises are performed	Main text p.9	*1) & *7)
WHEN, HOW MUCH: dosage	13	Detailed description of the exercise intervention including, but not limited to, number of exercise repetitions/sets/sessions, session duration, intervention/program duration etc	Main text p.9 & Suppl. Met. 1.5	*1) & *7)
TAILORING: what, how	14a	Describe whether the exercises are generic (one size fits all) or tailored whether tailored to the individual	Main text p.9	*1) & *2)
	14b	Detailed description of how exercises are tailored to the individual	NA	
	15	Describe the decision rule for determining the starting level at which people commence an exercise program (such as beginner, intermediate, advanced etc)	NA	
HOW WELL: planned, actual	16a	Describe how adherence or fidelity to the exercise intervention is assessed/measured	Main text p.9 & Suppl. Met. 1.5	*3) & *4)
	16b	Describe the extent to which the intervention was delivered as planned	Main text p.17,18	*7)

***It is recommended that this checklist is used in conjunction with the Explanation and Elaboration Statement which is a guide each item in the CERT Checklist**

The CERT Checklist is designed for reporting details of an exercise intervention. The CERT Checklist should be used in conjunction with a reporting checklist appropriate for the study type e.g. the CONSORT Statement (www.consort-statement.org) for randomised controlled trials, the SPIRIT Statement (www.spirit-statement.org) for a clinical trial protocol. For further guidance regarding reporting guidelines please consult the EQUATOR network (www.equator-network.org)

** Authors – please use N/A if an item is not applicable Reviewers – please use "?" if information is not provided or not/insufficiently reported

† If the information is not provided in the primary paper that is under consideration, please provide details of where this information is available e.g. in a published protocol, published papers (provide citation details) or on a website (provide the URL).

*__1)__ Recommended exercise group classes per exercise intensity group (translated from Dutch):

__*2)__ In collaboration with the sports scientists of the University Sports Center (USC), we provided a list of generic exercise group classes offered at the USC which were supervised by an experienced fitness instructor, that participants were allowed to choose from (Supplementary Materials 1.5; *1)).

***3)** Their presence and active engagement were monitored by tracking their sports center visits using an automated fingerprint entrance system and by using weekly questionnaires on exercise duration and activities. Additionally, participants received a HR monitor (Polar, Finland) to measure HR during each training session (Table 1).

***4)** For motivation purposes, participants were contacted regularly to check in on their progress and one experimenter joined them at least once during the intervention period to train with them (more detail in Supplementary Material 1.5).

***5)** No home-program or non-exercise components were added to the intervention.

***6)** No adverse events occurred during the exercise intervention in this study.

***7)** All participants were enrolled in an exercise program for 12 weeks, in which they were instructed to exercise three times a week for 45 minutes [Astorino et al., 2017; Haskell et al., 2007] at the university sports center (USC).

***8)** Hours spent exercising demonstrated high compliance with the exercise program in both exercise groups (Table 1). As expected, participants in the high-intensity condition spent significantly more time exercising in the intended higher HR regime than the low-intensity condition, which did not explain the change in VO2max ($t(40)=1.34$, $p=0.19$; Figure 3C). The low- and high-intensity exercise groups did not show a significant change from pre to post-intervention on the total score or scores for walking and intermediate-intensity activities as measured with the IPAQ questionnaire. However, a significant condition x time effect was found on vigorous-intensity activities ($\chi^2(1)=5.46, p=0.02$), indicating an increase in the high-intensity group but not in the low-intensity group (Supplementary Results 2.3). Nevertheless, contrary to our expectations, we found no condition x time effect on VO2max; instead, we found decisive evidence (BF>100) for an effect of time ($\chi^2(1)=15.43, p<0.001$; low-intensity: 4.7%, high-intensity: 12.65% change) (Figure 3A). Nevertheless, post-hoc tests revealed only a significant increase in the high-intensity condition (low: $t(49)=1.72$, $p=0.09$; high: $t(49)=4.20$, $p<0.01$). In line with the results on VO2max, we found no interaction effect, but decisive evidence (BF>100) for a main effect of time ($\chi^2(1)=38.92, p<0.001$) on the maximal resistance attained. Post-hoc tests revealed a significant increase in both conditions ($t(23)=4.67, p<0.01$; $t(24)=7.02, p<0.01$; Figure 3B). No effects on HR during the VO2max test were found (Supplementary Table 2).

Low-Intensity Group:

- BBB - The class consists of a short warm-up followed by various exercises that focus on the belly, buttocks, and legs.
- Body power* - This is a group workout to music using simple and effective exercises with barbells and dumbbells. It is focused on the muscular endurance of the whole body.
- Essentrics - At Essentrics you get an effective toning workout to music using dynamic stretches and fluid movements without using gear. The main goals are a slimmer silhouette, more flexibility, and better posture.
- Pilates - In this class the focus is on: posture and control, flexibility, breathing, and awareness. In Pilates, you do floor exercises that target all postural muscles in the body, especially the abdominal and back muscles. You do the exercises slowly, fluently and in collaboration with your breath. You concentrate on doing the exercises carefully and accurately, and not on the number of repetitions. The result is better posture and flexibility.
- TrippleShape barre workout - Ballet Barre workout is a combination of ballet, pilates, and yoga to contemporary music.
- Yoga basic - This is a basic yoga class. The focus lies on: stretching and holding postures.

- Kinesis - Kinesis Training takes traditional exercises (like chest press, lat pull, row) and combines them with functional movements (like reaching, squatting, bending). It develops balance, core/overall strength, and flexibility for people of all fitness levels.
- Abs - 15 minutes of abs training using own-body weight.
- Basic movement - In this class good technique of the most basic exercises using own-body weight is being practiced. This lesson focuses on questions such as: How do I perform the squat correctly? What is a pull-up and how do I do it? What variations are there for a deadlift?
- Mobility class - This lesson focuses on increasing flexibility in muscles and joints.
- Calisthenics - This is a class to practice exercises like a handstand, muscle up, human flag, front and back lever.
- Core*- This lesson focuses on posture and strengthening the abdominal and back muscles in a controlled manner. The training does not only consist of floor exercises but is also offered in challenging circuit forms.
- W.A.C.* - Weightlifting Aerobic Circuit: this workout is originally based on Olympic weightlifting, a valuable full-body workout.

* For Core, W.A.C., and body power, participants were only allowed to do a maximum of 1 of these 3 per week for this sports program.

High-Intensity Group:

- Combat - In this class you train your entire body during circuit training. It includes techniques from (kick) boxing, self-defense techniques, strength, and conditioning to increase your heart rate.
- Fitness training - This is circuit training in which all available materials are used. Walking and jumping are used a lot in this training, the intensity is largely determined by the participants themselves. A high-intensity training involving both strength and condition.
- Fit Fight - This is a powerful cardio workout inspired by Eastern martial arts such as karate, boxing, taekwondo, and Muay Thai to music.
- Kick & shape - This is an intensive class in which participants learn various punching and kicking techniques against the punching bag to music.
- Spinning - In this class participants sit on a spinning bike, on which, under the guidance of the teacher, they go on a 'bike tour' in which different speeds alternate. The resistance is chosen by the participants themselves.
- Step & dance - Step & dance is a choreographically challenging, advanced step class,

with the goal of increasing aerobic fitness.

- Total body workout - This class is a short piece of aerobics hi/low impact after a warm-up. This is followed by exercises for all muscle groups: back, arms, abdomen, shoulders, chest, and legs. This class improves the participant's overall fitness, both cardio and muscle endurance are trained.
- Synrgy - Synrgy is a large device, with rods, ropes, bells, and whistles. Participants can follow group training sessions of 30 minutes several times a day, in which they do high-intensity interval training in circuit form.
- H.I.I.T. - High-Intensity Interval Training consists of short periods of intense effort, followed by short recovery moments. Participants train their general condition but also strength and speed, using simple materials or body weight.
- CrossFit - This small group training is based on the principles of a fitness concept from the USA: functionality, variation, and high intensity. Participants train speed, (muscle) endurance, strength, flexibility, and coordination.

The TIDieR (Template for Intervention Description and Replication) Checklist*:

Information to include when describing an intervention and the location of the information

Item number	Item	Where located **	
		Primary paper (page or appendix number)	Other † (details)
1.	**BRIEF NAME** Provide the name or a phrase that describes the intervention.	p. 8	
2.	**WHY** Describe any rationale, theory, or goal of the elements essential to the intervention.	p. 3-5	
3.	**WHAT** Materials: Describe any physical or informational materials used in the intervention, including those provided to participants or used in intervention delivery or in training of intervention providers. Provide information on where the materials can be accessed (e.g. online appendix, URL).	p. 7-16	Supplements 1-1.5
4.	Procedures: Describe each of the procedures, activities, and/or processes used in the intervention, including any enabling or support activities.	p. 7-16	Supplements 1-1.5
5.	**WHO PROVIDED** For each category of intervention provider (e.g. psychologist, nursing assistant), describe their expertise, background and any specific training given.	p. 9 + 10	Supplements 1.5
6.	**HOW** Describe the modes of delivery (e.g. face-to-face or by some other mechanism, such as internet or telephone) of the intervention and whether it was provided individually or in a group.	p. 9 + 10	Supplements 1.5
7.	**WHERE** Describe the type(s) of location(s) where the intervention occurred, including any necessary infrastructure or relevant features.	p. 9	
8.	**WHEN and HOW MUCH** Describe the number of times the intervention was delivered and over what period of time including the number of sessions, their schedule, and their duration, intensity or dose.	p. 9 + 10	Supplements 1.5
9.	**TAILORING** If the intervention was planned to be personalised, titrated or adapted, then describe what, why, when, and how.	Not applicable	
10.‡	**MODIFICATIONS** If the intervention was modified during the course of the study, describe the changes (what, why, when, and how).	Not applicable	
11.	**HOW WELL** Planned: If intervention adherence or fidelity was assessed, describe how and by whom, and if any strategies were used to maintain or improve fidelity, describe them.	p. 9 + 10	Supplements 1.5
12.‡	Actual: If intervention adherence or fidelity was assessed, describe the extent to which the intervention was delivered as planned.	p. 17 + 18	Supplements 2.3

** **Authors** - use N/A if an item is not applicable for the intervention being described. **Reviewers** – use '?' if information about the element is not reported/not sufficiently reported.

† If the information is not provided in the primary paper, give details of where this information is available. This may include locations such as a published protocol or other published papers (provide citation details) or a website (provide the URL).

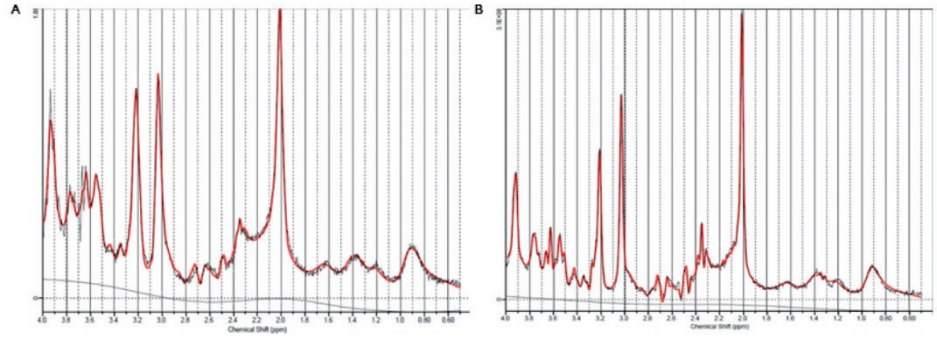
‡ If completing the TIDieR checklist for a protocol, these items are not relevant to the protocol and cannot be described until the study is complete.

* We strongly recommend using this checklist in conjunction with the TIDieR guide (see *BMJ* 2014;348:g1687) which contains an explanation and elaboration for each item.

* The focus of TIDieR is on reporting details of the intervention elements (and where relevant, comparison elements) of a study. Other elements and methodological features of studies are covered by other reporting statements and checklists and have not been duplicated as part of the TIDieR checklist. When a **randomised trial** is being reported, the TIDieR checklist should be used in conjunction with the CONSORT statement (see www.consort-statement.org) as an extension of **Item 5 of the CONSORT 2010 Statement**. When a **clinical trial protocol** is being reported, the TIDieR checklist should be used in conjunction with the SPIRIT statement as an extension of **Item 11 of the SPIRIT 2013 Statement** (see www.spirit-statement.org). For alternate study designs, TIDieR can be used in conjunction with the appropriate checklist for that study design (see www.equator-network.org).

Supplementary Methods

1H-MRS



Supplementary Figure 1. Representative spectra for one participant, measured in the A) hippocampus and B) dorsal anterior cingulate cortex (dACC).

Spectra were fitted using LCModel with a simulated basis set with a measured macromolecular baseline (using an sLASER dual inversion recovery sequence(Penner and Bartha, 2015)) and the following metabolites: alanine (Ala), aspartate (Asp), creatine (Cr), γ-aminobutyric acid (GABA), Gln, Glu, GSH, glycine (Gly), glycerophosphocholine (GPC), Inositol, Lac, phosphoethanolamine (PE), phosphocholine (PCh), phosphocreatine (PCr), inositol (Ins), choline (Cho), NAA, N-acetyl aspartyl glutamate (NAAG), taurine (Tau), scylloinositol (Sci), succinate (Suc), pyruvate (Pyr) and threonine (Thr).

The following control parameters were used for LCModel analyses:

```
deltat= 2.50e-04
 doecc= F
 dows= T
 hzpppm= 2.9804e+02
 nunfil= 2048
 ppmend= 0.5
 ppmst= 4.0
 DKNTMN= 0.5
 atth2o= 0.7
 attmet= 1.0
 chcomb(17)= 'Lac+Thr'
 chcomb(18)= 'Cho+GPC+PC'
 chomit(1)= 'MM09'
 chomit(2)= 'MM12'
 chomit(3)= 'MM14'
 chomit(4)= 'MM17'
 chomit(5)= 'MM20'
 chomit(6)= 'Ala'
 chomit(7)= 'Pyr'
 chomit(8)= 'Suc'
 ncombi= 18
 neach= 50
 nomit= 8
 wconc= individual values
 CHUSE1(1)='NAA'
 CHUSE1(2)='PCr'
 CHUSE1(3)='PC'
```

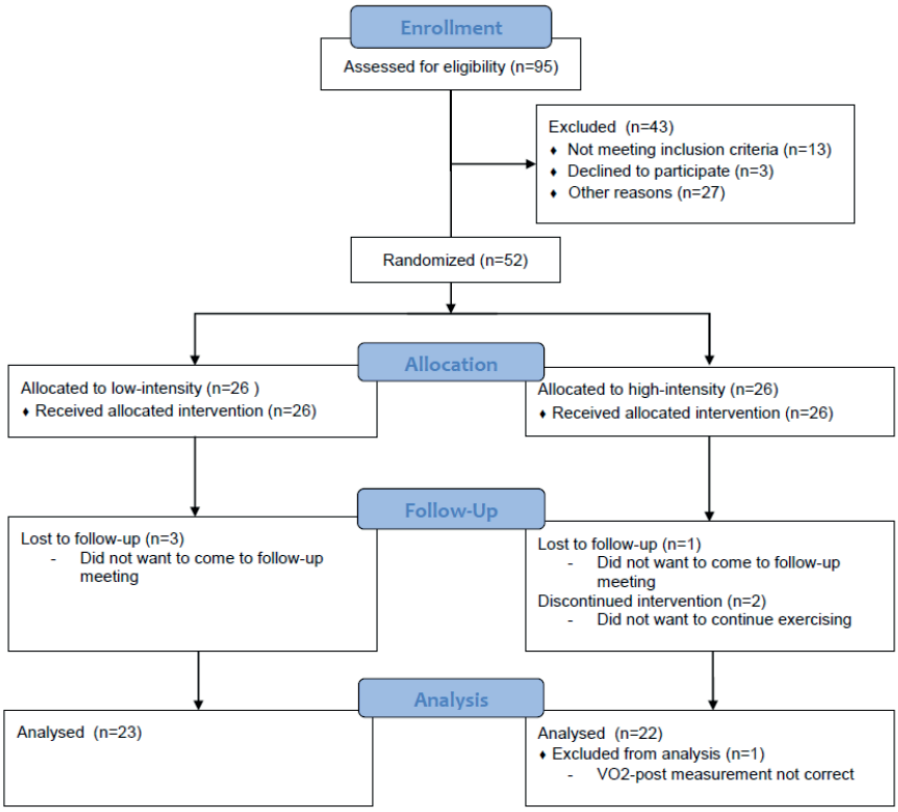
Statistical Analysis

Supplementary Table 1. Evidence Categories for Bayes Factor (BF) [Jeffrey, 1961; Wagenmakers, 2007].

Bayes Factor	Interpretation
> 100	Decisive evidence for H_A
30 - 100	Very strong evidence for H_A
10 - 30	Strong evidence for H_A
3 - 10	Substantial evidence for H_A
1 - 3	Anecdotal evidence for H_A
1	No evidence
1/3 - 1	Anecdotal evidence for H_0
1/10 – 1/3	Substantial evidence for H_0
1/30 – 1/10	Strong evidence for H_0
1/100 – 1/30	Very strong evidence for H_0
< 1/100	Decisive evidence for H_0

Sample Size Calculations

We aimed to investigate the neural mechanisms underlying brain volume changes resulting from exercise interventions. First, we determined the sample size to assess a hypothesized effect on neurometabolite signaling. A previous study investigating exercise effects on anterior cingulate cortex glutamate concentration changes reported an effect size of d=1.48 (Cohen d) in young participants [Maddock et al., 2016]. As this study used an acute exercise intervention, we expected a more moderate effect size for a longitudinal exercise intervention of d=1.0. To detect the effect of exercise, with a power of 80% and an α of 0.05, we would need 12 participants per exercise intervention group. Taking a 20% dropout into account (due to MRI data quality), we would need at least 15 subjects in each exercise intervention group. Secondly, we calculated the required sample size to assess the effect of exercise on vascular outcome measures. A previous study using contrast-enhanced MRI assessed the effect of a dietary intervention with flavanol on CBV in the hippocampus [Brickman et al., 2014]. This study detected an increase in CBV with an effect size of d=0.75. We expect the dietary intervention to have effects of a similar magnitude as the exercise intervention. With an effect size of d=0.75, a power of 80%, and an α of 0.05, we would need 16 subjects to detect a difference before and after the exercise intervention. Taking a 20% dropout into account, we would need at least 20 subjects in each exercise group.



Supplementary Figure 2: CONSORT flow chart

Exercise Intervention

Recommended exercise group classes per exercise intensity group (translated from Dutch):

Low-Intensity Group:

- BBB - The class consists of a short warm-up followed by various exercises that focus on the belly, buttocks, and legs.
- Body power* - This is a group workout to music using simple and effective exercises with barbells and dumbbells. It is focused on the muscular endurance of the whole body.
- Essentrics - At Essentrics you get an effective toning workout to music using dynamic stretches and fluid movements without using gear. The main goals are a slimmer silhouette, more flexibility, and better posture.

- Pilates - In this class the focus is on: posture and control, flexibility, breathing, and awareness. In Pilates, you do floor exercises that target all postural muscles in the body, especially the abdominal and back muscles. You do the exercises slowly, fluently and in collaboration with your breath. You concentrate on doing the exercises carefully and accurately, and not on the number of repetitions. The result is better posture and flexibility.
- TrippleShape barre workout - Ballet Barre workout is a combination of ballet, pilates, and yoga to contemporary music.
- Yoga basic - This is a basic yoga class. The focus lies on: stretching and holding postures.
- Kinesis - Kinesis Training takes traditional exercises (like chest press, lat pull, row) and combines them with functional movements (like reaching, squatting, bending). It develops balance, core/overall strength, and flexibility for people of all fitness levels.
- Abs - 15 minutes of abs training using own-body weight.
- Basic movement - In this class good technique of the most basic exercises using own-body weight is being practiced. This lesson focuses on questions such as: How do I perform the squat correctly? What is a pull-up and how do I do it? What variations are there for a deadlift?
- Mobility class - This lesson focuses on increasing flexibility in muscles and joints.
- Calisthenics - This is a class to practice exercises like a handstand, muscle up, human flag, front and back lever.
- Core*- This lesson focuses on posture and strengthening the abdominal and back muscles in a controlled manner. The training does not only consist of floor exercises but is also offered in challenging circuit forms.
- W.A.C.* - Weightlifting Aerobic Circuit: this workout is originally based on Olympic weightlifting, a valuable full-body workout.

* For Core, W.A.C., and body power, participants were only allowed to do a maximum of 1 of these 3 per week for this sports program.

High-intensity Group:

- Combat - In this class you train your entire body during circuit training. It includes techniques from (kick) boxing, self-defense techniques, strength, and conditioning to increase your heart rate.
- Fitness training - This is circuit training in which all available materials are used. Walking and jumping are used a lot in this training, the intensity is largely determined by the participants themselves. A high-intensity training involving both strength and condition.
- Fit Fight - This is a powerful cardio workout inspired by Eastern martial arts such as karate, boxing, taekwondo, and Muay Thai to music.

- Kick & shape - This is an intensive class in which participants learn various punching and kicking techniques against the punching bag to music.
- Spinning - In this class participants sit on a spinning bike, on which, under the guidance of the teacher, they go on a 'bike tour' in which different speeds alternate. The resistance is chosen by the participants themselves.
- Step & dance - Step & dance is a choreographically challenging, advanced step class, with the goal of increasing aerobic fitness.
- Total body workout - This class is a short piece of aerobics hi/low impact after a warm-up. This is followed by exercises for all muscle groups: back, arms, abdomen, shoulders, chest, and legs. This class improves the participant's overall fitness, both cardio and muscle endurance are trained.
- Synrgy - Synrgy is a large device, with rods, ropes, bells, and whistles. Participants can follow group training sessions of 30 minutes several times a day, in which they do high-intensity interval training in circuit form.
- H.I.I.T. - High-Intensity Interval Training consists of short periods of intense effort, followed by short recovery moments. Participants train their general condition but also strength and speed, using simple materials or body weight.
- Crossfit - This small group training is based on the principles of a fitness concept from the USA: functionality, variation, and high intensity. Participants train speed, (muscle) endurance, strength, flexibility, and coordination.

Details about the exercise intervention:

Exercise progression was monitored by tracking their sports center visits using an automated fingerprint entrance system and by using weekly questionnaires on exercise duration and activities. Additionally, participants received an HR monitor (Polar, Finland) to measure HR during each training session. In case the regularity of participants' training sessions was decreasing, participants were contacted and motivated to continue exercising by one of the experimenters. No home-program or non-exercise components were added to the intervention. No adverse events occurred during the exercise intervention in this study.

Supplementary Results

Quality control spectra

Quality metric	**Left Hippocampus** **BL** *Mean ± std*	**Post** *Mean ± std*	**dACC** **BL** *Mean ± std*	**Post** *Mean ± std*
SNR	30.76 ± 6.05	28.85 ± 7.03	46.64 ± 2.96	47.41 ± 2.79
FWHM	14.41 ± 2.26	15.68 ± 3.71	7.55 ± 0.71	7.55 ± 0.72
Glu CRLB	3.17 ± 0.66	3.49 ± 0.76	2.0 ± 0	2.02 ± 0.15
Gln CRLB	29.41 ± 6.19	27.91 ± 6.69	19.97 ± 6.04	20.72 ± 5.98
GSH CRLB	7.48 ± 1.91	7.13 ± 1.49	7.67 ± 0.81	7.74 ± 0.83
NAA CRLB	1.02 ± 0.15	1.05 ± 0.22	1.0 ± 0	1.0 ± 0

Exploratory Analysis:

Supplementary Table 2 | Statistical tests for all variables: Linear mixed-effects models were used to investigate the condition (high- vs. low-intensity exercise) x time (pre- vs. post-intervention) x sex (female vs. male) interaction in Rv.3.5.3 using the lme4 package. Multiple comparison corrections using Sidak's resulted in an α^*=0.02. Model selection was based on an adjusted top-down procedure, in which the resulting models were compared using the Bayesian information criterion (BIC); the model was consequently tested using chi-square (χ^2) tests.

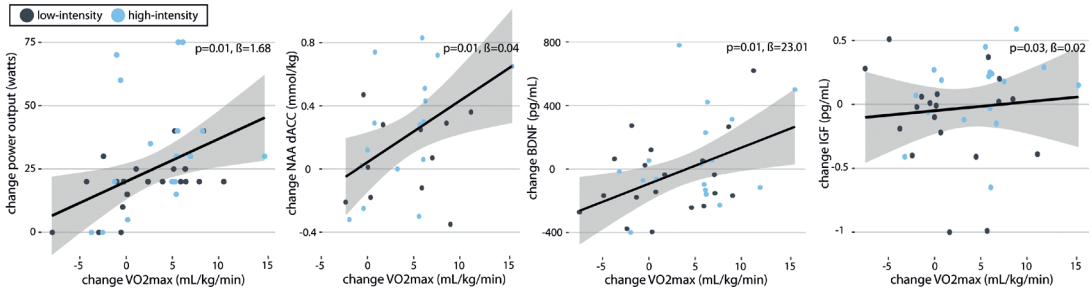
Variables	*Interaction effects* *Group * Time*	*Main effects* *Time*	*Sex*	*Tukey Post-Hoc test*
Fitness measures				
VO2max	$\chi^2(5)$=4.51, p=0.48, ΔBIC=-18.42	$\chi^2(1)$=15.43, **p<0.001**, ΔBIC=11.15	$\chi^2(1)$=21.26, **p<0.001**, ΔBIC=17.32	Female<Male; pre<post
Bike-resistance	$\chi^2(5)$=11.84, p=0.04, ΔBIC=-11.09	$\chi^2(1)$=38.92, **p<0.001**, ΔBIC=34.34	$\chi^2(1)$=36.23, **p<0.001**, ΔBIC=31.64	Female<Male; pre<post
HR during VO2max	$\chi^2(7)$=1.35, p=0.25, ΔBIC=-3.21	$\chi^2(1)$=4.03, p=0.04, ΔBIC=-0.53	$\chi^2(1)$<0.01, p=0.97, ΔBIC=-4.56	-
Volume measures				
Left Hippocampus				
Whole	$\chi^2(6)$=5.70, p=0.45, ΔBIC=-21.54	$\chi^2(1)$=2.68, p=0.10, ΔBIC=-1.82	$\chi^2(1)$=24.97, **p<0.001**, ΔBIC=21.54	Female<Male
Dentate Gyrus	$\chi^2(6)$=4.61, p=0.59, ΔBIC=-22.39	$\chi^2(1)$=2.14, p=0.14, ΔBIC=-2.35	$\chi^2(1)$=18.23, **p<0.001**, ΔBIC=14.27	Female<Male
CA1	$\chi^2(6)$=2.68, p=0.85, ΔBIC=-24.20	$\chi^2(1)$= 0.08, p=0.77, ΔBIC=-4.40	$\chi^2(1)$= 23.46, **p<0.001**, ΔBIC=19.00	Female<Male
CA3	$\chi^2(6)$=2.08, p=0.91, ΔBIC=-9.92	$\chi^2(1)$= 0.95, p=0.33, ΔBIC=-1.05	$\chi^2(1)$= 15.75, **p<0.001**, ΔBIC=11.26	Female<Male
WM	$\chi^2(5)$=7.55, p=0.18, ΔBIC=-14.83	$\chi^2(1)$= 5.18, **p=0.02**, ΔBIC=0.69	$\chi^2(1)$= 6.31, **p=0.01**, ΔBIC=1.83	Male<Female; Post<pre
GM	$\chi^2(6)$=2.83, p=0.83, ΔBIC=-24.17	$\chi^2(1)$= 0.04, p=0.83, ΔBIC=-4.46	$\chi^2(1)$= 31.79, **p<0.001**, ΔBIC=27.28	Female<Male
Right Hippocampus				
Whole	$\chi^2(5)$=5.00, p=0.43, ΔBIC=-18.39	$\chi^2(1)$=8.47, **p<0.01**, ΔBIC=4.03	$\chi^2(1)$= 22.72, **p<0.001**, ΔBIC=18.22	Female<Male; Post<pre
Dentate Gyrus	$\chi^2(6)$=10.59, p=0.10, ΔBIC=-16.41	$\chi^2(1)$= 4.08, p=0.14, ΔBIC=-2.08	$\chi^2(1)$= 13.04, **p<0.001**, ΔBIC=8.54	Female<Male
CA1	$\chi^2(6)$=7.45, p=0.28, ΔBIC=-5.40	$\chi^2(1)$= 0.84, p=0.36, ΔBIC=-4.30	$\chi^2(1)$= 23.46, **p<0.001**, ΔBIC=20.60	Female<Male
CA3	$\chi^2(6)$=2.08, p=0.91, ΔBIC=-24.85	$\chi^2(1)$= 0.95, p=0.33, ΔBIC=-3.66	$\chi^2(1)$= 15.75, **p<0.001**, ΔBIC=11.26	Female<Male
WM	$\chi^2(6)$=10.22, p=0.12, ΔBIC=-6.72	$\chi^2(1)$= 2.89, p=0.08, ΔBIC=-0.4	$\chi^2(1)$= 5.34, **p=0.02**, ΔBIC=0.96	Male<Female
GM	$\chi^2(6)$=3.74, p=0.71, ΔBIC=-23.26	$\chi^2(1)$= 0.50, p=0.48, ΔBIC=-4.00	$\chi^2(1)$= 27.06, **p<0.001**, ΔBIC=23.44	Female<Male
dACC				
	$\chi^2(6)$=7.03, p=0.95, ΔBIC=-20.10	$\chi^2(1)$= 0.005, p=0.95, ΔBIC=-5.5	$\chi^2(1)$= 5.4, **p=0.02**, ΔBIC=1.1	Female<Male
1H-MRS				
Hippocampus				
GLU	$\chi^2(7)$=4.22, p=0.74, ΔBIC=-26.44	$\chi^2(1)$=0.82, p=0.36, ΔBIC=-3.55	$\chi^2(1)$=0.84, p=0.36, ΔBIC=-3.53	-
GLN	$\chi^2(6)$=4.60, p=0.60, ΔBIC=-21.54	$\chi^2(1)$=1.92, p=0.17, ΔBIC=-3.56	$\chi^2(1)$=4.57, *p=0.03*, ΔBIC=0.21	*Female<Male*
NAA	$\chi^2(7)$=6.37, p=0.50, ΔBIC=-24.13	$\chi^2(1)$=2.71, p=0.10, ΔBIC=-1.65	$\chi^2(1)$=0.32, p=0.57, ΔBIC=-4.04	-
GSH	$\chi^2(6)$=6.37, p=0.46, ΔBIC=-21.46	$\chi^2(1)$=9.21, **p<0.01**, ΔBIC=4.83	$\chi^2(1)$=2.72, p=0.09, ΔBIC=-2.65	Pre<post
dACC				
GLU	$\chi^2(6)$=10.48, p=0.11, ΔBIC=-16.32	$\chi^2(1)$=1.85, p=0.17, ΔBIC=-2.61	$\chi^2(1)$=8.87, **p<0.01**, ΔBIC=4.41	Male<Female
GLN	$\chi^2(7)$=7.59, p=0.37, ΔBIC=-23.67	$\chi^2(1)$=0.01, p=0.91, ΔBIC=-5.05	$\chi^2(1)$=2.43, p=0.12, ΔBIC=-2.03	-
NAA	$\chi^2(6)$=5.52, p=0.48, ΔBIC=-22.72	$\chi^2(1)$=3.77, p=0.05, ΔBIC=-1.3	$\chi^2(1)$=6.05, **p=0.01**, ΔBIC=5.95	Male<Female
GSH	$\chi^2(7)$=5.68, p=0.58, ΔBIC=-34.41	$\chi^2(1)$=0.05, p=0.83, ΔBIC=-3.69	$\chi^2(1)$=2.27, p=0.13, ΔBIC=-2.81	-
Vasculature				
CBF				
Left Hippocampus	$\chi^2(6)$=5.59, p=0.47, ΔBIC=-21.13	$\chi^2(1)$=0.19, p=0.66, ΔBIC=-4.33	$\chi^2(1)$=0.74, p=0.39, ΔBIC=-4.32	-
Right Hippocampus	$\chi^2(7)$=5.65, p=0.58, ΔBIC=-25.36	$\chi^2(1)$=0.95, p=0.33, ΔBIC=-3.48	$\chi^2(1)$<0.01, p=0.94, ΔBIC=-4.42	-
GM	$\chi^2(7)$=13.60, p=0.06, ΔBIC=-18.67	$\chi^2(1)$=2.84, p=0.09, ΔBIC=-2.42	$\chi^2(1)$=0.85, p=0.36, ΔBIC=-4.43	-
CBV				
Left Hippocampus	$\chi^2(6)$=4.34, p=0.63, ΔBIC=-22.24	$\chi^2(1)$=5.97**, p=0.01**, ΔBIC=2.05	$\chi^2(1)$=15.21, **p<0.01**, ΔBIC=10.78	Male<Female
Right Hippocampus	$\chi^2(6)$=6.86, p=0.33, ΔBIC=-20.21	$\chi^2(1)$=3.57, p=0.06, ΔBIC=-1.12	$\chi^2(1)$=6.45, **p=0.01**, ΔBIC=2.00	Male<Female
GM	$\chi^2(7)$=8.23, p=0.31, ΔBIC=-22.70	$\chi^2(1)$=0.98, p=0.32, ΔBIC=-3.43	$\chi^2(1)$=1.71, p=0.19, ΔBIC=-3.31	-
Myelination (R1)				
WM left Hippocampus	$\chi^2(7)$=8.90, p=0.26, ΔBIC=-23.1	$\chi^2(1)$=0.20, p=0.65, ΔBIC=-5.6	$\chi^2(1)$=0.32, p=0.57, ΔBIC=-5.7	-
WM right Hippocampus	$\chi^2(7)$=12.76, p=0.08, ΔBIC=-21.0	$\chi^2(1)$=0.42, p=0.52, ΔBIC=-4.1	$\chi^2(1)$=0.63, p=0.43, ΔBIC=-4.1	-
Neurotrophic factors				
BDNF	$\chi^2(7)$=11.06, p=0.14, ΔBIC=-20.20	$\chi^2(1)$=0.95, p=0.33, ΔBIC=-4.50	$\chi^2(1)$=0.05, p=0.82, ΔBIC=-4.40	-
VEGF	$\chi^2(7)$=8.10, p=0.32, ΔBIC=-23.32	$\chi^2(1)$=0.40, p=0.53, ΔBIC=-4.09	$\chi^2(1)$=0.83, p=0.36, ΔBIC=-4.34	-
IGF	$\chi^2(7)$=4.49, p=0.72, ΔBIC=-17.06	$\chi^2(1)$=0.01, p=0.94, ΔBIC=-5.52	$\chi^2(1)$=1.59, p=0.21, ΔBIC=-3.10	-

Supplementary Table 3 | Exploratory analyses testing the association of all variables with VO2max, and hippocampal or dACC volume change were conducted using linear models in R, including the baseline measure of the explanatory variables, VO2max, and sex as covariates (α=0.05).

Variables	*Regression with VO2max*
SPORT	
VO2max	-
Bike-resistance	F(1,38)=7.46, p=0.01, β=1.68
Volume measures	
Left Hippocampus	
Whole	F(1,41)=0.51, p=0.48, β=1.74
Dentate Gyrus	F(1,42)=1.81, p=0.19, β=0.38
CA1	F(1,40)=0.82, p=0.37, β=-0.95
CA3	F(1,41)=0.88, p=0.36, β=0.17
WM	F(1,39)=0.06, p=0.80, β=-0.43
GM	F(1,41)=0.27, p=0.61, β=-1.06
Right Hippocampus	
Whole	F(1,40)=0.51, p=0.48, β=2.18
Dentate Gyrus	F(1,42)=1.80, p=0.19, β=1.76
CA1	F(1,40)=0.08, p=0.78, β=0.33
CA3	F(1,41)=0.88, p=0.36, β=-0.20
WM	F(1,40)=0.56, p=0.46, β=0.46
GM	F(1,39)=0.13, p=0.72, β=-3.47
dACC	F(1,40)=0.30, p=0.59, β=3.22
1H-MRS	
Hippocampus	
GLU	F(1,26)<0.01, p=0.95, β=-0.002
GLN	F(1,27)=0.38, p=0.55, β=0.02
NAA	F(1,25)=0.07, p=0.80, β=-0.006
GSH	F(1,25)=0.99, p=0.33, β=0.007
dACC	
GLU	F(1,25)=0.08, p=0.78, β=0.006
GLN	F(1,26)<0.01, p=0.98, β=0.001
NAA	F(1,27)=7.14, **p=0.01, β=0.04**
GSH	F(1,26)=0.26, p=0.61, β=0.002
Vasculature	
CBF	
Left Hippocampus	F(1,27)=1.08, p=0.31, β=-0.002
Right Hippocampus	F(1,27)<0.01, p=0.96, β=0.001
GM	F(1,28)=3.06, *p=0.06*, β=0.35
CBV	
Left Hippocampus	F(1,27)=0.63, p=0.44, β=-0.01
Right Hippocampus	F(1,27)=1.86, p=0.18, β=-0.02
GM	F(1,27)<0.01, p=0.94, β=0.01
Myelination (R1)	
Left	F(1,37)=0.02, p=0.88, β=0.001
Right	F(1,36)=0.33, p=0.57, β=-0.001
Neurotrophic factors	
BDNF	F(1,36)=6.84, **p=0.01**, β=23.01
VEGF	F(1,36)=0.02, p=0.89, β=-0.04
IGF	F(-1,36)=5.19, **p=0.03**, β=0.02

Supplementary Table 4 | Mean and standard deviation for pre- and post-exercise intervention per variable.

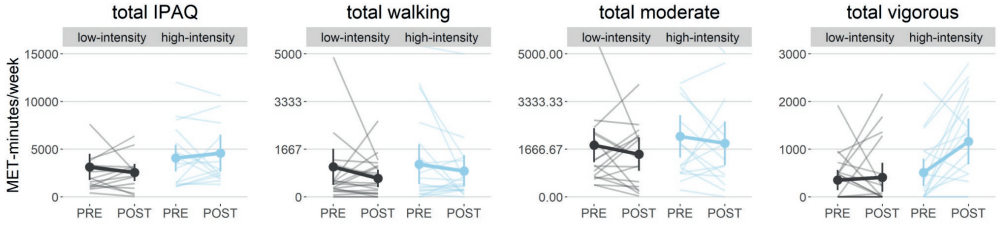
	HIGH-INTENSITY		LOW-INTENSITY	
	PRE	*POST*	*PRE*	*POST*
	Mean ± SD	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*
Participant characteristics:				
Age (years)	23.12 ± 2.52	23.25 ± 2.31	24.19 ± 3.25	24.43 ± 3.14
Body Mass Index (kg/m²)	23.30 ± 2.80	22.96 ± 2.75	23.96 ± 3.24	23.78 ± 3.17
Fitness measures:				
VO2max (kg/mL/min.)	37.21 ± 7.39	41.60 ± 6.69	37.32 ± 6.58	39.39 ± 7.38
Max. Heart Rate (beats/min.)	186.60 ± 8.41	186.01 ± 8.26	189.12 ± 7.53	187.48 ± 7.31
Resistance pre (watts)	240.33 ± 61.94	273.54 ± 67.56	244.62 ± 45.54	262.17 ± 45.45
Volume measures:				
Left hippocampus	3025.33 ± 428.42	3024.76 ± 432.52	3082.54 ± 350.55	3076.88 ± 346.14
Right hippocampus	3128.94 ± 484.38	3123.99 ± 461.99	3095.15 ± 334.10	3063.12 ± 335.44
dACC	3984.78 ± 832.14	4053.26 ± 848.16	4241.57 ± 682.49	4254.65 ± 875.18
Vasculature				
CBF				
Left Hippocampus	42.24 ± 7.85	43.81 ± 5.42	47.03 ± 9.40	44.88 ± 7.75
Right Hippocampus	41.08 ± 7.89	42.73 ± 6.07	45.28 ± 9.15	42.90 ± 8.17
GM	46.41 ± 7.29	46.61 ± 6.41	48.44 ± 6.43	46.33 ± 7.02
CBV				
Left Hippocampus	2.19 ± 0.96	2.15 ± 0.85	2.11 ± 1.08	1.74 ± 0.59
Right Hippocampus	2.13 ± 0.78	1.93 ± 0.75	1.90 ± 0.77	1.82 ± 0.56
GM	2.44 ± 0.57	2.86 ± 0.84	2.49 ± 0.43	2.59 ± 0.64
Myelination (R1)				
Left	8.49E-04 ± 2.90E-05	8.49E-04 ± 2.90E-05	8.51E-04 ± 3.97E-05	8.41E-04 ± 2.28E-05
Right	8.47E-04 ± 3.75E-05	8.47E-04 ± 3.75E-05	8.60E-04 ± 4.85E-05	8.49E-04 ± 3.00E-05
1H-MRS				
Hippocampus				
GLU	7.80 ± 0.77	7.80 ± 0.65	7.60 ± 0.51	7.75 ± 0.54
GLN	1.17 ± 0.56	1.31 ± 0.67	1.06 ± 0.39	1.28 ± 0.59
NAA	1.79 ± 0.38	1.92 ± 0.30	1.71 ± 0.31	1.97 ± 0.64
GSH	10.23 ± 0.99	10.20 ± 0.48	9.95 ± 0.55	10.12 ± 0.31
dACC				
GLU	11.67 ± 0.91	11.75 ± 0.93	11.89 ± 0.66	11.98 ± 0.60
GLN	1.56 ± 0.51	1.56 ± 0.48	1.53 ± 0.34	1.46 ± 0.40
NAA	0.92 ± 0.07	0.92 ± 0.10	0.90 ± 0.31	0.91 ± 0.63
GSH	10.88 ± 0.76	10.93 ± 0.69	10.87 ± 0.64	11.10 ± 0.08



Supplementary Figure 2 | Exploratory analysis – associations with VO2max: Significant associations of the change in power output during the VO2max test, NAA concentration in the dACC, blood BDNF levels, and blood IGF levels with change in VO2max.

Physical activity besides the intervention:

Minutes spent on physical activity did not change for either low- or high-intensity condition from pre to post (low: t(37)=0.26, p=0.79; high: t(36)=-0.12, p=0.91). When splitting these hours up into different categories we also did not find any changes from pre to post intervention in minutes spent walking (low: t(43)=0.85, p=0.40; high: t(45)=1.43, p=0.16); minutes spent on- intermediate activities (low: t(40)=0.80, p=0.43; high: t(41)=0.77, p=0.45). Minutes spent on vigorous activities significantly changed only in the high-intensity group (low: t(43)=-0.24, p=0.81; high: t(47)=3.46, p<0.01).



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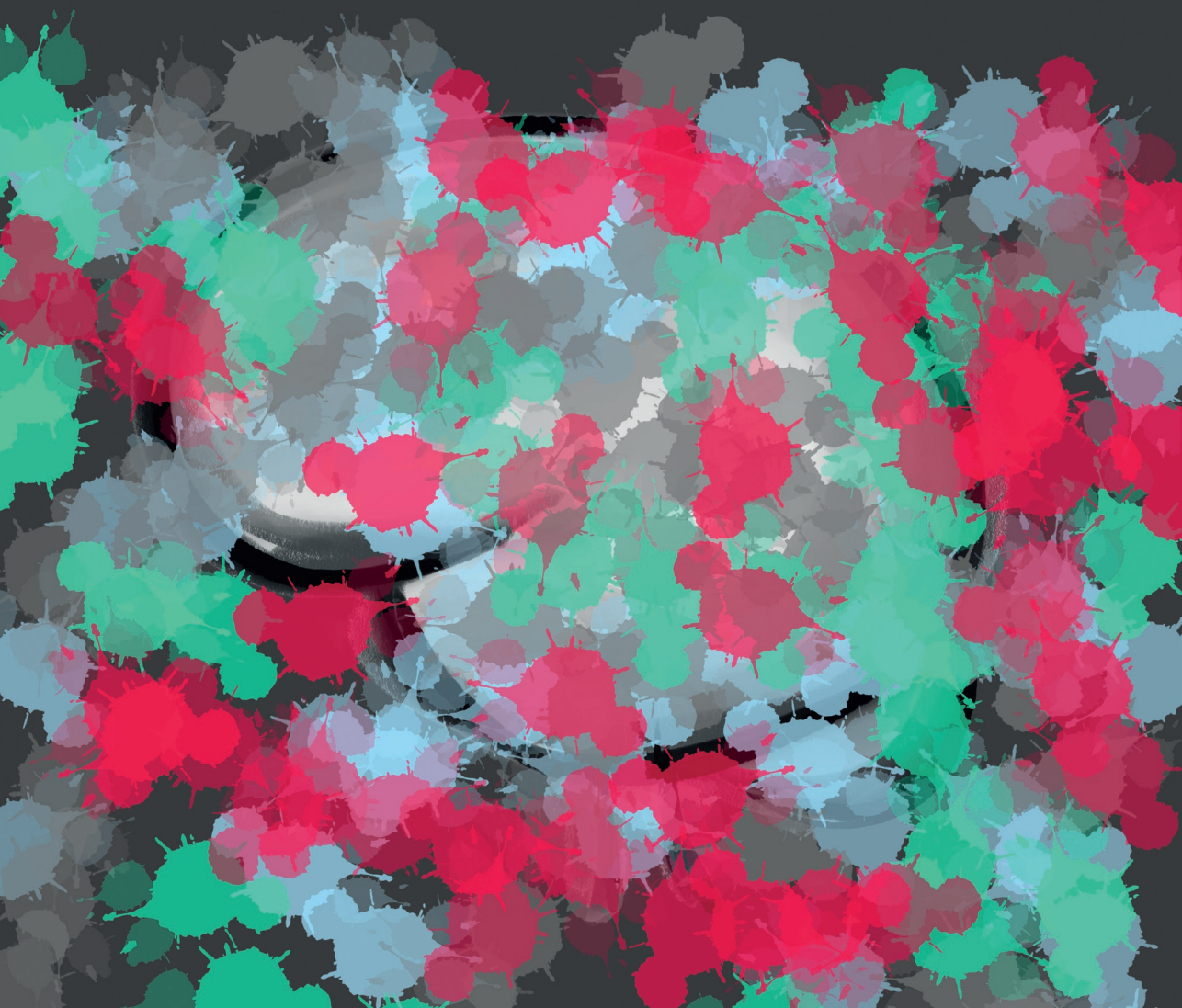
Chapter 6

General Discussion



General Discussion

In this thesis, the results of comprehensive MRI studies are reported that investigated the influence of different types of interventions on behavioral, cognitive and neurobiological readouts. Firstly, we studied cognitive task-based interventions as well as medication effects on brain functions and symptom clusters in individuals with ADHD. Secondly, we investigated the effects of a low- and high-intensity exercise intervention on various brain measures in young and healthy, but otherwise non-athletic adults.

In the following chapter, our findings in relation to current literature, and support for the specific choices we made in our studies will be discussed, and in addition recent methodological developments and possible future opportunities for this field of research will be highlighted.

Part I: cognitive and pharmacological interventions in ADHD

Emotional Dysregulation in ADHD

Inattention and hyperactivity are considered core symptoms of ADHD. In addition, individuals often exhibit impairments in emotion processing that are distinct from those caused by any comorbid conditions [Lenzi et al., 2018]. Both externalizing symptoms (e.g., aggression or conduct problems), and internalizing symptoms (e.g., anxiety and depression), have been linked to aberrant emotional processing in ADHD [Gillberg et al., 2004; Jarrett and Ollendick, 2008; Sciberras et al., 2014]. Interestingly, a recent meta-analysis indicated that most ADHD medication might have a limited efficacy for emotional dysregulation (ED) problems, especially in adults [Lenzi et al., 2018], resulting in therapeutic challenges.

Adults and children with ADHD often display multi-facetted symptoms of ED, including impulsivity, impatience, and emotional instability [Biedermann et al., 2012; Sobanski et al., 2010; Surman et al., 2013]. Because of the heterogeneity of the symptoms connected to it, recent studies on ED in children have argued that multimodal interventions (e.g. behavioral therapy or cognitive-behavioral-therapy (CBT) in combination with parental training) should be considered in order to achieve an effective reduction of symptoms, like irritability and symptoms of depression [Vacher et al., 2020]. In ***chapter 2 and 3,*** we studied emotional regulation issues in participants with ADHD, focusing on internalizing symptoms, i.e. depressive and anxiety symptoms, and their neural underpinnings using fMRI paradigms, as internalizing symptoms were previously found to relate to amygdala hyperreactivity [Posner et al., 2011b]. The emotion dysregulation spectrum is wide and therefore our assessment may have missed some aspects of emotional problems [Posner et al., 2011b]. Consequently, it would be of great value to have instruments available that can measure a more heterogeneous set of symptoms in

individuals, and e.g. distinguish emotional impulsivity and deficient emotional self-regulation from irritability [Faraone et al., 2019a].

Top-down vs. Bottom-up theory of ED

The "affectivity hypothesis" proposes that emotional lability in ADHD may emerge from a direct route of dysfunctional emotional processing itself [Posner et al., 2014]. Accordingly, previous studies in children with ADHD have proposed a hyperreactivity of the amygdala to negative emotional stimuli, as a potential underlying neural mechanism of emotion dysregulation [Brotman et al., 2010; Posner et al., 2011a; Posner et al., 2011b; Quinlan et al., 2017]. In contrast, in ***chapter 2 and 3,*** we did not find any differences in amygdala reactivity to emotional stimuli with our paradigms, when comparing participants with and without ADHD. This discrepancy with earlier studies may on one hand be due to interindividual differences in ED and on the other hand a result of a complex interplay between different brain regions. As mentioned before, ED in ADHD consists of a multi-faceted interplay of dimensions, including emotion recognition and emotional lability. Indeed, recent research has shown that not all individuals with ADHD experience ED in its full complexity and several subtypes of ED may even exist [Christiansen et al., 2019a].

Interestingly, a previous study has suggested that ADHD-related deficits in the prefrontal cortex may be responsible for a deficient integration of information by regions responsible for perception and emotion recognition, such as the amygdala [Winston et al., 2003]. We found some evidence supporting this hypothesis, as we report small, yet specific effects within cortico-limbic circuits during the emotional recognition task in our exploratory whole-brain connectivity analyses of children treated with MPH (***chapter 3)***. In line with this, we found in changes in top-down control processes rather than in the subcortex itself. These results are supported by the "dyscontrol hypothesis", which postulates that externalizing symptoms in ADHD are not emerging from direct dysfunctional emotional processing itself, but rather from an executive dysfunction, affecting top-down processes, such as the capacity to suppress responses that are evoked by emotional stimuli [Posner et al., 2014].

Age-related compensatory effects in ED

Compared to earlier studies in children with ADHD, research in adults has identified fewer regional group differences between individuals with ADHD and controls [Cortese et al., 2012]. One of the possible explanations for this are age-related compensatory effects, which may engage parietal, occipital, and subcortical systems (i.e., cerebellum and basal ganglia) to overcome possible impairments that may have started in early development [Cortese et al., 2012; Frazier et al., 2007]. Indeed, in both ***chapter 2 and 3,*** we failed to find any changes in amygdala reactivity to the emotional stimuli in adult ADHD participants.

Nevertheless, especially adults with ADHD report large functional impairments as a result of ED symptoms [Retz et al., 2012], which is in line with an earlier notion that emotional reactivity, a prominent aspect of ED in participants with ADHD, is influenced by many factors, including age, and is notably much more complex than originally thought [Graziano and Garcia, 2016].

It is therefore crucial to create future paradigms that can untangle the impacts of cognitive processes on ED in adult ADHD, especially since the characteristics of ED in adult ADHD are especially complex and multidimensional [Beheshti et al., 2020]. We may therefore speculate that the functional symptoms of ED presented in adults with ADHD may not be represented in just one particular brain region, but are more widespread across the whole brain.

Measuring mechanisms of ED with MRI

In order to investigate emotion processing, we made use of two different fMRI paradigms; in ***chapter 2,*** we chose to use a novel paradigm interleaving working memory (WM) blocks (n-back), with emotional and neutral picture blocks, (International Affective Picture System (IAPS)), whereas in ***chapter 3*** we used an emotional-face matching paradigm. Surprisingly, and in contrast to earlier findings [Brotman et al., 2010; Posner et al., 2011a; Posner et al., 2011b; Quinlan et al., 2017], we did not find amygdala hyper-reactivity to emotionally negative (non-scene) stimuli from the IAPS in the participants compared to without ADHD. Conversely, in ***chapter 3*** we chose a well-established emotional face-matching paradigm [Hariri et al., 2002], because the human amygdala had been suggested to exhibit a stronger BOLD fMRI response to fearful and threatening facial expressions, compared to non-face IAPS stimuli.

Additionally, because of its robustness, this task does not require the acquisition of many averages. Nevertheless, also with that task, we did not find any hyperreactivity of the amygdala. Interestingly, the only two other studies that investigated emotional reactivity in adult ADHD using IAPS images, also failed to find differences between participants with ADHD and TDCs [Hägele et al., 2016; Tajima-Pozo et al., 2018]. Consequently, functional impairments of the amygdala, and therefore also the influence of challenges or interventions thereon, may be a heterogeneous phenomenon, which seems to be highly dependent on the fMRI task used. The field should therefore reach a consensus on the (fMRI) tasks used to research well-defined cognitive and/or behavioral, in this case ED related, concepts.

MPH for ED

Previous studies indicated a lower efficacy of ADHD medications/stimulants for ED problems [Lenzi et al., 2018], whereas a small number of studies showed that stimulants could reduce part of the symptoms, in particular *internalizing* emotional symptoms

[Biederman et al., 2009; Coughlin et al., 2015]. Amygdala hyperreactivity and -connectivity in adolescents with ADHD (aged 11-16 years) was previously found to increase significantly after MPH discontinuation [Posner et al., 2011b]. However, the exact neural mechanisms underlying these changes remained unclear, particularly those related to long-term stimulant treatment. In ***chapter 3*** (as a secondary analysis of the ePOD trial), we therefore investigated how MPH treatment may modulate neural mechanisms that can underlie emotion regulation in ADHD.

In our study, long-term MPH treatment did not influence either internalizing symptoms, or the brain substrates underpinning emotional processing. Nevertheless, we showed that long-term MPH did reduce *ADHD* symptoms most effectively in those adults with the highest depression and anxiety symptoms at trial onset. This indicates that internalizing symptoms may possibly be used to predict MPH treatment effects on ADHD symptoms, particularly in (male) adults with ADHD. Based on ***chapter 2 and 3*** we could speculate that adults with ADHD that present with high levels of internalizing symptoms, might benefit most from both cognitive and pharmacological interventions. However, we did not find direct evidence for a beneficial effect on the *neural* correlates of emotion regulation of either intervention, nor did we identify differences between ADHD and TDC groups.

Importantly, preclinical studies had shown before that a prolonged treatment with MPH during adolescence induced anxiety and depressive-like behavior [Bolaños et al., 2003] and increased impulsivity later during adulthood [Somkuwar et al., 2016]. The most comprehensive study on long-term effects of ADHD medication to date, i.e. the multi-modal treatment study of ADHD (MTA), found that children treated with ADHD medication had higher rates of anxiety and depression (19.1%) than children receiving behavioral therapy only (4.3%), as measured 6 years after treatment onset. However, this effect had disappeared when studied after 8 years [Molina et al., 2009]. Interestingly, in ***chapter 3,*** we did not observe any increase in depressive nor anxiety symptoms in the MPH condition during the 16 weeks in this well-controlled trial. This is in line with another long-term (3-year) study, that found a reduced risk for developing depressive symptoms in association with previous medication [Chang et al., 2016]. It seems that the effect of MPH on internalizing symptoms is likely patient-specific. Also, for instance, Coughlin et al. [2015] have argued that the positive impact of MPH on anxiety symptoms outweighs the risk of psychostimulant use inducing anxiety in children with ADHD. In fact, we demonstrated that MPH improved ADHD symptoms the most in adults with the highest depressive and anxiety symptoms before treatment start.

Alternative therapy options in ADHD

Although MPH is usually recommended as the first pharmacological option for the treatment of adult ADHD, clinical trials focusing on the efficacy of MPH have shown a

wide variability of results, ranging from no effects at all to large improvements [Buitelaar et al., 2022; Castells et al., 2011]. It is therefore important to; 1) investigate where those heterogeneities come from, and preferably try to find biomarkers that could help predict and/or stratify patient responses and hence treatment allocation, and 2) find alternative therapies that might provide less side-effects and/or higher efficacy.

Also in other mental disorders, for example in anxiety or substance use disorders, emotional hyperreactivity occurs [Hofmann et al., 2012; Spence and Courbasson, 2012]. This was found to decrease when WM was activated during or before the exposure to emotionally relevant stimuli [Andrade et al., 2012; van den Hout et al., 2014; Kaag et al., 2018; Markus et al., 2016; May et al., 2010; McClelland et al., 2006]. WM training had also been demonstrated to improve emotional reactivity outcomes in both healthy subjects and other psychiatric disorders, but not yet in ADHD [Barkus, 2020; Schweizer et al., 2013]. In ***chapter 2***, we therefore investigated whether taxing of WM processes would have an influence on the neural mechanisms of emotional reactivity in adults with ADHD, with the potential to use WM training as a (add-on) treatment for individuals with ADHD. Interestingly, and in contrast to previous studies, we did not find any differences in amygdala reactivity to negative stimuli, and only weak evidence for a hypo-activation of WM related regions [Burgess et al., 2010; Cortese et al., 2012; Ko et al., 2013], when comparing adults with ADHD and typically developing controls (TDC). Probably as a consequence, we also did not find the WM task-load to influence the amygdala reactivity to emotional stimuli in either group.

As another alternative, non-pharmacological interventions such as exercise have been proposed [Christiansen et al., 2019b]. In **chapter 5**, we investigated the effects of a low- vs. high-intensity physical exercise training on several neuronal measure in young, healthy TDC participants. All in all, the benefits of physical activity were found to be likely not attributable to a single mechanism but they probably involve multiple biological changes within the body and brain that differ considerably across individuals. In our study, notably performed in a young population, exploratory analyses suggested that cardiovascular fitness was positively associated with whole-brain CBF and markers of neuronal viability, arguing that exercise does not benefit the hippocampus specifically, but rather involves several areas of the brain. As such, exercise training, might be beneficial for many brain disorders and mental health problems and deserves further study in the future. Such studies should consider multi-modal whole-brain approaches to not miss out on the potential wide-spread effects. Moreover, these more widespread beneficial effects of exercise could possibly be used to develop specific exercise regimens as a therapy for mental disorders. Indeed, aside from several beneficial peripheral effects of moderate exercise in large cohorts [del Pozo Cruz et al., 2022], specific studies have already shown beneficial effects for e.g. depression [Daley, 2008], anxiety [Aylett et al., 2018] and also ADHD in children and adolescence [Christiansen et

al., 2019b]. Nevertheless, large-scale clinical trials involving children as well as adults with ADHD, preferably medication-naive, are needed to investigate the exact effects of physical exercise on the ADHD brain.

Additionally, other training options have been proposed [Faraone et al., 2019b], including Parent Behavior Management Training, cognitive-behavioral therapies, mindfulness and meditation training, neurofeedback or Transcranial magnetic stimulation, but this is beyond the scope of this thesis and will not be discussed in detail.

Brain Networks in ADHD

Over the past years, ADHD is increasingly viewed as a disorder of brain-wide network dysconnectivity, as opposed to more region-specific deficits [Castellanos and Proal, 2012; Samea et al., 2019]. MPH is known to inhibit the reuptake of dopamine and noradrenaline in the brain [Cortese et al., 2017], and because the dopamine system undergoes considerable changes throughout development [Chen et al., 2010], we hypothesized that age might also modify the impact of MPH on functional connectivity. In ***chapter 4***, we therefore went on to study age-dependent effects of an *acute* dose of MPH on resting-state connectivity in dopamine sensitive regions. In ***chapter 4***, the same participants as described in ***chapter 3*** were measured before and after an *acute* dose of MPH, before they were randomized to one of the intervention groups. Additionally, MR scans of TDCs were obtained once without a medication dose. We used topological metrics, which not only enabled us to evaluate individual nodes or global connections, but also the relevance and integration within the global network before and after MPH administration.

Indeed, in accordance with our hypothesis, we observed opposite effects of acute MPH treatment on connectivity strength, and the relative importance of subcortical (dopamine-sensitive) regions in children vs. adults. These changes might potentially be attributable to maturational changes in the dopamine and noradrenergic systems [Chen et al., 2010]. Contrary to our expectations, MPH-induced modifications in the connectivity of the frontal brain regions were minimal. In these regions, no differences were identified between age groups. However, in the PFC, across groups, the global importance (i.e., the value as a hub) specifically increased within the network from before to after MPH. This might indicate a more important role for the frontal regions regarding information flow in the brain, after the intake of MPH [Farahani et al., 2019; Wang, 2010].

MPH has been hypothesized to cause changes in functional connectivity across multiple brain networks involved in ADHD [Pereira-Sanchez et al., 2021]. As an example, acute methylphenidate treatment has been shown to restore normal connections in fronto-parietal-cerebellar circuits in children with ADHD. The first study to notice this was An et al., [2013] who showed that a single dose of methylphenidate, compared to placebo, upregulated an abnormally decreased local connectivity in the bilateral ventral prefrontal cortices and the cerebellar vermis, while downregulating abnormally

increased local connectivity in the right parietal and visual areas in children with ADHD [An et al., 2013]. Similar results were seen in teenagers with ADHD when a single dose of methylphenidate was compared to placebo; they discovered that functional connectivity in occipital, temporal, and cerebellar regions as well as in visual, executive, and DMN, were normalized [Silk et al., 2017].

In recent years, it has been established that prolonged MPH treatment in medication-naive children with ADHD causes changes in fronto-parietal-cerebellar circuits [Yoo et al., 2018]. Finally, there is some evidence that this normalization may also occur in adults with ADHD [Cary et al., 2017; Picon et al., 2020]. We did not find normalizing effects of acute MPH in any of the measures in either age group, using topological outcome measures. This may suggest that the previously observed normalization towards a "control state" [Pereira-Sanchez et al., 2021] may exist only at the level of local connectivity, whereas MPH may cause a reorganization of function at the level of the global network. This suggests that the efficacy of stimulant therapy may not be based on normalization only, but rather depend on combinations of factors that return the network organization to the typical topology for some systems, while reorganizing others. In other words, it might be that altered networks in the brain do not need to return to the control state to function in the desired way. Additionally, if structural networks are changed in ADHD, the functional networks might possibly not be able to return to a control-like state. Future studies combining structural, anatomical connectivity with functional connectivity measures would aid in investigating this hypothesis.

As ADHD has been suggested to be more of a disorder of network connectivity dysfunction, it will be important to investigate the underlying neural mechanisms in a multi-modal, broad approach. This might facilitate the development of new interventions or trainings to help children and adults with ADHD struggling with internalizing as well as externalizing problems. According to a recent meta-analysis using rs-fMRI studies, the field of ADHD brain networks is still in its infancy. This is probably due to the majority of study results being based on small sample sizes, and in many areas, replication has not been achieved [Cortese et al., 2021]. While some results appear to be fairly consistent in replicated theory-driven studies (e.g., abnormalities within the DMN and in its connectivity) [Sutcubasi et al., 2020], data-driven approaches must be expanded in order to gain unbiased, comprehensive insights into the brain networks underlying ADHD. While we begin to comprehend the genetic underpinnings of these networks and the effects of treatment [Yoo et al., 2018], larger-scale investigations are required.

Context-dependence of task-based experiments

Research employing well-controlled but simplified paradigms with basic context-independent inputs has established some key aspects of brain architecture. In contrast to the static, single-sensory settings that the majority of studies attempt to apply, the

dynamic, multisensory real world demands a variety of top-down (attentional and otherwise) processes for us to function well in our daily lives. Therefore, one might argue that task-based neural reactivity is context-dependent, and hence that experimental set-ups might not be able to capture the full picture [Matusz et al., 2019]. As an example, ED in ADHD populations has been reported mainly on a behavioral level, for example in the social context, or in highly emotionally challenging periods of their life [Courbet et al., 2021; Purper-Ouakil et al., 2004]. Additionally, the neural mechanistic underpinnings are still mostly unknown and highly heterogeneous [Herrmann et al., 2010b]. This might partly be because showing context-independent stimuli in an experimental set-up might not induce the same emotional reaction that would occur when applied in more realistic, 'real-life' situations, or when stimuli would have been used that were adjusted to the specific individuals' life situation.

In ***chapter 5,*** we tried to apply a more naturalistic approach to the longitudinal exercise intervention. Participants were allowed to do the exercises in their own time, using a large list of possible classes they were allowed to do at the university sports center, only defining the frequency and intensity for them. This had a positive influence on adherence and made it more applicable for a comparison to daily life activities, but it may also have increased the heterogeneity within the exercise groups, thereby hampering the interpretation of the results. In fact, both exercise groups increased in fitness measures, even though one group was employed as a control condition, indicating that also mild levels of activity may already induce relevant brain changes in young adult participants. In future studies, the inclusion of a mostly sedentary control group would therefore be recommended to be able to exclude an effect of context completely.

On the other hand, the application of highly reproducible and controllable stimuli is needed in research in order to compare results between studies, but also within studies when it comes to longitudinal designs. In all chapters of this thesis, comparing results with the literature was often difficult, as many earlier studies had been inconsistent in their operationalization of stimuli as well as in outcome measures. Future studies could therefore consider applying more naturalistic, applicable, maybe even individual, stimuli in big sample sizes to generate new hypotheses, which then can be tested in a more controlled and standardized experiment. Especially the IAPS task used in ***chapter 2*** might e.g. be highly context dependent, as full scenes are depicted in it [Lang et al., 2005]. Nevertheless, this dataset was validated thoroughly and was found to clearly induce negative emotions in participants [Lang et al., 2005]. Nevertheless, in ***chapter 2***, we only used negative and neutral stimuli. In contrast, in individuals with ADHD, the processing of positive stimuli has been associated with lower ventral striatal activity [Herrmann et al., 2010a], decreased event-related potentials in EEG, and altered startle response modulation. Consistently, a deficient reward system was postulated as a mechanism behind these observations. Importantly, the deficit was revealed not just for

reward-related tasks but also for the processing of more general positive stimuli.

In summary, not only context makes a difference with respect to the influence of specific stimuli and interventions on the brain. Also, the heterogeneity within the enrolled participant groups plays a big role. In this thesis, we showed 1) age-specific effects of medication on symptom changes, and also on network connectivity in ***chapter 3 and 4***. We 2) showed a different influence of an exercise intervention in young adults (***chapter 5***) than in older adults as was found in previous studies [Firth et al., 2018]. In ***chapter 3 and 4,*** we concluded that medication might have a different influence on children than on adults with ADHD, because the brain is still in development. Maturation of several brain regions is going on until adolescence [Giedd et al., 1999], and medication might have a different influence *during* this much more sensitive phase of life [Xavier Castellanos et al., 2002] than when studied after development has been completed. In other words, neuronal plasticity is much higher during the brain development, than during adulthood. Nevertheless, neuroplasticity also plays a big role during aging, causing e.g. the hippocampus to decline in volume which could underlie cognitive functions to deteriorate as well [Hardcastle et al., 2020], which might explain why we did not find any effects of a physical exercise intervention in healthy, *young* adults in ***chapter 5***. Following this, one could conclude that interventions and task influences on the brain during phases of higher brain plasticity in life (e.g. in childhood and in older adults, or in disease) might be more effective and have bigger influence than in young adulthood, where the neuronal architecture of the brain may be less flexible.

Resting- vs. task-unrelated states

Some recent publications argue that functional connectivity can better be estimated by regressing the task effects out of task-based fMRI experiments. Despite substantial variation in the nature and design of the used tasks, it was observed that dispersed, task-induced modifications in functional connectivity predicted phenotype-independent task activation [Greene et al., 2020]. This might be the result of locking brain activity to a task, which would restrict it and synchronize it among participants [Buckner et al., 2013; Hasson et al., 2004]. Additionally, rs-fMRI paradigms require participants to lie still without a distraction (of e.g., a task) for quite a long time, which might increase the chances of motion. Task-induced resting-state fMRI approaches therefore might have the potential to increase inter-individual similarity and predicted accuracy by reducing inter-individual variability (e.g., head motion) that might influence the results substantially [Finn et al., 2017; Laumann et al., 2017]. These findings also support the widely held idea that diffuse brain circuitries, rather than specific regions of interest, underlie sophisticated complex cognitive functions [Turk-Browne, 2013].

Future studies could therefore use designs where a baseline per individual is acquired, which will then statistically be taken into account to explain the change

after a specific intervention, trigger or stimulus. Additionally, it would be beneficial if studies would not only take into account one measuring point, but several, or rather apply longer scanning sessions, to be able to find the exact moment of change after the intervention. These outcome measures could provide us with a more specific way to detect changes in brain responses in relation to environmental challenges, which could help develop potential biomarkers for mental disorders and may benefit the field by improving therapy response or develop new therapies and trainings.

Part II: Exercise as an intervention

Prolonged moderate to intense levels of physical exercise have been shown to have widespread beneficial effects on the body and mind [del Pozo Cruz et al., 2022]. Furthermore, in older adults, several studies showed positive effects of exercise on cognitive functioning and neuronal health, preventing further decreases in brain volume and cognitive decline [Firth et al., 2017]. Also in younger adults, exercise might have a positive influence on brain volume, but in a more rapid, temporary way, as increases were already found after 6 weeks of physical exercise training, but these returned to baseline levels again 6 weeks after the discontinuation of physical exercise [Thomas et al., 2016].

So far, the role of exercise intensity had received little attention [Lucas et al., 2015; Wilke, 2020], even though a recent meta- analysis stressed the importance of high-intensity training [heart- rate (HR) > 80% of maximum HR] for improving fitness in younger adults [Bacon et al., 2013; Wen et al., 2019]. Therefore, in ***chapter 5,*** we investigated what influence a low- (stretching and toning) vs. a high- intensity (aerobic) exercise intervention of 12 weeks would have on the hippocampal volume of young, healthy, but non-athletic adults. We additionally explored possible underlying neuronal mechanisms with comprehensive MRI methods. Overall, we observed no differential effects of either exercise intensity on hippocampal volume, vasculature, or neuro-metabolite markers, probably because cardiorespiratory fitness increased in both exercise groups.

Although the hippocampus is thought to benefit from exercise in general, exploratory analyses in our young cohort of ***chapter 5*** demonstrated that cardiovascular fitness exhibits beneficial relationships on whole-brain cerebral blood flow and indicators of neuronal survival and integrity, estimated with MR spectroscopy. In fact, the advantages of exercise might not be due to a single mechanism but rather include various biochemical changes inside the body and brain, some of which may be unique to each individual. These results showed that a multimodal approach is useful and maybe even necessary to disentangle the various effects that exercise might have on the (young) brain. It also highlights the value of a non-active, sedentary control group when

comparing exercise effects in young adult participants.

The most prominent advantages of exercise for the brain seem to apply mainly to middle-aged or older adults and diseased populations, and less so to younger, healthy adults, due to possible ceiling effects and an already optimal neuronal health. Nevertheless, it is important to mention that in ***chapter 5*** we studied a relatively homogenous group (comparable BMI, age, fitness), which might have made ceiling effects even more likely. Interestingly, evidence is accumulating that exercise effects might be transient and change relatively rapidly in young adults. For instance, Van Der Borght et al. [2009] found that the vasculature in rodents changed rapidly 3 days after exercise training but also declined again after 24h of inactive behavior. Thomas et al. [2016] even observed temporary changes in young healthy adults, but only in the anterior hippocampal volume and for measures of myelination. As these changes were temporary, a consistent and regular training scheme seems to be essential, which could have influenced our measures as well, as we, in our study, intentionally controlled for rapid exercise effects on perfusion, by guaranteeing a 24h gap between the last exercise session and MRI measurements, which allowed us to study mainly the *prolonged* effects of exercise intensity.

However, there is sufficient evidence to recommend structured exercise training as an effective first-line treatment in patients: e.g. for moderate depression and as an adjunctive intervention for improving symptomatic recovery in severe mental illness, as also adopted in the guidelines of the European Psychiatric Association on physical activity in mental illness [Stubbs et al., 2018]. Clinical practice recommendations for mood disorders [Malhi et al., 2015] from the Royal Australian and New Zealand College of Psychiatrists also identify exercise, smoking, food, and sleep as "step zero" targets, that are to be addressed before implementing medication and/or psychotherapy. However, systematic longitudinal studies with blinded experimenters and appropriate control groups should be implemented to confirm the assumed effects and likely help stratify subgroups who may benefit most.

Interventions as a challenge to help understand brain function

Challenging hemostasis

Testing interventions experimentally can also be done on a more methodologically relevant level. In recent years, a lot of MRI research has gone into the attempt of finding neurobiological markers to distinguish individuals with and without mental disorders, to improve diagnosis, but also for treatment allocation, but so far without any strong results. One theory is that comparing group averages (i.e. healthy control participants vs. individuals with a mental disorder) is not strong enough because of the heterogeneity that individuals with mental disorders display. Furthermore, studies have found that

specific tasks both increase inter-subject functional connectivity similarity and improve individual identifiability on the basis of functional connectivity patterns [Finn et al., 2017]. Moment-to-moment "events" in the BOLD signal have e.g. been found to explain substantial functional connectivity variance, which are highly synchronized across individuals during a task [Zamani Esfahlani et al., 2020].

Additionally, it has been suggested that, especially in neurochemical terms, the brain may not show detectable differences when in homeostasis, but when its function is challenged by a stimulus, the neurochemical *response* might become different. Maintaining homeostasis in the brain is likely to result in unaltered baseline metabolite concentrations; however, the response to a stress test or a complex, highly demanding cognitive task may reveal more specific metabolic anomalies [Duarte and Xin, 2018]. Several longitudinal investigations have failed to identify a correlation between baseline (i.e. pre-challenge) metabolite levels and a longitudinal, later reaction to the challenge [Brennan et al., 2017; Godlewska et al., 2019]. In spite of this, they hypothesized that the magnitude of change in metabolite levels during therapy may impact the degree of symptomatic improvement later on [Goff et al., 2002; de la Fuente-Sandoval et al., 2013]. These results are significant not just for the molecular insights they provide, but also because they reveal that changes in glutamate metabolite concentrations, for example, in the very beginning of the medication therapy may be predictive of longer-term clinical outcomes. Future clinical trials may examine change values as prospective predictors, since it is obvious that early treatment-response markers, such as neurometabolite changes, might guide treatment paradigms. In addition, a design like this would address concerns regarding the standardization of 1H-MRS acquisitions across different research sites, as it would focus on the within-subject variation in 1H-MRS metabolite levels rather than on a single measurement at a specific time point (provided that inter-site repeatability is stable enough) [Egerton, 2021].

Suggestions for future directions

Future studies should explore the influence of challenges on the brain further. For that is would be interesting to explore whether the influence could be increased by making the stimuli more individualized and adjusting them to the context in which, e.g., symptoms might arise in everyday life. For that purpose, it may be important to increase effect sizes in especially adult studies by either increasing sample sizes, by using more powerful tools (as described in more detail below) or by including a more homogeneous sample of participants, e.g. only including medication-naïve participants, or participants with the same symptom clusters. Furthermore, it might be important to pay closer attention to longitudinal statistical designs and models that can help describe the individual changes

per participant and can take into account baseline values, that might have a big influence on how the outcome measures will change over time [Twisk et al., 2018].As described before, MRI studies aiming to find new biomarkers for the improvement or development of new treatments for mental disorders are needed. One essential problem of such studies lies in the heterogeneity of patients with mental disorders, which causes effect sizes to be small. Several ways of dealing with this could be proposed.

Advances in MR measurements

First, the development of more accurate MRI sequences may allow the detection of smaller changes in the brain, that could further this effort. This could e.g. be achieved with the development of better spatial or temporal resolution, depending on the property that needs to be measured. MRI has already improved massively over the past decades and continues to improve until today.

The development and validation of specific MR-sequences, but also of methods for pre- and postprocessing of the data, are needed to improve the signal we can extract, and therefore the information we can obtain. Any imaging technique used to compare local metrics over time and/or across individuals is susceptible to errors and biases introduced by the imaged volume selection (especially in MRS), co-registration among images, and smoothing procedures, and these need to be taken into account [Triana et al., 2020].

Multi-modal approach

Additionally, combining several MRI contrasts might help to establish a more detailed profile of functional, structural, and biochemical alterations, which leads to a more complete picture of the underlying biology responsible for a specific behavioral readout. By combining many MRI contrasts, we may leverage the relative contributions of various disease substrates to selected MRI contrasts and significantly boost the sensitivity to specific substrates. There are possible approaches spanning from simply measuring several MRI parameters (thus e.g. using several sequences for different outcome measures) in the same individuals, to develop joint models, to using complex computational approaches to derive new measures [Cercignani and Bouyagoub, 2018]. Moreover, having this information, additional measures (i.e., cognitive or physiological) can be chosen more judiciously from there.

Unexploited potentials of established MR methods

Nevertheless, some acquisition strategies in MRI still present unexploited potential as stand-alone methods. For example, most studies employing 1H-MRS are using static acquisitions, i.e., several averages acquired at one time-point and consequently averaged, from a single relatively large voxel in the brain. Increasing research efforts are

spent on MR spectroscopic imaging (MRSI), which permits the simultaneous assessment of multiple voxels. Particularly, 1H-MRSI sequences that are currently available, can measure e.g. Glx [Ding et al., 2015; Gasparovic et al., 2011; Steel et al., 2020], glutamate and glutamine [Goryawala et al., 2016; Henning et al., 2009], and GABA [Moser et al., 2019] across substantial portions of the brain. This approach has the ability to map metabolite anomalies associated with, for example, certain diseases or mental disorders, allowing for the analysis of region-specific abnormalities between these groups.

Functional 1H-magnetic resonance spectroscopy (fMRS), which detects changes in metabolite concentration over time, examines how the 1H-MRS metabolite signal varies in response to an external stimulus. For example, multiple experimental studies have shown that glutamate concentration changes measured with fMRS can increase by an average of 7% [Mullins, 2018]. It remains yet unknown how precisely changes in metabolite signal are displayed, in other words what the shape and duration of their change function is, whether or not and to what extend habituation happens, and whether or not homeostatic processes may occur [Apvalka et al., 2015; Jelen et al., 2018]. These questions have large potential and are now being investigated to enhance the possible use of fMRS. The investigation of pharmacological manipulation of metabolite dynamics using fMRS will be an intriguing topic of future research. In line with this, improved recording methods and improved analysis techniques have inspired many groups to examine both non-stationary and stationary aspects of rs-fMRI on much shorter time scales than in the past, i.e., "dynamic rs-fMRI". In their review, Thompson et al. conclude that studies comparing dynamic rs-fMRI to behavior or disease have shown that these techniques can often distinguish changing brain states better than static rs-fMRI, indicating that indeed a more dynamic approach might be a promising avenue in the future [Thompson, 2018].

Potential of field strength

Not only different MR-sequences can be used to our advantage, also different field strengths of the MRI scanner can influence the ultimate measurement [Neuner et al., 2022]. The primary benefits of ultra-high-field (UHF; >7T) MRI include a high (temporal) signal-to-noise ratio (SNR), which results in an increased spatial resolution and contrast and enables the study of single participants individually [Dumoulin et al., 2018; Triantafyllou et al., 2005]. For example, with the availability of UHF MRI scanners for human research studies, it becomes increasingly possible to resolve lower concentration metabolites, such as GABA or glutathione, and to separate glutamate and glutamine resonances under optimal acquisition sequences [Godlewska et al., 2017].

The primary clinical benefit of using a higher field strength, namely 7T MR imaging, is problem solving when the resolution or signal of lower field strength magnets is not sufficient [Sydnor and Roalf, 2020]. Even though MR imaging at 7T provides greater signal-to-noise ratio, smaller voxel size, and/or faster scan times compared to lower field

strength scans, it comes at the expense of increased costs and artifacts in certain applications, including non-uniform radiofrequency fields, enhanced susceptibility artifacts [Ladd et al., 2018].

Potential of exploratory studies

More exploratory clinically applied cognitive neuroscience studies using MRI should be done, which could discover new targets and avenues, that consequently could be investigated in bigger, randomized controlled trials. In line, big datasets could be used together with artificial intelligence (AI) methods, to find new patterns and clusters in multi-modal datasets. In recent years, one of the most important theoretical advances in psychiatry and clinical psychology has been a move away from single-cause theories of mental disease [Kendler, 2019]. Many now agree that (1) the etiology of mental diseases is complex and multifaceted, (2) many of the processes that maintain mental disorders cross diagnostic boundaries, and (3) mental disorders require pluralist explanations [Borsboom et al., 2019] or other complexity-based approaches [van der Wal et al., 2021].

Potential of artificial intelligence (AI) in MR neuroimaging

In general, AI has found its way already into regular practice for cognitive and clinical neuroscience research. The applications to psychiatric neuroimaging may offer the possibility of developing robust and dependable illness biomarkers for monitoring everyday clinical practice [Davatzikos, 2019; Johnston et al., 2015]. MRI applications have focused amongst others on mental disorders, including bipolar disorders [Rubin-Falcone et al., 2018], autistic spectrum disorders [Wallace et al., 2013], conduct disorders [Zhang et al., 2018], schizophrenia [Nieuwenhuis et al., 2012], attention deficit hyperactivity disorder (ADHD) [Lim et al., 2013], and depressive disorders [Serpa et al., 2014; Wise et al., 2018]. Support vector regression [Valli et al., 2016], multivariate relevance vector regression [Hoexter et al., 2013], and multivariate pattern analysis [Cabral et al., 2016] are frequently used to improve the analysis of MRI for the detection and treatment of mental illnesses.

Overall, AI in MRI processing is expected to continue to evolve, helping clinical personnel with innovative technologies that are effective and efficient. Both mass-univariate and multivariate approaches of processing MRI data can provide information regarding the location of disease-related structural or functional changes. In reality, univariate approaches are simpler to interpret, but they may be less responsive to small changes in distributed systems [Brammer, 2009]. However, both approaches, if effectively executed, have the potential to yield valuable disease outcome maps.

However, one possible issue with AI methods might be the issue of overfitting, which could impair the generalizability of the results [Davatzikos, 2019]. Overfitting occurs when an algorithm discovers a solution that precisely parameterizes the existing dataset, but fails to appropriately classify additional data. One cause of overfitting is the

use of insufficient sample numbers for training data [Wen et al., 2018]. Also, appropriate machine learning tools require sufficient amounts of input data, which may not be trivial given the variability among mental health disorder cohorts and subsequent issues of data harmonization and potential biases introduced when using them to develop machine learning based tools aimed for universal use. Other limitations of AI based methods are most likely due to insufficient code and (training) data sharing. Consequently, machine- and deep-learning approaches are only justifiable when paired with large-scale open-access datasets and open-source software.

Consensus and collaboration

To achieve more comparability and reproducibility between studies, more consensus should be found between research sites. Open science, consensus papers, checklists and reporting standards could help with this endeavor. Additionally, standardized data processing and analysis pipeline should be developed and most importantly applied.

Furthermore, it could also be helpful to develop advanced artificial intelligence methods to improve the quality of existing datasets to make them comparable to more modern ones, or to recover data that may not meet certain quality control standards to increase the number of usable datasets.

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Chapter 7

English Summary
Nederlandse samenvatting
Deutsche Zusammenfassung

English Summary

Challenging the Brain : Insights from comprehensive structural & functional MRI studies.

Approximately 13% of people worldwide are affected by mental health disorders, that cause substantial reductions in quality of life and economic productivity. Unfortunately, most medications for mental disorders suffer from two major problems: 1) there is a strong heterogeneity in their efficacy between individuals, and often even treatment resistance to many treatments; 2) adverse side effects are relatively common. Therefore, efforts are undertaken to develop specific biomarkers or tools that would not only allow more insight into the underlying mechanisms, but could also be used to identify correlated, or even predictive, biological measures, which will aid patient stratification and thereby improve the efficacy and reliability of medication for specific subgroups of patients, and/or help to develop better ones.

In **this thesis**, MRI techniques were used to study functional and structural changes in the brain induced by different external stimuli. These included: taxing working memory (WM), emotional tasks, stimulant medication and an exercise intervention. MRI techniques were applied to measure the influences on functional reactivity and brain connectivity, on brain metabolite concentrations and on volumetric measures of specific subregions, cerebral blood flow and cerebral blood volume of the brain. Additionally, measures of behavior, cognition and peripheral physiology were applied and correlated to the neurobiological, MRI-based changes.

We studied these effects in individuals with Attention-Deficit/Hyperactivity Disorder (ADHD), one of the most common neurodevelopmental disorders. Even though it is most commonly diagnosed in childhood, ADHD can persist into adulthood. Regardless of the steadily improving methods in the field of imaging research, and the ever growing literature on ADHD, its underlying pathophysiological mechanisms remain elusive [Ghimire et al., 2020]. In addition to inattention and hyperactivity, which are considered to be the core symptoms of ADHD, individuals often also exhibit emotion dysregulation (ED) that consists of a multi-faceted interplay of dimensions, including emotion recognition and emotional lability.

The first-line treatment for ADHD is pharmacotherapy with stimulant medications, with methylphenidate (MPH) being most commonly prescribed. Its mechanism of action involves a direct inhibition of the dopamine and noradrenaline transporters. Although MPH is recommended for the treatment of adult ADHD as well, meta analyses have shown a wide variability of efficacy, ranging from no effects at all to large improvements in ADHD symptoms. It is therefore important to; 1) investigate where that variation comes from, and preferably try to find markers that could help patient stratification and

treatment allocation, and 2) find alternative therapies that might provide less side-effects or higher efficacy.

Chapter 2

In ***chapter 2***, we investigated whether the taxing of WM would affect the neural mechanisms of emotional reactivity in adults with ADHD. Previous studies in children with ADHD had found a hyperreactivity of the amygdala in response to negative emotional stimuli. In anxiety and substance use disorders, a decrease in emotional hyperreactivity has been found when presented with a WM task during or before seeing emotional stimuli. In addition, WM training was shown to improve emotional reactivity outcomes in both healthy individuals and those with mental health conditions, but results in ADHD were still lacking.

Therefore, we here tested if taxing WM in individuals with ADHD could help reduce their hyperreactivity to emotional stimuli, with the potential to use WM training as a (add-on) treatment for individuals with ADHD.

Interestingly, we did not find any differences in amygdala reactivity to negative vs. neutral stimuli between typically developing controls (TDC) and adults with ADHD. Also, only weak evidence was found for a hypo-activation of WM related regions, including the para-cingulate cortex and dorsolateral prefrontal cortex, in the TDC vs ADHD groups. Probably as a consequence, we also did not find any influence of the load of the WM task on amygdala reactivity in either group. This might have been due to the relatively low scores in the category of ED symptoms, including depressive and anxiety symptoms in the included participants. Additionally, because we included adults with ADHD who had been diagnosed in childhood, compensatory mechanisms might have developed over time, that may have reduced the emotion regulation problems in adulthood, therefore reducing the effects of our WM taxing paradigm.

Chapter 3

In ***chapter 3,*** we investigated effects of prolonged MPH treatment on the underlying neural mechanisms of emotion regulation in ADHD. In adolescents with ADHD (aged 11-16 years), amygdala hyperreactivity and -connectivity in response to fearful faces had been identified before, which significantly increased following MPH discontinuation. Additionally, an acute MPH challenge restored altered resting-state circuits in individuals with ADHD of all ages. Yet, the precise neural mechanisms underlying such changes in emotional processing in ADHD, remained unclear, particularly after longer stimulant treatment. Additionally, (pre-)clinical research had suggested that effects of ADHD medication are age-dependent.

Therefore, in this study (as the secondary analysis of the ePOD trial), we investigated the potential influence of a prolonged methylphenidate treatment on amygdala

reactivity and connectivity to an emotion recognition task. We used the longitudinal data from the ePOD trial, which included medication-naïve male children and adults with ADHD, randomized to either four months of methylphenidate, or placebo.

Long-term MPH treatment did not have any effect on either internalizing symptoms, nor on the brain substrates underpinning emotional processing. However, we did find that MPH reduced ADHD symptoms most effectively in those adults with the highest depression and anxiety symptoms before trial onset, indicating that adult ADHD patients with comorbidities might potentially benefit most from long-term therapy with MPH.

Chapter 4

ADHD is increasingly viewed as a disorder of brain-wide network dysconnectivity, as opposed to being due to region-specific deficits. In ***chapter 4***, we therefore studied age-dependent effects of an acute dose of MPH on resting-state connectivity in dopamine sensitive regions. MPH inhibits the reuptake of dopamine and noradrenaline in the brain and as the dopamine system undergoes considerable changes throughout development, we hypothesized that age might modify the impact of MPH on functional connectivity.

In ***chapter 4***, the same participants as described in ***chapter 3*** were measured before and after an acute dose of MPH, before they were randomized to one of the intervention groups. Additionally, MR scans of TDCs were obtained without medication dose. Therefore, we used topological metrics in ***chapter 4***, which not only enabled us to evaluate individual nodes or global connections, but also the relevance and integration of pre-specified nodes within the global network, before and after MPH administration.

In accordance with our hypothesis, we observed opposite effects of acute MPH administration on connectivity strength and the relative importance of the subcortical nodes in children vs adults. Contrary to our expectations, MPH-induced modifications in the connectivity of frontal brain regions were minimal. In these regions, no differences were found between the age groups, and across groups the global importance of these areas (i.e., their value as a hub) specifically increased within the network. This might indicate a more important role for frontal regions regarding information flow in the network and we conclude that the acute effects of MPH on connectivity metrics in dopamine-sensitive subcortical regions, but not cortical regions, are different in children and adults with ADHD. This is likely due to maturational changes in the dopamine and noradrenergic systems. Furthermore, we did not find any normalizing effects of acute MPH in either age group, suggesting that the previously observed normalization towards a “control state” may exist only at the level of local connectivity, whereas MPH may cause a reorganization of functions at the level of the global network.

These findings suggest that the efficacy of stimulant therapy may not be based on normalization only, but rather depend on combinations of factors that return the network organization to its typical topology for some systems while reorganizing

others. In other words, it might be that altered networks in the brain do not need to return to the control state to function in the desired way, a restructuring of function could be sufficient. Our findings also imply that the age of stimulant-naive individuals with ADHD determines the effect that MPH may have on subcortical connections. This is most likely due to the continued development of the dopamine and noradrenaline systems in the younger participants. These findings highlight the need for more investigations into the possible age-dependent effects of MPH.

Chapter 5

In ***chapter 5***, we studied the effects of physical exercise as an external stimulus that is known to induce widespread beneficial effects on the body and mind. Furthermore, in older adults, several studies showed a positive effect on cognitive functioning and neuronal health, preventing further decreases in brain volume and cognitive decline. Also in younger adults it has been suggested that exercise might have a positive influence on brain volume, increasing after 6 weeks of physical exercise training already, but also returning to baseline levels 6 weeks later.

In ***chapter 5,*** we investigated the influence of a low- (stretching and toning) vs. a high- intensity (aerobic) exercise intervention of 12 weeks on the hippocampal volume of young, healthy, but non-athletic adults, and, additionally explored the possible underlying neuronal mechanisms that could underly these volumetric changes with comprehensive MRI methods.

Overall, we observed no differential effects of the different exercise intensities on hippocampal volume, vasculature, or neuro-metabolite markers, probably because cardiorespiratory fitness increased in both groups. In fact, the advantages of exercise might not be due to a single mechanism, but rather include various biochemical changes inside the body and brain, some of which may be unique to each individual. Although the hippocampus is thought to benefit from exercise in general, exploratory analyses in our young cohort of ***chapter 5*** demonstrated that cardiovascular fitness exhibits beneficial relationships on whole-brain cerebral blood flow and indicators of neuronal survival and integrity, estimated with MR spectroscopy. These results show that a multimodal approach is useful and maybe even necessary to disentangle the various effects that exercise might have on the (young) brain.

Nederlandse Samenvatting

Challenging the Brain: Inzichten uit uitgebreide structurele en functionele MRI-studies.

Ongeveer 13% van de mensen wereldwijd heeft te maken met psychische problemen. Het is bekend dat die leiden tot een aanzienlijke vermindering van de kwaliteit van leven alsmede economische productiviteit. Helaas hebben de meeste medicijnen voor psychische stoornissen twee grote problemen: 1) er is een groot verschil in hun werkzaamheid (met ook vaak therapieresistentie); en 2) vervelende bijwerkingen komen relatief vaak voor. Daarom is het belangrijk om specifieke biomarkers en meettechnieken te ontwikkelen die de ten grondslag liggende biologische veranderingen in psychische stoornissen kunnen bloot leggen. Deze kennis zal niet alleen kunnen helpen in de keuze voor de meest geschikte behandeling of medicatie, maar ook bijdragen in het vergroten van de *evidence* waarop deze medicijnen worden voorgeschreven. Uiteindelijk zal hierdoor de werkzaamheid van bestaande medicijnen verbeteren, en het aantal bijwerkingen worden gereduceerd. Ook kan deze kennis gebruikt worden om betere medicijnen te ontwikkelen.

Een veel gebruikte methode in de neurowetenschappen om mechanismen (sneller) bloot te leggen is door het brein te 'challengen' door middel van een externe stressor van emotionele-, farmacologische of fysieke aard. In dit proefschrift werden om die reden verschillende externe stimuli toegepast om functionele en structurele veranderingen in de hersenen te induceren, welke vervolgens met verschillende MRI-technieken konden worden gemeten. Deze stimuli waren: een werkgeheugen taak, emotionele taken, stimulerende medicatie en een sportinterventie. We gebruikten zowel 3T- als 7T-MRI-technieken om de invloed van deze stimuli te meten op: functionele reactiviteit en connectiviteit in de hersenen, hersenmetaboliet-concentraties, hersenvolumes, hersen-doorbloeding en bloedvolume. Tegelijkertijd werden deze neurobiologische veranderingen gecorreleerd aan veranderingen in gedrag, cognitie en perifere fysiologie.

Ondanks verbeterde beeldvormende technieken en de steeds groeiende literatuur, blijven de onderliggende (neuro) biologische mechanismen van Attention-Deficit/ Hyperactivity Disorder (ADHD) slechts gedeeltelijk begrepen. Naast onoplettendheid en hyperactiviteit, die worden beschouwd als de kernsymptomen van ADHD, vertonen individuen vaak stoornissen in de verwerking van emoties (emotionele dysregulatie; ED), wat bestaat uit een veelzijdig samenspel van dimensies, waaronder emotieherkenning en emotionele labiliteit.

De eerstelijnsbehandeling van ADHD is farmacotherapie met stimulerende middelen. Methylfenidaat (MPH) is hierbij de meest voorgeschreven medicatie. De werkingsmechanismen van MPH bestaan voornamelijk uit heropname-remming van dopamine- en noradrenaline transporters. Hoewel MPH gewoonlijk ook wordt aanbevolen als de eerste farmacologische optie voor de behandeling van ADHD bij volwassenen, laten meta-analyses in die leeftijdsgroep een groot verschil in effectiviteit zien, variërend van überhaupt geen effect, tot grote verbeteringen. Het is daarom belangrijk om; 1) te onderzoeken welke mechanismen ten grondslag liggen aan die verschillen, en aan de hand hiervan biomarkers te vinden die kunnen helpen bij patient stratificatie, en 2) alternatieve therapieën te vinden die mogelijk minder bijwerkingen-, of een hogere werkzaamheid bieden.

Hoofdstuk 2

In **hoofdstuk 2** hebben we onderzocht of het stimuleren van het werkgeheugen invloed heeft op de mechanismen die ten grondslag liggen onder emotionele reactiviteit bij volwassenen met ADHD. Eerdere studies in kinderen met ADHD toonden een hyperreactiviteit van de amygdala op negatieve emotionele stimuli aan. Interessant is dat bij zowel angst- als middelengebruik stoornissen de emotionele hyperreactiviteit afneemt wanneer het werkgeheugen wordt geactiveerd tijdens of vóór de blootstelling aan emotioneel relevante stimuli. Emotionele reactiviteit verbeterde ook na werkgeheugentraining bij zowel gezonde personen als mensen met psychische aandoeningen, maar dit is nog niet eerder onderzocht bij mensen met ADHD.

Omdat werkgeheugentraining naast ene externe stimulus, ook een mogelijk (add-on) behandeling zou kunnen zijn in ADHD testten we in dit hoofdstuk of het stimuleren van het werkgeheugen ook zou kunnen helpen bij het verminderen van hyperreactiviteit op emotionele stimuli bij volwassen personen met ADHD.

Onverwacht, vonden we geen verschillen in amygdala-reactiviteit op negatieve versus neutrale stimuli tussen volwassenen met ADHD en normaal ontwikkelende controles (TDC). Ook werd slechts zwak bewijs gevonden voor hypo-activering van werkgeheugen gerelateerde regio's, waaronder de paracingulate cortex en dorsolaterale prefrontale cortex, in de ADHD-groep vergeleken met de TDC. Waarschijnlijk vonden we daarom ook geen invloed van het werkgeheugen taakje op de amygdala-reactiviteit op emotionele stimuli in beide groepen. Deze resultaten kunnen te wijten zijn aan relatief lage scores op ED symptomen, waaronder symptomen van depressie en angst bij de geïncludeerde deelnemers. Bovendien, omdat we volwassenen met ADHD onderzochten die in de kindertijd waren gediagnosticeerd met ADHD, is het wellicht zo dat compensatie-mechanismen de problemen met emotieregulatie op volwassen leeftijd verminderd, waardoor het effect van de stimulatie van het werkgeheugen niet heel groot was.

Hoofdstuk 3

In **hoofdstuk 3** onderzochten we daarentegen de effecten van een langdurige farmacologische behandeling met MPH op de onderliggende neurale mechanismen van emotieregulatie bij ADHD. Bij adolescenten met ADHD (11-16 jaar) waren eerder amygdala-hyperreactiviteit en -connectiviteit als een hyperreactiviteit op angstige gezichten aangetoond, wat significant verhoogde na het stoppen van MPH. Bovendien was aangetoond dat acute MPH-toediening de "resting-state" circuits herstelt bij personen met ADHD van alle leeftijden. Ondanks deze kennis, bleven de precieze neurale mechanismen die ten grondslag liggen aan veranderingen in emotionele verwerking bij ADHD, vooral na een langere behandeling met stimulerende middelen, onduidelijk. Bovendien hebben een aantal (pre)klinische onderzoeken aangetoond dat de effecten van ADHD-medicatie leeftijdsafhankelijk zijn. In deze studie (als secundaire analyse van de ePOD-studie) bestuderen we daarom de invloeden van een langdurige MPH-behandeling op amygdala-reactiviteit en connectiviteit met behulp van een emotieherkenningstaak. Daarvoor gebruikten we de longitudinale data van het ePOD-onderzoek, waarin medicatie-naïeve kinderen en volwassenen met ADHD werden gerandomiseerd naar vier maanden MPH-, of placebo behandeling.

We vonden dat MPH op de lange termijn geen effect heeft op internaliserende symptomen noch de onderliggende hersenveranderingen bij emotionele verwerking. We hebben wel aangetoond dat MPH de ADHD-symptomen het meest effectief vermindert bij die volwassenen met de hoogste depressie- en angstsymptomen (vóór de start van de studie).

Hoofdstuk 4

ADHD wordt in toenemende mate gezien als een stoornis van netwerkdisconnectiviteit, in tegenstelling tot regio specifieke afwijkingen. In **hoofdstuk 4** bestudeerden we daarom de connectiviteit in rusttoestand in dopaminegevoelige hersenregio's. MPH remt de heropname van dopamine en noradrenaline in de hersenen. Omdat het dopaminesysteem tijdens de ontwikkeling aanzienlijke veranderingen ondergaat, was onze verwachting dat leeftijd ook invloed zou hebben op de effecten van MPH op functionele connectiviteit in dopaminegevoelige hersenregio's. In **hoofdstuk 4** werden dezelfde deelnemers als in **hoofdstuk 3** gemeten, maar nu voor en na een *acute* (dus een enkele) dosis met MPH, en voordat ze werden gerandomiseerd naar een van de interventiegroepen. Bovendien werd eenmaal een MR-scan van leeftijd en geslacht gematchte TDC's gemaakt zonder medicatie. In onze analyses maakten we gebruik van topologische methodes, aangezien die ons niet alleen in staat stellen om individuele knooppunten of globale verbindingen te evalueren, maar ook de relevantie en integratie van vooraf gespecificeerde knooppunten binnen het globale netwerk te meten: en dit voor en na MPH-toediening.

In overeenstemming met onze hypothese vonden we tegenovergestelde effecten van acute MPH op de connectiviteitssterkte en het relatieve belang van de subcorticale knooppunten bij kinderen versus volwassenen. In tegenstelling tot onze verwachtingen echter waren de MPH-geïnduceerde modificaties in de connectiviteit van frontale hersengebieden minimaal. In deze regio's werden geen verschillen gevonden tussen de beide leeftijdsgroepen, en tussen de groepen nam het mondiale belang van deze gebieden (d.w.z. hun waarde als hub) specifiek binnen het netwerk toe. Dit zou kunnen wijzen op een belangrijkere rol voor frontale regio's met betrekking tot de informatiestroom in het netwerk. We concluderen dan ook dat de acute effecten van MPH op connectiviteitsstatistieken in dopamine-gevoelige subcorticale regio's, maar niet in corticale regio's, verschillend zijn bij kinderen en volwassenen met ADHD. Dit is vermoedelijk grotendeels toe te schrijven aan ontwikkelingsveranderingen in de dopamine- en nor-adrenerge systemen. Verder vonden we geen normaliserende effecten van acute MPH in beide leeftijdsgroepen, wat suggereert dat de eerder gevonden normalisatie naar een "controle status" mogelijk alleen op het niveau van lokale connectiviteit bestaat, terwijl MPH een reorganisatie van functie op het niveau van het hersenwijde netwerk lijkt te veroorzaken.

Deze bevindingen suggereren dat de werkzaamheid van stimulerende medicijnen niet alleen gebaseerd is op normalisatie. Met andere woorden, het kan zijn dat gewijzigde netwerken in de hersenen niet naar de controlestaat hoeven terug te keren om op de gewenste manier te functioneren, een herstructurering van de functie zou kunnen volstaan. Onze bevindingen tonen daarnaast aan dat de effecten van MPH op de connectiviteit van subcorticale regio's leeftijdsafhankelijk zijn bij niet eerder behandelde deelnemers met ADHD, waarschijnlijk als gevolg van de voortdurende ontwikkeling van het dopamine- en nor-adrenalinesystemen. Deze bevindingen benadrukken het belang voor toekomstige studies om een ontwikkelingsperspectief te nemen bij het bestuderen van de MPH effecten.

Hoofdstuk 5

In hoofdstuk 5 hebben we de stimulus 'langdurige lichamelijke inspanning' bestudeerd, waarvan bekend is dat deze uiteenlopende gunstige effecten heeft op lichaam en geest. Bovendien toonden verschillende onderzoeken bij ouderen een positief effect op het cognitief functioneren en de neuronale gezondheid, waardoor verdere afname van hersenvolume en cognitieve achteruitgang werd voorkomen. Ook bij jonge volwassenen bestaat het idee dat lichaamsbeweging een positieve invloed zou kunnen hebben op het hersenvolume.

In **hoofdstuk 5** onderzochten we daarom wat voor invloed een lage (stretching en toning) vs. een hoge intensiteit (aërobe) inspanningsinterventie van 12 weken zou hebben op het hippocampusvolume van jonge, gezonde, maar niet-atletische

volwassenen. Bovendien hebben we met uitgebreide MRI-methoden onderzocht wat de mogelijke neuronale mechanismen kunnen zijn die ten grondslag liggen aan de deze volumetrische veranderingen. Over het algemeen vonden we geen verschil van de twee trainingsintensiteiten op hippocampusvolume, onderliggende bloedvaten structuur of neuro-metabolietmarkers. Waarschijnlijk omdat de fysieke fitheid in beide trainingsgroepen toenam. In feite zijn de voordelen van lichaamsbeweging misschien niet te wijten aan een enkel mechanisme, maar eerder aan verschillende biochemische veranderingen in het lichaam en de hersenen. Hoewel men denkt dat de hippocampus baat heeft bij lichaamsbeweging in het algemeen, hebben verkennende analyses in ons jonge cohort van **hoofdstuk 5** aangetoond dat fitheid gunstige effecten vertoont op de cerebrale doorbloeding van de *gehele* hersenen naast op indicatoren van neuronale overleving en integriteit. Onze multimodale aanpak bleek derhalve nuttig en misschien zelfs noodzakelijk is om de verschillende effecten van beweging op het (jonge) brein te ontrafelen.

Deutsche Zusammenfassung

Challenging the Brain: Erkenntnisse aus umfassenden strukturellen und funktionellen MRT-Studien.

Etwa 13 % der Menschen weltweit leiden unter psychischen Gesundheitsproblemen. Es ist bekannt, dass diese zu einer erheblichen Verminderung der Lebensqualität sowie der wirtschaftlichen Produktivität führen. Leider haben die meisten Medikamente für psychische Störungen zwei Hauptprobleme; 1) es gibt einen großen Unterschied in ihrer Wirksamkeit (mit oft auch Behandlungsresistenz); und 2) unangenehme Nebenwirkungen sind relativ häufig. Daher ist es wichtig, spezifische Biomarker und Messtechniken zu entwickeln, die die zugrunde liegenden (neuro)biologischen Veränderungen bei psychischen Störungen aufdecken können. Dieses Wissen hilft bei der Auswahl der am besten geeigneten Behandlung oder Medikamenten. Letztendlich wird dies die Wirksamkeit bestehender Medikamente verbessern und die Zahl der Nebenwirkungen verringern. Dieses Wissen kann auch genutzt werden, um bessere, innovative Medikamente zu entwickeln.

Eine weit verbreitete Methode in den Neurowissenschaften, um neuronale Mechanismen (schneller) aufzudecken, ist die „Herausforderung" des Gehirns durch einen externen Stressor emotionaler, pharmakologischer oder physikalischer Natur. Daher wurden in dieser Arbeit verschiedene externe Stimuli verwendet, um funktionelle und strukturelle Veränderungen im Gehirn zu induzieren, die dann mit verschiedenen MRT-Techniken gemessen werden konnten. Diese Stimuli waren: eine Arbeitsgedächtnisaufgabe, emotionale Aufgaben, stimulierende Medikamente und eine Sportintervention. Wir haben sowohl 3T- als auch 7T-MRT-Techniken verwendet, um den Einfluss dieser Stimuli zu messen: funktionelle Reaktivität und Konnektivität im Gehirn, Konzentrationen von Gehirnmetaboliten, regionale Gehirnvolumen, zerebraler Blutfluss und Blutvolumen. Gleichzeitig wurden diese neurobiologischen Veränderungen mit Veränderungen im Verhalten, der Kognition und der peripheren Physiologie korreliert.

Trotz verbesserter MRT Methoden und der ständig wachsenden Literatur sind die zugrunde liegenden (neuro)biologischen Mechanismen der Aufmerksamkeitsdefizit-/Hyperaktivitätsstörung (ADHS) nur teilweise verstanden. Neben Unaufmerksamkeit und Hyperaktivität, die als Kernsymptome von ADHS gelten, zeigen Betroffene häufig Störungen in der emotionalen Verarbeitung (emotionale Dysregulation; ED), die aus einem vielschichtigen Zusammenspiel von Dimensionen besteht, darunter Probleme mit Emotionserkennung und emotionale Labilität.

Die erste-Wahl-Behandlung von ADHS Stimulanzien, so wie Methylphenidat (MPH), was das am häufigsten verschriebene Medikament ist. Die Wirkmechanismen von MPH bestehen hauptsächlich in der Hemmung von der Wiederaufnahme von Dopamin- und

Noradrenalin-Transportern. Während MPH normalerweise auch als erste pharmakologische Option zur Behandlung von ADHS bei Erwachsenen empfohlen wird, zeigen Metaanalysen in dieser Altersgruppe große Unterschiede in der Wirksamkeit, die von überhaupt keiner Wirkung bis zu erheblichen Verbesserungen reichen. Es ist daher wichtig; 1) um die Mechanismen zu untersuchen, die diesen Unterschieden zugrunde liegen, um Biomarker zu finden, die bei der Medikamentenzuweisung helfen können, und 2) um alternative Therapien zu finden, die weniger Nebenwirkungen oder eine höhere Wirksamkeit bieten können.

Kapitel 2

In **Kapitel 2** haben wir untersucht, ob die Stimulierung des Arbeitsgedächtnisses die Mechanismen beeinflusst, die der emotionalen Reaktivität bei Erwachsenen mit ADHS zugrunde liegen. Frühere Studien bei Kindern mit ADHS zeigten eine Hyperreaktivität der Amygdala auf negative emotionale Reize. Interessanterweise nimmt die emotionale Hyperreaktivität sowohl bei Angst- als auch bei Substanzgebrauchsstörungen ab, wenn das Arbeitsgedächtnis während oder vor der Bloßstellung zu emotional relevanten Reizen. Auch die emotionale Reaktionsfähigkeit verbesserte sich nach dem Training des Arbeitsgedächtnisses sowohl bei gesunden Personen als auch bei Menschen mit psychischen Erkrankungen, aber dies wurde bisher nicht bei Menschen mit ADHS untersucht. Da das Training des Arbeitsgedächtnisses eine mögliche (Zusatz-)Behandlung bei ADHS sein könnte, haben wir in diesem Kapitel getestet, ob die Stimulierung des Arbeitsgedächtnisses auch dazu beitragen kann, die Hyperreaktivität auf emotionale Reize bei erwachsenen Personen mit ADHS zu reduzieren.

Unerwarteterweise fanden wir keine Unterschiede in der Amygdala-Reaktivität auf negative vs. neutralen Stimuli zwischen Erwachsenen mit ADHS und normal entwickelnden Kontrollteilnehmern. Außerdem wurden in der ADHS-Gruppe im Vergleich zum normal entwickelnden Kontrollteilnehmern nur schwache Hinweise auf eine Hypoaktivierung von Regionen gefunden, die mit dem Arbeitsgedächtnis in Verbindung stehen, einschließlich des paracingulären Kortex und des dorsolateralen präfrontalen Kortex. Dies ist wahrscheinlich der Grund, warum wir in beiden Gruppen keinen Einfluss der Arbeitsgedächtnisaufgabe auf die Amygdala-Reaktivität zu emotionalen Reizen fanden. Diese Ergebnisse können auf relativ niedrige Werte bei ED-Symptomen zurückzuführen sein, einschließlich Symptomen von Depressionen und Angstzuständen in unseren Teilnehmern. Da wir Erwachsene mit ADHS untersucht haben, bei denen ADHS in der Kindheit diagnostiziert wurde, ist es außerdem möglich, dass Kompensationsmechanismen die Probleme mit Emotionsregulation im Erwachsenenalter reduzierten, so dass die Wirkung der Stimulation des Arbeitsgedächtnisses nicht sehr groß war.

Kapitel 3

Im Gegensatz dazu untersuchten wir in **Kapitel 3** die Auswirkungen einer pharmakologischen Langzeitbehandlung mit MPH auf die zugrunde liegenden neuronalen Mechanismen der Emotionsregulation bei ADHS. Bei Jugendlichen mit ADHS (11-16 Jahre) war zuvor eine Amygdala-Hyperreaktivität und Konnektivität auf ängstliche Gesichter nachgewiesen worden, die nach Absetzen von MPH signifikant zunahmen. Darüber hinaus hat sich gezeigt, dass die akute MPH-Verabreichung bei Personen mit ADHS aller Altersgruppen die Netzwerke im Gehirn im Ruhezustand wiederherstellt. Trotz dieses Wissens blieben die genauen neuronalen Mechanismen unklar, die den Veränderungen der emotionalen Verarbeitung bei ADHS zugrunde liegen, insbesondere nach längerer Stimulanzien Behandlung. Darüber hinaus haben eine Reihe von (prä) klinischen Studien gezeigt, dass die Wirkung von ADHS-Medikamenten altersabhängig ist. In dieser Studie (als Sekundäranalyse der ePOD-Studie) untersuchten wir daher die Einflüsse einer langfristigen MPH-Behandlung auf die Amygdala-Reaktivität und -Konnektivität während einer Emotionserkennungsaufgabe. Wir verwendeten die Langzeitdaten von der ePOD-Studie, in der Medikamenten naive Kinder und Erwachsene mit ADHS zu einer viermonatigen MPH- oder Placebo-Behandlung randomisiert wurden.

Wir fanden heraus, dass MPH keine langfristigen Auswirkungen auf die Symptome von Depression oder Angstzuständen hatte und auch nicht auf die zugrunde liegenden neuronalen Mechanismen der emotionalen Verarbeitung. Zusätzlich konnten wir zeigen, dass MPH bei der Reduzierung von ADHS-Symptomen bei den Erwachsenen mit den stärksten Symptomen von Depressionen und Angstzuständen (vor Beginn der Studie) am effektivsten war.

Kapitel 4

ADHS wird zunehmend als eine Störung gehirnweiter Netzwerk-konnektivität angesehen, im Gegensatz zu regionalspezifischen Defiziten. Wir haben in **Kapitel 4** die Konnektivität im Ruhezustand in Dopamin-empfindlichen Gehirnregionen untersucht. Methylphenidat hemmt die Wiederaufnahme von Dopamin und Noradrenalin im Gehirn, und da das Dopaminsystem im Laufe der Entwicklung erheblichen Veränderungen unterliegt, stellten wir die Hypothese auf, dass Alter ebenfalls einen Einfluss hat auf wie MPH die funktionelle Konnektivität verändert. In **Kapitel 4** werden die gleichen Teilnehmer wie in **Kapitel 3** beschrieben. Sie wurden vor und nach einer *akuten* MPH-Dosis gemessen, bevor sie einer der Interventionsgruppen zugeteilt wurden. Zusätzlich wurden MRT-Scans von Kontrollpersonen mit neurotypischer Entwicklung einmalig ohne Medikamentendosis gemacht. Wir haben topologische Metriken verwendet, die es uns nicht nur ermöglichten, einzelne Regionen/ globale Verbindungen des Gehirns

zu bewerten, sondern auch die Relevanz und Integration von vordefinierten Regionen innerhalb des globalen Netzwerks zu untersuchen, und das vor und nach der MPH-Verabreichung.

In Übereinstimmung mit unserer Hypothese beobachteten wir gegensätzliche Auswirkungen einer akuten MPH Dosis auf die Konnektivitätsstärke und die relative Wichtigkeit der subkortikalen Regionen bei Kindern gegenüber Erwachsenen. Entgegen unseren Erwartungen waren die MPH-induzierten Modifikationen in der Konnektivität der frontalen Hirnregionen minimal. In diesen Regionen wurden keine Unterschiede zwischen den Altersgruppen festgestellt. Über die Gruppen hinweg nahm die globale Bedeutung dieser Gebiete (d.h. ihr Wert als Knotenpunkt) innerhalb des Netzwerks gezielt zu. Dies kann auf eine wichtigere Rolle der frontalen Regionen im Hinblick auf den Informationsfluss im Netzwerk hindeuten. Wir folgern daraus, dass die akuten Auswirkungen von MPH auf Konnektivität in Dopamin-empfindlichen subkortikalen Regionen, nicht aber in kortikalen Regionen von Kindern und Erwachsenen mit ADHS unterschiedlich sind. Dies ist möglicherweise weitgehend auf Entwicklungsveränderungen im Dopamin- und noradrenergen System zurückzuführen.

Darüber hinaus fanden wir in beiden Altersgruppen keine normalisierenden Effekte durch akutes MPH. Das deutet darauf hin, dass die zuvor beobachtete Normalisierung in Richtung eines „Kontrollzustands" nur auf der Ebene der lokalen Konnektivität existiert, während MPH eine Reorganisation der Funktion auf der Ebene des globalen Netzwerks verursachen kann. Dies deutet darauf hin, dass die Wirksamkeit der Stimulanz Therapie möglicherweise nicht nur auf Normalisierung basiert. Sie hängt vielmehr von Faktorkombinationen ab, die die Netzwerkorganisation für einige Systeme zu einer typischen Topologie zurückführen, während sie andere reorganisieren. Mit anderen Worten, es kann sein, dass veränderte Netzwerke im Gehirn nicht in den Kontrollzustand zurückkehren müssen, um wie gewünscht zu funktionieren. Eine Umstrukturierung der Funktion kann ausreichen. Unsere Ergebnisse zeigen, dass die Auswirkungen von MPH auf die Konnektivität subkortikaler Regionen bei Stimulanzien-naiven Teilnehmern mit ADHS altersabhängig sind. Grund sind vermutlich die anhaltenden Entwicklungen von Dopamin- und Nor-Adrenalin-Systemen. Diese Ergebnisse unterstreichen, wie wichtig es für zukünftige Studien ist, bei Untersuchung der Auswirkungen der MPH-Behandlung Altersunterschiede in Betracht zu ziehen.

Kapitel 5

In **Kapitel 5** haben wir die Effekte eines körperlichen Sport-Trainings untersucht, was weitreichende positive Auswirkungen auf Körper und Geist hat. Darüber hinaus zeigen mehrere Studien bei älteren Erwachsenen eine positive Wirkung auf kognitive Funktionen und die neuronale Gesundheit. Die Abnahme des Gehirnvolumens und ein kognitiver Rückgang können dadurch verhindert werden. Auch bei jüngeren Erwachsenen wird

vermutet, dass Bewegung einen positiven Einfluss auf das Gehirnvolumen haben könnte. Aber es nimmt bereits nach 6 Wochen körperlichen Trainings zu, und kehrt 6 Wochen danach auch wieder zu den Ausgangswerten zurück.

In **Kapitel 5** haben wir untersucht, welchen Einfluss eine 12-wöchige Trainingsintervention mit niedriger (Dehnung und Kräftigung) vs. hoher Intensität (aerob) auf das Volumen des Hippocampus bei jungen, gesunden, aber nicht sportlichen Erwachsenen haben kann. Zusätzlich untersuchten wir mit umfassenden MRT-Methoden die möglicherweise zugrunde liegenden neuronalen Mechanismen, die diese volumetrischen Veränderungen verursachen können.

Insgesamt beobachteten wir keine unterschiedlichen Auswirkungen der verschiedenen Trainingsintensitäten auf Hippocampusvolumen, Gefäßsystem oder Neuro-metabolit Marker. Grund ist vermutlich die Zunahme der Fitness in beiden Trainingsgruppen. Tatsächlich sind die Vorteile des Trainings voraussichtlich nicht auf einen einzigen Mechanismus zurückzuführen, sondern umfassen vielmehr verschiedene biochemische Veränderungen in Körper und Gehirn. Einige davon können für jeden Menschen spezifisch sein. Es wird angenommen, dass der Hippocampus im Allgemeinen von Bewegung profitiert. Dies zeigen explorative Analysen in unserer jungen Kohorte von **Kapitel 5**. Sie zeigen einen positiven Zusammenhang von Fitness und zerebralen Blutfluss des *gesamten* Gehirns und Indikatoren für das Überleben und der Integrität von Neuronen auf. Diese Ergebnisse zeigen, dass ein multimodaler Ansatz nützlich und sogar notwendig ist, um die verschiedenen Auswirkungen zu entwirren, die Bewegung und Sport auf das (junge) Gehirn haben können.

Chapter 8

List of Publications
PhD Portfolio
Acknowledgements
Curriculum Vitae

List of Publications

1. Batelaan, N. M., Seldenrijk, A., van den Heuvel, O. A., van Balkom, A. J. L. M., **Kaiser, A.**, Reneman, L., & Tan, H. L. (2022). Anxiety, Mental Stress, and Sudden Cardiac Arrest: Epidemiology, Possible Mechanisms and Future Research. *Frontiers in Psychiatry*, *12*, 813518. https://doi.org/10.3389/FPSYT.2021.813518/FULL
2. **Kaiser, A.**, Broeder, C., Cohen, J. R., Douw, L., Reneman, L., & Schrantee, A. (2022). Effects of a single-dose methylphenidate challenge on resting-state functional connectivity in stimulant-treatment naive children and adults with ADHD. *Human Brain Mapping*, *May*, 1–12. https://doi.org/10.1002/hbm.25981
3. **Kaiser, A.**, Reneman, L., Solleveld, M. M., Coolen, B. F., Scherder, E. J. A., Knutsson, L., Bjørnerud, A., van Osch, M. J. P., Wijnen, J. P., Lucassen, P. J., & Schrantee, A. (2022). A Randomized Controlled Trial on the Effects of a 12-Week High- vs. Low-Intensity Exercise Intervention on Hippocampal Structure and Function in Healthy, Young Adults. *Frontiers in Psychiatry*, *12*(January), 1–15. https://doi.org/10.3389/fpsyt.2021.780095
4. **Kaiser, A.**, Reneman, L., Lucassen, P. J., de Vries, T. J., Schrantee, A., & Kaag, A. M. (2022). Targeting working memory to modify emotional reactivity in adult attention deficit hyperactivity disorder: a functional magnetic resonance imaging study. *Brain Imaging and Behavior*, *16*(2), 680–691. https://doi.org/10.1007/s11682-021-00532-6
5. Hilberdink, C. E., Zuiden, M. van, Schrantee, A., Korosi, A., **Kaiser, A.**, Zhutovsky, P., Ginty, A. T., Ensink, J. B. M., Lindauer, R. J. L., Vrijkotte, T. G. M., & Rooij, S. R. de. (2021). Dysregulated functional brain connectivity in response to acute social-evaluative stress in adolescents with PTSD symptoms. *Https://Doi.Org/10.1080/20008198.2021.1880727*, *12*(1).
6. Hof, M. Van den, Jellema, P. E. J., Haar, A. M. ter, Scherpbier, H. J., Schrantee, A., **Kaiser**, **A**., Caan, M. W. A., Majoie, C. B. L. M., Reiss, P., Wit, F. W. N. M., Mutsaerts, H.-J. M. M., & Pajkrt, D. (2021). Normal structural brain development in adolescents treated for perinatally acquired HIV: a longitudinal imaging study. *AIDS (London, England)*, *35*(8), 1221. https://doi.org/10.1097/QAD.0000000000002873
7. **Kaiser, A.**, Bottelier, M. A., de Ruiter, M. B., Solleveld, M. M., Tamminga, H. G. H., Bouziane, C., Geurts, H. M., Lindauer, R. J. L., Kooij, J. J. S., Lucassen, P. J., Schrantee, A., & Reneman, L. (2021). Effects of prolonged methylphenidate treatment on amygdala reactivity and connectivity: a randomized controlled trial in stimulant treatment-naive, male participants with ADHD. *Psychoradiology*, *1*(3), 152–163. https://doi.org/10.1093/psyrad/kkab013

PhD portfolio

1. PhD training

	Year	ECTS
General courses		
How to effectively supervise individual student projects? For PhD Candidates	2018	1
Specific courses		
The Amsterdam UMC World of Science	2017	0.7
Basic Legislation in Science (BROK)	2018	1.5
Scientific Writing in English for Publication	2017	1.5
Advanced Topics in Biostatistics	2018	2.1
MRI	2017	1.5
Emergency Response Officer	2019, 2020	2
Seminars, workshops and master classes		
ECNP Workshop on Neuropsychopharmacology for Early Career Scientists in Europe; Nice, France (poster presentation)	2019	2
(Inter)national conferences		
ISMRM=International Society for Magnetic Resonance in Medicine		
ECNP=European College of Neuropsychopharmacology		
MRS Workshop 2022; Lausanne (poster presentation)	2022	0.5
ISMRM; London (pitch at the Current Issues in Brain Function Study group)	2022	1
ISMRM; online (oral presentation)	2021	1
ISMRM benelux; online (oral presentation)	2020	1
Amsterdam Neuroscience; online	2020	0.5
ISMRM; online (poster presentation)	2020	0.5
ECNP; online (poster presentation)	2020	0.5
ISMRM benelux; online	2019	0.5
Amsterdam Neuroscience (poster presentation)	2019	0.5
ISMRM; Montreal (oral presentation)	2019	1
ISMRM benelux; Leiden (poster presentation)	2019	0.5
Amsterdam Neuroscience (poster presentation)	2018	0.5
ISMRM; Paris (poster presentation)	2018	0.5
ISMRM benelux; Antwerpen (oral presentation)	2018	1
Amsterdam Neuroscience (poster presentation)	2017	0.5
ECNP; Paris (poster presentation)	2017	0.5

2. Teaching

	Year	ECTS
Lecturing		
Guest lectures on MRI and fMRI physics; Masters	2018-2022	4
MRI demos; Bachelors, Masters, PhD	2018-2021	5
Tutorials/workgroups on fMRI and MRI analysis; Bachelors	2018-2022	4
Co-coordination of "Brain Organization and Cognition"; Masters	2021	2
Supervising		
Bachelor Thesis: 7	2017-2021	7
Master Thesis: 17	2017-2021	17

3. Parameters of Esteem

	Year
Organisational Activities	
International Conference for Cognitive Neuroscience (ICON)	2017
ISMRM Meeting Benelux Chapter	
Symposium and Networking Event – Amsterdam Brain and	2018
Cognition (ABC)	2020
Several Events with APROVE (PhD Association AMC)	2017-2020
Awards and Prizes	
Travel grant Amsterdam University Fund	2022
ISMRM Current Issues in Brain Function Study Group	2022
Presentation Award	
ISMRM Educational Stipend	2018, 2019, 2020
Moderating	
Research Day of the Radiology and Nuclear Medicine	2022
Department, Amsterdam UMC, location AMC	

4. Publications

	Year
Peer reviewed	
7	

Curriculum Vitae

Antonia Kaiser was born on the 3rd of May 1991 in Munich, Germany. After finishing her secondary education at the Christian-Dietrich-Grabbe Gymnasium in Detmold, Germany in 2010, she started her Bachelor's Degree in Cognitive Science at the University of Osnabrück, Germany. In her third year, Antonia completed an internship at the Semel Institute at the University of California Los Angeles, USA, about Obsessive Compulsive Disorder (OCD) in children. She obtained her Bachelor's Degree in 2014 with a thesis on the effects of a vibration belt, as a new sense, on orientation in young adults. After an additional voluntary internship at the Max Planck Institute for Human Development Berlin, Germany, she enrolled in the Master's program Brain and Cognitive Science at the University of Amsterdam, with a specialization in Cognitive Neuroscience. During her Master's, she completed her first internship at the MOVE department of the Vrije Universiteit Amsterdam in which she investigated the potential over and underestimation of movements in older participants. She completed her second internship at Amsterdam University Medical Center, location AMC, studying the effects of a randomized controlled trial of 12-week high- vs. low-intensity exercise on brain perfusion under the supervision of Prof. dr. Liesbeth Reneman, Prof. dr. Paul J. Lucassen and dr. Anouk Schrantee. This internship led to Antonia applying for a PhD position in the same department. Under the supervision of Prof. dr. Liesbeth Reneman, Prof. dr. Paul J. Lucassen, Dr. Anouk Schrantee, and dr. Marco Bottelier, she started her PhD on the potential influences of emotion dysregulation and medication in ADHD and exercise in healthy volunteers on the brain, measured with comprehensive structural and functional MRI methods. In September 2021, Antonia started working part-time as a postdoctoral researcher on a collaborative project between the department of Radiology and Nuclear Medicine at the Amsterdam UMC, location AMC and VUmc, applying deep neural networks in order to decontaminate low signal-to-noise ratio MR spectroscopy data. At the same time, she started working as a part-time lecturer for the Bachelor's Program Psychobiology at the University of Amsterdam, teaching a wide range of topics from Philosophy to Neurophysiology. In February 2023 Antonia will start working as a postdoctoral-researcher at the École polytechnique fédérale de Lausanne (EPFL) University of Lausanne and the Center for Biomedical Imaging (CIBM). She will be working on the project: "Advancing magnetic resonance spectroscopy: towards a sensitive tool for targeting neurometabolic alterations in psychiatric disorders".

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Thirteen years ago, I moved away from home to start studying Cognitive Science, beginning my journey of becoming a Neuroscientist. I have met a lot of absolutely incredible people all over the world: Detmold, Osnabrück, Los Angeles, Berlin, and Amsterdam.
In the following few pages I would like to thank all of you, for being there for me, living life together, going on adventures, and making life so much more fun. Because of all of you, I was able to start my adventure of an academic career, and had the endurance and motivation to finish this PhD. If I forgot to mention you by name, please know: all of you played an important role! Thank you!

In no particular order:

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amazing journey I will always look back to with a smile. I am also so happy to be able to call you a friend and hope that we will keep in touch and have many more evenings with a wine or a tea, and some games, or go on an unexpected adventure. Thank you so much!

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Fellow interns from when we all started at Z0 as students, **Marie-Elise, Gijs, Dirk, Sofie, Anne, David, Elena, Karen**, thanks for making my internship time a blast, we had so much fun together, and supported each other throughout writing our theses, thanks for that!
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Masters: Thanks to all my fellow Brainies. You guys got me excited about (cognitive) Neuroscience again after doubting if I wanted to follow this path. It was great to bond with you all on so many different levels. Because all of you were so passionate about all kind of things, I found my competitiveness again and made it this far. Special thanks go out to **Vincent** and **Anna** for running this program and making our two years so incredibly multifaceted, I guess you could call it interdisciplinary ;). I will always be looking back with a big smile. Also, very proud to have gotten the opportunity to contribute to this beautiful program as a lecturer.

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Dani, you are such a passionate and strong woman. I admire you for your strong opinions and big heart. Thank you for long and deep conversations that made me think and inspired me.

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worth, reminding me that being vulnerable is okay, listening to me, showing me that little things matter. Thank you so much for being my friend!

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---- Continued in German

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Ending words: Thank you to everyone who has been and will be part of my life. Without you guys, I couldn't have done it. You all inspire me to the fullest. Thank you!

Hakuna matata, diesen Spruch sag‘ ich gern
Hakuna matata, gilt stets als modern
Es heißt, die Sorgen bleiben dir immer fern
Keiner nimmt uns die Philosophie
Hakuna matata

- Lion King