Food for thought: Novel insights into childhood ADHD

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About the cover:
The typographic image of the title consists of different tracks within each letter. Each track contributes individually to the character of the letter. This image reflects the multiple tracks that have been followed in the research described in this dissertation, and the multiple aetiological tracks that may lead to ADHD.
On the cover both the foreground and the background play a prominent role, which emphasises the idea that there are multiple factors that play a role in ADHD. It also reflects a core deficit in ADHD, which is the difficulty to focus attention.

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Food for thought:
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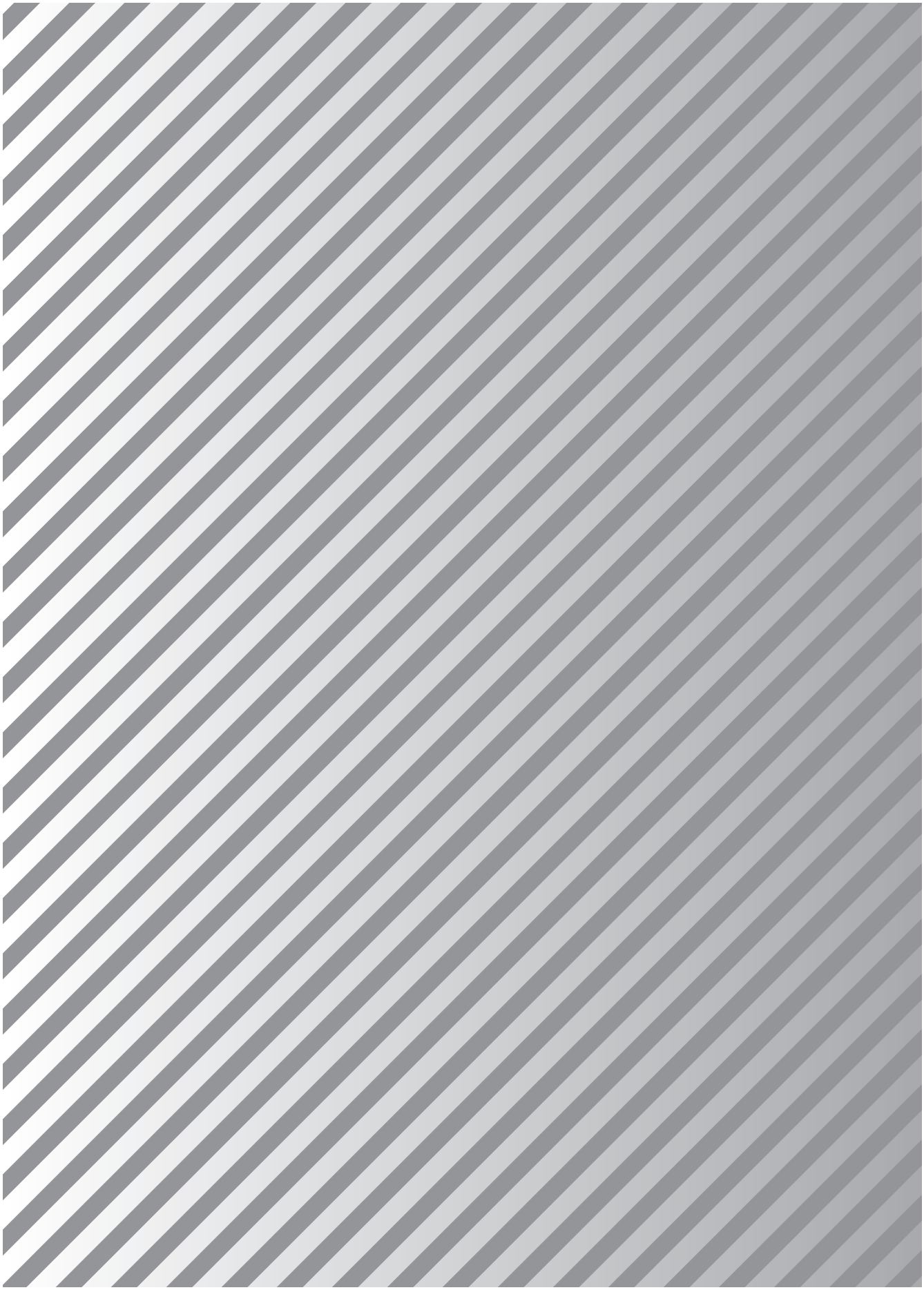
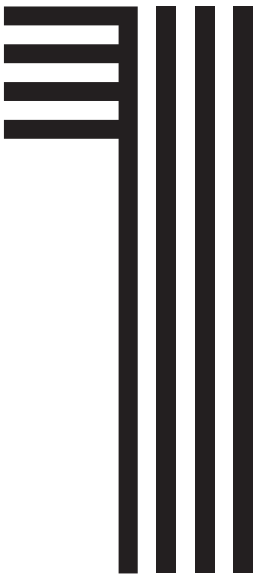
Contents



Chapter 1: General introduction



Diagnosis and prevalence

Attention-deficit/hyperactivity disorder (ADHD) is a childhood psychiatric disorder characterised by a persistent pattern of age-inappropriate inattention and/or hyperactivity-impulsivity (American Psychiatric Association, 2013). The diagnostic and statistical manual of mental disorders, fifth edition (DSM-5) describes the diagnostic criteria of ADHD. Individuals are fulfilling the criteria for a DSM-5 diagnosis of ADHD when at least six symptoms of inattention and/or at least six symptoms of hyperactivity-impulsivity have persisted for at least six months. Given that the DSM-5 defines nine symptoms on both symptoms domains, there are over 100.000 unique combinations of symptoms possible, varying from six symptoms of inattention and zero symptoms of hyperactivity-impulsivity, to nine symptoms on both symptom domains. The large variety in symptom combinations and symptom severity contributes to clinical heterogeneity in ADHD. Additional diagnostic criteria include the criterion of pervasiveness (symptoms being present in multiple settings, such as at home and at school) and the criterion of impairment (symptoms interfering with social or academic functioning). Furthermore, since the introduction of the DSM-5, symptoms should be present prior to the age of 12 years (American Psychiatric Association, 2013), compared to seven years old as age of onset in the DSM-IV (American Psychiatric Association, 2000). In the current dissertation DSM-IV criteria are used, since the studies described in this dissertation started prior to the introduction of the DSM-5.

ADHD is one of the most prevalent psychiatric disorders during childhood (Costello, Mustillo, Erkanli, Keeler, & Angold, 2003). Thus far, no prevalence rates of DSM-5 diagnoses of ADHD are available, but a meta-analysis estimated the worldwide prevalence of ADHD in children at 5.9 percent, when applying full DSM-IV diagnostic criteria (Willcutt, 2012). In case the criteria of pervasiveness and impairment are not fully assessed, prevalence rates of ADHD are as high as 8.8 to 13.3 percent in children (Willcutt, 2012). Clinical guidelines recommend the use of information obtained from parents and teachers, in order to determine whether the criteria of pervasiveness and impairment are met (American Academy of Pediatrics, 2011). It has been shown that in childhood, boys are about twice as likely as girls to meet criteria for a diagnosis of ADHD (Willcutt, 2012). A meta-analysis showed that by the age of 25 years old, 40 to 60 percent of individuals diagnosed with ADHD during childhood continue to have impairing symptoms of ADHD (Faraone, Biederman, & Mick, 2006). In addition, there is evidence for an adult-onset

form of ADHD (Moffitt et al., 2015). This may contribute to the fact that the prevalence rate of ADHD in young adults (estimated at 5 percent) is similar to the prevalence rate in children (Willcutt, 2012), even though part of the children with ADHD outgrow the full diagnosis of ADHD (Van Lieshout et al., 2016).

Aetiology

Regarding the aetiology of ADHD, many theories on risk factors have been proposed in the past decades, among which genetic, neurocognitive, metabolic and dietary risk factors — the latter two being studied in **chapter 2 and 3** of this dissertation. In the current chapter a non-limitative overview of existing literature on aetiological risk factors for ADHD is provided.

Genetic risk factors

One of the dominant theories on the aetiology of ADHD focuses on a model in which (interactions between) multiple genetic and environmental risk factors increase the susceptibility to ADHD (Faraone et al., 2015; Faraone et al., 2005). As studies performed in twins show that the heritability of ADHD is estimated at 76 percent, a large degree of the variability in ADHD in the population can be accounted for by genes (Faraone et al., 2005). Results have shown evidence for stable genetic risk factors that contribute to the onset of ADHD, but also for genetic factors that emerge during the development of children and adolescents, which may contribute to persistence of ADHD (Chang, Lichtenstein, Asherson, & Larsson, 2013). Many candidate gene studies have been performed to determine which genes influence the susceptibility to ADHD (Faraone et al., 2005). Based on at least three studies for each gene variant, there is evidence for an association between the following gene variants and ADHD: the dopamine D4 receptor (DRD4), the dopamine D5 receptor (DRD5), the dopamine transporter gene (DAT), dopamine beta-hydroxylase (DBH), the serotonin transporter gene (5-HTT), 5-hydroxytryptamine receptor 1B (HTR1B), and the gene encoding synaptosomal-associated protein 25 (SNAP-25). The majority of the genes related to ADHD consist of genes that are involved in the transport and binding of dopamine and serotonin. Altered functioning of these genes may explain altered dopamine concentrations in the brains of individuals with ADHD (Oades, 2008), and aberrant postsynaptic serotonin levels found in some individuals with ADHD (Oades, 2010). However, pooled odds ratios are small for all genes, suggesting that the vulnerability to ADHD is dependent on interactions between multiple genes of small effect (Faraone et al., 2005). In recent years, studies have

focused on polygenic risk scores for ADHD, which are aggregated scores of thousands of alleles associated with ADHD (Hamshere et al., 2013). Results showed that polygenic risk factors for ADHD were related to inattention and to hyperactivity-impulsivity (Martin, Hamshere, Stergiakouli, O'Donovan, & Thapar, 2014). It should be noted that a meta-analysis has shown that effects of genetic risk factors differed across symptom domains (Nikolas & Burt, 2010), suggesting that there are different genetic aetiological pathways in ADHD. More importantly, the aetiology of ADHD involves an interplay between genes and non-genetic factors, including prenatal exposure to maternal smoking (Thapar, Cooper, Eyre, & Langley, 2013).

Neurocognitive risk factors

During the past decades, researchers focused on detecting neurocognitive endophenotypes that mediate between genetic alterations and the behavioural phenotype of ADHD (Castellanos & Tannock, 2002; Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). Genetic risk factors have a negative influence on the structure and functioning of the brain of children with ADHD, which in turn may impair neurocognitive functioning (Faraone et al., 2015). For instance, a meta-analysis showed evidence for smaller volumes across several brain regions, including the right globus pallidus, right putamen and caudate nucleus (Frodl & Skokauskas, 2012), while another meta-analysis provided evidence for alterations in white matter integrity in multiple brain areas, including the right anterior corona radiata, right forceps minor, bilateral internal capsule, and left cerebellum (Van Ewijk, Heslenfeld, Zwiers, Buitelaar, & Oosterlaan, 2012). ADHD is associated with numerous neurocognitive deficits, such as impaired working memory (Willcutt et al., 2005) and poor inhibitory control (Barkley, 1997; Willcutt et al., 2005). Recently, studies started to focus on identifying subgroups of children with ADHD that share a neurocognitive profile, acknowledging that ADHD is an aetiologically heterogeneous disorder, with multiple pathways at the neurocognitive level resulting in the presence of ADHD symptoms. Thus far, three studies applied community detection procedures in individuals with ADHD, all showing multiple distinct neurocognitive profiles (Fair, Bathula, Nikolas, & Nigg, 2012; Mostert et al., 2015; Van Hulst, De Zeeuw, & Durston, 2015). It was found that the neurocognitive profiles in the ADHD samples were also observed in typically developing controls, with individuals with ADHD generally showing weaker neurocognitive performance within each profile (Fair et al., 2012; Mostert et al., 2015; Van Hulst et al., 2015). This finding suggests that individuals with ADHD reflect the extremes of normal neurocognitive heterogeneity. Therefore, low performance on

neurocognitive profiles may reflect a risk factor for ADHD. Since all three studies into neurocognitive profiling in ADHD used different selections of neurocognitive measures, profile characteristics differed across studies. In **chapter 4**, we apply community detection procedures in individuals with ADHD, to gain more insight into the number and type of neurocognitive profiles underlying ADHD. Further research is required to examine the clinical relevance of classifying children with ADHD into neurocognitive subgroups, given the limited predictive validity of neurocognitive functioning for persistence of ADHD (Van Lieshout, Luman, Buitelaar, Rommelse, & Oosterlaan, 2013). We address the predictive validity of neurocognitive profiling in children with ADHD in **chapter 4**, with a focus on predictive validity for functional outcomes (externalising problems, social problems and academic problems).

Metabolic risk factors

Several biochemical measures were reviewed as potential metabolic risk factors for ADHD, in order to provide more insight into the aetiology of ADHD, and to provide biomarkers that could be useful for diagnostic and therapeutic purposes. An extensive systematic review showed that there are several biomarkers that differentiate children with ADHD from controls (Scassellati, Bonvicini, Faraone, & Gennarelli, 2012). Five biomarkers were significantly related to ADHD, including norepinephrine (NE) and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) in urine, monoamine oxidase (MAO) in platelets, zinc in serum, plasma and urine, and cortisol in saliva. However, none of the biomarkers could unequivocally predict ADHD. The effects of the biomarkers that showed a difference between groups of children with ADHD and controls were too small for diagnostic purposes, and it is not clear whether these biomarkers are specific to ADHD (Scassellati et al., 2012). Therefore, other putative biomarkers for ADHD should be explored, which is the aim of **chapter 2 and 3** of this dissertation.

In **chapter 2** we explore the role of aromatic amino acids (AAAs) in blood in relation to ADHD. Given their impact on the synthesis of serotonin and dopamine, decreased blood concentrations of the AAAs tryptophan, tyrosine and phenylalanine may contribute to the expression of ADHD symptoms. While there are many other factors that affect the synthesis of dopamine and serotonin (including the transport of AAAs through the blood-brain barrier, and the availability of co-enzymes), normal circulating blood concentrations of AAAs are a first prerequisite for the synthesis of these neurotransmitters (Felger et al., 2013; O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015). We therefore hypothesise that

decreased AAA blood concentrations are a risk factor for ADHD, in line with three studies that reported lower plasma concentrations of tryptophan, tyrosine and phenylalanine in ADHD (Baker et al., 1991; Bornstein et al., 1990; Comings, 1990).

In **chapter 3** we focus on the contribution of another amino acid that is involved in the functioning of the brain: homocysteine. High concentrations of homocysteine have detrimental effects on neurocognitive functioning, by causing DNA damage, disturbed methylation or cell death, or by altering the functioning of glutamate receptors (Mattson & Shea, 2003). Homocysteine has been found associated with neurocognitive performance in patients with neurodegenerative diseases (Teunissen et al., 2005), in the normal aging population (Garcia & Zanibbi, 2004), as well as in psychiatric populations (Dias, Brissos, Cardoso, Andreazza, & Kapczinski, 2009; Ford, Flicker, Singh, Hirani, & Almeida, 2013). Given the overwhelming evidence of neurocognitive problems in ADHD, we explore whether homocysteine abnormalities were related to (neurocognitive deficiencies in) ADHD, and hypothesise that high homocysteine concentrations in blood are a risk factor for childhood ADHD.

Dietary risk factors

Other theories have focused on dietary abnormalities as risk factors for ADHD (Banerjee, Middleton, & Faraone, 2007). There is limited evidence for dietary deficiencies in ADHD, with some significant findings for zinc (Toren et al., 1996), folate (Durá-Travé & Gallinas-Victoriano, 2014), iron (Konofal, Lecendreux, Arnulf, & Mouren, 2004), and omega-6 fatty acids (Ng, Meyer, Reece, & Sinn, 2009). Although these topics require further research, the available evidence provides some empirical justification for nutritional interventions in ADHD (Hurt, Arnold, & Lofthouse, 2011). Other dietary risk factors that have received scientific interest are increased dietary intake of artificial food colours (Nigg, Lewis, Edinger, & Falk, 2012) and sugar (Wolraich, Wilson, & White, 1995). A meta-analysis showed that additive food colours increased parent-rated ADHD symptoms in children (Nigg et al., 2012). However, there were great individual differences in response to food colours, suggesting that this is a risk factor for some children (eight percent) only (Nigg et al., 2012). A meta-analysis of placebo-controlled studies showed that refined sugar did not increase behavioural problems, and did not decrease cognitive performance in children (Wolraich et al., 1995). In this dissertation we explore whether children with ADHD had lowered dietary intake of protein, as tryptophan, tyrosine and phenylalanine are constituents of protein in foods (**chapter 2**). A low dietary intake

of protein, in combination with abnormal AAA concentrations in children with ADHD, would be informative for a dietary risk factor for ADHD. Likewise, we explore whether children with ADHD had lowered dietary intake of vitamin B12 and folate (**chapter 3**), as a deficiency of folate or vitamin B12 leads to increased concentrations of homocysteine in the blood.

Functional impairments

Besides being impaired by ADHD symptoms, children with ADHD are often hampered by other difficulties; it appears that, unfortunately, for children with ADHD it never rains but it pours. Functional impairments that are associated with ADHD include, among others, behavioural problems, sleep disturbances, academic underachievement, social problems and emotion recognition deficiencies. In **chapter 5** of this dissertation, we study sleep disturbances in ADHD. In the current chapter a non-limitative overview of existing literature on functional impairments in ADHD is provided.

Behavioural problems

ADHD is associated with numerous problems at behavioural level, with 60 to 100 percent of children with ADHD having at least one other DSM-diagnosis (Gillberg et al., 2004). ADHD increases the risk of internalising disorders, such as anxiety disorder and depression, with comorbidity rates of 13 to 51 percent in children with ADHD. ADHD also increases the risk of externalising disorders, including oppositional defiant disorder (ODD) and conduct disorder (CD), with 43 to 93 percent of children with ADHD meeting criteria for ODD and/or CD (Gillberg et al., 2004; Jensen, Martin, & Cantwell, 1997). Another psychiatric comorbidity that is frequently reported in children with ADHD, is autism spectrum disorder (ASD), with 65 to 80 percent of children with ADHD showing symptoms of ASD (Gillberg et al., 2004). The varying presence of comorbid disorders across children with ADHD is, next to the variance in ADHD symptom combinations and symptom severity, another cause of clinical heterogeneity among children with ADHD. While some children with ADHD experience little comorbidity, others are hampered by a wide range of associated problems (Faraone et al., 2015). This heterogeneity may reflect differential aetiological pathways of ADHD. In **chapter 4** we explore whether certain neurocognitive profiles reflect an increased risk of comorbid behavioural problems in ADHD. This may provide insight into separate aetiological pathways at the neurocognitive level. Furthermore, given the great extent of psychiatric comorbidity in children with ADHD, it should be noted that these conditions may mediate or moderate

associations between ADHD and other functional outcomes. For instance, anxiety disorder, depression, ODD and CD seem to be a risk factor for sleep disturbances (Chervin, Dillon, Archbold, & Ruzicka, 2003; Chorney, Detweiler, Morris, & Kuhn, 2008; Corkum, Moldofsky, Hogg-Johnson, Humphries, & Tannock, 1999; Owens et al., 2009). It is therefore important to control for these comorbid conditions when studying sleep in ADHD. If internalising or externalising behaviour contributes to decreased sleep quality or quantity in children with ADHD, such a finding could be of relevance to the treatment of sleep problems in these children. Inadequate behaviour of children leading to sleep problems might be amenable to treatment (Hiscock et al., 2015). In **chapter 5** we study the effects of comorbid internalising and externalising problems on the association between ADHD and sleep problems.

Sleep disturbances

In addition to an increased risk of other psychiatric disorders, clinical observations suggest that ADHD is associated with an increased prevalence of sleep disturbances (Corkum, Tannock, & Moldofsky, 1998). A meta-analysis of studies using subjective measures of sleep quality (questionnaires filled out by parents) shows that children with ADHD have higher bedtime resistance, and more sleep onset difficulties, nocturnal awakenings, difficulties with arising in the morning and sleep disordered breathing compared to controls, although for all results considerable heterogeneity was reported across the studies (Cortese, Faraone, Konofal, & Lecendreux, 2009). A recent meta-analysis on sleep studies using actigraphy to measure sleep objectively, showed as well that non-medicated children with ADHD have increased sleep onset latency and decreased sleep efficiency, although, again, results were inconsistent (De Crescenzo et al., 2016). That same meta-analysis also showed that non-medicated children with ADHD do not suffer from altered sleep duration or increased wakefulness after sleep onset (De Crescenzo et al., 2016), in contrast to the findings of studies using subjective measures (Cortese et al., 2009; Yoon, Jain, & Shapiro, 2012). It is important to gain more insight into sleep problems in ADHD, as sleep quantity and quality may impact on inattentive behaviour (Beebe, 2011), executive functioning (Astill, Van der Heijden, Van IJzendoorn, & Van Someren, 2012), and academic performance (Curcio, Ferrara, & De Gennaro, 2006; Fallone, Acebo, Seifer, & Carskadon, 2005). Furthermore, it is important to explore factors that mediate or moderate sleep problems in children with ADHD, in order to assess which children are at risk of sleep disturbances. For instance, low socioeconomic status (SES) might increase the risk of lower sleep quality, due to lower parenting quality and poorer sleep

conditions (crowded, noisier and lower quality homes). Early and adequate intervention of sleep disturbances might prevent further detrimental effects. In **chapter 5** of this dissertation we explore sleep disturbances in medication-free children with ADHD, using objective measures of sleep quality and sleep quantity. We hypothesise that higher levels of internalising and externalising behaviour and low SES mediate or exacerbate the association between ADHD and sleep problems.

Academic underachievement

Another domain that is frequently affected in children with ADHD, is academic functioning (Loe & Feldman, 2007). It has been suggested that symptoms of ADHD have a negative effect on (a) learning and applying knowledge and (b) task performance at school (Loe & Feldman, 2007). These problems result in lower performance on standardised academic achievement tests (Frazier, Youngstrom, Glutting, & Watkins, 2007), increased prevalence of learning disabilities (Barry, Lyman, & Klinger, 2002), increased need for special education (Barry et al., 2002) and higher incidence of repeating a grade (Barbaresi, Katusic, Colligan, Weaver, & Jacobsen, 2007) in children with ADHD. There is evidence that only children with mainly symptoms of inattention show academic underachievement, while children with mainly hyperactive–impulsive symptoms, or symptoms in both symptom domains, do not show lower academic performance than typically developing children (Massetti et al., 2008). Given the great impact of academic underachievement, early detection of children with ADHD at risk of academic problems is important. In **chapter 4** of this dissertation we examine whether certain neurocognitive profiles translate into an increased risk of academic problems. We hypothesise that academic problems in children with ADHD are related to deficiencies in cool executive functions, including working memory and inhibitory control (Antonini et al., 2016).

Social problems

ADHD increases the risk of social problems, including peer rejection (McQuade & Hoza, 2008). Symptoms of ADHD may seriously interfere with social skills (Hoza, 2007). For instance, it has been suggested that socially inappropriate behaviour, such as not listening to others, initiating conversations at inappropriate times, and frequently interrupting on others, is a core feature of ADHD (Van der Oord et al., 2005). Furthermore, inattention may impair attending to cues that are important for social interaction (Hoza, 2007). In fact, severity of ADHD symptoms was found to be negatively related to social skills (Kaiser, McBurnett, & Pfiffner, 2011). As the underlying mechanisms of impaired social

functioning in children with ADHD remain unclear, and given that peer rejection may have a large detrimental impact on children, it is important to further explore risk factors for social impairment. In **chapter 4** of this dissertation we examine whether certain neurocognitive profiles translate into an increased risk of social problems. More specifically, we hypothesise that neurocognitive profiles that are characterised by emotion recognition deficiencies increase the risk of social problems (Trentacosta & Fine, 2010).

Emotion recognition deficiencies

Another core deficit of ADHD is a decreased ability to recognise facial emotional expressions, a central aspect of social cognition (Shaw, Stringaris, Nigg, & Leibenluft, 2014). However, the meta-analysis of Shaw et al. (2014) showed that there was significant heterogeneity across studies on emotion recognition in individuals with ADHD. It may be that results of facial emotion recognition studies in ADHD are influenced by the presence of comorbid symptoms of disorders that are characterised by emotion regulation deficiencies, such as ASD (Van der Meer et al., 2012) and CD (Cadesky, Mota, & Schachar, 2000). Furthermore, inconsistent results across studies into facial emotion recognition in children with ADHD may be explained by the use of different methodologies; while some paradigms used static pictures with a high expression intensity (Cadesky et al., 2000; Sinzig, Morsch, & Lehmkuhl, 2008), others used morphed pictures, in which the intensity of emotional expressions is manipulated (Pelc, Kornreich, Foisy, & Dan, 2006; Schwenck et al., 2013). Pictures with a high expression intensity provide insight into the ability of children to interpret the full display of an emotional expression. However, in daily life social interaction, children often need to process low-intensity emotional expressions or ambiguous emotional expressions. Using stimuli consisting of high-intensity emotional expressions might lead to a ceiling effect, and may therefore be invalid to detect more subtle emotion recognition deficiencies. Furthermore, in children, the ability to recognise emotions in other children's faces is particularly important in social interaction with peers (Nowicki & Mitchell, 1998). For the study in **chapter 7** a task was constructed consisting of pictures of children's faces. Children's faces are considered to be more ecologically valid when studying facial emotion recognition deficiencies that are relevant to peer problems in children with ADHD or other psychiatric populations (including ASD and CD). In **chapter 7** the construction of this new facial emotion recognition task is described, using stimuli that show children's faces that vary in emotional intensity.

Study design

The results reported in this dissertation are based on two studies that have been performed between February 2013 and July 2014. Study 1 was a case-control design, involving 83 children with ADHD and 72 typically developing primary school-aged children (6 to 13 years old). The results of Study 1 are described in **chapter 2-5**. The sample of typically developing children (*n*=72) was selected from a larger representative community-based sample (*n*=104), that is described in **chapter 6**. In **chapter 7** we report on a cross-sectional study (Study 2) involving another community-based sample (*n*=75).

Dissertation aims and outline

In the current dissertation we aim to provide novel insights into childhood ADHD, by addressing a wide range of topics relevant to ADHD. In **chapter 2 and 3** the potential role of amino acid abnormalities in the aetiology of ADHD is explored. The aim of **chapter 2** is to examine the role of aromatic amino acids in blood in relation to ADHD. Given their impact on the synthesis of serotonin and dopamine, decreased concentrations of tryptophan, tyrosine and phenylalanine in blood may contribute to the expression of ADHD symptoms. Decreased AAA blood concentrations, in turn, may be related to lowered dietary protein intake or to abnormal AAA excretion, as evidenced by increased urinary AAA concentrations. In **chapter 3** we focus on the role of homocysteine abnormalities in childhood ADHD. We examine whether homocysteine concentrations in children with ADHD are (a) positively related to symptoms of ADHD, (b) negatively related to neurocognitive functioning in ADHD, and (c) negatively related to intake of folate and vitamin B12. In **chapter 4** we describe a study into neurocognitive profiles in children with ADHD. Important aims of the study are to examine whether (a) neurocognitive profiles can be distinguished in children with ADHD and TD children, and (b) neurocognitive profiles predict externalising, social and academic problems in children with ADHD. The aim of **chapter 5** is to examine whether children with ADHD have an increased risk of sleep problems, using objective measures of sleep quality and quantity. We study confounding influences of comorbid internalising and externalising problems, and low SES, by exploring the mediating and moderating role of these factors in the association between ADHD and sleep problems. To control for the effects of stimulant medication use, all participants were tested free of medication. **Chapter 6** describes a study in which paediatric reference values are established for blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine. To our current knowledge, there

are no normative values available for blood spot concentrations of amino acids in primary school-aged children. In case aberrant blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine are involved in ADHD, it is important to have paediatric reference values available for clinical use. In **chapter 7** we aime to examine developmental effects on facial emotion recognition in primary school-aged children. We examine the effects of expression intensity, emotional condition, age, gender and IQ on facial emotion recognition. For this purpose, a facial emotion recognition task was developed, using pictures of children's faces that express different emotions (anger, fear, happiness, and sadness) at varying intensity levels. At the end of this dissertation (**chapter 8**), a summary and discussion of the findings of **chapter 2-7** can be found. Finally, new avenues for future research are provided.

References

American Academy of Pediatrics. (2011). ADHD: Clinical practice guideline for the diagnosis, evaluation, and treatment of attention-deficit/hyperactivity disorder in children and adolescents. *Pediatrics*, *128*(5), 1007-1022.

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders, fourth edition, text revision.* Washington: American Psychiatric Association.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders, fifth edition.* Arlington: American Psychiatric Association.

Antonini, T.N., Kingery, K.M., Narad, M.E., Langberg, J.M., Tamm, L., & Epstein, J.N. (2016). Neurocognitive and behavioral predictors of math performance in children with and without ADHD. *Journal of Attention Disorders, 20*(2), 108-118.

Astill, R.G., Van der Heijden, K.B., Van IJzendoorn, M.H., & Van Someren, E.J. (2012). Sleep, cognition, and behavioral problems in school-age children: A century of research meta-analyzed. *Psychological Bulletin, 138*(6), 1109–1138.

Baker, G.B., Bornstein, R.A., Rouget, A.C., Ashton, S.E., Van Muyden, J.C., & Coutts, R.T. (1991). Phenylethylaminergic mechanisms in attention-deficit disorder. *Biological Psychiatry, 29*(1), 15-22.

Banerjee, T.D., Middleton, F., & Faraone, S.V. (2007). Environmental risk factors for attention-deficit hyperactivity disorder. *Acta Paediatrica, 96*(9), 1269-1274.

Barbaresi, W.J., Katusic, S.K., Colligan, R.C., Weaver, A.L., & Jacobsen, S.J. (2007). Long-term school outcomes for children with attention-deficit/hyperactivity disorder: A population-based perspective. *Journal of Developmental & Behavioral Pediatrics, 28*(4), 265-273.

Barkley, R.A. (1997). *ADHD and the nature of self-control*. New York: The Guilford Press.

Barry, T.D., Lyman, R.D., & Klinger, L.G. (2002). Academic underachievement and attention-deficit/hyperactivity disorder: The negative impact of symptom severity on school performance. *Journal of School Psychology, 40*(3), 259-283.

Bornstein, R.A., Baker, G.B., Carroll, A., King, G., Wong, J.T., & Douglass, A.B. (1990). Plasma amino acids in attention deficit disorder. *Psychiatry Research, 33*(3), 301-306.

Cadesky, E.B., Mota, V.L., & Schachar, R.J. (2000). Beyond words: How do children with ADHD and/or conduct problems process nonverbal information about affect? *Journal of the American Academy of Child & Adolescent Psychiatry, 39*(9), 1160-1167.

Castellanos, F.X., & Tannock, R. (2002). Neuroscience of attention-deficit/hyperactivity disorder: The search for endophenotypes. N*ature Reviews Neuroscience, 3*(8), 617-628.

Chang, Z., Lichtenstein, P., Asherson, P.J., & Larsson, H. (2013). Developmental twin study of attention problems: High heritabilities throughout development. *JAMA Psychiatry, 70*(3), 311-318.

Chervin, R.D., Dillon, J.E., Archbold, K.H., & Ruzicka, D.L. (2003). Conduct problems and symptoms of sleep disorders in children. *Journal of the American Academy of Child & Adolescent Psychiatry, 42*(2), 201-208.

Chorney, D.B., Detweiler, M.F., Morris, T.L., & Kuhn, B.R. (2008). The interplay of sleep disturbance, anxiety, and depression in children. *Journal of Pediatric Psychology, 33*(4), 339-348.

Comings, D.E. (1990). Blood serotonin and tryptophan in Tourette syndrome. *American Journal of Medical Genetics, 36*(4), 418-430.

Corkum, P., Moldofsky, H., Hogg-Johnson, S., Humphries, T., & Tannock, R. (1999). Sleep problems in children with attention-deficit/hyperactivity disorder: Impact of subtype, comorbidity, and stimulant medication. *Journal of the American Academy of Child & Adolescent Psychiatry, 38*(10), 1285-1293.

Corkum, P., Tannock, R., & Moldofsky, H. (1998). Sleep disturbances in children with attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child & Adolescent Psychiatry, 37*(6), 637-646.

Cortese, S., Faraone, S.V., Konofal, E., & Lecendreux, M. (2009). Sleep in children with attention-deficit/hyperactivity disorder: Meta-analysis of subjective and objective studies. *Journal of the American Academy of Child & Adolescent Psychiatry, 48*(9), 894-908.

Costello, E.J., Mustillo, S., Erkanli, A., Keeler, G., & Angold, A. (2003). Prevalence and development of psychiatric disorders in childhood and adolescence. *Archives of General Psychiatry, 60*(8), 837-844.

Curcio, G., Ferrara, M., & De Gennaro, L. (2006). Sleep loss, learning capacity and academic performance. *Sleep Medicine Reviews, 10*(5), 323-337.

De Crescenzo, F., Licchelli, S., Ciabattini, M., Menghini, D., Armando, M., Alfieri, P., . . . Foti, F. (2016). The use of actigraphy in the monitoring of sleep and activity in ADHD: A meta-analysis. *Sleep Medicine Reviews, 26*, 9-20.

Dias, V.V., Brissos, S., Cardoso, C., Andreazza, A.C., & Kapczinski, F. (2009). Serum homocysteine levels and cognitive functioning in euthymic bipolar patients. *Journal of Affective Disorders, 113*(3), 285-290.

Durá-Travé, T., & Gallinas-Victoriano, F. (2014). Caloric and nutrient intake in children with attention deficit hyperactivity disorder treated with extended-release methylphenidate: Analysis of a cross-sectional nutrition survey. *Journal of the Royal Society of Medicine Open, 5*(2), 1-7.

Fair, D.A., Bathula, D., Nikolas, M.A., & Nigg, J.T. (2012). Distinct neuropsychological subgroups in typically developing youth inform heterogeneity in children with ADHD. *Proceedings of the National Academy of Sciences, 109*(17), 6769-6774.

Fallone, G., Acebo, C., Seifer, R., & Carskadon, M.A. (2005). Experimental restriction of sleep opportunity in children: Effects on teacher ratings. *SLEEP, 28*(12), 1561-1567.

Faraone, S.V., Asherson, P., Banaschewski, T., Biederman, J., Buitelaar, J.K., Ramos-Quiroga, J.A., . . . Franke, B. (2015). Attention-deficit/hyperactivity disorder. *Nature Reviews Disease Primers, 1*, 1-23.

Faraone, S.V., Biederman, J., & Mick, E. (2006). The age-dependent decline of attention deficit hyperactivity disorder: A meta-analysis of follow-up studies. *Psychological Medicine, 36*(2), 159-165.

Faraone, S.V., Perlis, R.H., Doyle, A.E., Smoller, J.W., Goralnick, J.J., Holmgren, M.A., & Sklar, P. (2005). Molecular genetics of attention-deficit/hyperactivity disorder. *Biological Psychiatry, 57*(11), 1313-1323.

Felger, J.C., Li, L., Marvar, P.J., Woolwine, B.J., Harrison, D.G., Raison, C.L., & Miller, A.H. (2013). Tyrosine metabolism during interferon-alpha administration: Association with fatigue and CSF dopamine concentrations. *Brain, Behavior, and Immunity, 31*, 153-160.

Ford, A.H., Flicker, L., Singh, U., Hirani, V., & Almeida, O.P. (2013). Homocysteine, depression and cognitive function in older adults. *Journal of Affective Disorders, 151*(2), 646-651.

Frazier, T.W., Youngstrom, E.A., Glutting, J.J., & Watkins, M.W. (2007). ADHD and achievement meta-analysis of the child, adolescent, and adult literatures and a concomitant study with college students. *Journal of Learning Disabilities, 40*(1), 49-65.

Frodl, T., & Skokauskas, N. (2012). Meta-analysis of structural MRI studies in children and adults with attention deficit hyperactivity disorder indicates treatment effects. *Acta Psychiatrica Scandinavica, 125*(2), 114-126.

Garcia, A., & Zanibbi, K. (2004). Homocysteine and cognitive function in elderly people. *Canadian Medical Association Journal, 171*(8), 897-904.

Gillberg, C., Gillberg, I.C., Rasmussen, P., Kadesjö, B., Söderström, H., Råstam, M., . . . Niklasson, L. (2004). Co–existing disorders in ADHD–implications for diagnosis and intervention. *European Child & Adolescent Psychiatry, 13*(1), i80-i92.

Hamshere, M.L., Langley, K., Martin, J., Agha, S.S., Stergiakouli, E., Anney, R.J., . . . Neale, B.M. (2013). High loading of polygenic risk for ADHD in children with comorbid aggression. *American Journal of Psychiatry, 170*(8), 909-916.

Hiscock, H., Sciberras, E., Mensah, F., Gerner, B., Efron, D., Khano, S., & Oberklaid, F. (2015). Impact of a behavioural sleep intervention on symptoms and sleep in children with attention deficit hyperactivity disorder, and parental mental health: Randomised controlled trial. *BMJ, 350*, h68.

Hoza, B. (2007). Peer functioning in children with ADHD. *Journal of Pediatric Psychology, 32*(6), 655-663.

Hurt, E.A., Arnold, L.E., & Lofthouse, N. (2011). Dietary and nutritional treatments for attention-deficit/hyperactivity disorder: Current research support and recommendations for practitioners. *Current Psychiatry Reports, 13*(5), 323-332.

Jensen, P.S., Martin, D., & Cantwell, D.P. (1997). Comorbidity in ADHD: Implications for research, practice, and DSM-V. *Journal of the American Academy of Child & Adolescent Psychiatry, 36*(8), 1065-1079.

Kaiser, N.M., McBurnett, K., & Pfiffner, L.J. (2011). Child ADHD severity and positive and negative parenting as predictors of child social functioning: Evaluation of three theoretical models. *Journal of Attention Disorders, 15*(3), 193-203.

Konofal, E., Lecendreux, M., Arnulf, I., & Mouren, M.-C. (2004). Iron deficiency in children with attention-deficit/hyperactivity disorder. *Archives of Pediatrics & Adolescent Medicine, 158*(12), 1113-1115.

Loe, I.M., & Feldman, H.M. (2007). Academic and educational outcomes of children with ADHD. *Journal of Pediatric Psychology, 32*(6), 643-654.

Martin, J., Hamshere, M.L., Stergiakouli, E., O'Donovan, M.C., & Thapar, A. (2014). Genetic risk for attention-deficit/hyperactivity disorder contributes to neurodevelopmental traits in the general population. *Biological Psychiatry, 76*(8), 664-671.

Massetti, G.M., Lahey, B.B., Pelham, W.E., Loney, J., Ehrhardt, A., Lee, S.S., & Kipp, H. (2008). Academic achievement over 8 years among children who met modified criteria for attention-deficit/hyperactivity disorder at 4–6 years of age. *Journal of Abnormal Child Psychology, 36*(3), 399-410.

Mattson, M.P., & Shea, T.B. (2003). Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. *Trends in Neurosciences, 26*(3), 137-146.

McQuade, J.D., & Hoza, B. (2008). Peer problems in attention deficit hyperactivity disorder: Current status and future directions. *Developmental Disabilities Research Reviews, 14*(4), 320-324.

Moffitt, T.E., Houts, R., Asherson, P., Belsky, D.W., Corcoran, D.L., Hammerle, M., . . . Polanczyk, G.V. (2015). Is adult ADHD a childhood-onset neurodevelopmental disorder? Evidence from a four-decade longitudinal cohort study. *American Journal of Psychiatry, 172*(10), 967-977.

Mostert, J.C., Hoogman, M., Onnink, A.M.H., Van Rooij, D., Von Rhein, D., Van Hulzen, K.J., . . . Norris, D.G. (2015). Similar subgroups based on cognitive performance parse heterogeneity in adults with ADHD and healthy controls. *Journal of Attention Disorders*. doi:10.1177/1087054715602332.

Ng, K.-H., Meyer, B.J., Reece, L., & Sinn, N. (2009). Dietary PUFA intakes in children with attention-deficit/hyperactivity disorder symptoms. *British Journal of Nutrition, 102*(11), 1635-1641.

Nigg, J.T., Lewis, K., Edinger, T., & Falk, M. (2012). Meta-analysis of attention-deficit/hyperactivity disorder or attention-deficit/hyperactivity disorder symptoms, restriction diet, and synthetic food color additives. *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(1), 86-97.e8.

Nikolas, M.A., & Burt, S.A. (2010). Genetic and environmental influences on ADHD symptom dimensions of inattention and hyperactivity: A meta-analysis. *Journal of Abnormal Psychology, 119*(1), 1-17.

Nowicki, S., & Mitchell, J. (1998). Accuracy in identifying affect in child and adult faces and voices and social competence in preschool children. *Genetic Social and General Psychology Monographs, 124*(1), 39-60.

O'Mahony, S., Clarke, G., Borre, Y., Dinan, T., & Cryan, J. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behavioural Brain Research, 277*, 32-48.

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

Oades, R.D. (2010). The role of serotonin in attention-deficit hyperactivity disorder (ADHD). In C. Müller & B. Jacobs (Eds.), *Handbook of the behavioral neurobiology of serotonin* (Vol. 21, pp. 565-584). Amsterdam: Elsevier.

Owens, J., Sangal, R.B., Sutton, V.K., Bakken, R., Allen, A.J., & Kelsey, D. (2009). Subjective and objective measures of sleep in children with attention-deficit/hyperactivity disorder. *Sleep Medicine, 10*(4), 446-456.

Pelc, K., Kornreich, C., Foisy, M.-L., & Dan, B. (2006). Recognition of emotional facial expressions in attention-deficit hyperactivity disorder. *Pediatric Neurology, 35*(2), 93-97.

Scassellati, C., Bonvicini, C., Faraone, S.V., & Gennarelli, M. (2012). Biomarkers and attention-deficit/hyperactivity disorder: A systematic review and meta-analyses. *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(10), 1003-1019.

Schwenck, C., Schneider, T., Schreckenbach, J., Zenglein, Y., Gensthaler, A., Taurines, R., . . . Romanos, M. (2013). Emotion recognition in children and adolescents with attention-deficit/hyperactivity disorder (ADHD). *ADHD Attention Deficit and Hyperactivity Disorders, 5*(3), 295-302.

Shaw, P., Stringaris, A., Nigg, J., & Leibenluft, E. (2014). Emotion dysregulation in attention deficit hyperactivity disorder. *American Journal of Psychiatry, 171*(3), 276-293.

Sinzig, J., Morsch, D., & Lehmkuhl, G. (2008). Do hyperactivity, impulsivity and inattention have an impact on the ability of facial affect recognition in children with autism and ADHD? *European Child & Adolescent Psychiatry, 17*(2), 63-72.

Teunissen, C.E., van Boxtel, M.P., Jolles, J., de Vente, J., Vreeling, F., Verhey, F., . . . Blom, H.J. (2005). Homocysteine in relation to cognitive performance in pathological and non-pathological conditions. *Clinical Chemical Laboratory Medicine, 43*(10), 1089-1095.

Thapar, A., Cooper, M., Eyre, O., & Langley, K. (2013). Practitioner review: What have we learnt about the causes of ADHD? *Journal of Child Psychology and Psychiatry, 54*(1), 3-16.

Toren, P., Eldar, S., Sela, B.-A., Wolmer, L., Weitz, R., Inbar, D., . . . Laor, N. (1996). Zinc deficiency in attention-deficit hyperactivity disorder. *Biological Psychiatry, 40*(12), 1308-1310.

Trentacosta, C.J., & Fine, S.E. (2010). Emotion knowledge, social competence, and behavior problems in childhood and adolescence: A meta-analytic review. *Social Development, 19*(1), 1-29.

Van der Meer, J.M., Oerlemans, A.M., van Steijn, D.J., Lappenschaar, M.G., de Sonneville, L.M., Buitelaar, J.K., & Rommelse, N.N. (2012). Are autism spectrum disorder and attention-deficit/hyperactivity disorder different manifestations of one overarching disorder? Cognitive and symptom evidence from a clinical and population-based sample. *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(11), 1160-1172.

Van der Oord, S., Van der Meulen, E.M., Prins, P.J.M., Oosterlaan, J., Buitelaar, J.K., & Emmelkamp, P.M.G. (2005). A psychometric evaluation of the social skills rating system in children with attention deficit hyperactivity disorder. *Behaviour Research and Therapy, 43*(6), 733-746.

Van Ewijk, H., Heslenfeld, D.J., Zwiers, M.P., Buitelaar, J.K., & Oosterlaan, J. (2012). Diffusion tensor imaging in attention deficit/hyperactivity disorder: a systematic review and meta-analysis. *Neuroscience & Biobehavioral Reviews, 36*(4), 1093-1106.

Van Hulst, B., De Zeeuw, P., & Durston, S. (2015). Distinct neuropsychological profiles within ADHD: A latent class analysis of cognitive control, reward sensitivity and timing. *Psychological Medicine, 45*, 735-745.

Van Lieshout, M., Luman, M., Buitelaar, J., Rommelse, N., & Oosterlaan, J. (2013). Does neurocognitive functioning predict future or persistence of ADHD? A systematic review. *Clinical Psychology Review, 33*(4), 539-560.

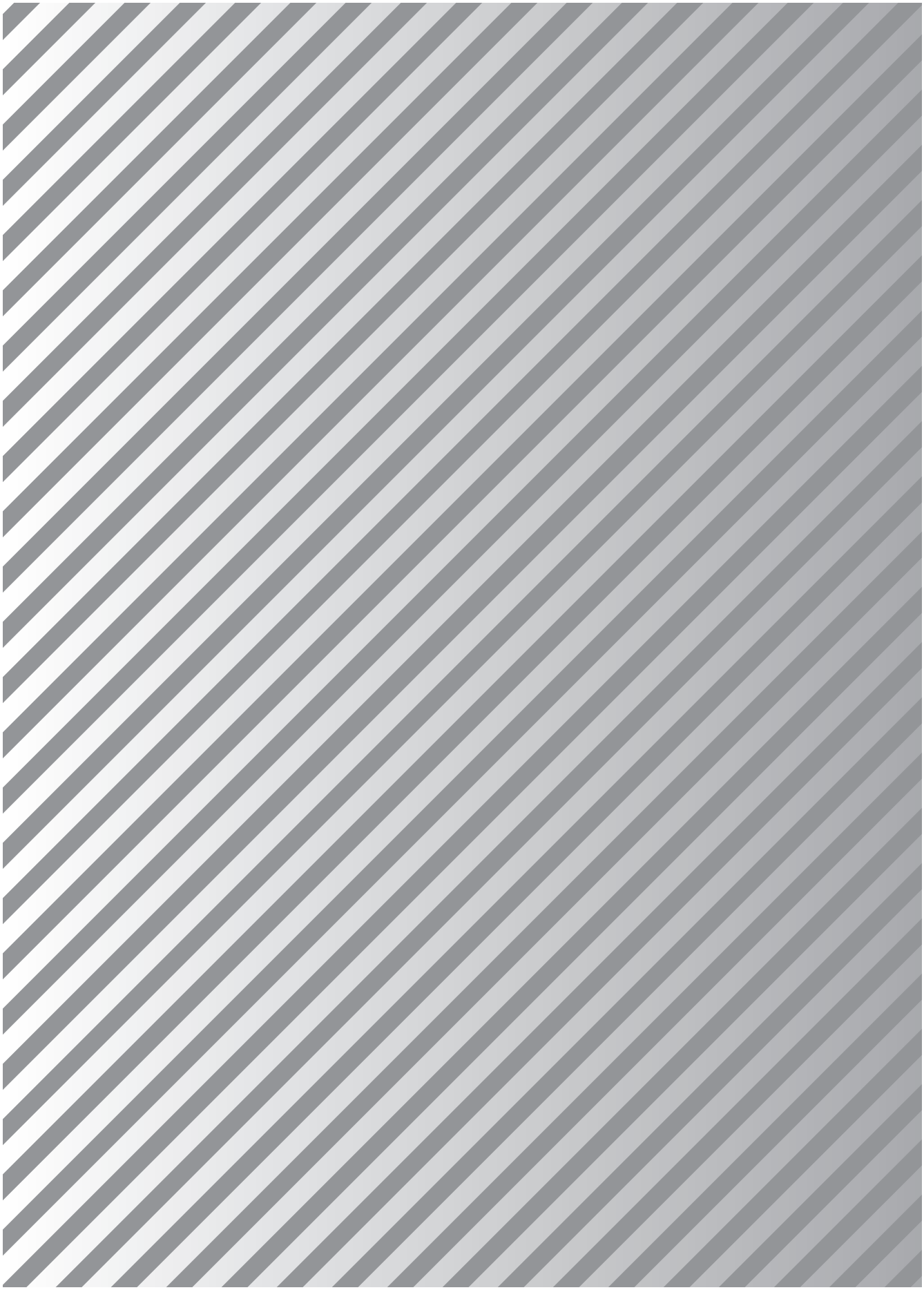
Van Lieshout, M., Luman, M., Twisk, J.W., Ewijk, H., Groenman, A.P., Thissen, A.J., . . . Oosterlaan, J. (2016). A 6-year follow-up of a large European cohort of children with attention-deficit/hyperactivity disorder-combined subtype: Outcomes in late adolescence and young adulthood. *European Child & Adolescent Psychiatry*, *25*(9), 1007–1017.

Willcutt, E.G. (2012). The prevalence of DSM-IV attention-deficit/hyperactivity disorder: A meta-analytic review. *Neurotherapeutics, 9*(3), 490-499.

Willcutt, E.G., Doyle, A.E., Nigg, J.T., Faraone, S.V., & Pennington, B.F. (2005). Validity of the executive function theory of attention-deficit/hyperactivity disorder: A meta-analytic review. *Biological Psychiatry, 57*(11), 1336-1346.

Wolraich, M.L., Wilson, D.B., & White, J.W. (1995). The effect of sugar on behavior or cognition in children: A meta-analysis. *JAMA, 274*(20), 1617-1621.

Yoon, S.Y.R., Jain, U., & Shapiro, C. (2012). Sleep in attention-deficit/hyperactivity disorder in children and adults: Past, present, and future. *Sleep Medicine Reviews, 16*(4), 371-388.



Chapter 2

No tryptophan, tyrosine and phenylalanine abnormalities in children with attention-deficit/hyperactivity disorder

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Abstract

Objective. The aim of the current study is to explore the role of aromatic amino acids (AAAs) in blood in relation to attention-deficit/hyperactivity disorder (ADHD). Given their impact on the synthesis of serotonin and dopamine, decreased concentrations of the AAAs tryptophan, tyrosine and phenylalanine in blood may contribute to the expression of ADHD symptoms. Decreased AAA blood concentrations, in turn, may be related to lowered dietary protein intake or to abnormal AAA excretion, as evidenced by increased urinary AAA concentrations.

Methods. Eighty-three children with ADHD (75% males) and 72 typically developing (TD) children (51% males), aged 6 to 13 years, participated in the study. AAA concentrations were assessed in blood spots and an 18-hour urinary sample. A nutritional diary was filled out by parents to calculate dietary protein intake. Parent and teacher questionnaires assessed symptoms of ADHD, oppositional defiant disorder, conduct disorder, and autism spectrum disorder.

Results. Children with ADHD showed normal AAA concentrations in blood spots and urine, as well as normal protein intake compared to controls. No associations between AAA concentrations and symptoms of ADHD or comorbid psychiatric disorders were found.

Conclusion. This study is the first to explore AAA metabolism in children with ADHD using a well-defined and relatively large sample. We found that AAA deficiencies are not related to ADHD. The results do not support treatment with AAA supplements in children with ADHD. Future studies regarding the cause of serotonin and dopamine alterations in ADHD should focus on other explanations, such as effects of altered transport of AAAs.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a childhood psychiatric disorder characterised by a persistent pattern of age-inappropriate inattention and/or hyperactivity-impulsivity (American Psychiatric Association, 2013). Several risk factors have been proposed for the disorder, including environmental (Banerjee, Middleton, & Faraone, 2007) and genetic (Faraone et al., 2005) factors. Environmental factors include, among others, dietary abnormalities and psychosocial adversity, although odds ratios obtained for these risk factors are small or not significant (Banerjee et al., 2007). Currently one of the main theories on genetic risk factors for ADHD involves aberrant dopaminergic neurotransmission (Madras, Miller, & Fischman, 2005; Swanson et al., 2007). Dopamine receptor and transporter genes play a significant role in ADHD (Li, Sham, Owen, & He, 2006; Spencer et al., 2013), which may explain decreased dopamine levels in ADHD (Oades, 2008). Other genetic studies provide evidence for an association between the serotonergic system and ADHD (Gizer, Ficks, & Waldman, 2009; Kiive & Harro, 2013; Oades et al., 2008), in line with aberrant postsynaptic serotonin levels found in some individuals with ADHD (Oades, 2010). Abnormal functioning of the dopamine and serotonin system has also been associated with neurocognitive deficits found in ADHD, such as cognitive impulsivity and poor executive attention (Oades, 2008). Similarly, dopamine and serotonin abnormalities have been associated with psychiatric disorders that are highly comorbid with ADHD, including oppositional defiant disorder (ODD; Lavigne et al., 2013), conduct disorder (CD; Van Goozen, Fairchild, Snoek, & Harold, 2007) and autism spectrum disorder (ASD; Gabriele, Sacco, & Persico, 2014).

While dopamine and serotonin hypotheses dominate current scientific work into ADHD, candidate gene study results are conflicting and effect sizes are small (Faraone et al., 2005). In addition to genetic risks of altered functioning of the neurotransmitter transporters and receptors, a potential interesting line of research focuses on the biosynthesis of dopamine and serotonin. Dopamine and serotonin are synthesised from aromatic amino acids (AAAs); the AAAs tyrosine and phenylalanine are precursors of dopamine and the AAA tryptophan is required for the synthesis of serotonin. While there are many other factors that affect the synthesis of dopamine and serotonin (including the transport of AAAs through the blood-brain barrier, and the availability of co-enzymes), normal circulating blood concentrations of AAAs are a first prerequisite for the synthesis of these neurotransmitters (Felger et al., 2013; O'Mahony, Clarke,

Borre, Dinan, & Cryan, 2015). Amino acids are constituents of protein in foods, such as meat, bananas and milk (Keszthelyi, Troost, & Masclee, 2009). Phenylalanine and tryptophan are both essential AAAs, and therefore must be obtained by dietary means, but tyrosine can also be synthesised in the body from phenylalanine (Harmer, McTavish, Clark, Goodwin, & Cowen, 2001). A lowered ingestion of protein or a malabsorption of AAAs may cause a decreased availability of AAAs (Keszthelyi et al., 2009). In the current study we explore the hypothesis that decreased AAA blood concentrations contribute to ADHD symptom expression, assuming a relation between AAA blood concentrations and aberrant neurotransmission of dopamine and serotonin in ADHD.

Thus far, five case-control studies have been published on AAA blood concentrations in individuals with ADHD (Baker et al., 1991; Bornstein et al., 1990; Comings, 1990; Hoshino, Ohno, & Yamamoto, 1985; Oades, Dauvermann, Schimmelmann, Schwarz, & Myint, 2010). Three studies, of which two describing the same sample (Baker et al., 1991; Bornstein et al., 1990), reported lower plasma concentrations of tryptophan, tyrosine and phenylalanine in ADHD (Baker et al., 1991; Bornstein et al., 1990; Comings, 1990). The other two studies, however, showed increased concentrations of free tryptophan in ADHD (Hoshino et al., 1985) and a trend towards increased serum concentrations of tryptophan in children with ADHD (Oades et al., 2010). All five studies are limited by non-standardised assessments of ADHD and small sample sizes (ranging from n=12 to n=48), and therefore further research into the availability of AAAs in ADHD is warranted. If blood concentrations of AAAs are decreased in an ADHD sample, this may be caused by reduced protein intake, malabsorption or increased excretion of AAAs. Although there is little evidence for dietary abnormalities in ADHD (Banerjee et al., 2007), thus far no studies have specifically examined protein intake in ADHD. Increased urinary concentrations of AAAs may be indicative of abnormal excretion (Bender, 1983; Kopple, 2007) and four studies have investigated this hypothesis in small ADHD samples (Baker et al., 1991; Bornstein et al., 1990; Dolina, Margalit, Malitsky, & Rabinkov, 2014; Zametkin, Karoum, Rapoport, Brown, & Wyatt, 1984). While there is no evidence for abnormal levels of urinary tyrosine and phenylalanine concentrations in ADHD (Baker et al., 1991; Bornstein et al., 1990; Zametkin et al., 1984), one study showed increased urinary tryptophan concentrations, suggesting abnormal AAA excretion (Dolina et al., 2014). Taken together, the currently available studies provide some evidence for an altered AAA availability in ADHD, although more research, with greater sample sizes

and standardised procedures to assess ADHD, is required to gain more insight into the potential contribution of AAAs to the expression of ADHD symptoms.

The hypothesis that AAA concentrations are related to ADHD symptoms is the basis for a number of depletion and supplementation studies. Depletion of dietary tryptophan was found to impair sustained attention in adults with ADHD (Mette et al., 2013), and to weaken behavioural inhibition in hostile children with ADHD (Zepf et al., 2008). Supplementation with tryptophan, on the other hand, resulted in a decrease of ADHD symptoms in children with ADHD (Nemzer, Arnold, Votolato, & McConnell, 1986). Tyrosine supplementation decreased ADHD symptoms in adults with ADHD (Reimherr, Wender, Wood, & Ward, 1987), but showed no effects on behavioural functioning in children with ADHD (Nemzer et al., 1986). Phenylalanine supplementation in adults with ADHD caused a decrease of restlessness and an increase on the ability to concentrate at trend level (Wood, Reimherr, & Wender, 1985), but in children no effects were reported for phenylalanine supplementation on ADHD symptoms (Zametkin, Karoum, & Rapoport, 1987). However, also these depletion and supplementation studies are limited by non-standardised assessments of ADHD and small sample sizes (ranging from *n*=10 to *n*=20), as well as the lack of control groups, hampering conclusions regarding the relation between AAAs and ADHD. Therefore, there is a need of further research to support the hypothesis that AAA concentrations may contribute to the expression of ADHD symptoms.

Another aspect that requires further research is the association between AAAs and symptoms of childhood psychiatric disorders that are highly comorbid with ADHD. As pointed out, dopamine and serotonin abnormalities have also been associated with ODD, CD and ASD. Indeed, tryptophan depletion have been shown to induce aggressive behaviour (Stadler et al., 2007; Zimmermann et al., 2012), and increased tryptophan levels have been found associated with childhood ASD (Hoshino et al., 1984), suggesting that AAA abnormalities might contribute to the expression of symptoms of ODD, CD and ASD. Given the inconsistent evidence for AAA abnormalities in ADHD, comorbid psychiatric conditions might act as possible confounding (mediating) or exacerbating (moderating) factors, and should therefore be taken into account when studying AAA concentrations in ADHD.

To summarise, there is inconsistent evidence that AAAs, acting as precursors of dopamine and serotonin, contribute to the expression of ADHD symptoms. The mostly outdated studies on this topic performed thus far are hampered by methodological shortcomings. Therefore, our aim is to explore concentrations of tryptophan, phenylalanine and tyrosine in a well-phenotyped sample of children with ADHD as compared to a control sample consisting of typically developing (TD) children. We firstly hypothesise that children with ADHD show decreased blood concentrations of tryptophan, tyrosine and phenylalanine compared to controls, and that below average AAA concentrations increase the risk of being diagnosed with ADHD. Secondly, we hypothesise that blood AAA concentrations are related to ADHD symptoms. Thirdly, we hypothesise that abnormal blood AAA concentrations are related to decreased protein ingestion or by aberrant AAA excretion, as evidenced by increased urinary AAA concentrations. Finally, we study the possible confounding effects of symptoms of ODD, CD and ASD on our findings.

Methods

Participants

Subjects were 83 children with ADHD (75 percent males) and 72 TD children (51 percent males), aged between 6 and 13 years. Inclusion criteria for the ADHD group were: (a) a clinical diagnosis of ADHD according to DSM-IV criteria, (b) confirmation of this diagnosis by the Diagnostic Interview Schedule for Children, fourth edition, administered to parents (DISC-IV-P; Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000), (c) significant ADHD symptoms, as indicated by parent ratings >90th percentile on at least one of the ADHD scales (Inattention and Hyperactivity/Impulsivity scales) of the Disruptive Behaviour Disorder Rating Scale (DBDRS; Pelham, Gnagy, Greenslade, & Milich, 1992), and (d) pervasive ADHD symptoms, as indicated by teacher ratings >75th percentile on at least one of the ADHD scales of DBDRS. Having a comorbid diagnosis (for example ODD or ASD) was no exclusion criterion, neither was treatment with stimulant medication. Children on stimulant medication (*n*=50, 60 percent of the ADHD group) discontinued drug use 24 hours before testing, in order to allow complete washout (Pelham et al., 1999), and during participation in our study. Inclusion criteria for the TD group were: (a) absence of a clinical diagnosis of any developmental or behavioural disorder (including ADHD and ODD), and (b) scores <90th percentile on both parent- and teacher-rated ADHD scales of the DBDRS.

Materials

Behaviour

Parents of children eligible for inclusion in the ADHD group were assessed with the disruptive behaviour disorder section of the DISC-IV-P. The DISC-IV-P is a widely used standardised diagnostic interview for the assessment of DSM-IV childhood psychiatric disorders, with adequate psychometric properties (Shaffer et al., 2000).

Parents and teachers of children in both the ADHD and TD group completed the DBDRS to assess ADHD symptoms and symptoms of ODD and CD. The DBDRS contains four scales measuring symptoms of inattention, hyperactivity/impulsivity, ODD and CD on a 4-point Likert scale (ranging from 0 to 3), with higher scores indicating worse symptoms. Adequate psychometric properties have been reported for the DBDRS (Oosterlaan et al., 2008).

The Strengths and Weaknesses of ADHD-symptoms and Normal Behaviour rating scale (SWAN; Hay, Bennett, Levy, Sergeant, & Swanson, 2007; Swanson et al., 2005) was completed by parents and teachers to assess symptoms of ADHD. This widely used questionnaire contains two subscales; the Inattention scale and the Hyperactivity/ Impulsivity scale, each comprising 9 items. Items are scored on a 7-point Likert scale (ranging from -3 to +3), with higher scores indicating worse symptoms. The items are based on the DSM-IV symptoms of ADHD, but reflect both ends (strong and weak) of the behaviour described in each ADHD symptom. Mean scores on both subscales were used as dependent variables. Adequate psychometric properties have been reported for the SWAN (Lakes, Swanson, & Riggs, 2012).

ASD symptoms were assessed using the 65–item Social Responsiveness Scale (SRS; Constantino et al., 2003; Constantino & Gruber, 2005), completed by parents and teachers. The items of the SRS are based on the DSM-IV symptom domains of ASD, including impairment in social interaction, communicative deficits and restricted/ stereotypic patterns of behaviours or interests. The SRS uses a 4–point Likert scale (ranging from 0 to 3), and the summed item score on the total SRS scale was used as dependent measure, with higher scores indicating worse symptoms. The SRS has adequate psychometric properties (Bölte, Poustka, & Constantino, 2008; Constantino et al., 2003).

Blood spots

To investigate blood concentrations of tryptophan, tyrosine and phenylalanine, a dried blood spot technique was used. Collecting blood spots is less invasive for children than taking venous blood samples and the dried blood spot technique is sufficiently robust and stable for diagnostic purposes (Chace, Sherwin, Hillman, Lorey, & Cunningham, 1998; Kand'ár & Žáková, 2009; Rashed et al., 1997). Blood spot AAA concentrations are highly correlated with serum AAA concentrations (rs ranging from .86 to .96) (Pecce, Scolamiero, Ingenito, Parenti, & Ruoppolo, 2013). A blood spot of each child was collected using a disposable safety lancet. Three blood drops were spotted onto a blood stain card. A 5.5mm punch of a dried blood spot was mixed with 100µl of an internal standard solution (containing 29µM L-phenylalanine-D5, 6µM L-tyrosine-D4 and 5µM L-tryptophan-D5) and 400µl methanol in a Gas Chromatography vial (GC-vial) and shaken for 15 minutes in an ultrasonic bath. The supernatant was transferred in another GC-vial and evaporated under nitrogen at 30°C. Subsequently, the sample was butylated with 100µl of 5.5% acetyl chloride (in n-butanol) at 60°C for 15 minutes. Afterwards, the butanol-layer was evaporated under nitrogen (at 30°C) and the residue was dissolved in 500µl acetonitrile. Blood spot concentrations of tryptophan, tyrosine and phenylalanine were determined by positive electrospray liquid chromatography–tandem mass spectrometry (LC-MS/MS), using an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA), coupled to a high-performance liquid chromatography (HPLC) system (Perkin Elmer Series 200, Shelton, USA). Three µl of the sample was injected on a symmetry C18 column (3.9*150mm, 5µm; Waters, Milford, MA, USA) and eluted with a flow rate of 1ml/min of 75% acetonitrile (containing 0.4% of formic acid). Tryptophan, tyrosine and phenylalanine eluted within 1 minute and were measured using the transitions: mass-to-charge ratio (m/z) 261.2→159.2 (tryptophan), m/z 238.2→136.2 (tyrosine) and m/z 222.2→120.2 (phenylalanine). All obtained LC-MS/MS data were acquired and processed using Analyst 1.4.2 software (Applied Biosystems, Foster City, CA, USA). The blood spot concentrations of tryptophan, tyrosine and phenylalanine were expressed in µmole/L. Reliability of the LC-MS/MS was confirmed by examining the inter-assay variance (being 5 to 10 percent), intra-assay variance (being 8 to 10 percent) and recovery (being 90 to 112 percent).

Dietary protein intake

Daily protein intake was assessed during three days, using a parent-reported nutritional diary. Standardised dietary records and instructions were provided. Parents were

instructed to register all consumed foods and drinks in the dietary record and to express the consumed amounts as accurate as possible. The amount of protein intake (grams/day) was calculated based on a computerised version of the Dutch food composition database (National Institute for Public Health and the Environment, 2013). The Dutch food composition database contains over 2000 food products with information about the nutritional composition of these food products (Westenbrink & Jansen-van der Vliet, 2013). The database is widely used for scientific purposes (e.g. Altorf-van der Kuil et al., 2013; Van der Zwaluw et al., 2014; Van Kernebeek, Oosting, Feskens, Gerber, & De Boer, 2014).

Urine

In order to examine urinary AAA concentrations, participants collected all urine excreted within 18 consecutive hours (after-school hours) in a urine collection container. During urine collection, the container was stored in a refrigerator (<5°C). A 10ml sample was sent to a laboratory for analysis, where the sample was stored at -20°C. An HPLC technique with fluorescence detection was used for the analysis of tryptophan in urine (Kema et al., 1993). The concentrations of tyrosine and phenylalanine in urine were determined using a Biochrom amino acid analyser (Fekkes, Voskuilen-Kooyman, Jankie, & Huijmans, 2000). The urinary concentrations of tryptophan, tyrosine and phenylalanine were expressed by a µmole to total urine volume ratio, to rule out effects of polyuria or oliguria. Reliability of the HPLC technique was confirmed by examining the precision of the analyses, being 2.25 percent for tryptophan (relative standard deviation), and 1.50 percent for tyrosine and phenylalanine. There are high correlations between the amino acid concentrations in 12-hour samples and 24-hour samples (Khalaf, Böcker, Kerp, & Petersen, 1991), indicating that there is no diurnal variation in the excretion of amino acids, validating the use of an 18-hour sample in the current study.

Procedure

This study received approval from the local medical ethical committee of the VU University Medical Center Amsterdam, the Netherlands (#NL39922.029.12), and has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained of parents of all children, and of children ≥12 years, prior to participation. Children with ADHD were recruited from mental health outpatient clinics, through the parental association for children with behavioural problems, and through a university research website. The

TD group was recruited from primary schools located throughout the country. Children on stimulant medication discontinued drug use one day prior to participation (day 0), to ensure complete washout, and during the assessment of blood, urine and food intake (day 1 to day 3). On day 1 the blood spot was collected in the early morning, to rule out the effects of diurnal variation of blood AAA concentrations. The same day, after school time, urine collection started and continued for the following 18 hours, until the child would return to school the next morning (day 2). In the early morning of day 1 parents received detailed instructions on how to fill out the dietary record and how to collect urine of their child. After the instruction, parents started recording their child's dietary intake, which continued for the following three days (day 1 to day 3). Parents and teachers were invited to fill out the questionnaires on a secured website. All data were collected between February 2013 and July 2014. The ADHD and TD group were recruited simultaneously, to control for possible seasonal effects on food intake or AAA metabolism.

Data analysis

All statistical analyses were performed using R, version 3.2.1. All variables were inspected on outliers and missing values for the ADHD and TD group separately. Winsorising was applied to outliers, these were replaced with a value one unit bigger (or smaller) than the previous most extreme score in the distribution of the group (Tabachnick & Fidell, 2001). Missing data in the urinary concentrations, dietary data and behavioural data were randomly distributed and replaced using group means. For the blood spots there were no missing data. All data were normally distributed, except for CD symptoms. Group differences in gender were examined using a chi-squared test, and group differences in age and behavioural functioning were examined using independent samples t-tests.

To test the first hypothesis, group differences in AAA blood spot concentrations were assessed using Analyses of Variances (ANOVAs) with group (ADHD or TD) as fixed factor. Effect sizes were calculated in terms of partial eta squared, and interpreted as small (>.01), medium (>.06) or large (>.14) (Cohen, 1988). In addition, odds ratios were calculated, which expressed the risk of being diagnosed with ADHD with below average AAA concentrations. Normative data for AAA concentrations were derived from a large representative community-based sample of primary school-aged children (*n*=104, 52 percent males); for sample information, see **chapter 6** of this dissertation. For each AAA, concentrations corresponding to the lowest 16th percentile (*M*-1 *SD*) of the normative

sample were used as a threshold value to define below average AAA concentrations (for tryptophan 45 µmole/L, tyrosine 39 µmole/L, and phenylalanine 47 µmole/L). Odds ratios were calculated with their 95 percent confidence interval (95%CI) and Fisher's Exact Test was performed to examine the significance of the odds ratios.

To test the second hypothesis, Pearson product-moment correlation coefficients investigated the relationship between blood spot AAA concentrations and both parent and teacher rated symptoms of ADHD. The magnitude of correlation coefficients was interpreted as small (>.10), medium (>.30) or large (>.50) (Cohen, 1988). Data of the ADHD group and TD group were combined to maximise variability in the ADHD symptom measures.

To test the third hypothesis, correlation analyses between blood spot AAA concentrations and protein ingestion and urinary AAA concentrations were performed in the whole sample. We also examined whether there were group differences in protein ingestion and urinary AAA concentrations using ANOVAs with group (ADHD or TD) as fixed factor. Lastly, correlational analyses evaluated whether blood spot AAA concentrations were related to parent- and teacher-reported symptoms of comorbid psychiatric disorders (Pearson product-moment correlation coefficients for ODD and ASD, Spearman's rank correlation coefficients for CD). If symptoms of ODD, CD or ASD were found related to the AAA concentrations, previous analyses were rerun with these symptoms entered as covariates. To correct for multiple testing, the alpha level of the correlation analyses was adjusted according to the Bonferroni procedure per outcome domain; ADHD symptoms (12 analyses, thus p=.004), potential determinants of AAA abnormalities in blood spots (12 analyses, thus p=.004), and symptoms of comorbid psychiatric disorders (18 analyses, thus p=.003). Bonferroni adjusted results are reported.

Results

No groups differences were found in terms of age, but groups differed in gender as well as symptoms of ADHD, ODD, CD and ASD, see Table 2.1. The ADHD group had a larger proportion of males and more parent- and teacher-rated symptoms of ADHD, ODD, CD and ASD than the TD group.

The DISC-IV-P indicated that in our ADHD sample, 65 children met DSM-IV criteria for the combined subtype of ADHD, 12 children met DSM-IV criteria for the predominantly

inattentive subtype, and six children met DSM-IV criteria for the predominantly hyperactive-impulsive subtype.

Table 2.1. Group characteristics of the ADHD group (*n*=83) and TD group (*n*=72)

	ADHD group *M (SD)*	TD group *M (SD)*	Statistic *(t/χ^2)*
Age in months	116.71 (19.86)	119.17 (20.69)	-.75, NS
Males *n* (%)	62 (74.70)	37 (51.39)	9.08**
Parent-rated symptoms			
Inattention[a]	17.47 (4.82)	3.31 (3.07)	22.11**
Hyperactivity/Impulsivity[a]	16.31 (5.97)	3.26 (2.71)	17.92**
Inattention[b]	1.20 (.80)	-.46 (.72)	13.64**
Hyperactivity/Impulsivity[b]	1.30 (.90)	-.39 (.90)	11.58**
ODD[a]	9.72 (4.93)	3.04 (2.71)	10.63**
CD[a]	2.45 (2.46)	.49 (.84)	6.83**
ASD[c]	61.77 (25.93)	29.26 (14.23)	9.84**
Teacher-rated symptoms			
Inattention[a]	14.71 (6.23)	1.85 (2.29)	17.49**
Hyperactivity/Impulsivity[a]	13.80 (7.39)	1.57 (2.29)	14.30**
Inattention[b]	1.04 (.85)	-.74 (1.02)	11.66**
Hyperactivity/Impulsivity[b]	1.08 (.95)	-.83 (1.08)	11.60**
ODD[a]	7.82 (6.04)	.89 (1.93)	9.89**
CD[a]	2.20 (3.03)	.18 (.66)	5.93**
ASD[c]	85.58 (21.84)	25.07 (14.18)	20.71**

Notes. [a]Disruptive Behaviour Disorder Rating Scale, [b]Strengths and Weaknesses of ADHD-symptoms and Normal Behaviour rating scale, [c]Social Responsiveness Scale. ***p*<.01. ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; CD, conduct disorder; NS, not significant; ODD, oppositional defiant disorder; TD, typically developing.

No significant group differences were observed for blood spot concentrations of tryptophan, tyrosine, or phenylalanine, see Table 2.2 (all *F*s<4.00 and *p*s>.05). A below average (<16th percentile) blood spot concentration of phenylalanine increased the risk of being diagnosed with ADHD by 2.22, although this effect just escaped conventional levels of significance (OR=2.22, 95%CI [.92–5.73], *p*=.07). A below average blood spot concentration of tryptophan (OR=2.09, 95%CI [.86–5.40], *p*=.11) or tyrosine (OR=1.83, 95%CI [.74–4.79], *p*=.22) did not increase the risk of being diagnosed with ADHD.

Table 2.2. Blood spot and urine concentrations of AAAs and protein ingestion in the ADHD group (*n*=83) and TD group (*n*=72)

	ADHD group *M (SD)*	TD group *M (SD)*	*F*	pη^2
Blood spots				
Tryptophan (µmole/L)	52.10 (10.06)	53.54 (9.10)	.87, NS	<.01
Tyrosine (µmole/L)	50.37 (15.92)	55.33 (15.88)	3.75, NS	.02
Phenylalanine (µmole/L)	56.40 (14.01)	57.46 (11.10)	.27, NS	<.01
Dietary intake				
Protein intake (g/day)	65.57 (16.05)	63.72 (13.86)	.58, NS	<.01
Urine				
Tryptophan (µmole/total urine)	38.09 (17.23)	38.26 (17.02)	<.01, NS	<.01
Tyrosine (µmole/total urine)	73.93 (33.25)	66.11 (29.72)	2.35, NS	.02
Phenylalanine (µmole/ total urine)	48.36 (17.90)	46.74 (20.78)	.27, NS	<.01

Notes. AAA, aromatic amino acid; ADHD, attention-deficit/hyperactivity disorder; NS, not significant; TD, typically developing.

In the combined group of children with ADHD and TD children, AAA blood spot concentrations were not significantly related to ratings of ADHD symptoms (all *r*s<.24 and *p*s>.004). Furthermore, blood spot concentrations of tryptophan, tyrosine or phenylalanine were not significantly related to protein intake or urinary AAA concentrations (all *r*s<.19 and *p*s>.004). There were no differences between the ADHD and TD group with regard to protein intake or to urinary concentrations of tryptophan, tyrosine, or phenylalanine, see Table 2.2.

No significant associations between AAAs and symptoms of comorbid psychiatric disorders were found (all *r*s<.20 and *p*s>.003) and therefore none of the previous analyses were repeated adjusting for the effects of symptoms of ODD, CD or ASD. Since the ADHD group consisted of considerably more males than the TD group, the group analyses were rerun with gender as a covariate. These analyses did not alter the results.

Discussion

The main objectives of this study are to examine whether children with ADHD have decreased AAA blood spot concentrations, and whether blood spot AAA concentrations

are related to symptoms of ADHD. In contrast to our hypothesis and some earlier studies on this topic (Baker et al., 1991; Bornstein et al., 1990; Comings, 1990), we did not find any differences in the AAA blood spot concentrations between the ADHD and TD group or associations between AAA blood spot concentrations and symptoms of ADHD. The finding that AAA alterations are not related to ADHD, argues against nutritional interventions with amino acid supplements for children with ADHD. In past years, some studies have examined the effects of AAA supplementation in children and adults with ADHD, with inconsistent results (Nemzer et al., 1986; Reimherr et al., 1987; Wood et al., 1985; Zametkin et al., 1984). The apparent lack of AAA deficiencies in ADHD might explain the conflicting results of amino acid supplementation on reducing ADHD symptoms (Hurt, Arnold, & Lofthouse, 2011). It might be that the association between AAA blood concentrations (being precursors of serotonin and dopamine) and the expression of ADHD symptoms is too indirect to detect. There are several other factors involved in the metabolism of dopamine and serotonin that could be aberrant in ADHD. It might be that an altered transport of tryptophan across the blood-brain barrier (Johansson et al., 2011) or an abnormal reuptake of dopamine and serotonin (Oades, 2010), moderate the association between AAA concentrations in blood and ADHD symptoms. Another explanation might be that AAA concentrations should be below a certain threshold before affecting the behaviour and functioning of children. In depletion studies, tryptophan concentrations drop by 60 to 90 percent (Booij, Van der Does, & Riedel, 2003) and tyrosine and phenylalanine concentrations by 74 percent (Montgomery, McTavish, Cowen, & Grasby, 2003), and are therefore much lower than baseline AAA concentrations, as measured in the current study. Clearly, the studies that focused hitherto on baseline concentrations of AAAs in ADHD were scarce and the results were inconsistent. Given our larger sample size, careful screening and correction for multiple comparisons, the current results challenge the hypothesis of AAA abnormalities in ADHD. Future studies regarding the serotonin and dopamine hypothesis in ADHD (Oades, 2010) may focus on other aspects of the serotonin and dopamine metabolism, such as the transport of AAAs (Johansson et al., 2011), rather than on decreased AAA concentrations in blood.

We did not find evidence for altered protein intake in ADHD or for an association between protein intake and blood spot AAA concentrations. Further, we did not find any evidence for an aberrant AAA excretion, since no increased urinary AAA concentrations were found in children with ADHD and urinary AAA concentrations were not significantly

related to blood spot AAA concentrations, in line with the results of previous studies on urinary AAA concentrations in ADHD (Baker et al., 1991; Bornstein et al., 1990; Zametkin et al., 1984). Therefore, we believe that abnormal AAA metabolism, due to a failure of the intestines to absorb AAAs into the bloodstream or increased excretion into the urine, are no plausible causes of ADHD symptoms. Lastly, we did not find a confounding role of ODD, CD or ASD symptoms in the association between AAAs and ADHD.

There are some limitations to the current study that should be noted. Firstly, we measured AAA concentrations in blood and urine, which represent only two factors in the metabolic pathway of serotonin and dopamine. Therefore, we can only draw conclusions regarding the presence of AAAs in the blood, which is a first prerequisite for an adequate biosynthesis of serotonin and dopamine. Secondly, while our study is the largest of its kind and had an adequate power to detect medium-sized effects, our study was not sufficiently powered to detect small-sized effects. We found a relatively high odds ratio of 2.2 for being diagnosed with ADHD when having low phenylalanine concentrations, but this result just escaped conventional levels of significance. Therefore, our study does not definitively rule out that low phenylalanine concentrations are present in (a subgroup of) children with ADHD. For instance, it might be that only children with severe deficiencies in executive functioning have decreased phenylalanine concentrations, as an altered dopamine functioning in the prefrontal cortex and the striatum is thought to impair executive functions, including sustained attention and interference control in ADHD (Del Campo, Chamberlain, Sahakian, & Robbins, 2011; Oades, 2008). Despite the limitations, our study is the first to explore AAA concentrations in children with ADHD using a well-defined and relatively large sample, showing that AAA abnormalities are not related to ADHD.

References

Altorf-van der Kuil, W., Engberink, M.F., De Neve, M., Van Rooij, F.J., Hofman, A., Van't Veer, P., . . . Geleijnse, J.M. (2013). Dietary amino acids and the risk of hypertension in a Dutch older population: The Rotterdam Study. *American Journal of Clinical Nutrition, 97*(2), 403-410.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders, fifth edition*. Arlington: American Psychiatric Association.

Baker, G.B., Bornstein, R.A., Rouget, A.C., Ashton, S.E., Van Muyden, J.C., & Coutts, R.T. (1991). Phenylethylaminergic mechanisms in attention-deficit disorder. *Biological Psychiatry, 29*(1), 15-22.

Banerjee, T.D., Middleton, F., & Faraone, S.V. (2007). Environmental risk factors for attention-deficit hyperactivity disorder. *Acta Paediatrica, 96*(9), 1269-1274.

Bender, D.A. (1983). Biochemistry of tryptophan in health and disease. *Molecular Aspects of Medicine, 6*(2), 101-197.

Bölte, S., Poustka, F., & Constantino, J.N. (2008). Assessing autistic traits: Cross-cultural validation of the social responsiveness scale (SRS). *Autism Research, 1*(6), 354-363.

Booij, L., Van der Does, A., & Riedel, W. (2003). Monoamine depletion in psychiatric and healthy populations: Review. *Molecular Psychiatry, 8*(12), 951-973.

Bornstein, R.A., Baker, G.B., Carroll, A., King, G., Wong, J.T., & Douglass, A.B. (1990). Plasma amino acids in attention deficit disorder. *Psychiatry Research, 33*(3), 301-306.

Chace, D.H., Sherwin, J.E., Hillman, S.L., Lorey, F., & Cunningham, G.C. (1998). Use of phenylalanine-to-tyrosine ratio determined by tandem mass spectrometry to improve newborn screening for phenylketonuria of early discharge specimens collected in the first 24 hours. *Clinical Chemistry, 44*(12), 2405-2409.

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Hillsdale: Erlbaum.

Comings, D.E. (1990). Blood serotonin and tryptophan in Tourette syndrome. *American Journal of Medical Genetics, 36*(4), 418-430.

Constantino, J., Davis, S.A., Todd, R.D., Schindler, M.K., Gross, M.M., Brophy, S.L., . . . Reich, W. (2003). Validation of a brief quantitative measure of autistic traits: Comparison of the social responsiveness scale with the autism diagnostic interview-revised. *Journal of Autism and Developmental Disorders, 33*(4), 427-433.

Constantino, J., & Gruber, C. (2005). *Social Responsiveness Scale (SRS)*. Los Angeles, CA: Western Psychological Services.

Del Campo, N., Chamberlain, S.R., Sahakian, B.J., & Robbins, T.W. (2011). The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. *Biological Psychiatry, 69*(12), e145-e157.

Dolina, S., Margalit, D., Malitsky, S., & Rabinkov, A. (2014). Attention-deficit hyperactivity disorder (ADHD) as a pyridoxine-dependent condition: Urinary diagnostic biomarkers. *Medical Hypotheses, 82*(1), 111-116.

Faraone, S.V., Perlis, R.H., Doyle, A.E., Smoller, J.W., Goralnick, J.J., Holmgren, M.A., & Sklar, P. (2005). Molecular genetics of attention-deficit/hyperactivity disorder. *Biological Psychiatry, 57*(11), 1313-1323.

Fekkes, D., Voskuilen-Kooyman, A., Jankie, R., & Huijmans, J. (2000). Precise analysis of primary amino acids in urine by an automated high-performance liquid chromatography method: Comparison with ion-exchange chromatography. *Journal of Chromatography B, 744*(1), 183-188.

Felger, J.C., Li, L., Marvar, P.J., Woolwine, B.J., Harrison, D.G., Raison, C.L., & Miller, A.H. (2013). Tyrosine metabolism during interferon-alpha administration: Association with fatigue and CSF dopamine concentrations. *Brain, Behavior, and Immunity, 31*, 153-160.

Gabriele, S., Sacco, R., & Persico, A.M. (2014). Blood serotonin levels in autism spectrum disorder: A systematic review and meta-analysis. *European Neuropsychopharmacology, 24*(6), 919-929.

Gizer, I.R., Ficks, C., & Waldman, I.D. (2009). Candidate gene studies of ADHD: a meta-analytic review. *Human Genetics, 126*(1), 51-90.

Harmer, C.J., McTavish, S.F.B., Clark, L., Goodwin, G.M., & Cowen, P.J. (2001). Tyrosine depletion attenuates dopamine function in healthy volunteers. *Psychopharmacology, 154*(1), 105-111.

Hay, D.A., Bennett, K.S., Levy, F., Sergeant, J., & Swanson, J. (2007). A twin study of attention-deficit/hyperactivity disorder dimensions rated by the strengths and weaknesses of ADHD-symptoms and normal-behavior (SWAN) scale. *Biological Psychiatry, 61*(5), 700-705.

Hoshino, Y., Ohno, Y., & Yamamoto, T. (1985). Plasma free tryptophan concentration in children with attention deficit disorder. *Folia Psychiatrica et Neurologica Japonica, 39*(4), 531-536.

Hoshino, Y., Yamamoto, T., Kaneko, M., Tachibana, R., Watanabe, M., Ono, Y., & Kumashiro, H. (1984). Blood serotonin and free tryptophan concentration in autistic children. *Neuropsychobiology, 11*(1), 22-27.

Hurt, E.A., Arnold, L.E., & Lofthouse, N. (2011). Dietary and nutritional treatments for attention-deficit/hyperactivity disorder: Current research support and recommendations for practitioners. *Current Psychiatry Reports, 13*(5), 323-332.

Johansson, J., Landgren, M., Fernell, E., Vumma, R., Ahlin, A., Bjerkenstedt, L., & Venizelos, N. (2011). Altered tryptophan and alanine transport in fibroblasts from boys with attention-deficit/hyperactivity disorder (ADHD): An in vitro study. *Behavioral and Brain Functions, 7*(40), 1-7.

Kand'ár, R., & Žáková, P. (2009). Determination of phenylalanine and tyrosine in plasma and dried blood samples using HPLC with fluorescence detection. *Journal of Chromatography B, 877*(30), 3926-3929.

Kema, I.P., Schellings, A.M., Hoppenbrouwers, C.J., Rutgers, H.M., de Vries, E.G., & Muskiet, F.A. (1993). High performance liquid chromatographic profiling of tryptophan and related indoles in body fluids and tissues of carcinoid patients. *Clinica Chimica Acta, 221*(1), 143-158.

Keszthelyi, D., Troost, F., & Masclee, A. (2009). Understanding the role of tryptophan and serotonin metabolism in gastrointestinal function. *Neurogastroenterology & Motility, 21*(12), 1239-1249.

Khalaf, A.-N., Böcker, J., Kerp, L., & Petersen, K.-G. (1991). Urine screening in outdoor volunteers: Day versus night versus 24 hour collection. *Clinical Chemistry and Laboratory Medicine, 29*(3), 185-188.

Kiive, E., & Harro, J. (2013). The effect of serotonin transporter gene promoter polymorphism on adolescent and adult ADHD symptoms and educational attainment: A longitudinal study. *European Psychiatry, 28*(6), 372-378.

Kopple, J.D. (2007). Phenylalanine and tyrosine metabolism in chronic kidney failure. *Journal of Nutrition, 137*(6), 1586S-1590S.

Lakes, K.D., Swanson, J.M., & Riggs, M. (2012). The reliability and validity of the English and Spanish Strengths and Weaknesses of ADHD and Normal Behavior Rating Scales in a preschool sample: Continuum measures of hyperactivity and inattention. *Journal of Attention Disorders, 16*(6), 510-516.

Lavigne, J.V., Herzing, L.B., Cook, E.H., LeBailly, S.A., Gouze, K.R., Hopkins, J., & Bryant, F.B. (2013). Gene X environment effects of serotonin transporter, dopamine receptor D4, and monoamine oxidase A genes with contextual and parenting risk factors on symptoms of oppositional defiant disorder, anxiety, and depression in a community sample of 4-year-old children. *Development and Psychopathology, 25*(2), 555-575.

Li, D., Sham, P.C., Owen, M.J., & He, L. (2006). Meta-analysis shows significant association between dopamine system genes and attention deficit hyperactivity disorder (ADHD). *Human Molecular Genetics, 15*(14), 2276-2284.

Madras, B.K., Miller, G.M., & Fischman, A.J. (2005). The dopamine transporter and attention-deficit/hyperactivity disorder. *Biological Psychiatry, 57*(11), 1397-1409.

Mette, C., Zimmermann, M., Grabemann, M., Abdel-Hamid, M., Uekermann, J., Biskup, C.S., . . . Kis, B. (2013). The impact of acute tryptophan depletion on attentional performance in adult patients with ADHD. *Acta Psychiatrica Scandinavica, 128*(2), 124-132.

Montgomery, A.J., McTavish, S.F., Cowen, P.J., & Grasby, P.M. (2003). Reduction of brain dopamine concentration with dietary tyrosine plus phenylalanine depletion: An [^{11}C]raclopride PET study. *American Journal of Psychiatry, 160*, 1887-1889.

National Institute for Public Health and the Environment. (2013). *Nederlands Voedingsstoffenbestand. NEVO-online versie 2013/4.0.*

Nemzer, E.D., Arnold, L.E., Votolato, N.A., & McConnell, H. (1986). Amino acid supplementation as therapy for attention deficit disorder. *Journal of the American Academy of Child Psychiatry, 25*(4), 509-513.

O'Mahony, S., Clarke, G., Borre, Y., Dinan, T., & Cryan, J. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behavioural Brain Research, 277*, 32-48.

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

Oades, R.D. (2010). The role of serotonin in attention-deficit hyperactivity disorder (ADHD). In C.P. Müller & B.L. Jacobs (Eds.), *Handbook of the behavioral neurobiology of serotonin* (pp. 565-584). London: Elsevier.

Oades, R.D., Dauvermann, M.R., Schimmelmann, B.G., Schwarz, M.J., & Myint, A.M. (2010). Attention-deficit hyperactivity disorder (ADHD) and glial integrity: S100B, cytokines and kynurenine metabolism - effects of medication. *Behavioral and Brain Functions, 6*(29), 1-14.

Oades, R.D., Lasky-Su, J., Christiansen, H., Faraone, S.V., Sonuga-Barke, E.J., Banaschewski, T., . . . Ebstein, R.P. (2008). The influence of serotonin-and other genes on impulsive behavioral aggression and cognitive impulsivity in children with attention-deficit/hyperactivity disorder (ADHD): Findings from a family-based association test (FBAT) analysis. *Behavioral and Brain Functions, 4*(48), 1-14.

Oosterlaan, J., Baeyens, D., Scheres, A., Antrop, I., Roeyers, H., & Sergeant, J. (2008). *VvGK6-16: Vragenlijst voor Gedragsproblemen bij Kinderen 6 tot en met 16 jaar.* Amsterdam: Harcourt Publishers.

Pecce, R., Scolamiero, E., Ingenito, L., Parenti, G., & Ruoppolo, M. (2013). Optimization of an HPLC method for phenylalanine and tyrosine quantization in dried blood spot. *Clinical Biochemistry, 46*(18), 1892-1895.

Pelham, W.E., Aronoff, H.R., Midlam, J.K., Shapiro, C.J., Gnagy, E.M., Chronis, A.M., . . . Waxmonsky, J. (1999). A comparison of Ritalin and Adderall: Efficacy and time-course in children with attention-deficit/hyperactivity disorder. *Pediatrics, 103*(4), e43.

Pelham, W.E., Gnagy, E.M., Greenslade, K.E., & Milich, R. (1992). Teacher ratings of DSM-III-R symptoms for the disruptive behavior disorders. J*ournal of the American Academy of Child & Adolescent Psychiatry, 31*(2), 210-218.

Rashed, M.S., Bucknall, M.P., Little, D., Awad, A., Jacob, M., Alamoudi, M., . . . Ozand, P.T. (1997). Screening blood spots for inborn errors of metabolism by electrospray tandem mass spectrometry with a microplate batch process and a computer algorithm for automated flagging of abnormal profiles. *Clinical Chemistry, 43*(7), 1129-1141.

Reimherr, F.W., Wender, P., Wood, D., & Ward, M. (1987). An open trial of L-tyrosine in the treatment of attention deficit disorder, residual type. *American Journal of Psychiatry, 144*(8), 1071-1073.

Shaffer, D., Fisher, P., Lucas, C.P., Dulcan, M.K., & Schwab-Stone, M.E. (2000). NIMH Diagnostic Interview Schedule for Children Version IV (NIMH DISC-IV): Description, differences from previous versions, and reliability of some common diagnoses. *Journal of the American Academy of Child & Adolescent Psychiatry, 39*(1), 28-38.

Spencer, T.J., Biederman, J., Faraone, S.V., Madras, B.K., Bonab, A.A., Dougherty, D.D., . . . Fischman, A.J. (2013). Functional genomics of attention-deficit/hyperactivity disorder (ADHD) risk alleles on dopamine transporter binding in ADHD and healthy control subjects. *Biological Psychiatry, 74*(2), 84-89.

Stadler, C., Zepf, F., Demisch, L., Schmitt, M., Landgraf, M., & Poustka, F. (2007). Influence of rapid tryptophan depletion on laboratory-provoked aggression in children with ADHD. *Neuropsychobiology, 56*(2-3), 104-110.

Swanson, J., Kinsbourne, M., Nigg, J., Lanphear, B., Stefanatos, G.A., Volkow, N., . . . Wadhwa, P.D. (2007). Etiologic subtypes of attention-deficit/hyperactivity disorder: Brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. *Neuropsychology Review, 17*(1), 39-59.

Swanson, J., Schuck, S., Mann, M., Carlson, C., Hartman, K., Sergeant, J., . . . McCleary, R. (2005). *Categorical and dimensional definitions and evaluations of symptoms of ADHD: The SNAP and the SWAN ratings scales.* Retrieved from http://www.adhd.net/SNAP_SWAN.pdf.

Tabachnick, B.G., & Fidell, L.S. (2001). *Using multivariate statistics* (4th ed.). Boston: Allyn and Bacon.

Van der Zwaluw, N.L., Van de Rest, O., Tieland, M., Adam, J.J., Hiddink, G.J., Van Loon, L.J., & De Groot, L.C. (2014). The impact of protein supplementation on cognitive performance in frail elderly. *European Journal of Nutrition, 53*(3), 803-812.

Van Goozen, S.H., Fairchild, G., Snoek, H., & Harold, G.T. (2007). The evidence for a neurobiological model of childhood antisocial behavior. *Psychological Bulletin, 133*(1), 149-182.

Van Kernebeek, H., Oosting, S., Feskens, E., Gerber, P., & De Boer, I. (2014). The effect of nutritional quality on comparing environmental impacts of human diets. *Journal of Cleaner Production, 73*, 88-99.

Westenbrink, S., & Jansen-van der Vliet, M. (2013). *NEVO-online 2013: Achtergrondinformatie [background information]*. Bilthoven, the Netherlands: Rijksinstituut voor Volksgezondheid en Milieu [National Institute for Public Health and the Environment].

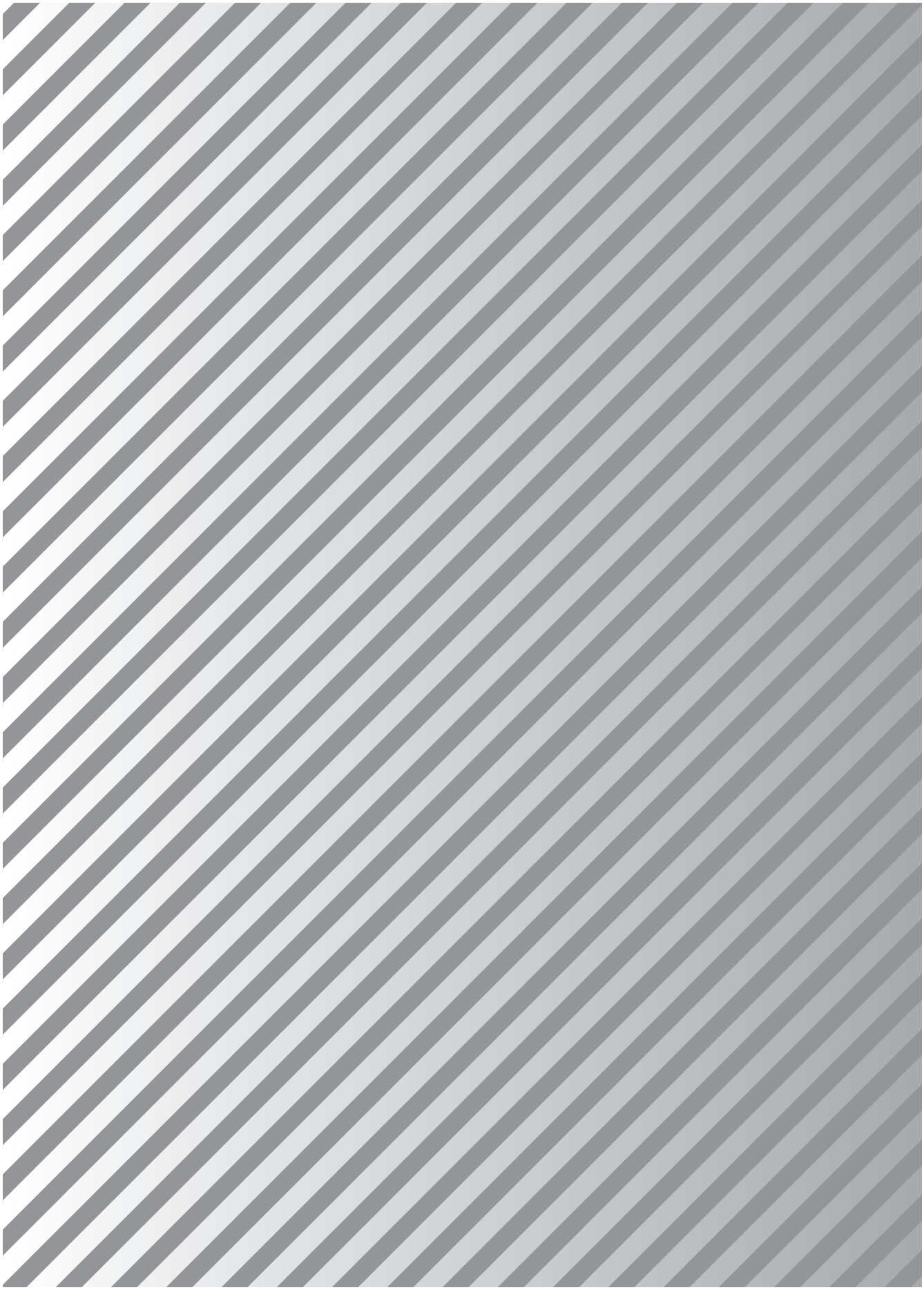
Wood, D.R., Reimherr, F.W., & Wender, P.H. (1985). Treatment of attention deficit disorder with DL-phenylalanine. *Psychiatry Research, 16*(1), 21-26.

Zametkin, A.J., Karoum, F., & Rapoport, J.L. (1987). Treatment of hyperactive children with {d}-phenylalanine. *American Journal of Psychiatry, 144*(6), 792-794.

Zametkin, A.J., Karoum, F., Rapoport, J.L., Brown, G.L., & Wyatt, R.J. (1984). Phenylethylamine excretion in attention deficit disorder. *Journal of the American Academy of Child Psychiatry, 23*(3), 310-314.

Zepf, F.D., Holtmann, M., Stadler, C., Demisch, L., Schmitt, M., Woeckel, L., & Poustka, F. (2008). Diminished serotonergic functioning in hostile children with ADHD: Tryptophan depletion increases behavioural inhibition. *Pharmacopsychiatry, 41*(2), 60-65.

Zimmermann, M., Grabemann, M., Mette, C., Abdel-Hamid, M., Ueckermann, J., Kraemer, M., . . . Zepf, F.D. (2012). The effects of acute tryptophan depletion on reactive aggression in adults with attention-deficit/hyperactivity disorder (ADHD) and healthy controls. *PLOS ONE, 7*(3), e32023.



Chapter 3

Homocysteine concentrations and neurocognitive functioning in children with attention-deficit/hyperactivity disorder

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Abstract

Objective. We investigated whether children with attention-deficit/hyperactivity disorder (ADHD) had increased blood concentrations of homocysteine compared to typically developing (TD) children. Secondly, we examined whether homocysteine concentrations in children with ADHD were related to symptoms of ADHD, which would lend further support to the role of homocysteine in ADHD, and to neurocognitive functioning within this group. Further, we studied if intake of folate and vitamin B12 (determinants of homocysteine) were associated with homocysteine concentrations.

Methods. In this observational case-control study homocysteine concentrations were assessed in blood spots of 55 children with ADHD and 54 TD children, aged 6 to 13 years. Parent and teacher questionnaires assessed symptoms of ADHD. Neurocognitive functioning was measured using the Digit Span Task, Grid Task and Flanker Task, targeting verbal and visuospatial working memory, interference control, variability in responding, and lapses of attention. Intake of folate and vitamin B12 was measured using nutritional diaries.

Results. Children with ADHD did not differ from controls in their homocysteine blood spot concentrations. Homocysteine concentrations were neither related to symptoms of ADHD nor to neurocognitive functioning in children with ADHD. Intake of folate and vitamin B12 was not associated with homocysteine concentrations.

Conclusion. A childhood diagnosis of ADHD and symptoms of the disorder seem unrelated to homocysteine blood spot concentrations, and increased homocysteine blood spot concentrations do not seem to play a role in neurocognitive problems in childhood ADHD.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a childhood psychiatric disorder characterised by a persistent pattern of age-inappropriate levels of inattention and/or hyperactivity-impulsivity (American Psychiatric Association, 2013). Neurocognitive dysfunctions play a central role in explanatory models of the disorder (Castellanos & Tannock, 2002; Rommelse & de Zeeuw, 2014; Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005); ADHD is associated with numerous neurocognitive deficits, such as impaired working memory (Willcutt et al., 2005) and poor inhibitory control (Barkley, 1997; Willcutt et al., 2005). It is important to investigate the aetiology of neurocognitive deficiencies in children with ADHD, in order to gain more insight into neurobiological causal pathways of ADHD. Research on the underlying mechanisms of impaired neurocognitive functioning in ADHD has predominantly focussed on alterations in the structure and function of the brain (Faraone et al., 2015).

An alternative approach to study the underlying mechanisms of impaired neurocognitive functioning in ADHD, is to investigate amino acid concentrations that are a prerequisite for healthy brain functioning. A potential candidate for such an approach is homocysteine, as high concentrations of homocysteine can have detrimental effects on neurocognitive performance, by causing DNA damage, disturbed methylation, cell death or by altering the functioning of glutamate receptors (Mattson & Shea, 2003). Homocysteine has been found associated with neurocognitive performance in neurodegenerative diseases (Teunissen et al., 2005), in the normal aging population (Garcia & Zanibbi, 2004), as well as in psychiatric populations (Dias, Brissos, Cardoso, Andreazza, & Kapczinski, 2009; Ford, Flicker, Singh, Hirani, & Almeida, 2013). Homocysteine is biosynthesised from methionine, an essential amino acid that is obtained by digestion of proteins. Compared to patients with bipolar disorder with normal homocysteine concentrations, patients with bipolar disorder with elevated concentrations of homocysteine, showed poorer performance on tasks measuring verbal working memory and interference control (Dias et al., 2009). Further, in studies with healthy elderly, negative associations have been found between homocysteine and cognitive performance (Dufouil, Alpérovitch, Ducros, & Tzourio, 2003; Mooijaart et al., 2005; Prins et al., 2002). For instance, healthy elderly with hyperhomocysteinemia (elevated levels of homocysteine) had lower scores on a global test of cognitive functioning and on a task measuring attention, compared to individuals with normal homocysteine concentrations (Dufouil et al., 2003). However, there are also

studies that showed no relation between homocysteine and neurocognitive functioning in either healthy adults (Dias et al., 2009) or healthy elderly subjects (Kalmijn et al., 1999). Strikingly, the neurocognitive functions that seem to be related to homocysteine concentrations (working memory, interference control and attention) (Dias et al., 2009; Teunissen et al., 2005), are exactly those that are impaired in (subgroups of) children with ADHD (Mullane, Corkum, Klein, & McLaughlin, 2009; Tamm et al., 2012; Willcutt et al., 2005). Thus far, the relation between homocysteine and neurocognitive functioning has not been studied in children or adults with ADHD. In the current study, we explored the hypothesis that neurocognitive deficits observed in ADHD may be related to high concentrations of homocysteine.

Increased homocysteine concentrations could be caused by dietary or genetic risk factors, such as a dietary deficiency or a genetic defect to produce adequate amounts of bioactive folate and vitamin B12 (Blom, Shaw, Den Heijer, & Finnell, 2006; Bottiglieri et al., 2000). Vitamin B12 and in particular folate are strong determinants of homocysteine, since the conversion of homocysteine to methionine is dependent on the cofactors folate and vitamin B12. A deficiency of folate or vitamin B12 therefore leads to increased concentrations of homocysteine in the blood (Mattson & Shea, 2003). In the current study, we investigated whether the hypothesised increased homocysteine concentrations in children with ADHD are related to dietary deficiencies, as there is some evidence for decreased folate intake in children with ADHD (Durá-Travé & Gallinas-Victoriano, 2014).

Even though some studies focused on homocysteine concentrations in healthy children (Van Beynum et al., 2005), thus far, no studies have examined whether increased homocysteine concentrations are involved in childhood ADHD. Recently a study in adults showed that plasma concentrations of homocysteine were decreased in adults with ADHD as compared to normal controls (Karababa et al., 2014), which was contrary to expectations. However, these results should be interpreted with caution, given the small sample size and limited diagnostic screening of participants. In the current study it is examined whether children with ADHD have increased concentrations of homocysteine compared to typically developing (TD) children. Further, we examine whether homocysteine concentrations in children with ADHD are (a) positively related to symptoms of ADHD, (b) negatively related to neurocognitive functioning in ADHD, which would lend further support to the role of homocysteine in ADHD, and (c) negatively related to intake of folate and vitamin B12, which would be informative for a dietary

risk factor for ADHD. The results might provide insight into one potential underlying mechanism of neurocognitive deficits found in this population.

Methods

Participants

Subjects were 55 children with ADHD (76 percent males) and 54 TD children (52 percent males), aged between 6 and 13 years. Inclusion criteria for the ADHD group were: (a) a clinical diagnosis of ADHD according to DSM-IV criteria, (b) confirmation of this diagnosis by the Diagnostic Interview Schedule for Children, fourth edition, administered to parents (DISC-IV-P) (Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000), (c) significant ADHD symptoms, as indicated by scores >90th percentile on at least one of the ADHD scales (Inattention and Hyperactivity/Impulsivity scales) of the parent version of the Disruptive Behaviour Disorder Rating Scale (DBDRS) (Pelham, Gnagy, Greenslade, & Milich, 1992), and (d) pervasive ADHD symptoms, as indicated by scores >75th percentile on at least one of the ADHD scales of the teacher version of the DBDRS. Having a comorbid diagnosis (for example autism spectrum disorder or learning disability) was no exclusion criterion, neither was treatment with stimulant medication. Children on stimulant medication (60 percent of the ADHD group) discontinued drug use 24 hours before testing, in order to allow complete washout (Pelham et al., 1999). Inclusion criteria for the TD group were: (a) absence of a clinical diagnosis of any developmental or behavioural disorder (including ADHD, established by parent report), and (b) scores <90th percentile on both parent- and teacher-rated ADHD scales of the DBDRS. Children of both groups with an IQ<70 were excluded.

Materials

Diagnostic assessment

Parents of children eligible for inclusion in the ADHD group were assessed with the ADHD section of the DISC-IV-P (Shaffer et al., 2000). The DISC-IV-P is a widely used standardised diagnostic interview for the assessment of DSM-IV childhood psychiatric disorders.

ADHD symptom severity was assessed in both groups using the DBDRS, filled out by one of the parents and teacher (Pelham et al., 1992). The DBDRS measures the DSM-IV symptoms of ADHD (and other externalising disorders) using a 4-point Likert scale. Scores on the Inattention and Hyperactivity/Impulsivity scales were used, with higher scores indicating worse symptom levels.

Full scale IQ was estimated using a short form of the Wechsler Intelligence Scale for Children-III (WISC-III; Wechsler, 1991), comprising the subtests Vocabulary, Arithmetic, Block Design and Picture Arrangement. This short form of four subtests has an excellent reliability (*r*=.95) and validity (*r'*=.90) (Sattler, 2008).

Neurocognitive functioning

To measure verbal working memory, the backward condition of the Digit Span Task of the WISC-III was used (Wechsler, 1991). In the backward condition, the child is required to repeat in reversed order a sequence of numbers expressed verbally by the interviewer. There were seven sequence levels, starting from a span of two digits up to a span of eight digits. The product of the number of correct responses and the highest obtained span served as measure of verbal working memory (Kessels, Van Zandvoort, Postma, Kappelle, & De Haan, 2000).

Visuospatial working memory was measured using the backward condition of the Grid Task (Bergman Nutley, Soderqvist, Bryde, Humphreys, & Klingberg, 2010), in which the child is required to repeat in reversed order a sequence of visual stimuli (yellow dots) presented on a computer screen in a four by four grid. The computer mouse was used to respond. There were nine sequence levels, starting from two dots up to a span of ten dots. Each level consisted of two sublevels; sublevel A in which the sequence followed a logical pattern and the more difficult sublevel B in which the sequence showed no clear pattern. The product of the number of correct responses and the highest obtained span served as measure of visuospatial working memory (Kessels et al., 2000), with 0.5 point added to the highest obtained span when sublevel B was reached (Bergman Nutley et al., 2010).

Interference control, variability in responding and lapses of attention were measured using an adapted version of the Eriksen Flanker Task (Eriksen & Eriksen, 1974; Scheres et al., 2003). In each trial, a target stimulus (black arrow) appeared at the centre of a computer screen, pointing either to the left or right, and the child was asked to press the corresponding response button (left or right button). The target was flanked by two neutral items (rectangles) in the neutral condition, by arrows pointing in the same direction in the congruent condition, and by arrows pointing in the opposite direction in the incongruent condition. Forty-eight neutral, 48 congruent and 48 incongruent trials were presented in random order. Difference scores between congruent and

incongruent trials were calculated for latency on correct trials and for accuracy, which served as measures of interference control (Mullane et al., 2009). Individual response time distributions derived from the correct neutral trials were used to measure (a) variability in responding, using the SD (sigma) of the normal component of the ex-Gaussian distribution, and (b) attentional lapses, using the *M* plus *SD* of the exponential component of the ex-Gaussian distribution (tau) (Whelan, 2010).

Homocysteine

To examine total homocysteine concentrations, a dried blood spot technique was used. Collecting blood spots is less invasive for children than taking venous blood samples. Validity of the dried blood spot technique is supported by a strong relationship between homocysteine in blood spots and in plasma (r=.94) (Bowron, Barton, Scott, & Stansbie, 2005). Homocysteine concentrations measured in dried blood spots are circa 40 percent lower than homocysteine concentrations obtained by plasma samples, caused by haemolysis during the drying of the blood spot (Bowron et al., 2005). Since no adequate paediatric reference values for blood spot homocysteine concentrations are available, concentrations in the ADHD group are compared to concentrations in a group of TD children. A blood spot of each child was collected using a disposable safety lancet. The blood drop was spotted onto a specially designed blood stain card. Concentrations of total homocysteine in blood spots (in µmol/L) were determined in the clinical chemistry laboratory of our academic medical center, using liquid chromatography–mass spectrometry (LC-MS), based on the protocol of Gempel, Gerbitz, Casetta, and Bauer (2000). Reliability of the LC-MS/MS was confirmed by examining the inter-assay variance (being 11 percent), intra-assay variance (being 10 percent) and recovery (being 105.5 percent).

Nutrition

The ingestion of folate and vitamin B12 was assessed during three days, using a parent reported nutritional diary. Standardised dietary records and instructions were provided. Parents were instructed to register all consumed foods and drinks in the dietary record and to express the consumed amounts as accurate as possible. The amount of folate and vitamin B12 intake (in µg/day) was calculated by a dietician based on a computerised version of a food composition database (National Institute for Public Health and the Environment, 2013). The food composition database contains over 2000 food products with information about the nutritional composition of these food products (Westenbrink

& Jansen-van der Vliet, 2013). The database is widely used for scientific purposes (e.g. Altorf-van der Kuil et al., 2013; Van der Zwaluw et al., 2014; Van Kernebeek, Oosting, Feskens, Gerber, & De Boer, 2014). The use of a three-day parent reported nutritional diary has been validated in a study of primary school-aged children, with a low mean level (four percent) of overestimation (O'Connor et al., 2001).

Procedure

This study received approval from the local medical ethical committee (#NL39922.029.12) and has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki. Written informed consent was obtained of parents of all children, and of children ≥12 years, prior to participation. Children with ADHD were recruited from mental health outpatient clinics, through the parental association for children with behavioural problems, and through a university research website. The TD group was recruited from regular primary schools throughout the country. The data were collected between February 2013 and July 2014. The ADHD and TD group were recruited simultaneously, to control for possible seasonal effects on food intake or homocysteine concentrations. Children were tested at their own school. The blood spot was collected in the early morning of the test day, to rule out the effects of diurnal variation of homocysteine concentrations (Guttormsen, Schneede, Fiskerstrand, Ueland, & Refsum, 1994). Fasting was not required prior to assessment of blood spots, as blood spots were collected within a short time span (<2 hours) after breakfast. Registration of dietary food intake started in the morning of the testing day, and continued for the subsequent three days (day 1 to day 3). Children on stimulant medication discontinued drug use one day prior to participation (day 0), and during the assessment of blood and food intake (day 1 to day 3), to ensure complete washout during the assessments.

Data analysis

All statistical analyses were performed using R, version 3.2.1. Initially a total of 142 children participated in the current study. However, in 22 children with ADHD and 11 TD children the level of homocysteine was too low to be detected (under 1.0 µmol/L). Since the proportion of participants with an undetectable level of homocysteine was similar in the ADHD and TD groups (χ^2=2.68, p=.10), it was concluded that the extremely low concentrations of homocysteine were not specific to ADHD or controls. It was chosen to exclude these 33 children from further analyses to avoid the risk of artificially deflated means. All dependent variables were inspected on outliers and missing values within the

ADHD and TD group. Winsorising was applied to outliers in homocysteine concentrations (*n*=2), the intake of folate and vitamin B12 (*n*=3), and neurocognitive measures (*n*=4) (Tabachnick & Fidell, 2001). Missing data in the intake of folate and vitamin B12 (*n*=3), the measures obtained by the Flanker Task (*n*=1) and the DBDRS (*n*=2), due to technical errors or to non-adherence of parents to the protocol, were randomly distributed and replaced using group means. Group differences in gender were examined using a chi-squared test, and group differences in age, IQ and ADHD symptoms were examined using one-way analysis of variance (ANOVA).

Firstly, it was investigated whether the groups differed in homocysteine concentrations and neurocognitive functioning, using ANOVA. Effect sizes were calculated in terms of partial eta squared, and interpreted as small (>.01), medium (>.06) or large (>.14) (Cohen, 1988). Secondly, using Pearson's correlations it was investigated whether homocysteine concentrations in children with ADHD were related to (a) symptoms of ADHD (using the parent- and teacher-rated ADHD scales on the DBDRS), (b) neurocognitive functioning and (c) folate and vitamin B12 intake. In case of significant correlation coefficients, regression analyses were performed to examine the potential confounding effects of age, gender and IQ on the association between homocysteine concentrations and the significant outcome measures.

Results

For group characteristics and dependent variables, see Table 3.1. The ADHD and TD group did not differ significantly in mean age and IQ. The ADHD group consisted of considerably more males and showed more parent- and teacher-rated ADHD symptoms than the TD group. To examine the potential confounding effect of gender differences, all group analyses were rerun with gender as covariate. The DISC-IV-P indicated that 43 children met DSM-IV criteria for the combined subtype of ADHD, 8 children for the predominantly inattentive subtype, and 4 children for the predominantly hyperactive-impulsive subtype.

Table 3.1. Group characteristics of the ADHD group (*n*=55) and TD group (*n*=54)

	ADHD group		TD group		Statistic (χ^2/*F*)
	M (SD)	Range	*M (SD)*	Range	
Males *n* (%)	42 (76)		28 (52)		7.12*
Age (in years)	9.73 (1.72)	6.33 - 13.42	9.90 (1.70)	6.67 - 12.42	.27, NS
Estimated IQ	101.33 (13.67)	70 - 132	104.11 (13.88)	71 - 129	1.11, NS
Parent-rated symptoms					
Inattention[a]	17.51 (5.22)	6 - 27	3.41 (3.14)	0 - 11	290.60**
Hyperactivity/Impulsivity[a]	16.13 (5.93)	1 - 27	3.31 (2.77)	0 - 10	207.60**
Teacher-rated symptoms					
Inattention[a]	15.09 (5.69)	2 - 27	2.13 (2.62)	0 - 11	231.80**
Hyperactivity/Impulsivity[a]	13.60 (7.13)	0 - 27	1.69 (2.40)	0 - 9	135.80**
Homocysteine (µmol/L)	4.16 (2.46)	1.00 - 11.54	3.65 (1.95)	1.10 - 8.00	1.47, NS
Folate (intake, µg/day)	159.56 (49.42)	70 - 271	166.65 (53.77)	90 - 322	.51, NS
Vitamin B12 (intake, µg/day)	3.27 (1.45)	.76 - 7.10	2.85 (1.00)	.69 - 5.19	2.99, NS
Verbal working memory	14.71 (9.12)	4 - 42	16.69 (8.05)	4 - 36	1.44, NS
Visuospatial working memory	43.81 (22.55)	4.0 - 97.5	55.24 (26.00)	18.0 - 133.1	6.02*
Interference control latency (ms)	78.80 (68.36)	-16.38 - 246.67	67.26 (38.10)	-.83 - 150.02	1.18, NS
Interference control accuracy (%)	-2.00 (3.47)	-12 - 4	-1.65 (2.66)	-10 - 6	.35, NS
Variability in responding (sigma in ms)	90.36 (54.40)	10.02 - 248.83	70.86 (32.36)	16.15 - 166.94	5.15*
Attentional lapses (tau in ms)	156.93 (75.96)	32.38 - 320.06	100.60 (47.11)	22.39 - 241.72	21.55**

Notes. [a]Disruptive Behaviour Disorder Rating Scale. *p<.05, **p<.01. ADHD, attention-deficit/hyperactivity disorder; NS, not significant; TD, typically developing.

Our results showed that all homocysteine concentrations in blood spots in our sample were under 12 µmol/L. No group differences were found in homocysteine concentrations. The ADHD group performed significantly worse on visuospatial working memory (F(1,107)=6.02, p=.02, pη^2=.05), showed more variability in responding, as evidenced by an increased sigma (F(1,107)=5.15, p=.03, pη^2=.05), and had more attentional lapses, as evidenced by an increased tau (F(1,107)=21.55, p<.01, pη^2=.17). No group differences were found for the other neurocognitive measures (verbal working memory and interference control), see Table 3.1. Including gender as covariate did not change the findings. Repeating the group analyses including the 33 participants with an undetectable level of homocysteine did not alter the results.

Associations between homocysteine concentrations and measures of (a) symptoms of ADHD, (b) neurocognitive functioning and (c) folate and vitamin B12 intake were examined in the ADHD group. All measures were normally distributed. Results showed that homocysteine concentrations were neither significantly related to ADHD symptoms, nor to any of the neurocognitive measures, nor to intake of folate and vitamin B12, see Table 3.2. Repeating the analyses including the 33 participants with an undetectable level of homocysteine did not alter the results.

Table 3.2. Pearson correlations between homocysteine and measures of ADHD symptoms, neurocognitive functioning and nutrition in the ADHD group (n=55)

	r	*p*
ADHD symptoms (DBDRS)		
Inattention[a]	.00	.99
Hyperactivity/Impulsivity[a]	.13	.34
Inattention[b]	-.07	.63
Hyperactivity/Impulsivity[b]	-.03	.82
Neurocognitive functioning		
Verbal working memory	.15	.28
Visuospatial working memory	-.21	.12
Interference control latency (ms)	.07	.59
Interference control accuracy (%)	.20	.14
Variability in responding (sigma in ms)	-.14	.30
Attentional lapses (tau in ms)	-.04	.79
Nutrition		
Folate (intake, µg/day)	-.12	.38
Vitamin B12 (intake, µg/day)	-.06	.65

Notes. [a]Parent-rated, [b]Teacher-rated. ADHD, attention-deficit/hyperactivity disorder; DBDRS, Disruptive Behaviour Disorder Rating Scale.

Discussion

This is the first study investigating homocysteine concentrations in children with ADHD. Our first objective is to examine whether children with ADHD have increased homocysteine concentrations. In contrast to our hypothesis, we neither found a difference in homocysteine concentrations between the ADHD and TD group, nor an association between homocysteine concentrations and symptoms of ADHD. Our study therefore does not support the hypothesis that increased homocysteine concentrations are involved in the aetiology of ADHD. This is in line with the single available study into homocysteine carried out in adults with ADHD (Karababa et al., 2014), that did not find increased homocysteine concentrations in ADHD either. The study in adults showed decreased homocysteine concentrations in adults with ADHD (Karababa et al., 2014), which should be interpreted with caution, given the small sample size and limited diagnostic screening of participants. Further, the authors of that study suggested that decreased homocysteine concentrations may relate to increased folate concentrations (due to altered dietary preferences) (Karababa et al., 2014), which was not supported by our study; in our childhood ADHD sample homocysteine concentrations seemed unrelated to folate and vitamin B12 intake.

We further examined whether homocysteine is negatively related to neurocognitive functioning in the ADHD group. We replicated previously reported deficiencies in visuospatial working memory, variability in responding and lapses of attention in ADHD, but did not find any deficits in the domains of verbal working memory and interference control. The latter is in accordance with the growing consideration that certain neurocognitive dysfunctions are not universally present in children with ADHD, but are only present in a minority of children with ADHD (Coghill, Seth, & Matthews, 2014). Against our hypothesis, we did not find any evidence for the contribution of homocysteine to neurocognitive deficiencies in children with ADHD. Our results contrast with the existing literature regarding the associations between homocysteine and neurocognitive functioning in adults and elderly (Teunissen et al., 2005). Possibly, homocysteine concentrations should reach a certain threshold before being toxic and affecting neurocognitive functioning. In the current study, all homocysteine concentrations were under 12 µmol/L and mean homocysteine concentrations in the ADHD and TD samples were 33 to 41 percent lower than total plasma homocysteine concentrations found in normative data of 6 to 10 year old children (Van Beynum et al.,

2005). This is in line with the literature that shows that homocysteine concentrations in dried blood spots are generally 40 percent lower than in plasma samples (Bowron et al., 2005). Our findings suggest that homocysteine concentrations in blood spots of children with ADHD can be considered normal. In studies that did show associations between homocysteine and cognition, homocysteine concentrations possibly reached toxic levels, with higher average concentrations (10 to 20 µmol/L) than found in our sample (Dufouil et al., 2003; Prins et al., 2002; Teunissen et al., 2005).

Another explanation might be that the association between homocysteine and neurocognitive functioning found in some previous studies, is mediated or moderated by factors that were not controlled for in these studies, including cardiovascular risk factors (Dufouil et al., 2003) and smoking history (Ford et al., 2013). It might be that these factors, that are less likely to affect the results in a study of primary school-aged children, have confounded previous research on homocysteine and neurocognitive functioning in adults and elderly.

A third explanation that we would like to put forward, is that the effects of high homocysteine concentrations might be more detrimental in vulnerable life-stages, including late adulthood (Teunissen et al., 2005), and during the early stage of prenatal development (Ars et al., 2016). For example, a recent study showed negative effects of high plasma homocysteine concentrations in mothers during early pregnancy on intelligence, language and visuospatial processing in 6 to 8 year old children (Ars et al., 2016).

Lastly, we examined whether homocysteine concentrations were negatively related to dietary intake of folate and vitamin B12 in children with ADHD. We did not find significant associations between homocysteine and the intake of folate and vitamin B12, and our results therefore are not indicative for a dietary risk factor for ADHD. In the past decades there has been a growing interest in nutritional interventions for ADHD (Hurt, Arnold, & Lofthouse, 2011). Our results do not provide support for nutritional interventions with folate (Ghanizadeh, Sayyari, & Mohammadi, 2013) or vitamin B12 (Ghanizadeh et al., 2013; Patel & Curtis, 2007) for children with ADHD, given that we neither found evidence for decreased intake of folate or vitamin B12, nor for increased homocysteine concentrations in children with ADHD. Our results further emphasise the limited theoretical foundation and empirical evidence for these interventions.

There are some limitations to the current study that should be noted. Firstly, we did not measure blood concentrations of determinants of homocysteine, such as folate and vitamin B12, and therefore provide limited insight into the homocysteine metabolism of children with ADHD. Secondly, even though our three-day dietary record provides sufficient insight into the intake of folate and vitamin B12, as the dietary pattern of children is relatively stable across years (Frémeaux et al., 2011), we suggest that in future studies intake of folate and vitamin B12 will be measured during the days prior to assessment of homocysteine concentrations. Finally, blood concentrations of folate and vitamin B12 may have been more reliable to study the intake of these nutrients.

Despite the limitations, our study is the first to examine the association between homocysteine and neurocognitive deficiencies in childhood ADHD. Our results suggest that homocysteine abnormalities do not seem to be related to childhood ADHD and that elevated homocysteine concentrations do not seem to contribute to the aetiology of neurocognitive dysfunctions in ADHD.

References

Altorf-van der Kuil, W., Engberink, M.F., De Neve, M., Van Rooij, F.J., Hofman, A., Van't Veer, P., . . . Geleijnse, J.M. (2013). Dietary amino acids and the risk of hypertension in a Dutch older population: The Rotterdam Study. *American Journal of Clinical Nutrition, 97*(2), 403-410.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders, fifth edition*. Arlington: American Psychiatric Association.

Ars, C., Nijs, I., Marroun, H., Muetzel, R., Schmidt, M., Steenweg-de Graaff, J., . . . Steegers, E. (2016). Prenatal folate, homocysteine and vitamin B12 levels and child brain volumes, cognitive development and psychological functioning: The Generation R Study. *British Journal of Nutrition.* doi:10.1017/S0007114515002081.

Barkley, R.A. (1997). Behavioral inhibition, sustained attention, and executive functions: Constructing a unifying theory of ADHD. *Psychological Bulletin, 121*(1), 65-94.

Bergman Nutley, S., Soderqvist, S., Bryde, S., Humphreys, K., & Klingberg, T. (2010). Measuring working memory capacity with greater precision in the lower capacity ranges. *Developmental Neuropsychology, 35*(1), 81-95.

Blom, H.J., Shaw, G.M., Den Heijer, M., & Finnell, R.H. (2006). Neural tube defects and folate: Case far from closed. *Nature Reviews Neuroscience, 7*(9), 724-731.

Bottiglieri, T., Laundy, M., Crellin, R., Toone, B.K., Carney, M.W., & Reynolds, E.H. (2000). Homocysteine, folate, methylation, and monoamine metabolism in depression. *Journal of Neurology, Neurosurgery & Psychiatry, 69*(2), 228-232.

Bowron, A., Barton, A., Scott, J., & Stansbie, D. (2005). Blood spot homocysteine: A feasibility and stability study. *Clinical Chemistry, 51*(1), 257-258.

Castellanos, F.X., & Tannock, R. (2002). Neuroscience of attention-deficit/hyperactivity disorder: The search for endophenotypes. *Nature Reviews Neuroscience, 3*(8), 617-628.

Coghill, D., Seth, S., & Matthews, K. (2014). A comprehensive assessment of memory, delay aversion, timing, inhibition, decision making and variability in attention deficit hyperactivity disorder: Advancing beyond the three-pathway models. *Psychological Medicine, 44*(9), 1989-2001.

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Hillsdale: Erlbaum.

Dias, V.V., Brissos, S., Cardoso, C., Andreazza, A.C., & Kapczinski, F. (2009). Serum homocysteine levels and cognitive functioning in euthymic bipolar patients. *Journal of Affective Disorders, 113*(3), 285-290.

Dufouil, C., Alpérovitch, A., Ducros, V., & Tzourio, C. (2003). Homocysteine, white matter hyperintensities, and cognition in healthy elderly people. *Annals of Neurology, 53*(2), 214-221.

Durá-Travé, T., & Gallinas-Victoriano, F. (2014). Caloric and nutrient intake in children with attention deficit hyperactivity disorder treated with extended-release methylphenidate: Analysis of a cross-sectional nutrition survey. *Journal of the Royal Society of Medicine Open, 5*(2), 1-7.

Eriksen, B.A., & Eriksen, C.W. (1974). Effects of noise letters upon the identification of a target letter in a nonsearch task. *Perception & Psychophysics, 16*(1), 143-149.

Faraone, S.V., Asherson, P., Banaschewski, T., Biederman, J., Buitelaar, J.K., Ramos-Quiroga, J.A., . . . Franke, B. (2015). Attention-deficit/hyperactivity disorder. *Nature Reviews Disease Primers, 1*, 1-23.

Ford, A.H., Flicker, L., Singh, U., Hirani, V., & Almeida, O.P. (2013). Homocysteine, depression and cognitive function in older adults. *Journal of Affective Disorders, 151*(2), 646-651.

Frémeaux, A.E., Hosking, J., Metcalf, B.S., Jeffery, A.N., Voss, L.D., & Wilkin, T.J. (2011). Consistency of children's dietary choices: Annual repeat measures from 5 to 13 years (EarlyBird 49). *British Journal of Nutrition, 106*(5), 725-731.

Garcia, A., & Zanibbi, K. (2004). Homocysteine and cognitive function in elderly people. *Canadian Medical Association Journal, 171*(8), 897-904.

Gempel, K., Gerbitz, K.-D., Casetta, B., & Bauer, M.F. (2000). Rapid determination of total homocysteine in blood spots by liquid chromatography-electrospray ionization-tandem mass spectrometry. *Clinical Chemistry, 46*(1), 122-123.

Ghanizadeh, A., Sayyari, Z., & Mohammadi, M.R. (2013). Effect of methylphenidate and folic acid on ADHD symptoms and quality of life and aggression: A randomized double blind placebo controlled clinical trial. *Iranian Journal of Psychiatry, 8*(3), 108.

Guttormsen, A.B., Schneede, J., Fiskerstrand, T., Ueland, P.M., & Refsum, H.M. (1994). Plasma concentrations of homocysteine and other aminothiol compounds are related to food intake in healthy human subjects. *Journal of Nutrition, 124*(10), 1934-1941.

Hurt, E.A., Arnold, L.E., & Lofthouse, N. (2011). Dietary and nutritional treatments for attention-deficit/hyperactivity disorder: Current research support and recommendations for practitioners. *Current Psychiatry Reports, 13*(5), 323-332.

Kalmijn, S., Launer, L.J., Lindemans, J., Bots, M.L., Hofman, A., & Breteler, M.M. (1999). Total homocysteine and cognitive decline in a community-based sample of elderly subjects. *American Journal of Epidemiology, 150*(3), 283-289.

Karababa, I.F., Savas, S.N., Selek, S., Cicek, E., Cicek, E.I., Asoglu, M., . . . Ulas, T. (2014). Homocysteine levels and oxidative stress parameters in patients with adult ADHD. *Journal of Attention Disorders.* doi:10.1177/1087054714538657

Kessels, R.P., Van Zandvoort, M.J., Postma, A., Kappelle, L.J., & De Haan, E.H. (2000). The Corsi block-tapping task: Standardization and normative data. *Applied Neuropsychology, 7*(4), 252-258.

Mattson, M.P., & Shea, T.B. (2003). Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. *Trends in Neurosciences, 26*(3), 137-146.

Mooijaart, S.P., Gussekloo, J., Frölich, M., Jolles, J., Stott, D.J., Westendorp, R.G., & de Craen, A.J. (2005). Homocysteine, vitamin B-12, and folic acid and the risk of cognitive decline in old age: The Leiden 85-Plus study. *American Journal of Clinical Nutrition, 82*(4), 866-871.

Mullane, J.C., Corkum, P.V., Klein, R.M., & McLaughlin, E. (2009). Interference control in children with and without ADHD: A systematic review of Flanker and Simon task performance. *Child Neuropsychology, 15*(4), 321-342.

National Institute for Public Health and the Environment. (2013). *Nederlands Voedingsstoffenbestand [Dutch Food Composition Database]. NEVO-online version 2013/4.0.*

O'Connor, J., Ball, E.J., Steinbeck, K.S., Davies, P.S., Wishart, C., Gaskin, K.J., & Baur, L.A. (2001). Comparison of total energy expenditure and energy intake in children aged 6–9 y. *American Journal of Clinical Nutrition, 74*(5), 643-649.

Patel, K., & Curtis, L.T. (2007). A comprehensive approach to treating autism and attention-deficit hyperactivity disorder: A prepilot study. *Journal of Alternative and Complementary Medicine, 13*(10), 1091-1098.

Pelham, W.E., Aronoff, H.R., Midlam, J.K., Shapiro, C.J., Gnagy, E.M., Chronis, A.M., . . . Waxmonsky, J. (1999). A comparison of Ritalin and Adderall: Efficacy and time-course in children with attention-deficit/hyperactivity disorder. *Pediatrics, 103*(4), e43.

Pelham, W.E., Gnagy, E.M., Greenslade, K.E., & Milich, R. (1992). Teacher ratings of DSM-III-R symptoms for the disruptive behavior disorders. *Journal of the American Academy of Child & Adolescent Psychiatry, 31*(2), 210-218.

Prins, N., Den Heijer, T., Hofman, A., Koudstaal, P., Jolles, J., Clarke, R., & Breteler, M. (2002). Homocysteine and cognitive function in the elderly: The Rotterdam Scan Study. *Neurology, 59*(9), 1375-1380.

Rommelse, N., & de Zeeuw, P. (2014). Neurobiological measures to classify ADHD: A critical appraisal. *European Child & Adolescent Psychiatry, 23*(5), 243-246.

Sattler, J.M. (2008). *Resource guide to accompany assessment of children: Cognitive foundations.* La Mesa, CA: Jerome M. Sattler, Publisher, Inc.

Scheres, A., Oosterlaan, J., Swanson, J., Morein-Zamir, S., Meiran, N., Schut, H., . . . Sergeant, J.A. (2003). The effect of methylphenidate on three forms of response inhibition in boys with AD/HD. *Journal of Abnormal Child Psychology, 31*(1), 105-120.

Shaffer, D., Fisher, P., Lucas, C.P., Dulcan, M.K., & Schwab-Stone, M.E. (2000). NIMH Diagnostic Interview Schedule for Children Version IV (NIMH DISC-IV): Description, differences from previous versions, and reliability of some common diagnoses. *Journal of the American Academy of Child & Adolescent Psychiatry, 39*(1), 28-38.

Tabachnick, B.G., & Fidell, L.S. (2001). *Using multivariate statistics* (4th ed.). Boston: Allyn and Bacon.

Tamm, L., Narad, M.E., Antonini, T.N., O'Brien, K.M., Hawk Jr, L.W., & Epstein, J.N. (2012). Reaction time variability in ADHD: A review. *Neurotherapeutics, 9*(3), 500-508.

Teunissen, C.E., van Boxtel, M.P., Jolles, J., de Vente, J., Vreeling, F., Verhey, F., . . . Blom, H.J. (2005). Homocysteine in relation to cognitive performance in pathological and non-pathological conditions. *Clinical Chemical Laboratory Medicine, 43*(10), 1089-1095.

Van Beynum, I.M., Den Heijer, M., Thomas, C.M., Afman, L., Oppenraay-van Emmerzaal, D., & Blom, H.J. (2005). Total homocysteine and its predictors in Dutch children. *American Journal of Clinical Nutrition, 81*(5), 1110-1116.

Van der Zwaluw, N.L., Van de Rest, O., Tieland, M., Adam, J.J., Hiddink, G.J., Van Loon, L.J., & De Groot, L.C. (2014). The impact of protein supplementation on cognitive performance in frail elderly. *European Journal of Nutrition, 53*(3), 803-812.

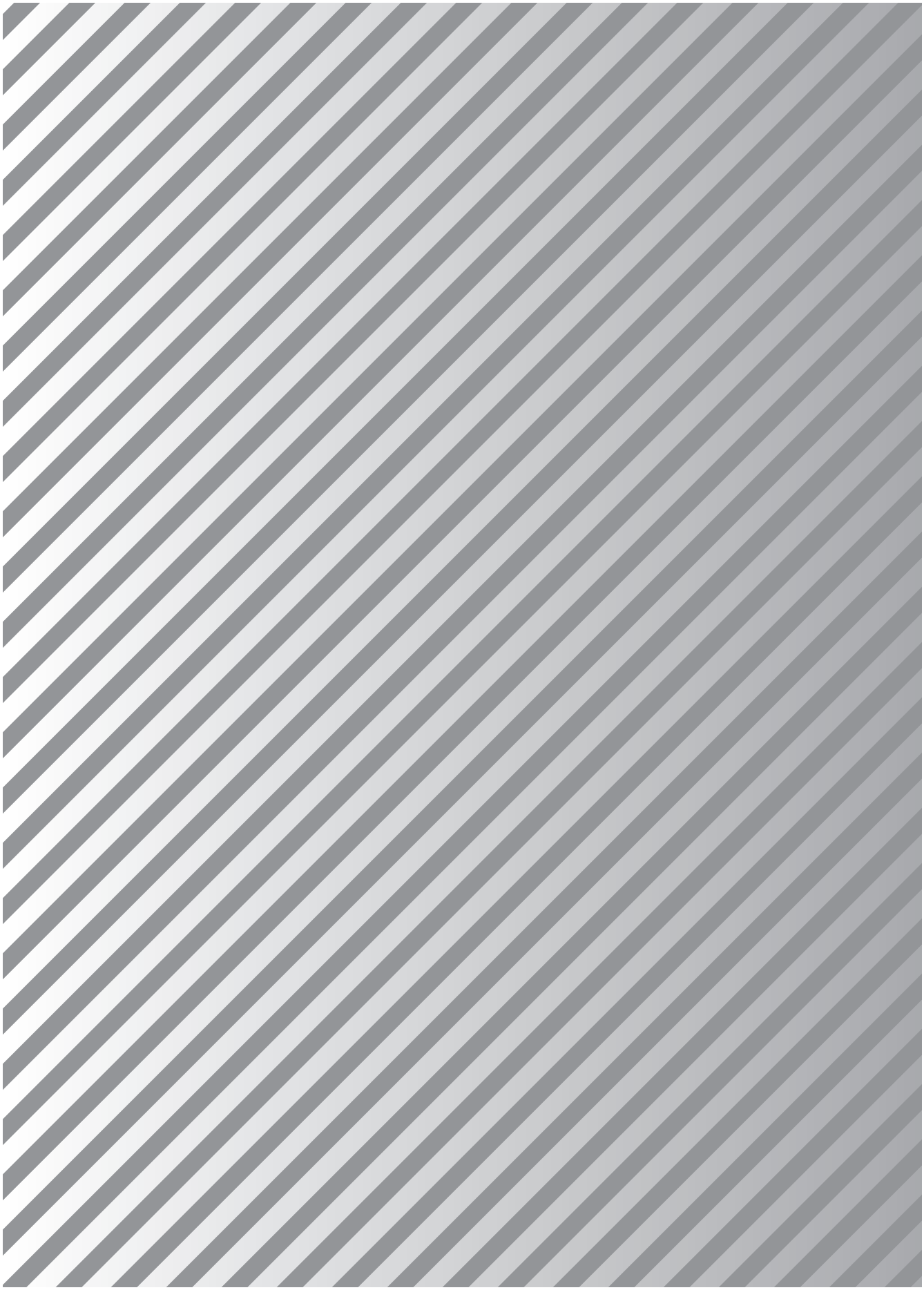
Van Kernebeek, H., Oosting, S., Feskens, E., Gerber, P., & De Boer, I. (2014). The effect of nutritional quality on comparing environmental impacts of human diets. *Journal of Cleaner Production, 73*, 88-99.

Wechsler, D. (1991). *Wechsler intelligence scale for children, third edition: Manual*. San Antonio: The Psychological Corporation.

Westenbrink, S., & Jansen-van der Vliet, M. (2013). *NEVO-online 2013: Achtergrondinformatie [background information].* Bilthoven, the Netherlands: Rijksinstituut voor Volksgezondheid en Milieu [National Institute for Public Health and the Environment].

Whelan, R. (2010). Effective analysis of reaction time data. *The Psychological Record, 58*(3), 475–482.

Willcutt, E.G., Doyle, A.E., Nigg, J.T., Faraone, S.V., & Pennington, B.F. (2005). Validity of the executive function theory of attention-deficit/hyperactivity disorder: A meta-analytic review. *Biological Psychiatry, 57*(11), 1336-1346.



Chapter 4

Neurocognitive profiles in children with attention-deficit/hyperactivity disorder and their predictive value for functional outcomes

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Abstract

Objective. In the current study we examine whether neurocognitive profiles can be distinguished in children with attention-deficit/hyperactivity disorder (ADHD) and typically developing (TD) children, and whether neurocognitive profiles predict externalising, social and academic problems in children with ADHD.

Methods. Neurocognitive data of 81 children with ADHD and 71 TD children were subjected to confirmatory factor analysis. The resulting factors were used for community detection in the ADHD and TD group.

Results. Four subgroups were detected in the ADHD group, characterised by (1) poor emotion recognition, (2) poor interference control, (3) slow processing speed, or (4) increased attentional lapses and fast processing speed. In the TD group three subgroups were detected, closely resembling subgroups 1-3. Neurocognitive subgroups in the ADHD sample did not differ in externalising, social and academic problems.

Conclusion. We found a neurocognitive profile unique to ADHD. The clinical validity of neurocognitive profiling is questioned, given the lack of associations with functional outcomes.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is the most common mental health disorder diagnosed in children and adolescents (Willcutt, 2012). The disorder is characterised by a persistent pattern of age-inappropriate levels of inattention and/or hyperactivity-impulsivity (American Psychiatric Association, 2013) and is aetiologically heterogeneous (Nigg, Willcutt, Doyle, & Sonuga-Barke, 2005; Sonuga-Barke & Halperin, 2010). Three aetiological neurocognitive pathways have been identified in ADHD, with deficiencies present in temporal processing, inhibitory control, or delay aversion (Sonuga-Barke, Bitsakou, & Thompson, 2010). Recently, a paradigm shift took place from identifying subgroups of children with ADHD based on a single neurocognitive deficit, towards identifying subgroups that share a neurocognitive profile, acknowledging that neurocognitive functioning consists of a complex interplay of strengths and weaknesses. Thus far three studies applied community detection procedures to distinguish subgroups of individuals with ADHD, all showing distinct neurocognitive profiles (Fair, Bathula, Nikolas, & Nigg, 2012; Mostert et al., 2015; Van Hulst, De Zeeuw, & Durston, 2015). In the study of Fair et al. (2012), subgroups of children with ADHD were characterised by either (a) high levels of response variability, (b) reduced working memory, memory span and processing speed, (c) inaccurate temporal information processing, or (d) suboptimal arousal. The results of Van Hulst et al. (2015) in children with ADHD showed subgroups characterised by either (a) fast reaction times and high cognitive control, (b) poor cognitive control, or (c) slow and variable timing. Mostert et al. (2015) showed that in adults with ADHD subgroups were either characterised by (a) impaired attention and inhibition, (b) impaired delay discounting, or (c) impaired fluency and memory. Since all three studies used different selections of neurocognitive measures, profile characteristics differed across studies. In all studies the detected profiles in the ADHD group were also observed in typically developing controls, with individuals with ADHD generally showing weaker performance (Fair et al., 2012; Mostert et al., 2015; Van Hulst et al., 2015). This finding suggests that individuals with ADHD reflect the extremes of normal neurocognitive heterogeneity.

One step forward in the approach of profiling is to include a more extensive set of measures that reflect core neurocognitive alterations in ADHD. One of the most consistently reported deficits in ADHD is an increase in intra-individual reaction time variability, including increased variability in responding and attentional lapses as

measured by ex-Gaussian modelling (Tamm et al., 2012). However, thus far ex-Gaussian measures of intra-individual reaction time variability have not been addressed in neurocognitive profiling of children with ADHD. Another core deficit in ADHD, that has been omitted thus far in neurocognitive profiling studies, is the ability to recognise facial emotional expressions, a central aspect of social cognition (Shaw, Stringaris, Nigg, & Leibenluft, 2014).

Further, the clinical value of neurocognitive profiling has not been addressed, since studies into neurocognitive profiling were solely aimed at investigating aetiological pathways of ADHD. More specifically, it was argued that distinct neurocognitive profiles could be indicative of separate neurobiological pathways (Fair et al., 2012; Mostert et al., 2015; Van Hulst et al., 2015). Even though evidence for the predictive value of neurocognitive functioning for persistence of ADHD is relatively weak (Van Lieshout, Luman, Buitelaar, Rommelse, & Oosterlaan, 2013), it has been argued that neurocognitive deficiencies in individuals with ADHD result in significant functional impairments (Coghill, Seth, & Matthews, 2014). Therefore, associations should be studied between profiles and key functional outcomes of the disorder. Although ADHD increases the risk of associated externalising problems (including oppositional defiant disorder [ODD] and conduct disorder [CD]; Gillberg et al., 2004), social problems (McQuade & Hoza, 2008) and academic problems (Loe & Feldman, 2007), not all children with ADHD are impaired in terms of these functional outcomes. Neurocognitive profiles may act as moderators, explaining differences in adverse outcomes. More specifically, emotion recognition deficiencies have been found to increase the risk of ODD (Noordermeer et al., 2015) and CD (Cadesky, Mota, & Schachar, 2000), and of decreased social functioning (Trentacosta & Fine, 2010). Further, academic problems in children with ADHD may be related to deficiencies in cool executive functions (EF), including working memory and inhibitory control (Antonini et al., 2016).

The current study seeks to replicate previous work on community detection in samples of children with ADHD and typically developing (TD) children. In line with Fair et al. (2012), we expect to find distinct neurocognitive profiles based on children's performance on measures of cool EF (memory span, working memory, interference control). The present study extends previous research by adding ex-Gaussian parameters of intra-individual reaction time variability (processing speed, variability in responding and lapses of attention), as well as a measure of social cognition (emotion recognition). In

line with previous studies (Fair et al., 2012; Van Hulst et al., 2015), we expect to find similar neurocognitive profiles in children with ADHD and TD children, with weaker performance for the ADHD group. We also examine whether the neurocognitive profiles within the ADHD group reflect differential risk factors for functional outcomes: associated externalising, social and academic problems.

Methods

Participants

Subjects were 81 children with ADHD (74 percent males) and 71 TD children (52 percent males), aged between 6 and 13 years. Inclusion criteria for the ADHD group were: (a) a clinical diagnosis of ADHD according to DSM-IV criteria, (b) confirmation of this diagnosis by the Diagnostic Interview Schedule for Children, fourth edition, administered to parents (DISC-IV-P; Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000), (c) significant ADHD symptoms, as indicated by scores >90th percentile on at least one of the ADHD scales (Inattention and Hyperactivity/Impulsivity scales) of the parent version of the Disruptive Behaviour Disorder Rating Scale (DBDRS; Pelham, Gnagy, Greenslade, & Milich, 1992), and (d) pervasive ADHD symptoms, as indicated by scores >75th percentile on at least one of the ADHD scales of the teacher version of the DBDRS. Having a comorbid diagnosis (for example ODD) was no exclusion criterion, neither was treatment with stimulant medication. Children on stimulant medication (59 percent of the ADHD group) discontinued drug use 24 hours before testing, in order to allow complete washout. Inclusion criteria for the TD group were: (a) absence of a clinical diagnosis of ADHD or ODD as obtained from parent information, and (b) scores <90th percentile on both parent- and teacher-rated ADHD scales of the DBDRS. Children with an IQ<70 were excluded.

Materials

Diagnostic assessment

Parents of children eligible for inclusion in the ADHD group were assessed with the ADHD section of the DISC-IV-P (Shaffer et al., 2000). The DISC-IV-P is a widely used standardised diagnostic interview for the assessment of DSM-IV childhood psychiatric disorders.

ADHD symptom severity was assessed in both groups using the DBDRS, filled out by one of the parents and teacher (Pelham et al., 1992). The DBDRS measures the DSM-IV symptoms of ADHD (and other externalising disorders) using a 4-point Likert scale.

Scores on the Inattention and Hyperactivity/Impulsivity scales were used, with higher scores indicating worse symptoms.

Full scale IQ was estimated using a short form of the Wechsler Intelligence Scale for Children-III (WISC-III; Wechsler, 1991), comprising the subtests Vocabulary, Arithmetic, Block Design and Picture Arrangement. This short form of four subtests has an excellent reliability (r=.95) and validity (r'=.90) (Sattler, 2008).

Neurocognitive functioning

To measure verbal working memory, the backward condition of the Digit Span Task of the WISC-III was used (Wechsler, 1991). In the backward condition, the child is required to repeat in reversed order a sequence of numbers expressed verbally by the interviewer. There were seven sequence levels, starting from a span of two digits up to a span of eight digits. The product of the total number of correct responses and the highest obtained span served as measure of verbal working memory (Kessels, Van Zandvoort, Postma, Kappelle, & De Haan, 2000).

Visuospatial working memory was measured using the backward condition of the Grid Task (Bergman Nutley, Soderqvist, Bryde, Humphreys, & Klingberg, 2010), in which the child is required to repeat in reversed order a sequence of visual stimuli (yellow dots) presented on a computer screen in a four by four grid. The computer mouse was used to respond. There were nine sequence levels, starting from two up to a span of ten dots. Each level consisted of sublevel A, in which the sequence followed a logical pattern, and the more difficult sublevel B, in which the sequence showed no clear pattern. The product of the total number of correct responses and the highest obtained span served as measure of visuospatial working memory (Kessels et al., 2000), with 0.5 point added to the highest obtained span when sublevel B was reached (Bergman Nutley et al., 2010).

For interference control, processing speed, variability in responding and lapses of attention, an adapted version of the Eriksen Flanker Task was used (Eriksen & Eriksen, 1974; Scheres et al., 2003). In each trial, a target stimulus (black arrow) appeared at the centre of a computer screen, pointing either to the left or right side, and the child was asked to press the spatially corresponding response button (left or right button). In the neutral condition, the target was flanked by two neutral items (rectangles), in the congruent condition by arrows pointing in the same direction, and in the incongruent

condition by arrows pointing in the opposite direction. In total, 48 neutral, 48 congruent and 48 incongruent trials were presented in random order. Difference scores between congruent and incongruent trials were calculated for latency on correct trials and accuracy, and served as measures of interference control (Mullane, Corkum, Klein, & McLaughlin, 2009). Further, individual response time distributions derived from the correct neutral trials were examined. The mean (mu) and standard deviation (sigma) of the normal component of the ex-Gaussian distribution served as measures of processing speed and variability in responding, and the mean plus standard deviation of the exponential component of the ex-Gaussian distribution (tau) as measure of attentional lapses (Whelan, 2010).

Recognition of facial emotional expressions was examined using the Children's Emotion Recognition Task (CERT), a computerised task developed for the current study. The CERT is an adaptation of a previously validated paradigm (Nowicki & Duke, 1994), but instead of using pictures of adult faces, the CERT consists of solely pictures of children's faces, to make the task more ecologically valid. The CERT contains 100 pictures of children's faces with neutral facial expressions, and four basic emotional expressions (happy, fear, anger, sadness); 20 stimuli per emotional expression, presented in random order. Pictures were selected from the validated National Institute of Mental Health Child Emotional Faces Picture Set database, based on their distinctive character (Egger et al., 2011). Upon presentation of the stimulus, participants had to indicate the corresponding emotion with a computer mouse: angry, frightened, happy, sad or neutral. The task was self-paced. For all five expressions, inverse efficiency scores were calculated, by dividing the mean reaction time by the proportion of correct responses (Townsend & Ashby, 1983).

Externalising problems

Associated externalising problems were examined using the ODD and CD scales of the parent- and teacher-rated DBDRS (Pelham et al., 1992).

Social functioning

Sociometric data were collected for all participating children (Coie & Dodge, 1983). All children in the classroom of a participant were required to nominate classroom peers they liked most (positive rating) and classroom peers they disliked most (negative rating). Children were free to nominate as many peers for both categories as they wanted. For each participant, we summed up the number of times the child was positively rated and

the number of times the child was negatively rated. In order to adjust for classroom size, sum scores were divided by the number of classroom peers, yielding percentages of positive ratings and negative ratings, which were used as indicators of social acceptance and social rejection, respectively (Coie & Dodge, 1983).

Social problems (such as getting teased) were examined using the 11–item Social Problems scale of the Child Behavior Checklist (CBCL) and Teacher Rating Form (TRF), completed by parents and teachers, respectively (Achenbach, 1991). For both scales, items were rated on a three-point Likert-scale and summed up.

Academic functioning

Academic functioning was examined using data collected by teachers for the national pupil monitoring system, containing measures of reading comprehension (Staphorsius & Krom, 1998), spelling (de Wijs, Krom, & van Berkel, 2006) and mathematics (Janssen, Verhelst, Engelen, & Scheltens, 2010). These tests are administered twice a year and provide ability scores, which are standardised scores per academic domain, across the school grades.

Procedure

Children with ADHD were recruited from mental health outpatient clinics, through the parental association for children with behavioural problems, and through a university research website. The TD group was recruited from primary schools located throughout the country. Children were tested at their own school. Prior to participation, written informed consent was obtained of parents of all children, and of children ≥12 years. This study received approval from the local medical ethical committee (#NL39922.029.12).

Data analysis

All statistical analyses were performed using R, version 3.2.1. All neurocognitive dependent variables were transformed into z-scores, to have all measures on the same metric scale, and if necessary reverse-scored, to ensure that for all variables higher scores were indicative of better performance.

The number of neurocognitive measures per domain was reduced by performing confirmatory factor analyses. Based on the model of Fair et al. (2012), it was hypothesised which of the neurocognitive measures were representative of the same

latent factor. Our conceptual model consisted of seven latent factors; memory span, working memory, interference control, processing speed, variability in responding, lapses of attention and emotion recognition. We acknowledged that there might be better fitting models, and therefore we also examined a six-factor model (6A) in which memory span and working memory were combined into one latent factor (memory); another six-factor model (6B) in which variability in responding and lapses of attention were combined into one latent factor (response time variability); and a five-factor model consisting of memory, response time variability, interference control, processing speed and emotion recognition. Correlations between neurocognitive measures >.80 were interpreted as signs of multicollinearity (Field, Miles, & Field, 2012). Fit of all models was evaluated using chi-square (χ^2), comparative fit index (CFI), Tucker-Lewis index (TLI), root mean square error of approximation (RMSEA) and standardised root mean square residual (SRMR) (Hu & Bentler, 1998). The most parsimonious model with adequate fit was selected and factor scores were calculated, serving as measures of neurocognitive functioning.

It was examined whether distinct neurocognitive profiles could be detected in the ADHD and TD group separately, using community detection procedures (see Fair et al. (2012) for a detailed description). Briefly, in each group, participants were assigned to detected subgroups using the Louvain algorithm (modularity_louvain_und_sign.m by Rubinov and Sporns (2011)). For both groups correlation matrices between subjects' neurocognitive factor scores were created, providing insight into connections between clusters of participants. Subgroup assignment of each participant was based on group assignment across 100 runs of the modularity algorithm, applying the most frequent group assignment (mode). Robustness of the community structure was determined based on the quality index (Q), with values >.40 being interpreted as indication of distinct subgroups (Fortunato & Barthelemy, 2007). For both the ADHD and the TD group, characteristics of the subgroups were examined by comparing the neurocognitive subgroups to each other, using analysis of variance (ANOVA) and post hoc comparisons per factor score. Visual inspection of the plots was carried out to examine whether the profiles were similar in the ADHD and TD group (Fair et al., 2012). In case of similar profiles, it was investigated whether children with ADHD differed from TD children in factor scores, using multivariate analysis of variance (MANOVA). These analyses were done for each profile separately. Post hoc comparisons tested group differences on the factor scores.

To study the predictive validity of the neurocognitive profiles, it was investigated whether the neurocognitive subgroups in the ADHD group differed from each other on measures of externalising, social and academic functioning, using ANOVA and post hoc comparisons between subgroups. To correct for multiple testing, the alpha level was adjusted according to the Bonferroni procedure per outcome domain; externalising (four analyses, p=.013), social (four analyses, p=.013), and academic problems (three analyses, p=.017).

Results

For group characteristics, see Table 4.1. Analyses showed that the ADHD and TD group did not differ significantly in mean age and IQ. The ADHD group showed more ADHD symptoms and consisted of considerably more males than the TD group. The DISC-IV-P indicated that 64 children met the DSM-IV criteria for the combined subtype of ADHD, 11 children for the predominantly inattentive subtype, and six children for the predominantly hyperactive-impulsive subtype.

Table 4.1. Group characteristics of the ADHD group (n=81) and TD group (n=71)

	ADHD group *M (SD)*	TD group *M (SD)*	Statistic (t/χ^2)
Age in months	116.52 (19.96)	118.87 (20.68)	-.71, NS
Estimated IQ	100.23 (13.98)	104.24 (14.04)	-1.76, NS
Males *n* (%)	60 (74.07)	37 (52.11)	7.90**
Parent-rated symptoms			
Inattention	17.56 (4.83)	3.34 (3.08)	21.88**
Hyperactivity/Impulsivity	16.52 (5.87)	3.27 (2.73)	18.20**
Teacher-rated symptoms			
Inattention	14.72 (6.11)	1.87 (2.45)	17.40**
Hyperactivity/Impulsivity	14.02 (7.15)	1.56 (2.31)	14.83**

Notes. **p<.01. ADHD, attention-deficit/hyperactivity disorder; NS, not significant; TD, typically developing.

Correlation analyses showed no sign of multicollinearity (rs .10-.80). Fit indices were not satisfactory for the five factor model (χ^2(68)=147.5, CFI=.92, TLI=.90, RMSEA=.09, SRMR=.06) and for six-factor model 6B (χ^2(63)=137.6, CFI=.93, TLI=.90, RMSEA=.09, SRMR=.06). All fit indices were satisfactory for the other two models; for six-factor

model 6A (χ^2(65)=101.0, CFI=.97, TLI=.95, RMSEA=.06, SRMR=.06) and for the seven-factor model (χ^2(59)=88.0, CFI=.97, TLI=.96, RMSEA=.06, SRMR=.05). Out of the two well-fitting models, we selected the most parsimonious model, the six-factor model that is depicted below in Figure 4.1.

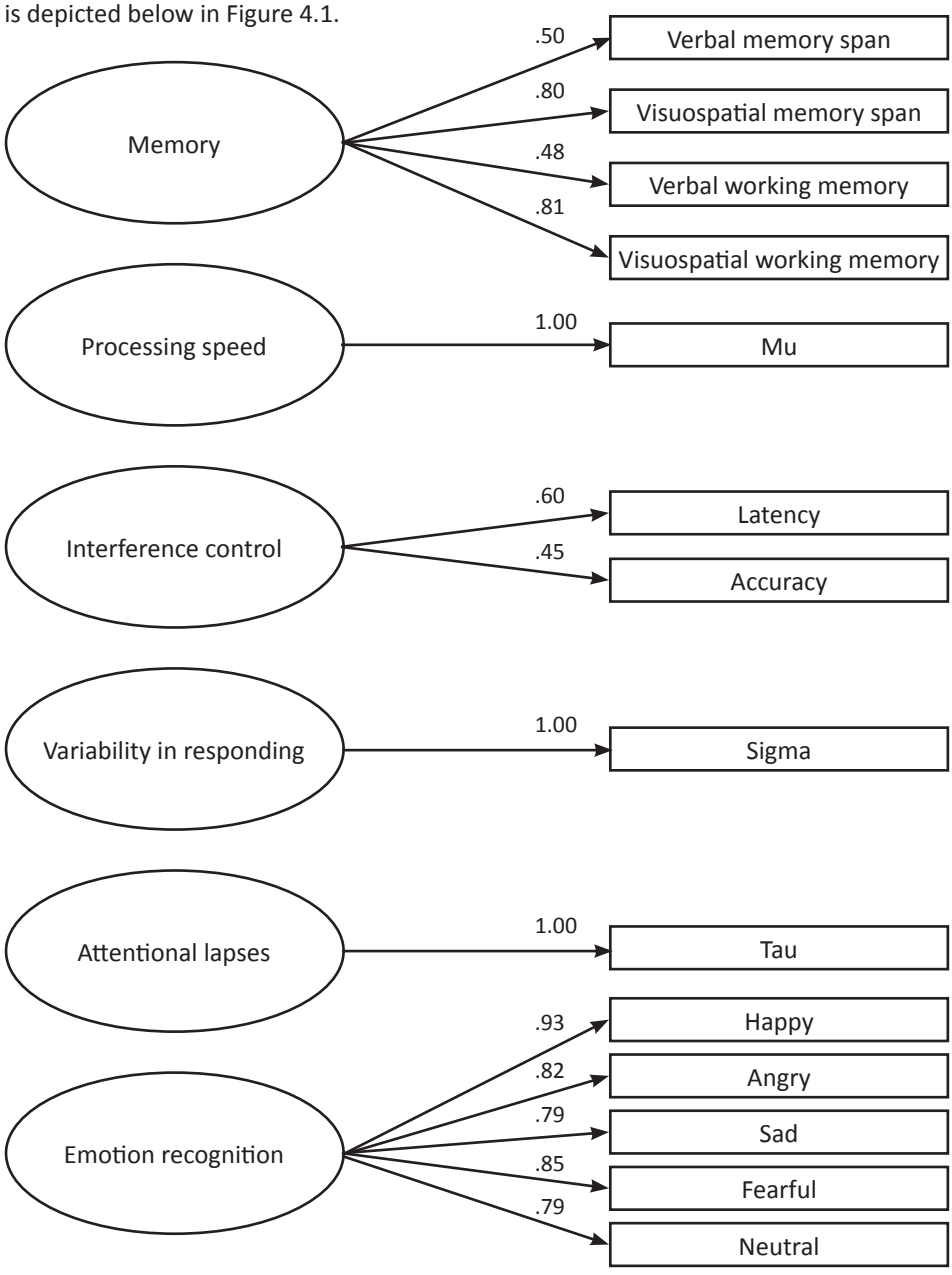


Figure 4.1. Overview of the six-factor model, created for data reduction. The numbers represent factor loadings.

Community detection analysis within the ADHD sample yielded four neurocognitive subgroups, each representing a neurocognitive profile (see Figure 4.2, panel a). The quality index (Q=.45) showed that the identified neurocognitive subgroups were strongly distinct from each other. Compared to the other subgroups, subgroup 1 (n=20) was characterised by poor emotion recognition (F(3,77)=6.59, p<.01), subgroup 2 (n=9) by poor interference control (F(3,77)=6.49, p<.01), and subgroup 3 (n=26) by slow processing speed (F(3,77)=13.56, p<.01). Subgroup 4 (n=26) was characterised by increased attentional lapses (F(3,77)=8.91, p<.01) and fast processing speed (F(3,77)=13.56, p<.01), compared to subgroups 1 and 3. Characteristics of the four subgroups are summarised in Table 4.2 and comparisons between the subgroups are shown in Table 4.3. There were no differences in IQ between subgroups. Subgroup 3 had less parent-rated symptoms of inattention compared to subgroups 1 and 4 and less parent-rated symptoms of hyperactivity/impulsivity compared to subgroup 1. Subgroup 4 had a higher mean age compared to subgroups 1 and 3.

Community detection analysis within the TD sample yielded three neurocognitive subgroups, each representing a neurocognitive profile (see Figure 4.2, panel b). The quality index (Q=.49) showed that the identified neurocognitive subgroups were strongly distinct from each other. Upon visual inspection it is clear that the three subgroups in the TD group closely resembled the first three subgroups in the ADHD group, see Figure 4.3. Analyses using MANOVA showed that, for all three subgroups, children with ADHD showed weaker neurocognitive performance than TD children. Post hoc analyses showed that children with ADHD had weaker neurocognitive performance than the TD children on one to four factors scores per subgroup (see Figure 4.3 and Table 4.3). Since the ADHD group consisted of considerably more males, analyses were rerun with gender as covariate. All group differences remained significant after adjusting for the effect of gender. The fourth subgroup obtained in the ADHD group, characterised by increased attentional lapses and fast processing speed, was not replicated in the TD group.

We examined whether differences in neurocognitive profiles in the ADHD sample were related to differences in functional outcomes, but found no significant differences between the neurocognitive subgroups on measures of associated externalising (Fs<1.20, ps>.34), social (Fs<3.20, ps>.03) and academic problems (Fs<1.70, ps>.19).

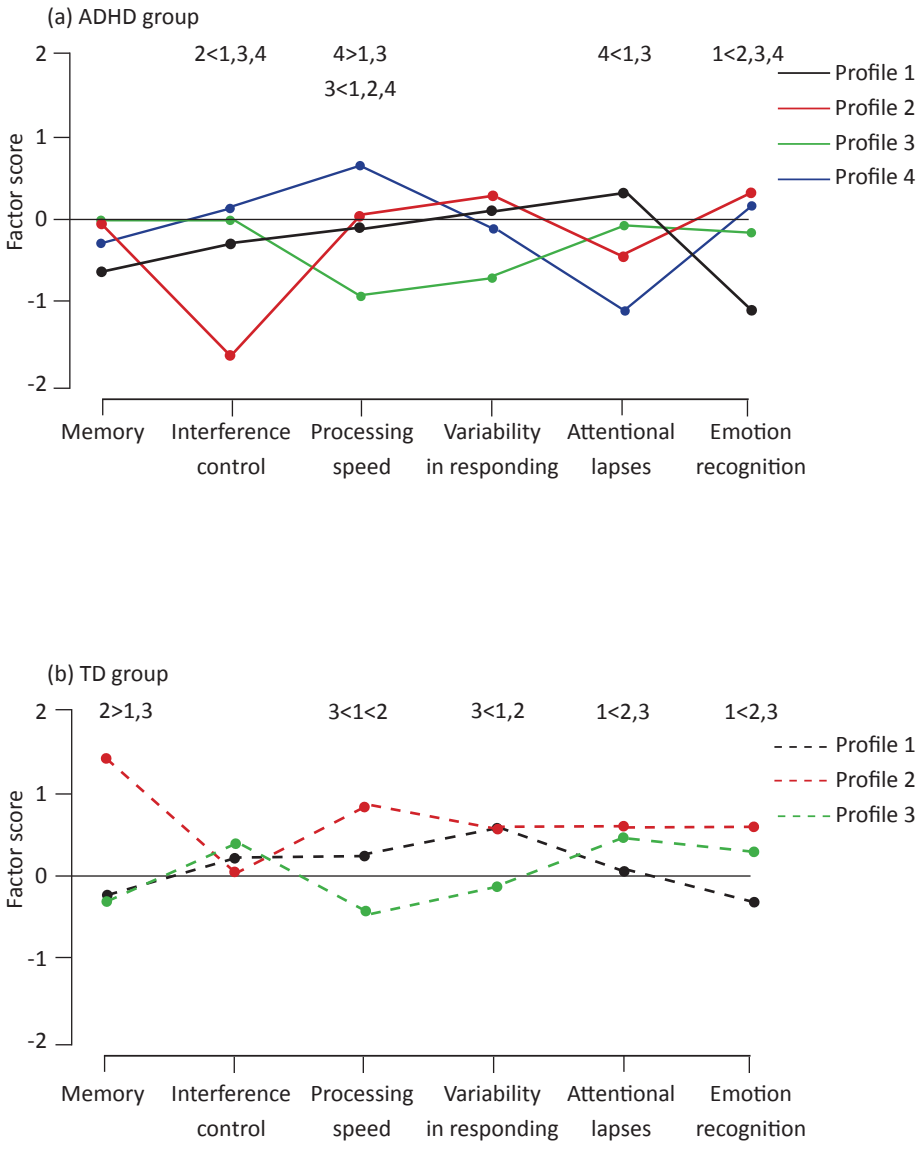


Figure 4.2. Neurocognitive profiles in children with ADHD in panel (a) and in TD children in panel (b). Symbols of comparison (< and >) indicate significant differences between profiles on the factor of interest.

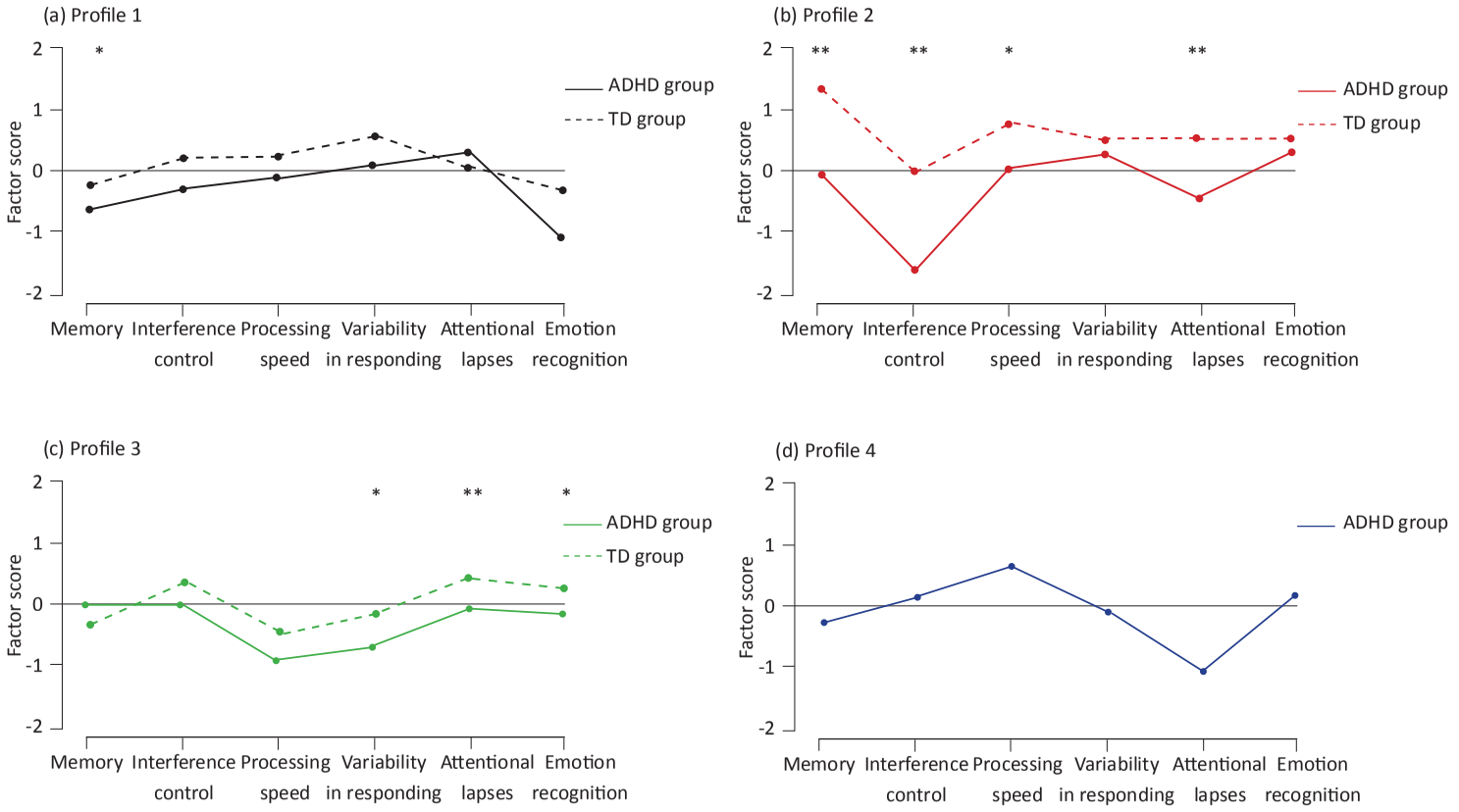


Figure 4.3. Neurocognitive profiles in children with ADHD and TD children. In panels (a), (b) and (c) a comparison in factor scores between the ADHD and TD group per profile is shown (*p<.05, **p<.01). Panel (d) shows a unique neurocognitive profile in the ADHD group.

Table 4.2. Characteristics of the neurocognitive subgroups in the ADHD group

	Profile 1 Poor emotion recognition	**Profile 2** Poor interference control	**Profile 3** Slow processing speed	**Profile 4** Increased attentional lapses / fast processing speed	Statistic (F/χ^2)	Pairwise comparison
	M (SD)	*M (SD)*	*M (SD)*	*M (SD)*		
n	20	9	26	26		
Age in months	110.90 (19.22)	111.89 (23.51)	112.38 (18.54)	126.58 (17.81)	3.58*	4>1;4>3
Males *n* (%)	17 (85)	9 (100)	15 (58)	19 (73)	8.04	1=2=3=4
Estimated IQ	100.10 (15.89)	99.00 (18.93)	102.38 (11.47)	98.62 (13.40)	.34	1=2=3=4
Parent-rated symptoms						
Inattention[a]	19.15 (5.32)	18.89 (4.65)	14.69 (3.91)	18.73 (4.29)	5.22**	3<1;3<4
Hyperactivity/ Impulsivity[a]	18.55 (5.70)	17.00 (6.56)	13.81 (4.95)	17.50 (5.95)	3.14*	3<1
Teacher-rated symptoms						
Inattention[a]	15.30 (6.36)	14.67 (6.69)	13.27 (6.25)	15.73 (5.64)	.78	1=2=3=4
Hyperactivity/ Impulsivity[a]	15.55 (5.93)	14.00 (10.37)	12.77 (6.81)	14.12 (7.25)	.56	1=2=3=4

Notes. [a]Disruptive Behaviour Disorder Rating Scale. *p<.05, **p<.01. ADHD, attention-deficit/hyperactivity disorder.

Table 4.3. Comparisons between children with ADHD and TD children per neurocognitive profile

	MANOVA	Memory	Interference control	Processing speed	Variability in responding	Attentional lapses	Emotion recognition
Profile 1 – Poor emotion recognition							
ADHD (*n*=20)		-.63 (.62)	-.27 (.95)	-.11 (.78)	.08 (.95)	.29 (.64)	-1.12 (1.53)
TD (*n*=15)		-.23 (.47)	.25 (.63)	.22 (.55)	.57 (.51)	.05 (.68)	-.32 (.47)
Difference (*F*)	2.59*	4.29*	3.45	1.91	3.16	1.15	3.78
Profile 2 – Poor interference control							
ADHD (*n*=9)		-.07 (1.38)	-1.64 (1.64)	.07 (.89)	.29 (1.04)	-.45 (1.39)	.31 (.52)
TD (*n*=24)		1.40 (.89)	.01 (.70)	.80 (.68)	.58 (.63)	.59 (.56)	.60 (.58)
Difference (*F*)	3.29*	13.17**	16.92**	6.32*	.94	9.72**	1.72
Profile 3 – Slow processing speed							
ADHD (*n*=26)		.00 (1.00)	-.01 (1.04)	-.91 (1.02)	-.70 (1.11)	-.10 (.82)	-.14 (.97)
TD (*n*=32)		-.32 (.60)	.38 (.57)	-.45 (.77)	-.16 (.70)	.49 (.44)	.28 (.61)
Difference (*F*)	4.43**	2.26	3.25	3.80	5.11*	11.82**	4.08*
Profile 4 – Increased attentional lapses / fast processing speed							
ADHD (*n*=26)		-.25 (.64)	.16 (1.03)	.66 (.84)	-.12 (1.25)	-1.15 (1.21)	.17 (.86)

Notes. *p<.05, **p<.01. ADHD, attention-deficit/hyperactivity disorder; MANOVA; multivariate analysis of variance; TD, typically developing. For each profile, values in the first two rows represent factor scores in *M (SD)*.

Discussion

The main aim of the current study is to gain more insight into neurocognitive profiles of children with ADHD. We found four distinct subgroups in the ADHD sample, with one subgroup characterised by poor interference control, one by slow processing speed, one by poor emotion recognition, and one by increased attentional lapses and fast processing speed. Our results partially replicate the findings of studies into neurocognitive profiling in children with ADHD, as our subgroup characterised by slow processing speed, was also found by Fair et al. (2012), and our subgroup characterised by fast processing speed also emerged in the study of Van Hulst et al. (2015). On the other hand, our slow processing speed subgroup, was not characterised by an additional reduction in working memory and memory span, as found by Fair et al. (2012), and our high processing speed subgroup, was not characterised by high cognitive control, as found by Van Hulst et al. (2015). Further, we observed two subgroups, characterised by poor interference control and poor emotion recognition, respectively, which are new findings. The latter is in accordance with the growing consideration that cool EF and social cognition are dissociable neurocognitive domains, with different aetiological pathways at the neurobiological level (Zelazo & Carlson, 2012). Our results also confirm that heterogeneity in observed neurocognitive profiles across studies at least partially stems from using different sets of neurocognitive measures as dependent measures. This violation of measurement invariance, due to the selection of different neurocognitive constructs or similar constructs assessed by different instruments, limits the commensurability of neurocognitive profiling in ADHD. This hampers the possibility to derive final conclusions regarding the number and type of neurocognitive profiles being core to ADHD.

In line with earlier work (Fair et al., 2012; Van Hulst et al., 2015), we found neurocognitive subgroups in the ADHD group that were also observed in the TD group, with children with ADHD showing generally weaker neurocognitive performance compared to the TD children. These findings suggest that the heterogeneity in childhood ADHD is nested within the normal variation of neurocognitive functioning in children. Although children with ADHD reflected the extremes of normal neurocognitive heterogeneity on some neurocognitive factors within each subgroup, on other factors their performance overlapped with the performance of TD children. The latter might be explained by the large variance in neurocognitive performance in children with ADHD, showing that not

all children with ADHD within a subgroup had weaker neurocognitive functioning on all factors.

Remarkably, in the TD group we did not find a subgroup characterised by fast processing speed and increased attentional lapses, suggesting this to be a unique neurocognitive subgroup in children with ADHD. This profile reflects a combination of fast responses at some moments, which could be indicative of impulsive responding (Hervey et al., 2006), and occasional lapses in attention at other moments. This neurocognitive profile could reflect one of many possible aetiological pathways of symptoms of hyperactivity/impulsivity and inattention. Our findings are in line with previous work on ex-Gaussian parameters, showing that children with the combined presentation of ADHD had fast processing speed and increased attentional lapses (Hervey et al., 2006; Tamm et al., 2012). This combination of fast responses and occasional attentional lapses is explained by complex brain activity during task performance. Greater right temporal-parietal junction activity and greater right inferior frontal gyrus activity have been found prior to fast responses, facilitating stimulus-triggered reorienting of attention after attentional lapses (Weissman, Roberts, Visscher, & Woldorff, 2006). Further, occasional attentional lapses have been related to reduced activity in the frontal cortex, prior to stimulus presentation (Weissman et al., 2006). Further research could focus on the underpinnings of the specificity of this reaction pattern in ADHD.

In terms of examining the clinical value of neurocognitive profiling in ADHD, our results showed no significant associations between any of the neurocognitive subgroups and measures of externalising, social and academic problems that are often found in ADHD. This lack of findings questions the clinical relevance of classifying children with ADHD into neurocognitive subgroups, in line with the limited predictive validity of neurocognitive functioning for persistency of ADHD (Van Lieshout et al., 2013). We might conclude that neurocognitive problems in ADHD could be seen as epiphenomena, potentially sharing the same aetiological factors as the ADHD symptoms, but that neurocognitive deficiencies do not mediate or moderate the association between ADHD and problems in various functional outcomes.

There are several limitations to the current study. Firstly, although several aspects of neurocognitive functioning were included, our neurocognitive assessment is non-exhaustive and did not tap into some important neurocognitive domains that have been

found altered in ADHD, such as temporal information processing (Toplak, Dockstader, & Tannock, 2006) and reward sensitivity (Luman, Oosterlaan, & Sergeant, 2005). However, our selection of neurocognitive measures is larger and more diverse than the neurocognitive measures used in previous studies. Secondly, although the size of our sample of children with ADHD was similar to the sample (*n*=96) of Van Hulst et al. (2015), replication in a larger sample of children with ADHD is warranted, as the subgroups had small sample sizes. Thirdly, our study was cross-sectional and therefore focused on the current externalising, social and academic problems of children with ADHD. In order to gain more insight into the predictive validity of neurocognitive profiles on future functioning, a longitudinal approach is recommended.

To conclude, in the current study we were able to replicate the results of previous work on subtyping children with ADHD based on neurocognitive functioning, showing that in children with ADHD neurocognitive subgroups can be found, that are also present in a group of TD children. We also found a unique subgroup in children with ADHD, characterised by fast processing speed and increased attentional lapses, which was not present in TD children. In the current study no meaningful relationship was found between neurocognitive subgroups and functional outcomes.

References

Achenbach, T.M. (1991). *Manual for the Child Behavior Checklist/4-18 and 1991 profile*. Burlington: Department of Psychiatry, University of Vermont.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders, fifth edition.* Arlington: American Psychiatric Association.

Antonini, T.N., Kingery, K.M., Narad, M.E., Langberg, J.M., Tamm, L., & Epstein, J.N. (2016). Neurocognitive and behavioral predictors of math performance in children with and without ADHD. *Journal of Attention Disorders, 20*(2), 108-118.

Bergman Nutley, S., Soderqvist, S., Bryde, S., Humphreys, K., & Klingberg, T. (2010). Measuring working memory capacity with greater precision in the lower capacity ranges. *Developmental Neuropsychology, 35*(1), 81-95.

Cadesky, E.B., Mota, V.L., & Schachar, R.J. (2000). Beyond words: How do children with ADHD and/or conduct problems process nonverbal information about affect? *Journal of the American Academy of Child & Adolescent Psychiatry, 39*(9), 1160-1167.

Coghill, D., Seth, S., & Matthews, K. (2014). A comprehensive assessment of memory, delay aversion, timing, inhibition, decision making and variability in attention deficit hyperactivity disorder: Advancing beyond the three-pathway models. *Psychological Medicine, 44*(9), 1989-2001.

Coie, J.D., & Dodge, K.A. (1983). Continuities and changes in children's social status: A five-year longitudinal study. *Merrill-Palmer Quarterly, 29*(3), 261-282.

De Wijs, A., Krom, R., & van Berkel, S. (2006). *Spelling.* Arnhem, the Netherlands: CITO.

Egger, H.L., Pine, D.S., Nelson, E., Leibenluft, E., Ernst, M., Towbin, K.E., & Angold, A. (2011). The NIMH Child Emotional Faces Picture Set (NIMH-ChEFS): A new set of children's facial emotion stimuli. *International Journal of Methods in Psychiatric Research, 20*(3), 145-156.

Eriksen, B.A., & Eriksen, C.W. (1974). Effects of noise letters upon the identification of a target letter in a nonsearch task. *Perception & Psychophysics, 16*(1), 143-149.

Fair, D.A., Bathula, D., Nikolas, M.A., & Nigg, J.T. (2012). Distinct neuropsychological subgroups in typically developing youth inform heterogeneity in children with ADHD. *Proceedings of the National Academy of Sciences, 109*(17), 6769-6774.

Field, A., Miles, J., & Field, Z. (2012). *Discovering statistics using R*. London: Sage.

Fortunato, S., & Barthelemy, M. (2007). Resolution limit in community detection. *Proceedings of the National Academy of Sciences, 104*(1), 36-41.

Gillberg, C., Gillberg, I.C., Rasmussen, P., Kadesjö, B., Söderström, H., Råstam, M., . . . Niklasson, L. (2004). Co–existing disorders in ADHD–implications for diagnosis and intervention. *European Child & Adolescent Psychiatry, 13*(1), i80-i92.

Hervey, A.S., Epstein, J.N., Curry, J.F., Tonev, S., Eugene Arnold, L., Keith Conners, C., . . . Hechtman, L. (2006). Reaction time distribution analysis of neuropsychological performance in an ADHD sample. *Child Neuropsychology, 12*(2), 125-140.

Hu, L.-t., & Bentler, P.M. (1998). Fit indices in covariance structure modeling: Sensitivity to underparameterized model misspecification. *Psychological Methods, 3*(4), 424-453.

Janssen, J., Verhelst, N., Engelen, R., & Scheltens, F. (2010). *Wetenschappelijke verantwoording van de toetsen LOVS Rekenen-Wiskunde voor groep 3 tot en met 8 [Scientific justification of the mathematics tests for Grade 1 to 6]*. Arnhem, the Netherlands: CITO.

Kessels, R.P., Van Zandvoort, M.J., Postma, A., Kappelle, L.J., & De Haan, E.H. (2000). The Corsi block-tapping task: Standardization and normative data. *Applied Neuropsychology, 7*(4), 252-258.

Loe, I.M., & Feldman, H.M. (2007). Academic and educational outcomes of children with ADHD. *Journal of Pediatric Psychology, 32*(6), 643-654.

Luman, M., Oosterlaan, J., & Sergeant, J.A. (2005). The impact of reinforcement contingencies on AD/HD: A review and theoretical appraisal. *Clinical Psychology Review, 25*(2), 183-213.

McQuade, J.D., & Hoza, B. (2008). Peer problems in attention deficit hyperactivity disorder: Current status and future directions. *Developmental Disabilities Research Reviews, 14*(4), 320-324.

Mostert, J.C., Hoogman, M., Onnink, A.M.H., Van Rooij, D., Von Rhein, D., Van Hulzen, K.J., . . . Norris, D.G. (2015). Similar subgroups based on cognitive performance parse heterogeneity in adults with ADHD and healthy controls. *Journal of Attention Disorders*. doi:10.1177/1087054715602332.

Mullane, J.C., Corkum, P.V., Klein, R.M., & McLaughlin, E. (2009). Interference control in children with and without ADHD: A systematic review of Flanker and Simon task performance. *Child Neuropsychology, 15*(4), 321-342.

Nigg, J.T., Willcutt, E.G., Doyle, A.E., & Sonuga-Barke, E.J. (2005). Causal heterogeneity in attention-deficit/hyperactivity disorder: Do we need neuropsychologically impaired subtypes? *Biological Psychiatry, 57*(11), 1224-1230.

Noordermeer, S.D.S., Luman, M., Buitelaar, J.K., Hartman, C.A., Hoekstra, P.J., Franke, B., . . . Oosterlaan, J. (2015). Neurocognitive deficits in attention-deficit/hyperactivity disorder with and without comorbid oppositional defiant disorder. Journal of Attention Disorders. doi:10.1177/1087054715606216.

Nowicki, S., & Duke, M.P. (1994). Individual differences in the nonverbal communication of affect: The Diagnostic Analysis of Nonverbal Accuracy Scale. *Journal of Nonverbal Behavior, 18*(1), 9-35.

Pelham, W.E., Gnagy, E.M., Greenslade, K.E., & Milich, R. (1992). Teacher ratings of DSM-III-R symptoms for the disruptive behavior disorders. *Journal of the American Academy of Child & Adolescent Psychiatry, 31*(2), 210-218.

Rubinov, M., & Sporns, O. (2011). Weight-conserving characterization of complex functional brain networks. *Neuroimage, 56*(4), 2068-2079.

Sattler, J.M. (2008). *Resource guide to accompany assessment of children: Cognitive foundations*. La Mesa, CA: Jerome M. Sattler, Publisher, Inc.

Scheres, A., Oosterlaan, J., Swanson, J., Morein-Zamir, S., Meiran, N., Schut, H., . . . Sergeant, J.A. (2003). The effect of methylphenidate on three forms of response inhibition in boys with AD/HD. *Journal of Abnormal Child Psychology, 31*(1), 105-120.

Shaffer, D., Fisher, P., Lucas, C.P., Dulcan, M.K., & Schwab-Stone, M.E. (2000). NIMH Diagnostic Interview Schedule for Children Version IV (NIMH DISC-IV): Description, differences from previous versions, and reliability of some common diagnoses. *Journal of the American Academy of Child & Adolescent Psychiatry, 39*(1), 28-38.

Shaw, P., Stringaris, A., Nigg, J., & Leibenluft, E. (2014). Emotion dysregulation in attention deficit hyperactivity disorder. *American Journal of Psychiatry, 171*(3), 276-293.

Sonuga-Barke, E., Bitsakou, P., & Thompson, M. (2010). Beyond the dual pathway model: Evidence for the dissociation of timing, inhibitory, and delay-related impairments in attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child & Adolescent Psychiatry, 49*(4), 345-355.

Sonuga-Barke, E.J., & Halperin, J.M. (2010). Developmental phenotypes and causal pathways in attention deficit/hyperactivity disorder: Potential targets for early intervention? *Journal of Child Psychology and Psychiatry, 51*(4), 368-389.

Staphorsius, G., & Krom, R. (1998). *CITO toetsen begrijpend lezen [CITO Reading Comprehension Tests]*. Arnhem, the Netherlands: CITO.

Tamm, L., Narad, M.E., Antonini, T.N., O'Brien, K.M., Hawk Jr, L.W., & Epstein, J.N. (2012). Reaction time variability in ADHD: A review. *Neurotherapeutics, 9*(3), 500-508.

Toplak, M.E., Dockstader, C., & Tannock, R. (2006). Temporal information processing in ADHD: Findings to date and new methods. *Journal of Neuroscience Methods, 151*(1), 15-29.

Townsend, J.T., & Ashby, F.G. (1983). *Stochastic modeling of elementary psychological processes.* Cambridge: Cambridge University Press.

Trentacosta, C.J., & Fine, S.E. (2010). Emotion knowledge, social competence, and behavior problems in childhood and adolescence: A meta-analytic review. *Social Development, 19*(1), 1-29.

Van Hulst, B., De Zeeuw, P., & Durston, S. (2015). Distinct neuropsychological profiles within ADHD: A latent class analysis of cognitive control, reward sensitivity and timing. *Psychological Medicine, 45*, 735-745.

Van Lieshout, M., Luman, M., Buitelaar, J., Rommelse, N., & Oosterlaan, J. (2013). Does neurocognitive functioning predict future or persistence of ADHD? A systematic review. *Clinical Psychology Review, 33*(4), 539-560.

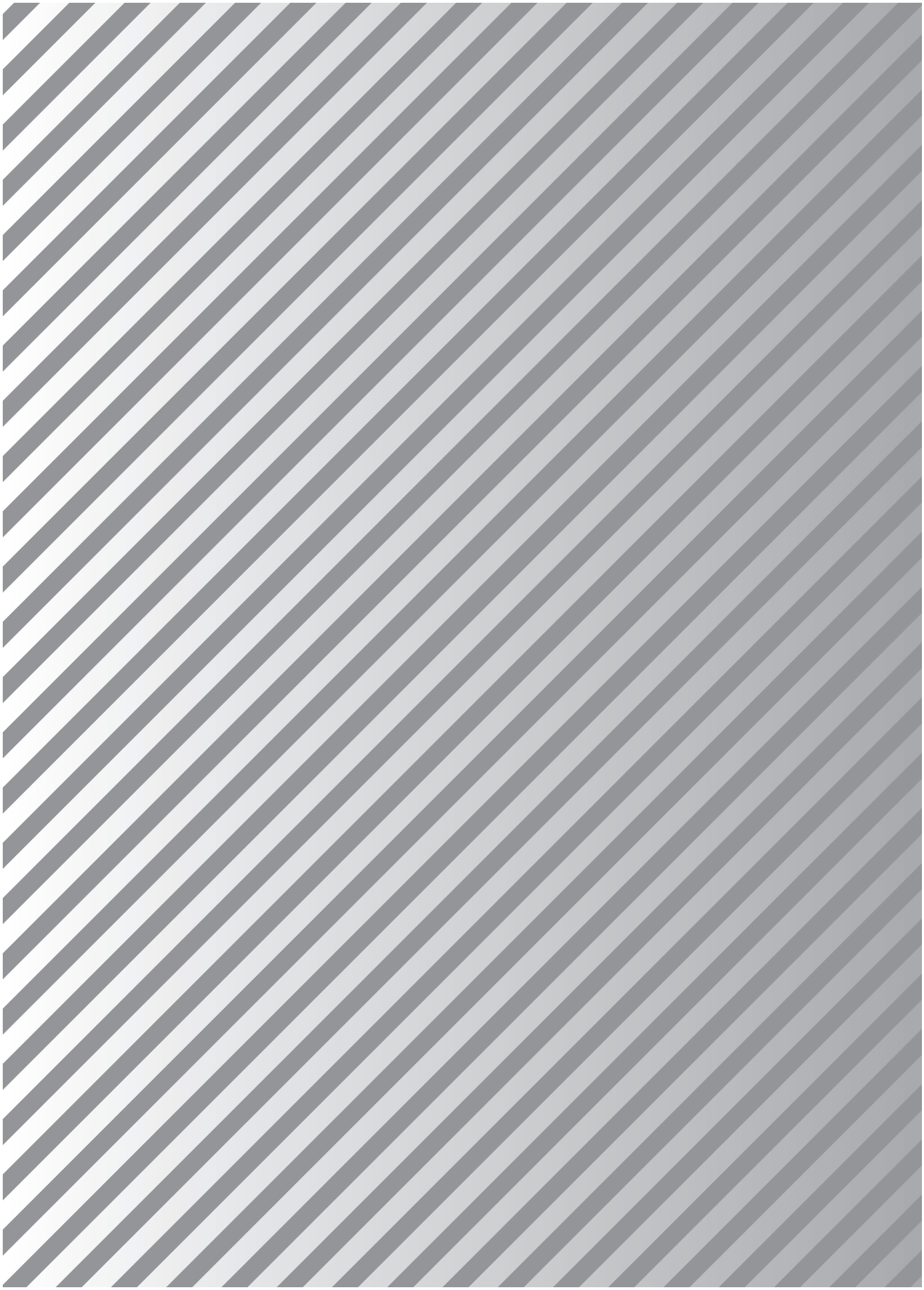
Wechsler, D. (1991). *Wechsler intelligence scale for children, third edition: Manual*. San Antonio: The Psychological Corporation.

Weissman, D., Roberts, K., Visscher, K., & Woldorff, M. (2006). The neural bases of momentary lapses in attention. *Nature Neuroscience, 9*(7), 971-978.

Whelan, R. (2010). Effective analysis of reaction time data. *The Psychological Record, 58*(3), 475-482.

Willcutt, E.G. (2012). The prevalence of DSM-IV attention-deficit/hyperactivity disorder: A meta-analytic review. *Neurotherapeutics, 9*(3), 490-499.

Zelazo, P.D., & Carlson, S.M. (2012). Hot and cool executive function in childhood and adolescence: Development and plasticity. *Child Development Perspectives, 6*(4), 354-360.



Chapter 5

No objectively measured sleep disturbances in children with attention-deficit/hyperactivity disorder

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Abstract

Objective. The main goal of this study is to gain more insight into sleep disturbances in children with attention-deficit/hyperactivity disorder (ADHD), using objective measures of sleep quality and quantity. The evidence for sleep problems in children with ADHD thus far is inconsistent, which might be explained by confounding influences of comorbid internalising and externalising problems, and low socioeconomic status. We therefore investigate the mediating and moderating role of these factors in the association between ADHD and sleep problems. To control for the effects of stimulant medication use, all participants are tested free of medication.

Methods. Sixty-three children with ADHD and 61 typically developing (TD) children, aged 6 to 13 years, participated. Sleep was monitored for one to three school nights using actigraphy. Parent and teacher questionnaires assessed symptoms of ADHD, internalising behaviour, oppositional defiant disorder and conduct disorder.

Results. Results showed no differences between the ADHD and TD group in any sleep parameter. Within the ADHD group, severity of ADHD symptoms was not related to sleep quality or quantity. Moderation analyses in the ADHD group showed an interaction effect of internalising and externalising behaviour on total sleep time, time in bed and average sleep bout duration.

Conclusion. The results of our study suggest that having ADHD is not a risk factor for sleep problems. Internalising and externalising behaviour moderate the association between ADHD and sleep, indicating a complex interplay between psychiatric symptoms and sleep.

Introduction

The most common childhood psychiatric disorder, attention-deficit/hyperactivity disorder (ADHD), is characterised by a persistent pattern of age-inappropriate levels of inattention and/or hyperactivity-impulsivity (American Psychiatric Association, 2013). Besides suffering from ADHD symptoms, children with ADHD are often hampered by other difficulties, including internalising and externalising behaviour and learning problems (Gillberg et al., 2004). In addition, clinical observations suggest that ADHD is associated with an increased prevalence of sleep disturbances (Corkum, Tannock, & Moldofsky, 1998). A meta-analysis of studies using subjective measures of sleep quality (questionnaires filled out by parents) shows that children with ADHD have higher bedtime resistance, more sleep onset difficulties, nocturnal awakenings, difficulties with arising in the morning and sleep disordered breathing compared to controls, although for all results considerable heterogeneity was reported across the studies (Cortese, Faraone, Konofal, & Lecendreux, 2009). A recent meta-analysis on sleep studies using actigraphy to objectively measure sleep, showed as well that non-medicated children with ADHD have increased sleep onset latency and decreased sleep efficiency, although, again, results were inconsistent (De Crescenzo et al., 2016). However, it also showed that non-medicated children with ADHD do not suffer from altered sleep duration or increased wakefulness after sleep onset (De Crescenzo et al., 2016), in contrast to the findings of studies using subjective measures (Cortese et al., 2009; Yoon, Jain, & Shapiro, 2012).

A possible reason for the inconsistencies in results between studies measuring sleep in children with ADHD is the presence of comorbid psychiatric disorders. ADHD increases the risk of internalising disorders, such as anxiety disorder and depression, with comorbidity rates of 13 to 51 percent in children with ADHD, and of externalising disorders, including oppositional defiant disorder (ODD) and conduct disorder (CD), with 43 to 93 percent of children with ADHD meeting criteria for ODD and/or CD (Gillberg et al., 2004; Jensen, Martin, & Cantwell, 1997). Comorbid psychiatric conditions might act as possible confounding (mediating) or exacerbating (moderating) factors in sleep problems in ADHD. For instance, having an anxiety disorder or depression was related to an increased sleep onset latency or increased awake time in children (Chorney, Detweiler, Morris, & Kuhn, 2008). Furthermore, having comorbid ODD in children with ADHD was associated with an increased risk of bedtime resistance, increased sleep

onset latency and difficulty in arising (Corkum, Moldofsky, Hogg-Johnson, Humphries, & Tannock, 1999). Children with ADHD and ODD had more parent-rated sleep problems than children with ADHD without ODD (Owens et al., 2009). CD has been associated with increased sleep disordered breathing and periodic leg movements during sleep (Chervin, Dillon, Archbold, & Ruzicka, 2003). It is therefore important to control for these comorbid conditions when studying sleep in ADHD. In case we would find that internalising or externalising behaviour contributes to decreased sleep quality or quantity in children with ADHD, such a finding could be of relevance for the treatment of sleep problems in ADHD. Inadequate behaviour of children leading to sleep problems might be amenable to treatment and successful treatment may lead to benefits for the child and family (Hiscock et al., 2015).

A second factor that may explain the inconsistencies in results of studies investigating sleep problems in children with ADHD is socioeconomic status (SES). Coming from a low SES environment is related to a greater risk of ADHD (Willcutt, 2012) and to poorer parenting quality (Dodge, Pettit, & Bates, 1994). Parenting quality in turn is an important factor in the sleep quality of children, given that parental harshness, inconsistent parenting and negative parent-child interactions are risk factors for sleep disturbances in children (Erath & Tu, 2011). Additional factors that add to lower sleep quality in low SES children are poorer sleep conditions due to crowded, noisier and lower quality homes (Evans, 2004). We therefore hypothesise that having a low SES might play a mediating or moderating role in sleep problems in children with ADHD and therefore this factor should be taken into account when studying sleep in ADHD.

The main goal of the current study is to gain more insight into sleep problems in ADHD using objective measures of sleep quality. Our study aims at filling a niche in the literature, by providing relatively large and well-phenotyped samples, in which a broad array of objective measures of sleep is assessed and in which potential confounding or exacerbating risk factors for sleep problems are taken into account. We hypothesise that higher levels of internalising and externalising behaviour and low SES mediate or exacerbate and hence moderate the association between ADHD and sleep problems. All participants are tested free of medication, to control for possible confounding effects of methylphenidate on sleep onset latency (Corkum, Panton, Ironside, MacPherson, & Williams, 2008) and sleep efficiency (Schwartz et al., 2004). It is important to examine whether children with ADHD are at increased risk of sleep problems, as sleep quantity

and quality may impact on academic performance (Curcio, Ferrara, & De Gennaro, 2006; Fallone, Acebo, Seifer, & Carskadon, 2005), executive functioning (Astill, Van der Heijden, Van IJzendoorn, & Van Someren, 2012) and inattentive behaviour (Beebe, 2011). Lastly, we explore the stability of the sleep-wake system, as there is some evidence for an increased instability of the sleep-wake system in children with ADHD (Gruber & Sadeh, 2004; Gruber, Sadeh, & Raviv, 2000).

Methods

Participants

Sixty-three children with ADHD (75 percent boys) and 61 typically developing (TD) children (52 percent boys), aged 6 to 13 years old, participated. Inclusion criteria for the ADHD group were: (a) a clinical diagnosis of ADHD according to DSM-IV criteria, (b) confirmation of this diagnosis by the parent version of the Diagnostic Interview Schedule for Children, fourth edition (DISC-IV-P; Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000), (c) severity of ADHD symptoms as indicated by scores >90th percentile on at least one of the ADHD scales (Inattention and Hyperactivity/Impulsivity scales) of the parent version of the Disruptive Behaviour Disorder Rating Scale (DBDRS; Pelham, Gnagy, Greenslade, & Milich, 1992), and (d) pervasiveness of ADHD symptoms as indicated by scores >75th percentile on at least one of the ADHD scales of the teacher version of the DBDRS. The cut-off criterion of the 90th percentile for the parent questionnaire was chosen to ensure that children in our ADHD group had significant levels of ADHD symptoms. The cut-off criterion for the teacher questionnaire was more liberal than for the parent questionnaire, since (a) the DSM-IV requires some of the symptoms to be present in at least two situations and (b) ADHD symptoms of the majority of our ADHD sample (62 percent) were often tempered at school due to stimulant medication use.

Inclusion criteria for the TD group were: (a) absence of any developmental or behavioural disorder (including ADHD and ODD, established by parent report), and (b) and scores <90th percentile on both parent- and teacher-rated ADHD scales of the DBDRS. The cut-off criterion of the 90th percentile was chosen to ensure that children in our TD sample had no significant levels of ADHD symptoms.

Materials

Sleep measures

Sleep quality and quantity were measured using actiwatches (Actiwatch 2, Philips Respironics), placed on the non-dominant wrist of children. Participants were instructed to wear the actiwatch for 72 consecutive hours. This time period was chosen to minimise the methylphenidate-free period of medicated participants with ADHD, while still collecting reliable and valid sleep data (Littner et al., 2003). Although a minimum of four to five nights would be recommended to enhance reliability of our measures of sleep (Acebo et al., 1999), this would have resulted in a biased sample of children with ADHD, due to drop out of children whose parents or teachers would object to discontinuation of stimulant medication for more than four school days. Data were collected for 15 seconds epochs. Only data derived of nights prior to school days ('school nights') were used, to control for shifts in sleep patterns during weekends and holidays. Excluding the data of non-school nights, in combination with data loss due to non-compliance or technical errors, resulted in groups of participants for whom data of three school nights (n=48), two school nights (n=42) or one school night (n=34) was available, evenly distributed over the ADHD and TD group. There were no significant differences between these three groups for any sleep parameter in the ADHD and TD group (ps>.35). Therefore, all available data were used, applying a minimum of one school night.

Within the actiware software (Philips Actiware 5.71) a validated sleep-wake algorithm (Kushida et al., 2001) is applied to determine whether an epoch is scored as wake or sleep, by comparing activity counts for the epoch in question and the 8 epochs immediately surrounding it, with a medium sensitivity threshold. For sleep onset the default setting was used, that identifies the first ten minutes for which all epochs but one are scored as immobile and then selects the first epoch of that group as sleep onset. Similar settings were used to determine sleep end, by identifying the last ten minutes for which all epochs but one are scored as immobile. Sleep end is then set to the last epoch of the period satisfying these requirements. Dependent variables were calculated for school nights and then averaged and included: (1) time in bed (minutes between lights out and on); (2) total sleep time (minutes scored as sleep between lights out and on); (3) nocturnal motor activity (activity count per minute between sleep onset and sleep end); (4) sleep onset latency (minutes between lights out and sleep onset); (5) morning arising latency (minutes between sleep end and lights on); (6) average wake bout duration

(minutes scored as wake between sleep intervals); and (7) average sleep bout duration (minutes scored as sleep between wake intervals). The last two parameters reflect the stability of the sleep pattern in children during the night, as a short average sleep bout duration shows that a child has an increased amount of night awakenings and a long average wake bout duration shows that a child has longer episodes of night awakenings. For our exploratory analyses, we examined the night-to-night variability in sleep onset latency and total sleep time, using the standard deviations of these measures over multiple nights.

Possible mediators and moderators

Internalising behaviour was assessed using the Child Behavior Checklist (CBCL) and Teacher Rating Form (TRF; Achenbach, 1991), completed by parents and teachers, respectively. Raw scores on the Internalising Problems scale of both questionnaires served as dependent measures. Externalising behaviour was measured using raw scores on the ODD and CD scales of both the parent and teacher DBDRS. Both for measures of internalising and externalising behaviour raw scores were used, to capture all available variation between individuals. By using raw scores of behaviour, we did not adjust for effects of gender or age, similarly to our sleep parameters that were also not adjusted for effects of gender and age.

Data regarding parental education and parental occupation were collected to determine SES. The classification of the national center for statistical information was used to determine the highest level of education and occupation on 6-point scales of both the father and mother (Statistics Netherlands, 2006, 2010). SES was calculated as the average of the education and occupation scores of both parents in case of two caretakers and as the average of the education and occupation scores of one parent in case of a single caretaker.

Procedure

Children with ADHD were recruited by the first author, from mental health outpatient clinics, through the parent association for children with behavioural problems, and through a university research website. Recruitment via a diverse capture area and multiple recruitment sources resulted in a heterogeneous ADHD sample, consisting of children from clinical as well as non-clinical settings, including children from all parts of the country and reflecting great heterogeneity in terms of ADHD symptom severity

and presence of comorbid psychiatric disorders. The TD group was recruited by the first author, with the help of research assistants, from primary schools located throughout the Netherlands. The data were collected between February 2013 and July 2014. The ADHD and TD group were recruited simultaneously, to control for seasonal effects on sleep quality. Prior to participation, written informed consent was obtained from parents of all children, and from children ≥12 years. Children using simulant medication or melatonin discontinued use at least 24 hours before participating in the current study. Parents and teachers filled out questionnaires on a secured website. The current study is part of a study into the relationship between ADHD and nutrients, which received approval from the local medical ethical committee (#NL39922.029.12).

Data Analysis

Group differences in sleep parameters were assessed using Analysis of Variance (ANOVA). Mediation and moderation analyses were conducted in the ADHD sample. For these analyses an aggregated measure of ADHD symptoms was calculated, which was the mean of the Inattention and Hyperactivity/Impulsivity scales of both the parent and teacher DBDRS. Mediation was tested by calculating the path coefficients between (a) the aggregated ADHD measure and seven sleep parameters, (b) the aggregated ADHD measure and seven possible mediators, and (c) the possible mediators and the sleep parameters while correcting for the presence of ADHD symptoms, using regression analyses (Baron & Kenny, 1986). It was subsequently tested whether the direct associations between the aggregated ADHD measure and sleep parameters were still significant after controlling for the indirect (mediated) effects. Moderation was tested using multiple hierarchical regression analyses for each sleep parameter. In the first step the aggregated ADHD measure was inserted as independent variable, in the second step the seven potential moderators were included, and in the third step the interaction effects between the aggregated ADHD measure and each of the seven possible moderators were entered separately.

Results

For group characteristics, see Table 5.1. Groups were equal in terms of age, but differed in gender and ADHD symptoms. The ADHD group had a larger proportion of males than the TD group. The DISC-IV-P showed that the majority of the participants with ADHD (*n*=49) met criteria for the combined subtype, with ten children meeting the criteria for the inattentive subtype and four for the hyperactive/impulsive subtype. According to the

DISC-IV-P, 26 of the participants with ADHD fulfilled diagnostic criteria for a diagnosis of ODD and/or CD. Prior to participation 39 children with ADHD (62 percent) used stimulant medication and seven children with ADHD (11 percent) used melatonin.

Table 5.1. Group characteristics of the ADHD group (*n*=63) and TD group (*n*=61)

	ADHD group *M (SD)*	TD group *M (SD)*	Statistic (t/χ^2)
Age in months	117.38 (19.47)	121.74 (19.28)	-1.25, NS
Males *n* (%)	47 (74.60)	32 (52.46)	6.57*
Parent-rated symptoms			
Inattention	16.98 (4.92)	3.56 (3.14)	18.18**
Hyperactivity/Impulsivity	15.98 (5.84)	3.38 (2.79)	15.41**
Teacher-rated symptoms			
Inattention	14.59 (6.10)	2.05 (2.58)	14.99**
Hyperactivity/Impulsivity	12.89 (6.91)	1.70 (2.39)	12.12**

Notes. [a]Disruptive Behaviour Disorder Rating Scale. *p<.05, **p<.01. ADHD, attention-deficit/hyperactivity disorder; NS, not significant; TD, typically developing.

Firstly, it was examined whether children with ADHD differed from TD children on objective measures of sleep, see Table 5.2 for results. Groups did not differ significantly in time in bed, total sleep time or nocturnal motor activity. Further, children with ADHD did not have an increased sleep onset latency or increased morning arising latency compared to typically developing children. Also no group differences were found with regard to average wake bout duration or average sleep bout duration. Exploratory analyses showed that the ADHD group (*n*=45) did not have a significantly increased night-to-night variability in sleep onset latency or total sleep time compared to the TD group (*n*=45). Since the ADHD group consisted of considerably more males than the TD group, the group analyses were rerun with gender as a covariate. These analyses did not alter the results.

Secondly, mediation analyses were carried out in the ADHD group. None of the path coefficients of associations between the aggregated measure of ADHD and sleep parameters were significant (all βs<.23, all ps>.05). Given that there were no direct associations between ADHD symptoms and sleep problems, further steps in the mediation analyses were not carried out.

Table 5.2. Actigraphic measures of sleep quality in the ADHD group (*n*=63) and TD group (*n*=61)

	ADHD group *M (SD)*	TD group *M (SD)*	*F*	*p*	pη^2
Time in bed	613.06 (54.87)	594.34 (49.26)	3.99	.05	.03
Total sleep time	518.02 (42.40)	506.14 (45.62)	2.26	.14	.02
Nocturnal motor activity	15.61 (7.16)	13.67 (5.13)	3.00	.09	.02
Sleep onset latency	19.39 (13.39)	21.12 (13.64)	.51	.48	<.01
Morning arising latency	25.55 (20.98)	23.45 (16.29)	.39	.54	<.01
Average wake bout duration	.90 (.28)	.82 (.24)	3.17	.08	.03
Average sleep bout duration	9.92 (2.46)	10.01 (2.39)	.05	.83	<.01
Variability in sleep onset latency	11.72 (9.30)[a]	10.48 (8.18)[a]	.45	.51	.01
Variability in total sleep time	31.11 (21.57)[a]	29.38 (17.92)[a]	.17	.68	<.01

Notes. [a]*n*=45. ADHD, attention-deficit/hyperactivity disorder; TD, typically developing.

Thirdly, using multiple hierarchical regression analyses, the possible moderating effects of internalising and externalising behaviour and low SES on the associations between the aggregated ADHD measure and sleep parameters were examined. For time in bed, significant interaction effects were found between the aggregated ADHD measure and (1) parent-rated ODD (β=.31, p<.05), (2) parent-rated CD (β=.52, p<.01), and (3) teacher-rated CD (β=.54, p<.01). For total sleep time, there were significant interactions between the aggregated ADHD measure and (1) parent-rated internalising behaviour (β=.36, p<.01), (2) parent-rated ODD (β=.31, p<.05), (3) parent-rated CD (β=.45, p<.01), and (4) teacher-rated CD (β=.38, p<.05). Finally, for average sleep bout duration, the aggregated ADHD measure interacted with teacher-rated CD (β=-.46, p<.01). All other interaction terms were not significant. For significant interaction effects, scatterplots were inspected and correlation analyses were carried out to examine whether the associations between the aggregated ADHD measure and sleep parameters differed across children with normal (percentile scores <90) or (sub)clinical levels (percentile scores ≥90) of the moderators in the ADHD sample. The slopes for the normal and (sub) clinical groups were inspected in scatterplots of ADHD symptoms and sleep parameters. Results showed that in children with ADHD and normal levels of parent-rated ODD symptoms there was a trend towards a negative association between ADHD symptoms and total sleep time (r=-.28, p=.07), while in children with ADHD and (sub)clinical levels of parent-rated ODD symptoms there was a trend towards a positive association between ADHD symptoms and total sleep time (r=.43, p=.05). Further, we found that in children

with ADHD and normal levels of parent-rated ODD symptoms and CD symptoms there was a negative association between ADHD symptoms and time in bed (r=-.38 and r=-.32 respectively, p<.05), whereas in children with ADHD and (sub)clinical levels of parent-rated ODD symptoms and CD symptoms there was no significant association between ADHD symptoms and time in bed. For the other significant interactions between our moderators and ADHD symptoms in the association with sleep parameters there was no clear evidence for significant differences in regression slopes between groups of children with ADHD and normal and (sub)clinical levels of internalising and externalising behaviour. We did not find the expected moderation of SES on the associations between ADHD symptoms and sleep parameters.

Discussion

The aim of the current study is to gain more insight into sleep problems in children with ADHD using objective measures of sleep. The main finding is that medication-free children with ADHD have normal sleep quality and quantity. This is further supported by the finding that ADHD symptoms are not related to any of the sleep parameters within the ADHD sample. Given the relatively large sample sizes used in the current study and the well-phenotyped samples of children with ADHD and typically developing controls, our results challenge the hypothesis of ADHD being significantly related to sleep problems. This conclusion is in accordance with the meta-analysis on actigraphic sleep studies in non-medicated children with ADHD, that showed that non-medicated children with ADHD do not suffer from altered sleep duration or increased wakefulness after sleep onset (De Crescenzo et al., 2016). Our study hereby contributes to the growing body of literature showing no evidence for objectively measured sleep problems in medication-free children with ADHD.

The discrepancy between the evidence in support of sleep problems as reported by subjective studies (Cortese et al., 2009) and the absence of evidence for objective sleep problems in our study (and other studies) using actigraphy, questions the association between parent-perceived sleep problems and objectively measured sleep problems. This is in line with studies that found parent-rated problems in children with ADHD, but limited or no problems in actigraphic measures of sleep in the same sample (Corkum, Tannock, Moldofsky, Hogg-Johnson, & Humphries, 2001; Owens et al., 2009). We suggest that other factors than disordered sleep play a role in the perception of parents of children with ADHD regarding the sleep quality of their child. Problematic behaviour of children

with ADHD throughout the day may cause a negative halo effect resulting in a negative perception of parents of the sleep pattern of their child (Cortese et al., 2009). Often, the behaviour of a child with ADHD is more demanding throughout the day, resulting in increased parental stress levels (Anastopoulos, Guevremont, Shelton, & DuPaul, 1992). This might decrease the ability of parents to focus on positive child behaviour and increase the tendency to overreact to negative child behaviour (Anastopoulos et al., 1992). Further research is required to explore the putative cause of parent-reported sleep problems in children with ADHD.

An explanation for the discrepancy between absence of sleep problems in children with ADHD in our study and evidence of sleep problems in some other studies (Yoon et al., 2012), might be that the children who participated in the current study withdrew from medication use during the study, whereas in other studies children continued using stimulant medication (Yoon et al., 2012). Although studies have reported that methylphenidate has a (direct) negative impact on sleep quality as compared to placebo (Corkum et al., 2008; Schwartz et al., 2004), our findings suggest that the discontinuation of methylphenidate use, even for a short period, may ameliorate possible negative effects of methylphenidate use on sleep quality in ADHD. Our findings could also suggest that medicated children with ADHD have developed some healthy sleep habits to diminish the negative effects of methylphenidate on sleep onset latency; however this hypothesis requires further research.

Furthermore, our moderation analyses showed some interaction effects between ADHD symptoms and internalising and externalising behaviour on time in bed, total sleep time and average sleep bout duration, although the interactions could not be easily explained when comparing sleep patterns in the ADHD group with (sub)clinical levels of psychiatric symptoms to the sleep patterns of children with ADHD and normal levels of this behaviour. Nevertheless, our findings indicate a complex interplay between ADHD symptoms and comorbid psychiatric symptoms, which might at least partly explain the heterogeneity within the current literature on sleep quality and quantity in ADHD.

There are several limitations to the current study that should be noted. Firstly, although actigraphy is a commonly used objective measure of sleep quality, it does not provide insight in parent-child interactions that might affect the perception of parents regarding the sleep quality of their child. We recommend to use videosomnography in future

studies, to gain insight in the behavioural aspects of sleep (Ipsiroglu et al., 2015). Secondly, we might not have captured sleep problems in children with ADHD using the data of only one to three nights. Given that a minimum of four or five nights of data collection is recommended in terms of reliability of the sleep measures (Acebo et al., 1999), the use of data of one to three nights should be seen as a limitation to our study. Alternative approaches that could be applied in future studies are (a) requiring medicated children with ADHD to withdraw from stimulant medication for a week or (b) recruiting medication naïve children with ADHD, although the risk of selection bias should be taken into account when using these alternative approaches.

Notwithstanding the limitations, the results of our study suggest that having ADHD is not a risk factor for sleep problems. We are confident that our sample of children with ADHD is an adequate representation of the ADHD population, as result of recruitment via a diverse capture area and multiple recruitment sources, and therefore expect that 25 to 50 percent of our ADHD sample had parent-perceived sleep problems (Corkum et al., 1998). As we generalise our results to the ADHD population, we assume that many parents of children with ADHD report sleep problems in the absence of objectively measurable sleep problems. For non-medicated children with ADHD, we suggest that alternative causes should be explored to explain the increased prevalence of parent-reported sleep problems. As suggested by Cortese et al. (2009), problematic behaviour of children with ADHD throughout the day may cause a negative halo effect, resulting in a negative perception of parents of the sleep pattern of their child. We suggest that clinicians, presented with a non-medicated child with ADHD and parent-rated sleep problems, explore whether the reported sleep problems might be caused by a biased perception of parents. Viewing parent-reported sleep problems of non-medicated children with ADHD as a possible sign of a negative parent-child interaction, could affect the treatment options for sleep problems. Currently a relatively large proportion of children with ADHD is treated with melatonin when faced with sleep disturbances. Melatonin treatment appears to be an effective intervention for children with ADHD treated with methylphenidate and suffering from insomnia (Weiss, Wasdell, Bomben, Rea, & Freeman, 2006). However, the results of our study question the adequacy of melatonin treatment in non-medicated children with ADHD.

References

Acebo, C., Sadeh, A., Seifer, R., Tzischinsky, O., Wolfson, A., Hafer, A., & Carskadon, M. (1999). Estimating sleep patterns with activity monitoring in children and adolescents: How many nights are necessary for reliable measures? *SLEEP, 22*(1), 95-103.

Achenbach, T.M. (1991). *Manual for the Child Behavior Checklist/4-18 and 1991 profile.* Burlington: Department of Psychiatry, University of Vermont.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders, fifth edition.* Arlington: American Psychiatric Association.

Anastopoulos, A.D., Guevremont, D.C., Shelton, T.L., & DuPaul, G.J. (1992). Parenting stress among families of children with attention deficit hyperactivity disorder. *Journal of Abnormal Child Psychology, 20*(5), 503-520.

Astill, R.G., Van der Heijden, K.B., Van IJzendoorn, M.H., & Van Someren, E.J. (2012). Sleep, cognition, and behavioral problems in school-age children: A century of research meta-analyzed. *Psychological Bulletin, 138*(6), 1109–1138.

Baron, R.M., & Kenny, D.A. (1986). The moderator–mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *Journal of Personality and Social Psychology, 51*(6), 1173-1182.

Beebe, D.W. (2011). Cognitive, behavioral, and functional consequences of inadequate sleep in children and adolescents. *Pediatric Clinics of North America, 58*(3), 649-665.

Chervin, R.D., Dillon, J.E., Archbold, K.H., & Ruzicka, D.L. (2003). Conduct problems and symptoms of sleep disorders in children. *Journal of the American Academy of Child & Adolescent Psychiatry, 42*(2), 201-208.

Chorney, D.B., Detweiler, M.F., Morris, T.L., & Kuhn, B.R. (2008). The interplay of sleep disturbance, anxiety, and depression in children. *Journal of Pediatric Psychology, 33*(4), 339-348.

Corkum, P., Moldofsky, H., Hogg-Johnson, S., Humphries, T., & Tannock, R. (1999). Sleep problems in children with attention-deficit/hyperactivity disorder: Impact of subtype, comorbidity, and stimulant medication. *Journal of the American Academy of Child & Adolescent Psychiatry, 38*(10), 1285-1293.

Corkum, P., Panton, R., Ironside, S., MacPherson, M., & Williams, T. (2008). Acute impact of immediate release methylphenidate administered three times a day on sleep in children with attention-deficit/hyperactivity disorder. *Journal of Pediatric Psychology, 33*(4), 368-379.

Corkum, P., Tannock, R., & Moldofsky, H. (1998). Sleep disturbances in children with attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child & Adolescent Psychiatry, 37*(6), 637-646.

Corkum, P., Tannock, R., Moldofsky, H., Hogg-Johnson, S., & Humphries, T. (2001). Actigraphy and parental ratings of sleep in children with attention-deficit/hyperactivity disorder (ADHD). *SLEEP, 24*(3), 303-312.

Cortese, S., Faraone, S.V., Konofal, E., & Lecendreux, M. (2009). Sleep in children with attention-deficit/hyperactivity disorder: Meta-analysis of subjective and objective studies. *Journal of the American Academy of Child & Adolescent Psychiatry, 48*(9), 894-908.

Curcio, G., Ferrara, M., & De Gennaro, L. (2006). Sleep loss, learning capacity and academic performance. *Sleep Medicine Reviews, 10*(5), 323-337.

De Crescenzo, F., Licchelli, S., Ciabattini, M., Menghini, D., Armando, M., Alfieri, P., . . . Foti, F. (2016). The use of actigraphy in the monitoring of sleep and activity in ADHD: A meta-analysis. *Sleep Medicine Reviews, 26*, 9-20.

Dodge, K.A., Pettit, G.S., & Bates, J.E. (1994). Socialization mediators of the relation between socioeconomic status and child conduct problems. *Child Development, 65*(2), 649-665.

Erath, S.A., & Tu, K.M. (2011). The parenting context of children's sleep. In M. El-Sheikh (Ed.), *Sleep and development: Familial and socio-cultural considerations* (pp. 29-47). New York: Oxford University Press.

Evans, G.W. (2004). The environment of childhood poverty. *American Psychologist, 59*(2), 77-92.

Fallone, G., Acebo, C., Seifer, R., & Carskadon, M.A. (2005). Experimental restriction of sleep opportunity in children: Effects on teacher ratings. *SLEEP, 28*(12), 1561-1567.

Gillberg, C., Gillberg, I.C., Rasmussen, P., Kadesjö, B., Söderström, H., Råstam, M., . . . Niklasson, L. (2004). Co–existing disorders in ADHD–implications for diagnosis and intervention. *European Child and Adolescent Psychiatry, 13*(1), i80-i92.

Gruber, R., & Sadeh, A. (2004). Sleep and neurobehavioral functioning in boys with attention-deficit/hyperactivity disorder and no reported breathing problems. *SLEEP, 27*(2), 267-273.

Gruber, R., Sadeh, A., & Raviv, A. (2000). Instability of sleep patterns in children with attention-deficit/hyperactivity disorder. J*ournal of the American Academy of Child & Adolescent Psychiatry, 39*(4), 495-501.

Hiscock, H., Sciberras, E., Mensah, F., Gerner, B., Efron, D., Khano, S., & Oberklaid, F. (2015). Impact of a behavioural sleep intervention on symptoms and sleep in children with attention deficit hyperactivity disorder, and parental mental health: Randomised controlled trial. *BMJ, 350*, h68.

Ipsiroglu, O.S., Hung, Y.-H.A., Forson Chan, M.L.R., Veer, D., Soo, S., Ho, G., . . . Kloesch, G. (2015). "Diagnosis by behavioral observation" home-videosomnography: A rigorous ethnographic approach to sleep of children with neurodevelopmental conditions. *Frontiers in Psychiatry.* doi: 10.3389/fpsyt.2015.00039.

Jensen, P.S., Martin, D., & Cantwell, D.P. (1997). Comorbidity in ADHD: Implications for research, practice, and DSM-V. *Journal of the American Academy of Child & Adolescent Psychiatry, 36*(8), 1065-1079.

Kushida, C.A., Chang, A., Gadkary, C., Guilleminault, C., Carrillo, O., & Dement, W.C. (2001). Comparison of actigraphic, polysomnographic, and subjective assessment of sleep parameters in sleep-disordered patients. *Sleep Medicine, 2*(5), 389-396.

Littner, M., Kushida, C.A., Anderson, W.M., Bailey, D., Berry, R.B., Davila, D.G., . . . Johnson, S.F. (2003). Practice parameters for the role of actigraphy in the study of sleep and circadian rhythms: An update for 2002. *SLEEP, 26*(3), 337-341.

Owens, J., Sangal, R.B., Sutton, V.K., Bakken, R., Allen, A.J., & Kelsey, D. (2009). Subjective and objective measures of sleep in children with attention-deficit/hyperactivity disorder. *Sleep Medicine, 10*(4), 446-456.

Pelham, W.E., Gnagy, E.M., Greenslade, K.E., & Milich, R. (1992). Teacher ratings of DSM-III-R symptoms for the disruptive behavior disorders. *Journal of the American Academy of Child & Adolescent Psychiatry, 31*(2), 210-218.

Schwartz, G., Amor, L.B., Grizenko, N., Lageix, P., Baron, C., Boivin, D.B., & Joober, R. (2004). Actigraphic monitoring during sleep of children with ADHD on methylphenidate and placebo. *Journal of the American Academy of Child & Adolescent Psychiatry, 43*(10), 1276-1282.

Shaffer, D., Fisher, P., Lucas, C.P., Dulcan, M.K., & Schwab-Stone, M.E. (2000). NIMH Diagnostic Interview Schedule for Children Version IV (NIMH DISC-IV): Description, differences from previous versions, and reliability of some common diagnoses. *Journal of the American Academy of Child & Adolescent Psychiatry, 39*(1), 28-38.

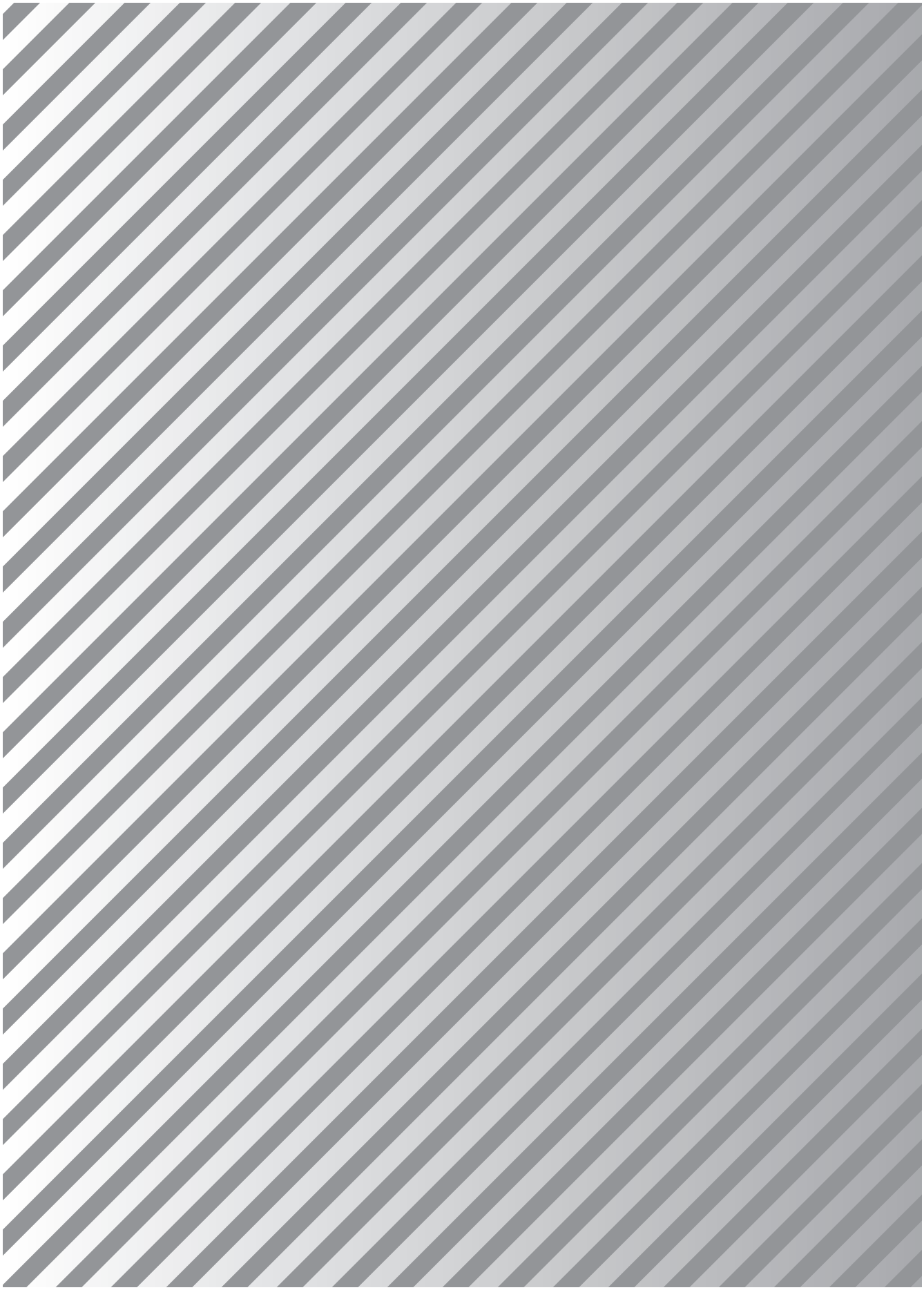
Statistics Netherlands. (2006). *Standard Educational Classification*. Den Haag/Heerlen, the Netherlands.

Statistics Netherlands. (2010). *Standard Occupational Classification.* Den Haag/Heerlen, the Netherlands.

Weiss, M.D., Wasdell, M.B., Bomben, M.M., Rea, K.J., & Freeman, R.D. (2006). Sleep hygiene and melatonin treatment for children and adolescents with ADHD and initial insomnia. *Journal of the American Academy of Child & Adolescent Psychiatry, 45*(5), 512-519.

Willcutt, E.G. (2012). The prevalence of DSM-IV attention-deficit/hyperactivity disorder: A meta-analytic review. *Neurotherapeutics, 9*(3), 490-499.

Yoon, S.Y.R., Jain, U., & Shapiro, C. (2012). Sleep in attention-deficit/hyperactivity disorder in children and adults: Past, present, and future. *Sleep Medicine Reviews, 16*(4), 371-388.



Chapter 6

Paediatric reference values for total homocysteine, tryptophan, tyrosine and phenylalanine in blood spots

This chapter is under review for publication as:

Bergwerff, C.E., Luman, M., Blom, H.J., & Oosterlaan, J. Paediatric reference values for total homocysteine, tryptophan, tyrosine and phenylalanine in blood spots.

Abstract

Objective. Determining blood concentrations of the amino acids homocysteine, tryptophan, tyrosine and phenylalanine in children is often required in clinical practice. Over the past decades, the use of blood spot samples to examine amino acid concentrations has increased, in particular in newborn screening. Especially in children the use of blood spot samples is of great clinical relevance, as this method is less invasive than taking venous blood samples. Currently, no paediatric reference values are available for amino acids in blood spots. The aim of the current study is to establish reference values for blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine in primary school-aged children.

Methods. Dried blood spots were obtained in a community sample of 104 healthy children, aged 6 to 12 years old (52% males). Blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine were determined by positive electrospray liquid chromatography–tandem mass spectrometry. Parents of participants completed questions regarding demographic characteristics. It was examined whether blood spot amino acid concentrations were related to gender and age.

Results. Our sample consisted of healthy children from various ethnic backgrounds, with varying levels of socioeconomic status, in line with the composition of the Dutch society. Blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine were similar in males and females, and independent of age.

Conclusion. Paediatric reference values for blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine were established, which could be used in clinical practice.

Introduction

Analysis of amino acid concentrations in blood of children is often used in clinical practice for diagnostic purposes and to make decisions regarding treatment (Lepage et al., 2006). Adequate reference values are required to determine which amino acid concentrations should be considered abnormal, and are thereby essential for clinical decision-making. Abnormal amino acid concentrations may reflect an imbalanced diet or infections (Suliman et al., 2005). For instance, high levels of homocysteine may reflect a vitamin B12 or folate deficiency (Mattson & Shea, 2003). Further, aberrant concentrations of amino acids can be indicative of metabolic disorders in children, such as decreased concentrations of tryptophan in plasma being indicative of Hartnup disease (Keszthelyi, Troost, & Masclee, 2009), and increased concentrations of phenylalanine and decreased concentrations of tyrosine in plasma being biomarkers for phenylketonuria (Lepage et al., 2006). One way to examine amino acid concentrations, is by analysing blood samples, obtained by venipuncture (Lepage, McDonald, Dallaire, & Lambert, 1997). However, in children this method is suboptimal, given that this invasive procedure can invoke pain, anxiety and distress. Over the past decades, the use of blood spot samples to examine amino acid concentrations has increased, in particular in newborn screening (Fingerhut & Olgemöller, 2009; Zytkovicz et al., 2001). Collecting blood spots, by means of a finger prick, is less invasive for children than taking venous blood samples, and the dried blood spot technique is sufficiently robust and stable for diagnostic purposes (Chace, Sherwin, Hillman, Lorey, & Cunningham, 1998; Kand'ár & Žáková, 2009; Rashed et al., 1997). In addition, blood spots can be assessed at home, as they can be stored at room temperature and be sent by regular mail. Blood spot amino acid concentrations are highly correlated with amino acids concentrations obtained in venous blood (rs ranging from .86 to .96) (Bowron, Barton, Scott, & Stansbie, 2005; Pecce, Scolamiero, Ingenito, Parenti, & Ruoppolo, 2013). As amino acid concentrations measured in dried blood spots are generally lower than amino acid concentrations obtained in venous blood samples, caused by haemolysis during the drying of the blood spot (Bowron et al., 2005; Pecce et al., 2013), it is important to create reference values specifically for blood spot concentrations. There are some reference values published on blood spot amino acid concentrations in infants, required in newborn screening (Rashed et al., 1997; Zytkovicz et al., 2001). However, to our current knowledge, there are no normative values available for blood spot concentrations of amino acids in primary school-aged children.

A challenge in providing reference values for amino acids in primary school-aged children is to establish normative data obtained from a representative sample of healthy children. Papers describing reference values often lack information regarding recruitment methods and demographic characteristics of the samples, such as ethnicity and socioeconomic status (SES). Due to the ethical challenge of acquiring samples from healthy children from the community, many paediatric reference values are obtained from hospitalised children (Lepage et al., 1997; Parvy, Bardet, Rabier, & Kamoun, 1988). Another challenge in establishing reference values, is to take into account the potential effects of age (Held, White, & Pasquali, 2011; Lepage et al., 1997; Van Beynum et al., 2005; Venta, Prieto, & Alvarez, 2002) and gender (Jung & Adeli, 2009) on individual differences in amino acid metabolism.

The current study aims at establishing reference values for blood spot amino acid concentrations in healthy primary school-aged children. In addition, we examine whether gender and age affect amino acid concentrations.

Methods

Participants

Subjects were a community sample of healthy primary school-aged children (*n*=104; 52 percent males). The mean age of the sample was 9 years and 9 months (range: 6 to 12 years). Parents of participants were asked to complete questions regarding age, gender, ethnicity and medical condition of the child, as well as questions regarding family status, education and occupation of the parents. Exclusion criteria were (a) a history of chronic metabolic, renal, hepatic and cardiac diseases, (b) prescribed medication use that could interfere with amino acid metabolism, and (c) acute illness, as reported by parents. Children were recruited from various primary schools located throughout the Netherlands. Recruitment throughout the country resulted in a heterogeneous sample, consisting of children living in different geographic environments (rural and urban areas). The level of SES was determined based on the highest level of education and the current occupation of parents on 6-point scales (Statistics Netherlands, 2006, 2010). SES was calculated as the average of the education and occupation scores of both parents in case of two caretakers and as the average of the education and occupation scores of one parent in case of a single caretaker (Cirino et al., 2002).

Materials

To investigate blood concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine, a dried blood spot technique was used. A blood spot of each child was collected using a disposable safety lancet. Three blood drops were spotted onto a blood stain card and analysed by the clinical chemistry laboratory of our academic medical center. Disks with a diameter of 5.5mm were punched out of the blood spots, each containing 8.1µL blood. Concentrations of total homocysteine in blood spots (in µmol/L) were determined using liquid chromatography–mass spectrometry (LC-MS/MS), based on the protocol of Gempel, Gerbitz, Casetta, and Bauer (2000). Reliability of the LC-MS/MS was confirmed by examining the inter-assay variance (being 11 percent), intra-assay variance (being 10 percent) and recovery (being 105.5 percent). While blood spot concentrations of tryptophan, tyrosine and phenylalanine were expressed in whole units, blood spot concentrations of total homocysteine were expressed in one decimal, since the LC-MS technique allowed for a more precise measure. For the analysis of tryptophan, tyrosine and phenylalanine concentrations in blood spots (in µmol/L), a 5.5mm punch of a dried blood spot was mixed with 100µl of an internal standard solution (containing 29µM L-phenylalanine-D5, 6µM L-tyrosine-D4 and 5µM L-tryptophan-D5) and 400µl methanol in a Gas Chromatography vial (GC-vial) and shaken for 15 minutes in an ultrasonic bath. The supernatant was transferred in another GC-vial and evaporated under nitrogen at 30°C. Subsequently, the sample was butylated with 100µl of 5.5% acetyl chloride (in n-butanol) at 60°C for 15 minutes. Afterwards, the butanol-layer was evaporated under nitrogen (at 30°C) and the residue was dissolved in 500µl acetonitrile. Blood spot concentrations were determined by positive electrospray liquid chromatography–tandem mass spectrometry (LC-MS/MS), using an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA), coupled to a high-performance liquid chromatography (HPLC) system (Perkin Elmer Series 200, Shelton, USA). Three µl of the sample was injected on a symmetry C18 column (3.9*150mm, 5µm; Waters, Milford, MA, USA) and eluted with a flow rate of 1ml/min of 75% acetonitrile (containing 0.4% of formic acid). Tryptophan, tyrosine and phenylalanine eluted within 1 minute and were measured using the transitions: mass-to-charge ratio (m/z) 261.2→159.2 (tryptophan), m/z 238.2→136.2 (tyrosine) and m/z 222.2→120.2 (phenylalanine). All obtained LC-MS/MS data were acquired and processed using Analyst 1.4.2 software (Applied Biosystems, Foster City, CA, USA). Reliability of the LC-MS/MS was confirmed by examining the inter-

assay variance (being 5 to 10 percent), intra-assay variance (being 8 to 10 percent) and recovery (being 90 to 112 percent).

Procedure

This study is part of a research project on the relation between amino acids (total homocysteine, tryptophan, tyrosine and phenylalanine) and ADHD, and received approval from the local medical ethical committee (#NL39922.029.12). The study has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained of parents of all children, and of 12 year old children, prior to participation. Blood spots were collected in the early morning of the test day, to rule out possible effects of diurnal variation of amino acid blood spot concentrations. Fasting was not required prior to assessment of blood spots, as blood spots were collected within a short time span (<2 hours) after breakfast.

Data analysis

All statistical analyses were performed using R, version 3.2.1. Outliers were replaced using winsorising (Tabachnick & Fidell, 2001). For nine children blood spot total homocysteine concentrations were missing due to insufficient blood spots collected in some participants. These data were not replaced. In none of the other variables there were missing values.

Firstly, it was investigated whether there were interactions between gender and age on amino acid concentrations, using multiple linear regression analyses. In case of non-significant interactions, the main effects of gender and age on amino acid concentrations were examined, using analysis of variance or by calculating Pearson product-moment correlation coefficients, respectively. When appropriate, reference values were partitioned.

Secondly, reference values were established for the total group, using percentile scores (5^{th}, 50^{th} and 95^{th}). The 5 to 95 percentile interval represents the central ninety percent of the sample distribution, which is common in a community sample (Refsum et al., 2004). Further, the 10^{th} and 90^{th} percentiles were presented, which are more stable measures in a sample of circa 100 participants (Lepage et al., 1997). In case of significant effects of gender or age, stratified reference values were defined.

Results

Inspection of our sample revealed that the children represented diverse cultural backgrounds; of our sample 81 children (78 percent) had a Dutch background, eight children (eight percent) had a non-Dutch Western background and 15 children (14 percent) had a non-Western background. The children in our sample had varying levels of SES; of our sample 38 children (37 percent) were classified as coming from a low SES family, 41 children (39 percent) as coming from a medium SES family and 25 children (24 percent) as coming from a high SES family.

Regression analyses showed no interaction effect between gender and age for any of the amino acid concentrations (all βs<.10, all *p*s>.55). Also, we did not find main effects of gender (all *F*s<1.00, all *p*s>.30) and age (all *r*s<.20, all *p*s>.05) on blood spot amino acid concentrations. Therefore, reference values for blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine were presented for the whole group of primary school-aged children, see Table 6.1.

Table 6.1. Reference values for blood spot amino acid concentrations, based on a sample of 104 healthy children

	5th percentile	10th percentile	50th percentile	90th percentile	95th percentile
Total homocysteine (µmol/L)[a]	.3	.5	2.5	6.0	7.5
Tryptophan (µmol/L)	40	42	52	63	65
Tyrosine (µmol/L)	34	34	52	77	81
Phenylalanine (µmol/L)	41	44	55	71	80

Note. [a]Based on blood spot concentrations of a smaller sample (*n*=95).

Discussion

The main purpose of the current study is to establish paediatric reference values for blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine. A key finding of our study is that we did not find an effect of age on amino acid blood spot concentrations, which is contrary to expectations based on previous studies (Lepage et al., 1997; Van Beynum et al., 2005). The absence of age differences might be explained by the age range studied in the present study, which was limited to 6 to 12 year. Age differences might emerge during more critical developmental stages, such as infancy

and puberty (Lepage et al., 1997; Van Beynum et al., 2005). We furthermore did not find any gender effects on blood spot amino acid concentrations, and therefore our results do not support the hypothesis of differences in amino acid concentrations between males and females, as was proposed in previous work (Jung & Adeli, 2009). As gender effects may reflect variations in amino acid concentrations during different stages of the menstrual cycle in females, our study does not rule out the need for gender-partitioned reference values in samples including children in puberty.

There are some limitations to the current study that should be noted. While our study provides reference values for primary school-aged children (a group of children that is often seen in clinical practice), in future studies the age range of our study should be expanded, to provide reference values for children under six years old and for adolescents. An age range of zero to 18 year would allow for examining gender and age effects on amino acids concentrations more thoroughly. Another limitation to the current study is the focus on the four amino acids total homocysteine, tryptophan, tyrosine and phenylalanine, while there are numerous other amino acids that are indicative of metabolic disorders in children (Lepage et al., 2006).

The current study also has several strengths. Normative data were obtained in a community sample of healthy children, who were recruited through primary schools located in diverse geographical areas. This recruitment method decreased the possibility of a biased sample, that might be present in studies selecting hospitalised children (Lepage et al., 1997; Parvy et al., 1988). Further, our sample consisted of children from various ethnic backgrounds, with proportions of immigrants in line with the composition of the Dutch society, as 10 percent of the Dutch population consists of Western immigrants and 12 percent of non-Western immigrants (Centraal Bureau voor de Statistiek, 2015). An ethnically representative sample is importance when establishing reference values, as it has been suggested that there are differences in amino acid metabolism between ethnic groups, due to genetic or nutritional factors (Van Beynum et al., 2005). The obtained reference values allow for a step forward in clinical practice, enabling clinicians to use blood spots more often, as alternative to venipuncture for the analysis of amino acid concentrations in primary school-aged children.

References

Bowron, A., Barton, A., Scott, J., & Stansbie, D. (2005). Blood spot homocysteine: A feasibility and stability study. *Clinical Chemistry, 51*(1), 257-258.

Centraal Bureau voor de Statistiek. (2015). *Population; sex, age, origin and generation*. Den Haag/Heerlen: CBS.

Chace, D.H., Sherwin, J.E., Hillman, S.L., Lorey, F., & Cunningham, G.C. (1998). Use of phenylalanine-to-tyrosine ratio determined by tandem mass spectrometry to improve newborn screening for phenylketonuria of early discharge specimens collected in the first 24 hours. *Clinical Chemistry, 44*(12), 2405-2409.

Cirino, P.T., Chin, C.E., Sevcik, R.A., Wolf, M., Lovett, M., & Morris, R.D. (2002). Measuring socioeconomic status reliability and preliminary validity for different approaches. *Assessment, 9*(2), 145-155.

Fingerhut, R., & Olgemöller, B. (2009). Newborn screening for inborn errors of metabolism and endocrinopathies: An update. *Analytical and Bioanalytical Chemistry, 393*(5), 1481-1497.

Gempel, K., Gerbitz, K.-D., Casetta, B., & Bauer, M.F. (2000). Rapid determination of total homocysteine in blood spots by liquid chromatography-electrospray ionization-tandem mass spectrometry. *Clinical Chemistry, 46*(1), 122-123.

Held, P.K., White, L., & Pasquali, M. (2011). Quantitative urine amino acid analysis using liquid chromatography tandem mass spectrometry and aTRAQ® reagents. *Journal of Chromatography B, 879*(26), 2695-2703.

Jung, B., & Adeli, K. (2009). Clinical laboratory reference intervals in pediatrics: The CALIPER initiative. *Clinical Biochemistry, 42*(16), 1589-1595.

Kand'ár, R., & Žáková, P. (2009). Determination of phenylalanine and tyrosine in plasma and dried blood samples using HPLC with fluorescence detection. *Journal of Chromatography B, 877*(30), 3926-3929.

Keszthelyi, D., Troost, F., & Masclee, A. (2009). Understanding the role of tryptophan and serotonin metabolism in gastrointestinal function. *Neurogastroenterology & Motility, 21*(12), 1239-1249.

Lepage, N., Li, D., Kavsak, P.A., Bamforth, F., Callahan, J., Dooley, K., & Potter, M. (2006). Incomplete pediatric reference intervals for the management of patients with inborn errors of metabolism. *Clinical Biochemistry, 39*(6), 595-599.

Lepage, N., McDonald, N., Dallaire, L., & Lambert, M. (1997). Age-specific distribution of plasma amino acid concentrations in a healthy pediatric population. *Clinical Chemistry, 43*(12), 2397-2402.

Mattson, M.P., & Shea, T.B. (2003). Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. *Trends in Neurosciences, 26*(3), 137-146.

Parvy, P., Bardet, J., Rabier, D., & Kamoun, P. (1988). Age-related reference values for free amino acids in first morning urine specimens. *Clinical Chemistry, 34*(10), 2092-2095.

Pecce, R., Scolamiero, E., Ingenito, L., Parenti, G., & Ruoppolo, M. (2013). Optimization of an HPLC method for phenylalanine and tyrosine quantization in dried blood spot. *Clinical Biochemistry, 46*(18), 1892-1895.

Rashed, M.S., Bucknall, M.P., Little, D., Awad, A., Jacob, M., Alamoudi, M., . . . Ozand, P.T. (1997). Screening blood spots for inborn errors of metabolism by electrospray tandem mass spectrometry with a microplate batch process and a computer algorithm for automated flagging of abnormal profiles. *Clinical Chemistry, 43*(7), 1129-1141.

Refsum, H., Grindflek, A.W., Ueland, P.M., Fredriksen, Å., Meyer, K., Ulvik, A., . . . Kase, B.F. (2004). Screening for serum total homocysteine in newborn children. *Clinical Chemistry, 50*(10), 1769-1784.

Statistics Netherlands. (2006). *Standard Educational Classification*. Den Haag/Heerlen, the Netherlands.

Statistics Netherlands. (2010). *Standard Occupational Classification*. Den Haag/Heerlen, the Netherlands.

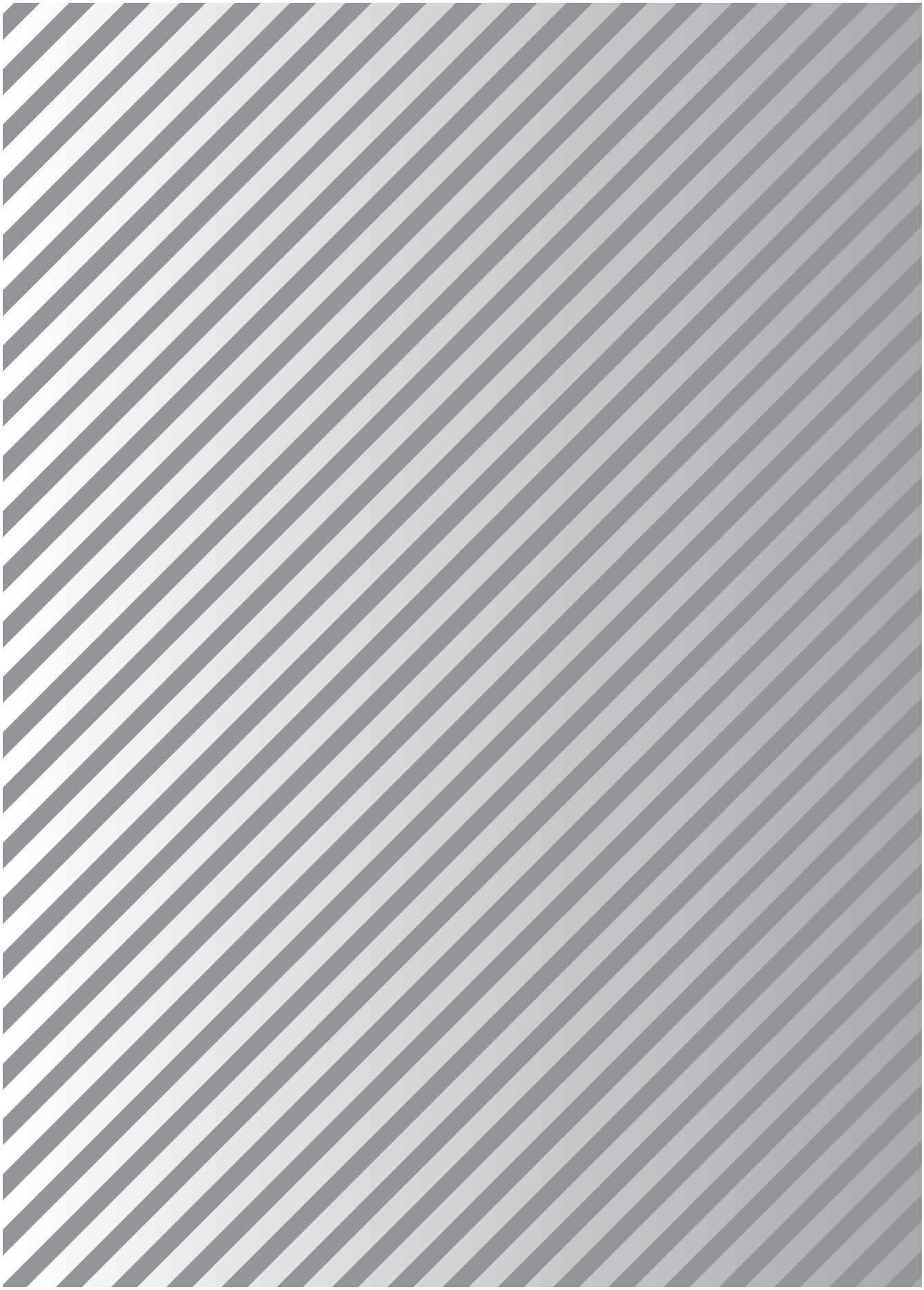
Suliman, M.E., Qureshi, A.R., Stenvinkel, P., Pecoits-Filho, R., Bárány, P., Heimbürger, O., . . . Alvestrand, A. (2005). Inflammation contributes to low plasma amino acid concentrations in patients with chronic kidney disease. *American Journal of Clinical Nutrition, 82*(2), 342-349.

Tabachnick, B.G., & Fidell, L.S. (2001). *Using multivariate statistics* (4th ed.). Boston: Allyn and Bacon.

Van Beynum, I.M., Den Heijer, M., Thomas, C.M., Afman, L., Oppenraay-van Emmerzaal, D., & Blom, H.J. (2005). Total homocysteine and its predictors in Dutch children. *American Journal of Clinical Nutrition, 81*(5), 1110-1116.

Venta, R., Prieto, B., & Alvarez, F.V. (2002). Regression-based reference limits for urinary amino acids in a pediatric population. *Clinical Chemistry and Laboratory Medicine, 40*(4), 383-390.

Zytkovicz, T.H., Fitzgerald, E.F., Marsden, D., Larson, C.A., Shih, V.E., Johnson, D.M., . . . Grady, G.F. (2001). Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: A two-year summary from the New England newborn screening program. *Clinical Chemistry, 47*(11), 1945-1955.



Chapter 7

Measuring social cognition in school-aged children using a morphed facial emotion recognition task

This chapter is under review for publication as:

Bergwerff, C.E., Luman, M., Meffert, H., Blair, R.J.R., & Oosterlaan, J. Measuring social cognition in school-aged children using a morphed facial emotion recognition task.

Abstract

Objective. In children, the ability to recognise emotions in other children's faces is important in social interaction with peers. A task using varying levels of emotional intensity may detect subtle facial emotion recognition deficiencies. We investigate differences in performance across emotional conditions and levels of expression intensity, examine developmental effects in facial emotion recognition, and explore whether facial emotion recognition is influenced by gender and IQ.

Methods. The Morphed Facial Emotion Recognition Task (MFERT) was constructed, consisting of photographs of children's faces depicting four basic emotions (anger, fear, happiness and sadness). High-intensity expressions (100%) were morphed with neutral expressions, resulting in 240 stimuli, varying in emotional intensity (10-100%). Dependent measures were accuracy scores per intensity per emotional condition. The MFERT was assessed in a community sample of 75 children, aged 6-12 years (45% males). IQ was estimated using a short form of the Wechsler Intelligence Scale for Children.

Results. For all emotional conditions, emotion intensity had a linear effect on accuracy. Accuracy was highest for happy expressions, followed by angry and frightened expressions. Accuracy was lowest for sad expressions. Age was related to emotion recognition at some intensity levels. Girls had higher accuracy at middle-intensity emotional expressions than boys, but not at low- and high-intensity expressions. IQ was not related to accuracy.

Conclusion. We found evidence for the sensitivity of our task to detect age- and gender-related differences in facial emotion recognition at some intensity levels. This makes the MFERT suitable to detect subtle emotion recognition deficiencies in clinical populations.

Introduction

A key aspect of social cognition is the ability to interpret emotional cues in social situations, including the facial emotional expressions of others (Leppänen & Hietanen, 2001), as understanding other's emotional state is essential to adapt to social interactions. In children, the ability to recognise emotions in other children's faces is particularly important in social interaction with peers (Nowicki & Mitchell, 1998). Deficiencies in facial emotion recognition in children and adolescents are thought to contribute to impairments in reciprocal social interaction skills and communication skills (Kothari, Skuse, Wakefield, & Micali, 2013), to aggressive and antisocial behaviour (Blair, Colledge, Murray, & Mitchell, 2001; Fairchild, Van Goozen, Calder, Stollery, & Goodyer, 2009; Rogers, Viding, Blair, Frith, & Happe, 2006), and to social phobia (Simonian, Beidel, Turner, Berkes, & Long, 2001). Emotion recognition deficiencies can be targeted in psychotherapy, when individuals display inadequate social behaviour (Penton-Voak et al., 2013).

The development of facial emotion recognition during childhood has received much scientific interest, although results are somewhat inconsistent on the extent and range of the change in emotion recognition ability (Herba & Phillips, 2004). A potential explanation for differences across studies into the developmental pathway of facial emotion recognition during childhood, is that the development is emotion-dependent (Mancini, Agnoli, Baldaro, Ricci Bitti, & Surcinelli, 2013; Rodger, Vizioli, Ouyang, & Caldara, 2015). It has been suggested that the recognition of facial expressions of happiness and sadness reaches a mature level earliest, followed by the recognition of angry expressions, with the recognition of frightened and disgusted expressions developing latest (Durand, Gallay, Seigneuric, Robichon, & Baudouin, 2007; Herba & Phillips, 2004). However, there is no consensus on the exact developmental pathway of the specific emotions. For instance, recent work showed that the recognition of happy, sad and angry facial expressions was similar between 6- and 16-year olds (Lawrence, Campbell, & Skuse, 2015). These findings contrast with a study of Mancini et al. (2013), who showed an age-related increase in the recognition of sad expressions in 8- to 11-year-olds, and with the results of Rodger et al. (2015), who showed an increase in the recognition of sad and angry expressions from the age of 5 years until adulthood. Furthermore, there is evidence that the recognition of frightened expressions develops from childhood (7 to 13 years) through adulthood (Thomas, De Bellis, Graham, & LaBar, 2007), although Rodger et al. (2015) showed that

the recognition of frightened expressions remains stable from the age of 5 years old until adulthood. Given the disparate findings across studies, it is unclear whether the ability to recognise specific emotions continues to develop during childhood. It is therefore important to further explore effects of age on facial emotion recognition in children for specific emotions separately, which is one of the aims of the current study.

As such, research into potential moderators of emotion recognition is required to get a better understanding of the development of these abilities. For example, there is evidence for gender effects, confirmed by a meta-analysis showing a small female advantage in facial emotion recognition in children (McClure, 2000). In this study, we further explored the role of gender effects on facial emotion recognition in children. In addition to gender, intelligence might possibly moderate facial emotion recognition (Buitelaar, Van der Wees, Swaab-Barneveld, & Van der Gaag, 1999). Buitelaar et al. (1999) found that performance IQ predicted facial emotion recognition in children. However, studies show inconsistent results on associations between IQ and facial emotion recognition, with significant associations only found in certain age groups (Lawrence et al., 2015). It is therefore important to further explore effects of IQ on facial emotion recognition.

The inconsistencies across studies may also be explained by the use of different methodologies; while some paradigms used static pictures with a high expression intensity (e.g., Lawrence et al., 2015; Mancini et al., 2013), others used morphed pictures, in which the intensity of emotional expressions is manipulated (e.g., Herba, Landau, Russell, Ecker, & Phillips, 2006; Thomas et al., 2007). Pictures with high expression intensity provide insight into the ability of children to interpret the full display of an emotional expression. However, in many social interactions, children may need to process low-intensity emotional expressions or ambiguous emotional expressions. Using stimuli only consisting of high-intensity emotional expressions might lead to a developmental ceiling effect and may leave subtle developmental changes in facial emotion recognition undetected (Thomas et al., 2007). A previous study showed that with increasing intensity of emotional expressions, the ability to recognise facial emotions increases with a linear trend, implying a gradual increase in performance (Gao & Maurer, 2009). However, in a subsequent study, ceiling effects were found for high-intensity angry and happy expressions, suggesting a quadratic trend (Gao & Maurer, 2010). A task using varying levels of emotional intensity, such as the task used in the current study, may therefore be more sensitive for detecting facial emotion recognition deficiencies.

Further, inconsistencies in the literature of the development of facial emotion recognition in children might be caused by other variations in task demands. In many studies performed thus far in children, tasks were based on pictures of adult faces, using the well-validated Ekman-Friesen Pictures of Facial Affect (e.g., Herba et al., 2006; Lawrence et al., 2015). However, children may perform better in recognising facial emotions expressed by children than by adults, due to the so-called own-age bias (Hills & Lewis, 2011; Proietti, Macchi Cassia, & Mondloch, 2015). When examining the abilities that are required for children in social interaction with peers, a task using pictures of children's faces would therefore be more ecologically valid. This is confirmed by a study showing that in preschool girls, the ability to recognise facial emotions expressed by children is related to social competence in the interaction with peers, while misjudging facial emotions expressed by adults is unrelated to the ability to get along with peers (Nowicki & Mitchell, 1998). Some studies have used the Diagnostic Analysis of Nonverbal Accuracy (DANVA), an instrument to measure facial emotion recognition, which encompasses a subset of stimuli of children's faces (Nowicki & Duke, 1994). However, the number of stimuli based on children's faces (*n*=24) is too small to examine developmental effects for specific emotional conditions. In other studies morphed pictures of children's faces were used, however, participants were presented with only 2 to 4 stimuli per intensity level of each emotion and only three emotional conditions per block (Gao & Maurer, 2009, 2010), which limits the variability in results. Further, in that paradigm stimulus presentation was self-paced, enabling participants to look at each emotional expression as long as they wanted, which may have added to ceiling effects (Gao & Maurer, 2009, 2010). In the current study we developed a facial emotion recognition task that improves on previous paradigms by using a larger set of stimuli of morphed emotional expressions in children's faces. We also decided to limit the stimulus presentation latency, as in daily life social interaction, children often need to process emotional expressions quickly (Herba & Phillips, 2004).

The aim of the current study is to examine developmental effects on emotion recognition in primary school-aged children. We examine the effect of expression intensity on facial emotion recognition and expect a linear effect for frightened and sad expressions (Gao & Maurer, 2009), and a quadratic effect for angry and happy expressions (Gao & Maurer, 2010). We explore the effect of emotional condition (angry, frightened, happy and sad expressions) and expect highest performance on expressions of happiness and sadness, followed by the recognition of angry expressions, with the recognition of frightened

expressions being lowest (Durand et al., 2007; Herba & Phillips, 2004). Furthermore, we explore the effects of age on emotion recognition, and hypothesise that the ability to recognise negatively valenced emotions (anger, fear and sadness) increases with age (Gao & Maurer, 2010), though the recognition of happy expressions might not (cf. Gao & Maurer, 2009). We also explore whether facial emotion recognition is influenced by gender and IQ. We expect girls to outperform boys (McClure, 2000), and IQ to be positively associated with facial emotion recognition (Buitelaar et al., 1999).

Methods

Participants

In the current study a community sample of 75 primary school-aged children (6 to 12 years old) participated; see Table 7.1 for group characteristics. Inclusion criteria were (a) attending primary school (grade 1 to 6), and (b) IQ≥70. Full scale IQ was estimated using a short form of the Wechsler Intelligence Scale for Children-III (WISC-III; Wechsler, 1991), consisting of the subtests Vocabulary, Arithmetic, Block Design and Picture Arrangement. This short form of four subtests has an excellent reliability (r_{ss}=.95) and validity (r'_{pw}=.90) (Sattler, 2008). Children were recruited from primary schools located throughout the Netherlands, in varying geographic environments (rural and urban areas). Parents of participants were asked to complete questions regarding age, gender and ethnicity of the child, as well as questions regarding ethnicity, family status, and education of the parents. Children represented diverse ethnic backgrounds and varying levels of socioeconomic status (SES). SES was determined based on the highest level of education of parents on a 6-point scale (Statistics Netherlands, 2006). The SES score was the average of the education scores of both parents in case of two caretakers, or the education score of one parent in case of a single caretaker.

Materials

For the current study the Morphed Facial Emotion Recognition Task (MFERT) was constructed, consisting of photographs of children's faces depicting four basic emotions (anger, fear, happiness and sadness). Pictures from six different child actors (three males, three females) from the validated NIMH Child Emotional Faces Pictures Set (NIMH-ChEFS) were used (Egger et al., 2011). Each actor contributed five pictures, featuring the four high-intensity emotions (100%) and a neutral expression (0%), all with a direct gaze. The pictures that were selected were originally validated in terms of an inter-rater agreement >.80 (Egger et al., 2011). The stimuli of the MFERT were created by morphing

each of the four high-intensity pictures of each actor with the neutral expression of the same actor in nine steps of 10% increments, resulting in stimuli varying in emotional intensity from 10% (90% neutral) to 100% (0% neutral). Morphs were created using Abrosoft FantaMorph software (Abrosoft, USA), see Figure 7.1.

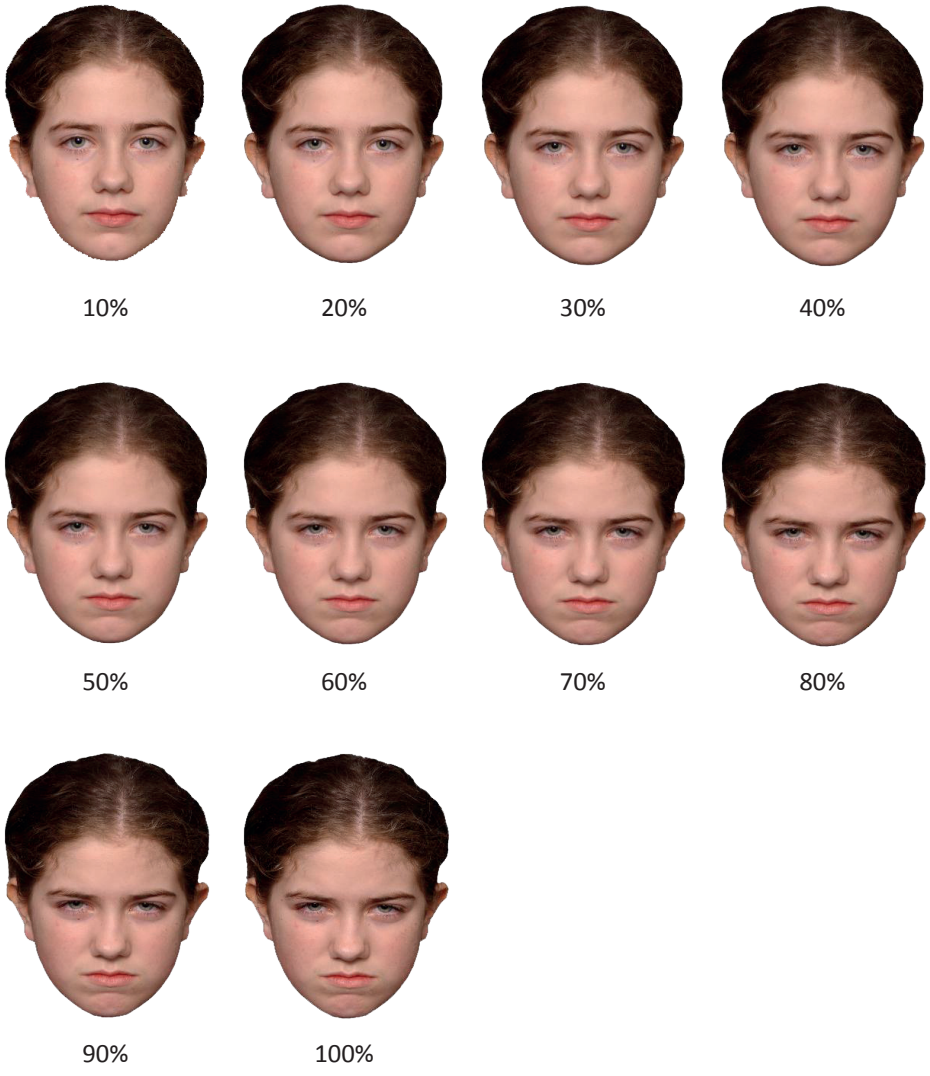


Figure 7.1. Examples of angry expressions at varying intensity levels.

The MFERT was programmed within E-prime, version 2.0 (Schneider, Eschman, & Zuccolotto, 2002). The task consists of 240 target stimuli (four emotional conditions by

ten intensity levels by six actors), presented in random order. Prior to each target (400 ms), a fixation cross (250 ms) appeared in the centre of the screen (see Figure 7.2 for the course of a trial). After each target, a response screen appeared, with five text boxes representing the emotion labels angry, frightened, happy, sad and neutral, respectively. Participants had to indicate the emotion corresponding to the target stimulus, by clicking on one of the five emotion labels with a computer mouse. The task was self-paced and the trials started without delay. Total task duration was on average 14.46 minutes (SD=3.15). The task was preceded by 30 practice trials, consisting of trials similar to the experimental trials, including the four high-intensity expressions and the neutral expression of each of the six actors. The practice trials were used to verify the ability of children to read the emotion labels and the ability to correctly use the computer mouse. For each trial, response selection (one of the five text boxes) was recorded. Dependent measures were accuracy scores (percentage correct) per intensity level per emotional condition.

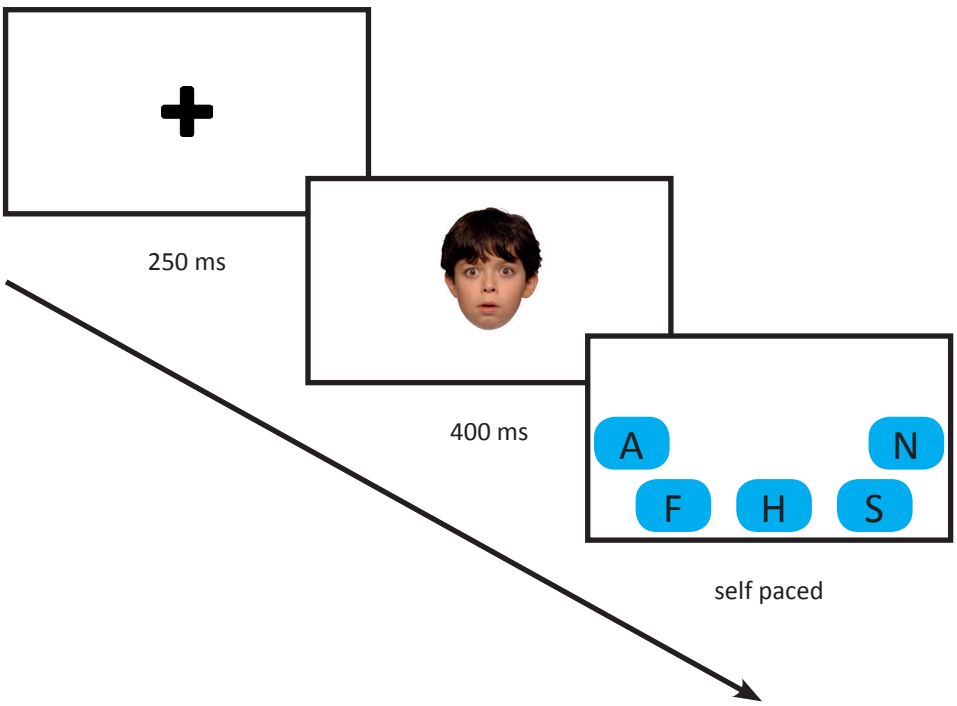


Figure 7.2. Course of a trial. Abbreviations in the text boxes in the third screen represent the emotion labels angry (A), frightened (F), happy (H), sad (S) and neutral (N).

Procedure

Prior to participation written informed consent was obtained of parents of all children, and of 12 year old children. During the test session participants completed the four subtests of the WISC-III, followed by the MFERT. Parents completed the questionnaire on demographic characteristics on a secured website.

Data Analysis

Statistical analyses were performed using R, version 3.2.1 and SPSS, version 24. There were no missing values in our dataset. Firstly, we explored whether our task manipulation was successful, in terms of increasing task performance with increasing expression intensity. For each emotional condition, the effect of expression intensity on accuracy scores was tested using trend analysis, using ANOVA with polynomial contrasts. These analyses tested whether a linear and quadratic trend could accurately describe the effects of emotional intensity on accuracy scores.

Secondly, we examined whether emotional condition, expression intensity, age, gender and IQ affected task performance using repeated measures analysis of covariance (ANCOVA). In this analysis, emotional condition (four levels) and expression intensity (ten levels) were entered as within-subjects factors, gender (boys and girls) as between-subjects factor, and age and IQ as covariates. In case of significant main or interaction effects, post hoc analyses were performed, using Bonferroni-corrected post hoc contrasts (for emotional condition and expression intensity), correlation analyses (for age and IQ) or independent samples t-tests (for gender).

Results

Group characteristics are shown in Table 7.1. Of our sample 58 children (77 percent) had an indigenous Dutch background (both parents born in the Netherlands), three children (four percent) had a non-Dutch Western background (at least one of the parents born in another country of Europe or the United States) and 14 children (19 percent) had a non-Western background (at least one of the parents born outside Europe and the United States). Of our sample 33 children (44 percent) had a low SES, 21 children (28 percent) had a medium SES and 21 children (28 percent) a high SES.

Table 7.1. Group characteristics of the sample

	M (SD)	Range
Males *n* (%)	34 (45)	
Age (in years)	9.66 (1.54)	6.58-12.67
Socioeconomic status	4.06 (1.06)	1-6
IQ	102.61 (13.96)	71-132

Note. For a full description of all measures, see the Methods section.

Firstly, trend analyses using ANOVA with polynomial contrasts were conducted to model the effects of expression intensity on performance level. For all emotional conditions, expression intensity had a significant linear effect on accuracy scores. In addition, significant quadratic effects of expression intensity on accuracy scores were found for angry, frightened and happy expressions, but not for sad expressions (see Table 7.2). These results indicate that with increasing intensity, there was an increase in accuracy. Linear trends provided the best description of the effects, since quadratic effects were smaller than linear effects, and the linear effects provide a more parsimonious description. Upon visual inspection of the graphs depicted in Figure 7.3, it is clear that, at least for the recognition of angry, frightened and happy expressions, there are ceiling effects in the high-intensity expressions, with accuracy approaching 100 percent.

Table 7.2. Facial emotion recognition in children: Effects of emotion intensity on accuracy scores

	Linear effect	Quadratic effect
Angry expressions	46.18**	-10.36**
Frightened expressions	41.38**	-17.97**
Happy expressions	46.87**	-25.77**
Sad expressions	37.45**	1.60, NS

Notes. **p<.01. Values in the cells represent t-values for the main effect of emotional intensity in ANOVAs with polynomial contrasts. NS, non-significant.

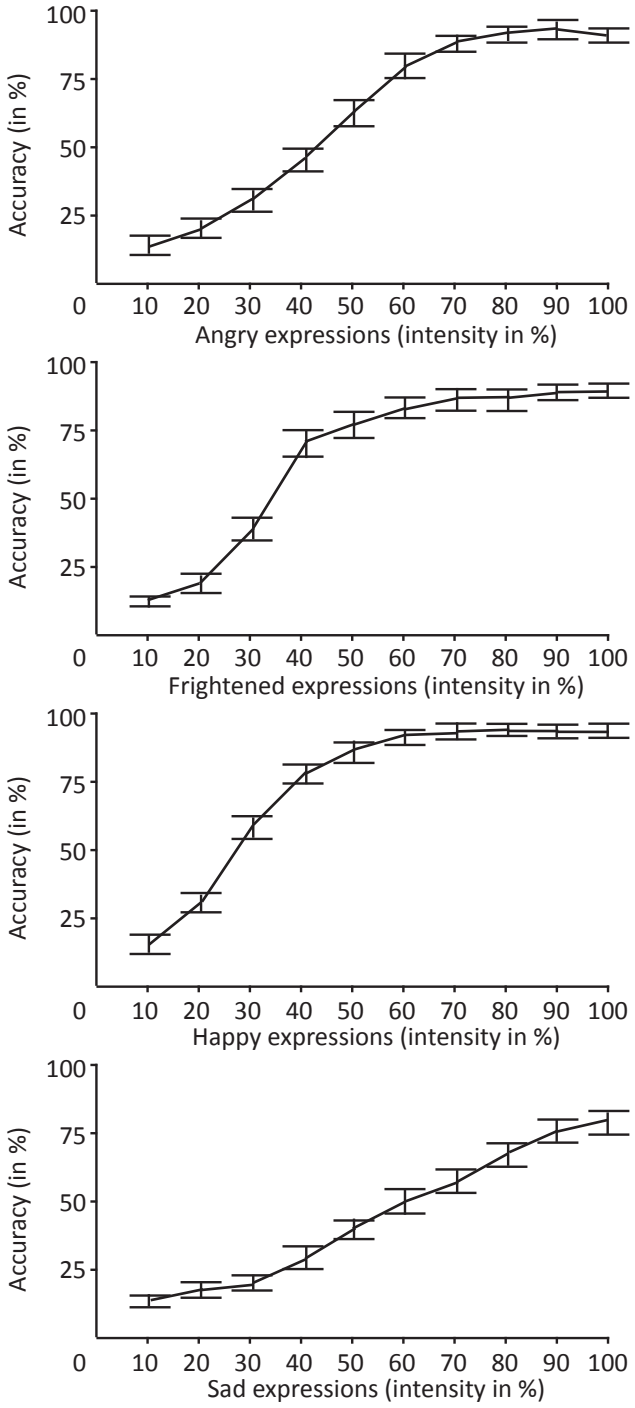


Figure 7.3. Line graphs depicting effects of emotional intensity on emotion recognition of angry, frightened, happy and sad expressions.

Secondly, we examined whether emotional condition, expression intensity, age, gender and IQ affected task performance using repeated measures ANCOVA. Mauchly's test indicated that the assumption of sphericity was violated ($\chi^2(377)=573.18$, $p<.01$), and therefore the degrees of freedom were corrected, using Huynh-Feldt estimates of sphericity (ε=.89). All results are shown in Table 7.3.

There was a main effect of condition ($F(2.95,209.53)=6.31$, $p<.01$). Post hoc tests using the Bonferroni correction revealed that accuracy was significantly lower for sad expressions ($M=40.69$, $SD=10.89$) than for angry ($M=61.38$, $SD=9.26$, $p<.01$), frightened ($M=64.04$, $SD=11.05$, $p<.01$) and happy expressions ($M=72.98$, $SD=9.29$, $p<.01$). There was no significant difference between accuracy for angry and for frightened expressions ($p=.65$). Accuracy was higher for happy expressions than for all negatively valenced emotions (all ps<.01).

There was a main effect of intensity ($F(6.35,450.72)=4.39$, $p<.01$). Post hoc tests using the Bonferroni correction revealed that accuracy was significantly lower at 10% ($M=8.61$, $SD=7.36$) than at all higher intensity levels, which was also true for accuracy at 20% ($M=17.45$, $SD=9.48$), 30% ($M=33.89$, $SD=11.51$), 40% ($M=54.11$, $SD=11.51$), 50% ($M=65.39$, $SD=12.10$) and 60% ($M=75.56$, $SD=11.28$). Accuracy at 70% ($M=80.89$, $SD=12.12$) did not significantly differ from accuracy at 80% ($M=84.89$, $SD=11.27$), but was lower than accuracy at 90% ($M=88.22$, $SD=10.12$) and 100% ($M=88.72$, $SD=10.41$). There were no significant differences between accuracy at 80%, 90% and 100%.

We found an interaction between condition and intensity ($F(24.05,1707.39)=1.77$, $p=.01$). Post hoc analyses revealed that results per condition were similar to the main effect of intensity. The main difference involved the ceiling effects across conditions. While in the angry condition there were no significant differences between accuracy at 70%, 80%, 90% and 100%, in the frightened and happy condition there were no significant differences between accuracy at 60%, 70%, 80%, 90% and 100%, suggesting a lower ceiling in the frightened and happy condition than in the angry condition. In the sad condition there was a higher ceiling than in all other conditions, as there was a ceiling at 90% (no difference in accuracy at 90% and at 100%).

We found a main effect of age (F(1,71)=5.31, p=.02), with increasing overall performance with increasing age (r=.22, p=.05). We also found an interaction effect between condition and age on accuracy (F(2.95,209.53)=4.34, p=.01). Age was related to recognition of frightened expressions (r=.34, p<.01), but not significantly related to recognition of angry, happy or sad expressions, see Table 7.3. We further found an interaction effect between intensity and age on accuracy (F(6.35,450.72)=5.56, p<.01). Post hoc analyses showed that age was significantly related to accuracy at 10% (r=-.42, p<.01), 50% (r=.27, p=.02), 60% (r=.33, p<.01), 70% (r=.27, p=.02), 80% (r=.24, p=.04), 90% (r=.22, p=.05) and 100% intensity (r=.27, p=.02). There were no age effects at the other low-intensity levels, see Table 7.3. There was a significant three-way interaction between condition, intensity and age (F(24.05,1707.39)=1.77, p=.01). Post hoc analyses revealed significant negative associations between age and accuracy at 10% (r=-.37, p<.01), and 30% intensity (r=-.39, p<.01) in the angry condition and at 10% in the happy condition (r=-.26, p=.02), and significant positive associations between age and accuracy at 30%, 50%, 90% and 100% intensity in the frightened condition (rs ranging from .26 to .37, ps<.05), at 80% (r=.28, p=.01) in the happy condition, and at 50% (r=.29, p=.01) and 60% (r=.27, p=.02) in the sad condition. We found no significant associations at the other intensity levels (all rs<.23, all ps>.05).

We found a main effect of gender (F(1,71)=9.76, p<.01), with girls (M=61.77, SD=5.93) outperforming boys (M=57.37, SD=7.34) on overall accuracy. There was no significant interaction effect between condition and gender on accuracy and no significant three-way interaction between condition, intensity and gender, see Table 7.3. However, we found an interaction effect between intensity and gender on accuracy (F(6.35,450.72)=3.51, p<.01). Post hoc analyses showed that females had higher accuracy scores than males at 30% (t=2.65, p<.01), 40% (t=3.52, p<.01), 50% (t=3.51, p<.01) and 60% intensity (t=2.04, p=.045). There were no significant gender differences at the low- and high-intensity levels, see Table 7.3.

We found no significant main effect of IQ, no significant interaction effect between condition and IQ, no significant interaction effect between intensity and IQ and no significant three-way interaction between condition, intensity and IQ, see Table 7.3.

Table 7.3. Results of repeated measures ANCOVA

	F	*p*	Post hoc comparison
Within-subjects effects			
Condition	6.31	<.01	Sad<Angry=Frightened<Happy
Condition X age[a]	4.34	.01	Angry *r*=-.11, NS; Frightened *r*=.34**; Happy *r*=.13, NS; Sad *r*=.21, NS
Condition X gender	.11	.95	N/A
Condition X IQ	1.13	.34	N/A
Intensity	4.39	<.01	10%<20%<30%<40%<50%<60%=70%=80%=90%=100%; 60%<80%; 70%<90%
Intensity X age[a]	5.56	<.01	10% *r*=-.42**; 20% *r*=-.04, NS; 30% *r*=-.02, NS; 40% *r*=.11, NS; 50% *r*=.27*; 60% *r*=.33**; 70% *r*=.27*; 80% *r*=.24*; 90% *r*=.22*; 100% *r*=.27*
Intensity X gender[a]	3.51	<.01	10% *t*=-1.00, NS; 20% *t*=.75, NS; 30% *t*=2.65**; 40% *t*=3.52**; 50% *t*=3.51**; 60% *t*=2.04*; 70% *t*=1.11, NS; 80% *t*=1.61, NS; 90% *t*=1.86, NS; 100% *t*=.84, NS
Intensity X IQ	1.33	.24	N/A
Condition X intensity	1.77	.01	Angry: 10%=20%<30%<40%<50%<60%=70%=80%=90%=100%; 10%<30%; 60%<80% Frightened: 10%=20%<30%<40%<50%=60%=70%=80%=90%=100%; 10%<30%; 50%<70% Happy: 10%<20%<30%<40%<50%=60%=70%=80%=90%=100%; 50%<80% Sad: 10%=20%=30%<40%<50%<60%=70%<80%=90%=100%; 10%<40%; 20%<40%; 60%<80%; 80%<100%
Condition X intensity X age[a]	1.77	.01	Angry 10% *r*=-.37**; Angry 30% *r*=-.39**; Frightened 30% *r*=.35**; Frightened 50% *r*=.27*; Frightened 90% *r*=.37**; Frightened 100% *r*=.26*; Happy 10% *r*=-.26*; Happy 80% *r*=.28*; Sad 50% *r*=.29*; Sad 60% *r*=.27*; other intensity levels NS
Condition X intensity X gender	1.43	.08	N/A
Condition X intensity X IQ	1.02	.44	N/A
Between-subjects effects			
Age	5.31	.02	*r*=.22*
Gender	9.76	<.01	Females>males
IQ	.43	.52	N/A

Notes. [a]*p*-values in these post hoc comparisons are not adjusted for multiple tests. *p<.05, **p<.01. N/A, not applicable; NS, not significant.

DISCUSSION

The aim of the current study is to examine developmental effects on emotion recognition in primary school-aged children. We examined the effects of expression intensity, emotional condition, age, gender and IQ on facial emotion recognition. A main finding of our study is the evidence for emotion-specific facial emotion recognition in primary school-aged children. We found that children performed better in recognising happy expressions, than in recognising angry, frightened or sad expressions. Our findings are in line with previous studies that found a better recognition of happy expressions in children than negatively valenced emotional expressions (Mancini et al., 2013; Rodger et al., 2015). We further found that children performed better in the recognition of angry expressions in children's faces than in the recognition of sad expressions. We expect that in peer-interactions, especially happy and angry expressions elicit strong responses in children, as these expressions on the face of peers provide positive or negative feedback, respectively (Gao & Maurer, 2010). Positive feedback by peers (happy expressions) seems to be important for social acceptance (DeWall, Maner, & Rouby, 2009), while negative feedback by peers (angry expressions) is related to peer rejection (Hubbard, 2001). The relatively better ability to recognise frightened expressions than sad expressions may be explained by a greater importance for children to detect a signal of a potential threat (Rodger et al., 2015), resulting in self-protective behaviour, than to detect sadness in a peer, resulting in prosocial behaviour. This effect is also found in the expression of emotions; children tend to show more self-protective than prosocial display rules in the choice to display negative emotions (Zeman & Garber, 1996). In contrast to high accuracy found in the recognition of sad expressions on adult faces by previous studies (Lawrence et al., 2015), we found relatively low accuracy in the recognition of sad expressions on children's faces. In social interaction with peers, children tend to mask expressions of sadness more often than expressions of anger, as they expect peers to provide negative feedback (such as teasing) to emotional behaviour (Zeman & Shipman, 1997). It may therefore be that children are less familiar with sad expressions of peers and therefore show more difficulty in recognising sad expressions. Improving the ability to recognise sad expressions might be beneficial, as misreading (subtle signals of) sadness may cause decreased empathy towards peers.

Another main finding of our study is the evidence for the sensitivity of our task to detect subtle differences in facial emotion recognition. Results showed age- and gender-related

differences in the recognition of facial emotional expressions at some intensity levels, whereas no effects of age, or gender were found for other intensity levels. The benefit of using varying intensity levels of facial emotional expressions to detect emotion recognition deficiencies, is further corroborated by the finding of ceiling effects in the recognition of angry, frightened and happy expressions at high-intensity levels. Our newly developed task, that encompasses varying intensity levels for four emotional expressions, therefore allows for a sensitive measure of facial emotion recognition in primary school-aged children. Our paradigm showed similar results in terms of increasing task performance with increasing emotional intensity as previous studies (Gao & Maurer, 2009; Thomas et al., 2007). We expect that the MFERT may detect subtle facial emotion recognition deficiencies in youth with neurodevelopmental disorders, including children with attention-deficit/hyperactivity disorder (Shaw, Stringaris, Nigg, & Leibenluft, 2014), adolescents with conduct disorder (Blair et al., 2001; Fairchild et al., 2009), and children with autism spectrum disorder (Harms, Martin, & Wallace, 2010). For instance, it has been suggested that high-functioning individuals with autism spectrum disorder, who seem to accurately recognise high-intensity facial emotional expressions, have more difficulty in recognising low-intensity expressions (Harms et al., 2010).

Although we found support for small effects of age on the recognition of facial emotional expressions at some intensity levels, we did not find any age effects on the majority of the intensity levels of these emotions. The latter suggests that the development of facial emotion recognition starts at a young age (under six years), and that this ability remains stable across primary school-age. Our findings are in line with the results of Lawrence et al. (2015), who showed that 6-year-olds performed at the same level as 16-year-olds in the recognition of angry, happy and sad facial expressions.

Further, although we found support for a small advantage in girls at the middle-intensity levels, we did not find any gender effects at the low- and high-intensity levels. Gender effects were not specific to a certain emotional condition. We suggest that gender differences in facial emotion recognition are more prevalent in infancy and preschool children, due to mainly social interactions with adults during the early years of development, causing a stronger effect of emotion socialisation by parents (McClure, 2000). From the age of six years onwards, it seems that boys catch up in the maturation of facial emotion recognition, which might be explained by an increase in social interactions with peers in primary school-aged children. An increased exposure

to facial expressions of peers may facilitate the development of emotion recognition in boys, resulting in a decrease of the gap between boys and girls (Mancini et al., 2013).

Further, we did not find any IQ effects on the recognition of facial emotional expressions. We suggest that effects of intelligence are more prevalent in low-functioning individuals (Herba & Phillips, 2004), while in our sample children with an IQ below 70 were excluded. Our results imply that facial emotion recognition in a community sample of primary school-aged children does not need to be interpreted in the light of general cognitive ability, and that deficiencies in facial emotion recognition do not automatically reflect a general cognitive delay.

There are some limitations to the current study that should be noted. While our study provides insight in facial emotion recognition across primary school-age, in future studies the age range of our study (6 to 12 year) should be expanded, to provide more insight in the developmental pathways of emotion recognition in children under six years old and in adolescents. An age range of zero to 18 year would allow for examining gender and age effects more thoroughly. Another limitation to our study is that we did not assess the ecological validity of the MFERT. We suggest that the MFERT will be assessed in larger samples, both in typically developing children and in psychiatric populations, to examine whether facial emotion recognition, assessed by the MFERT, is a predictor of social acceptance by peers.

There are several strengths to our study, that add to the adequacy of our newly developed MFERT. Firstly, the paradigm was evaluated in a community sample of primary school-aged children, who were recruited through primary schools located in diverse geographical areas. Our sample appears to be a representative sample of the Dutch population of primary school-aged children, as there were children from various ethnic and socioeconomic backgrounds. A second strength to our MFERT is that we used pictures of children's faces. As there is evidence for an own-age bias (Hills & Lewis, 2011; Proietti et al., 2015), paradigms using adult's faces might overestimate facial emotion recognition deficiencies in children. It is important to detect deficiencies in children that are specific to recognising facial expressions in peers, as the ability to interpret facial emotional expressions of peers is relevant in social interactions with peers (Nowicki & Mitchell, 1998).

To conclude, we have developed a task that allows for assessment of facial emotion recognition in primary school-aged children across the full range of emotion expressions. Even subtle deficiencies can be detected, as task performance increases with increasing expression intensity. Further research is required to examine the ecological validity of the MFERT in psychiatric populations.

References

Blair, R.J.R., Colledge, E., Murray, L., & Mitchell, D. (2001). A selective impairment in the processing of sad and fearful expressions in children with psychopathic tendencies. *Journal of Abnormal Child Psychology, 29*(6), 491-498.

Buitelaar, J.K., Van der Wees, M., Swaab-Barneveld, H., & Van der Gaag, R.J. (1999). Verbal memory and performance IQ predict theory of mind and emotion recognition ability in children with autistic spectrum disorders and in psychiatric control children. *Journal of Child Psychology and Psychiatry, 40*(6), 869-881.

DeWall, C.N., Maner, J.K., & Rouby, D.A. (2009). Social exclusion and early-stage interpersonal perception: Selective attention to signs of acceptance. *Journal of Personality and Social Psychology, 96*(4), 729-741.

Durand, K., Gallay, M., Seigneuric, A., Robichon, F., & Baudouin, J.-Y. (2007). The development of facial emotion recognition: The role of configural information. *Journal of Experimental Child Psychology, 97*(1), 14-27.

Egger, H.L., Pine, D.S., Nelson, E., Leibenluft, E., Ernst, M., Towbin, K.E., & Angold, A. (2011). The NIMH Child Emotional Faces Picture Set (NIMH-ChEFS): A new set of children's facial emotion stimuli. *International Journal of Methods in Psychiatric Research, 20*(3), 145-156.

Fairchild, G., Van Goozen, S.H., Calder, A.J., Stollery, S.J., & Goodyer, I.M. (2009). Deficits in facial expression recognition in male adolescents with early-onset or adolescence-onset conduct disorder. *Journal of Child Psychology and Psychiatry, 50*(5), 627-636.

Gao, X., & Maurer, D. (2009). Influence of intensity on children's sensitivity to happy, sad, and fearful facial expressions. *Journal of Experimental Child Psychology, 102*(4), 503-521.

Gao, X., & Maurer, D. (2010). A happy story: Developmental changes in children's sensitivity to facial expressions of varying intensities. J*ournal of Experimental Child Psychology, 107*(2), 67-86.

Harms, M.B., Martin, A., & Wallace, G.L. (2010). Facial emotion recognition in autism spectrum disorders: A review of behavioral and neuroimaging studies. *Neuropsychology Review, 20*(3), 290-322.

Herba, C.M., Landau, S., Russell, T., Ecker, C., & Phillips, M.L. (2006). The development of emotion-processing in children: Effects of age, emotion, and intensity. *Journal of Child Psychology and Psychiatry, 47*(11), 1098-1106.

Herba, C.M., & Phillips, M. (2004). Annotation: Development of facial expression recognition from childhood to adolescence: Behavioural and neurological perspectives. *Journal of Child Psychology and Psychiatry, 45*(7), 1185-1198.

Hills, P.J., & Lewis, M.B. (2011). The own-age face recognition bias in children and adults. *The Quarterly Journal of Experimental Psychology, 64*(1), 17-23.

Hubbard, J.A. (2001). Emotion expression processes in children's peer interaction: The role of peer rejection, aggression, and gender. *Child Development, 72*(5), 1426-1438.

Kothari, R., Skuse, D., Wakefield, J., & Micali, N. (2013). Gender differences in the relationship between social communication and emotion recognition. *Journal of the American Academy of Child & Adolescent Psychiatry, 52*(11), 1148-1157.

Lawrence, K., Campbell, R., & Skuse, D. (2015). Age, gender, and puberty influence the development of facial emotion recognition. *Frontiers in Psychology.* doi:10.3389/fpsyg.2015.00761.

Leppänen, J.M., & Hietanen, J.K. (2001). Emotion recognition and social adjustment in school–aged girls and boys. *Scandinavian Journal of Psychology, 42*(5), 429-435.

Mancini, G., Agnoli, S., Baldaro, B., Ricci Bitti, P.E., & Surcinelli, P. (2013). Facial expressions of emotions: Recognition accuracy and affective reactions during late childhood. *Journal of Psychology, 147*(6), 599-617.

McClure, E.B. (2000). A meta-analytic review of sex differences in facial expression processing and their development in infants, children, and adolescents. *Psychological Bulletin, 126*(3), 424-453.

Nowicki, S., & Duke, M.P. (1994). Individual differences in the nonverbal communication of affect: The Diagnostic Analysis of Nonverbal Accuracy Scale. *Journal of Nonverbal Behavior, 18*(1), 9-35.

Nowicki, S., & Mitchell, J. (1998). Accuracy in identifying affect in child and adult faces and voices and social competence in preschool children. *Genetic Social and General Psychology Monographs, 124*(1), 39-60.

Penton-Voak, I.S., Thomas, J., Gage, S.H., McMurran, M., McDonald, S., & Munafò, M.R. (2013). Increasing recognition of happiness in ambiguous facial expressions reduces anger and aggressive behavior. *Psychological Science, 24*(5), 688–697.

Proietti, V., Macchi Cassia, V., & Mondloch, C.J. (2015). The own-age face recognition bias is task dependent. *British Journal of Psychology, 106*(3), 446-467.

Rodger, H., Vizioli, L., Ouyang, X., & Caldara, R. (2015). Mapping the development of facial expression recognition. *Developmental Science, 18*(6), 926-939.

Rogers, J., Viding, E., Blair, R.J., Frith, U., & Happe, F. (2006). Autism spectrum disorder and psychopathy: Shared cognitive underpinnings or double hit? *Psychological Medicine, 36*(12), 1789-1798.

Sattler, J.M. (2008). *Resource guide to accompany assessment of children: Cognitive foundations.* La Mesa, CA: Jerome M. Sattler, Publisher, Inc.

Schneider, W., Eschman, A., & Zuccolotto, A. (2002). *E-prime* (Version 2.0). Pittsburgh: Psychology Software Tools Inc.

Shaw, P., Stringaris, A., Nigg, J., & Leibenluft, E. (2014). Emotion dysregulation in attention deficit hyperactivity disorder. *American Journal of Psychiatry, 171*(3), 276-293.

Simonian, S.J., Beidel, D.C., Turner, S.M., Berkes, J.L., & Long, J.H. (2001). Recognition of facial affect by children and adolescents diagnosed with social phobia. *Child Psychiatry and Human Development, 32*(2), 137-145.

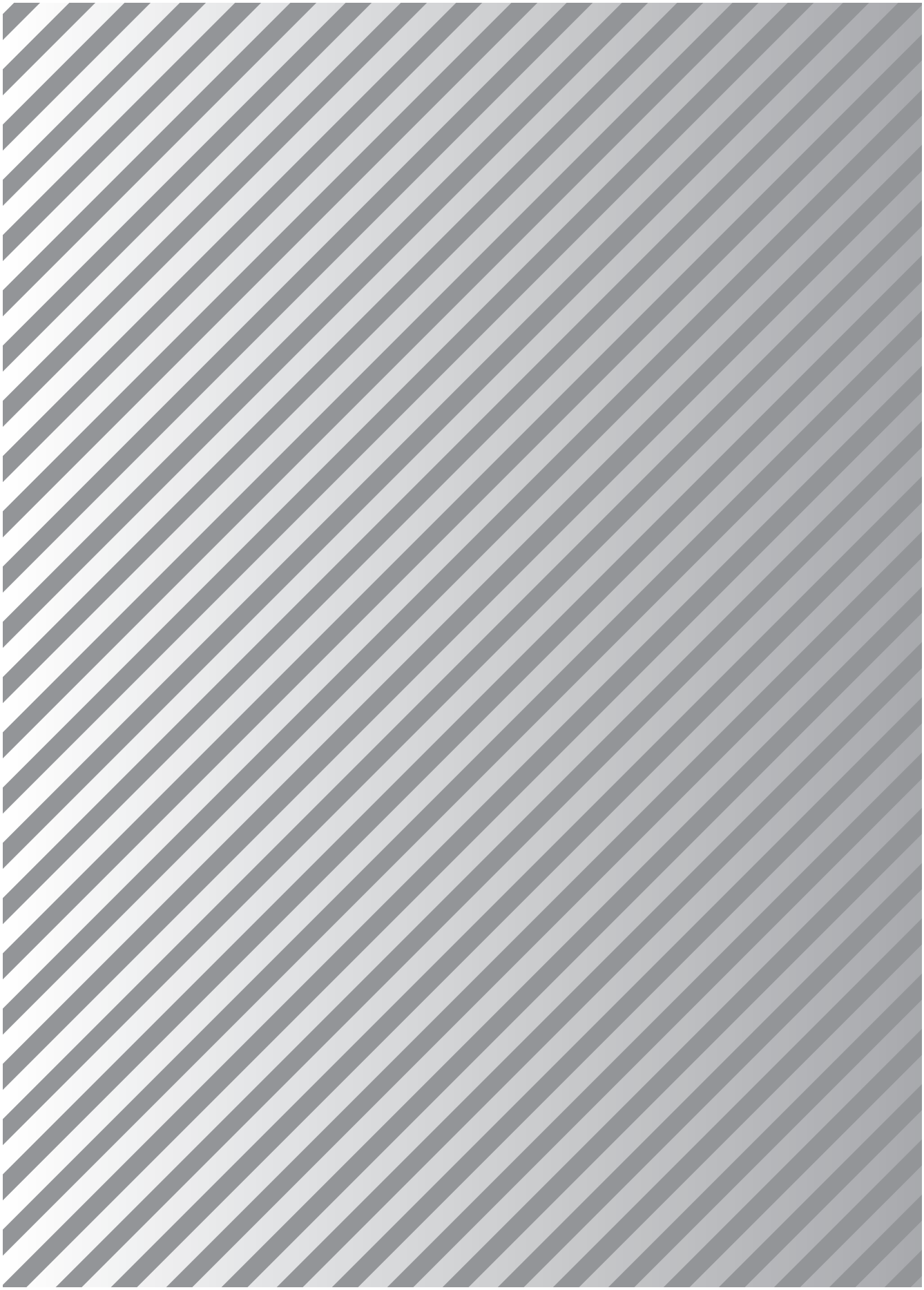
Statistics Netherlands. (2006). *Standard Educational Classification.* Den Haag/Heerlen, the Netherlands.

Thomas, L.A., De Bellis, M.D., Graham, R., & LaBar, K.S. (2007). Development of emotional facial recognition in late childhood and adolescence. *Developmental Science, 10*(5), 547-558.

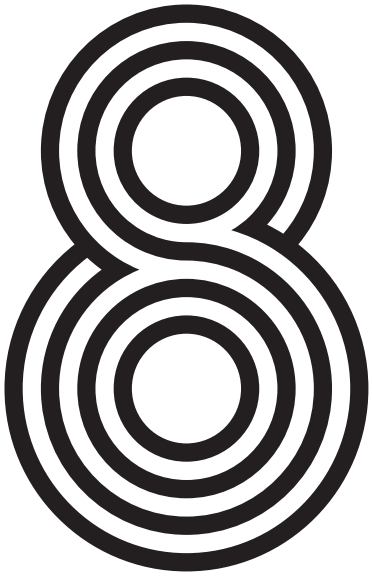
Wechsler, D. (1991). *Wechsler intelligence scale for children, third edition: Manual*. San Antonio: The Psychological Corporation.

Zeman, J., & Garber, J. (1996). Display rules for anger, sadness, and pain: It depends on who is watching. *Child Development, 67*(3), 957-973.

Zeman, J., & Shipman, K. (1997). Social-contextual influences on expectancies for managing anger and sadness: The transition from middle childhood to adolescence. *Developmental Psychology, 33*(6), 917-924.



Chapter 8
Summary and discussion



Summary of main findings

In this dissertation we aim to provide novel insights into childhood ADHD, by addressing a wide range of topics relevant to ADHD. Below, the main results from **chapter 2-7** of this dissertation are summarised (see also Table 8.1 for an overview). Subsequently, findings are critically evaluated, and new avenues for future research are provided.

The main objectives of **chapter 2** are to examine whether children with ADHD have decreased blood spot aromatic amino acid (AAA) concentrations, and whether blood spot AAA concentrations are related to symptoms of ADHD. AAAs, constituents of protein in foods, are involved in the biosynthesis of serotonin and dopamine, and therefore aberrant AAA concentrations may contribute to altered dopamine levels in ADHD (Oades, 2008), and to aberrant postsynaptic serotonin levels found in some individuals with ADHD (Oades, 2010). Given the central role of dopamine and serotonin alterations in ADHD (Oades, 2008), we hypothesise that decreased concentrations of tryptophan, tyrosine and phenylalanine in blood might contribute to the expression of ADHD symptoms. Based on the current literature, there is inconsistent evidence that AAAs are related to ADHD. The studies on this topic performed thus far are mostly outdated and hampered by methodological shortcomings (Baker et al., 1991; Bornstein et al., 1990; Comings, 1990; Hoshino, Ohno, & Yamamoto, 1985; Oades, Dauvermann, Schimmelmann, Schwarz, & Myint, 2010). We further explore whether abnormal blood spot AAA concentrations are related to decreased protein ingestion or by aberrant AAA excretion, as evidenced by increased urinary AAA concentrations. In the study 83 children with ADHD (75 percent males) and 72 typically developing (TD) children (51 percent males), aged 6 to 13 years, participated. AAA concentrations were assessed in blood spots and an 18-hour urinary sample. A nutritional diary was filled out by parents to calculate dietary protein intake. Parent and teacher questionnaires assessed symptoms of ADHD. In contrast to our hypothesis and some earlier studies on this topic (Baker et al., 1991; Bornstein et al., 1990; Comings, 1990), we did not find any differences in blood spot concentrations of tryptophan, tyrosine and phenylalanine between children with ADHD and TD children. In addition, below average (<16th percentile) blood spot AAA concentrations did not significantly increase the risk of being diagnosed with ADHD. In the combined sample of children with ADHD and TD children, no significant associations were found between AAA blood spot concentrations and ADHD symptoms. There was no difference between the ADHD and TD group in dietary protein intake or urinary AAA

concentrations, and AAA concentrations were not significantly related to protein intake and urinary AAA concentrations.

The objective of **chapter 3** is to examine whether increased homocysteine concentrations are related to childhood ADHD. High concentrations of homocysteine can have detrimental effects on neurocognitive performance, by causing DNA damage, disturbed methylation, cell death or by altering the functioning of glutamate receptors (Mattson & Shea, 2003). A deficiency of folate or vitamin B12 leads to increased concentrations of homocysteine in the blood, since the conversion of homocysteine to methionine is dependent on the cofactors folate and vitamin B12 (Mattson & Shea, 2003). Homocysteine has been found associated with neurocognitive performance in neurodegenerative diseases (Teunissen et al., 2005), in the normal aging population (Garcia & Zanibbi, 2004), as well as in psychiatric populations (Dias, Brissos, Cardoso, Andreazza, & Kapczinski, 2009; Ford, Flicker, Singh, Hirani, & Almeida, 2013). Strikingly, the neurocognitive functions that seem to be related to homocysteine concentrations (working memory, interference control and attention) (Dias et al., 2009; Teunissen et al., 2005), are exactly those that are impaired in (subgroups of) children with ADHD (Mullane, Corkum, Klein, & McLaughlin, 2009; Tamm et al., 2012; Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). Thus far, no studies have investigated the role of homocysteine concentrations in childhood ADHD. We hypothesise that children with ADHD have increased concentrations of homocysteine compared to TD children. We examine whether homocysteine concentrations in children with ADHD are (a) positively related to symptoms of ADHD, (b) negatively related to neurocognitive functioning in ADHD, which would lend further support to the role of homocysteine in ADHD, and (c) negatively related to intake of folate and vitamin B12, which would be informative for a dietary risk factor for ADHD. Homocysteine concentrations were assessed in blood spots of 55 children with ADHD and 54 TD children, aged 6 to 13 years. Parent and teacher questionnaires assessed symptoms of ADHD. Neurocognitive functioning was measured using the Digit Span Task, Grid Task and Flanker Task, targeting verbal and visuospatial working memory, interference control, variability in responding, and lapses of attention. Intake of folate and vitamin B12 was measured using nutritional diaries. In contrast to our hypothesis, we neither found a difference in homocysteine concentrations between children with ADHD and TD children, nor an association between homocysteine concentrations and symptoms of ADHD. Against our hypothesis, we did not find any evidence for the contribution of homocysteine to neurocognitive deficiencies in children

with ADHD. Furthermore, we did not find significant associations between homocysteine and the intake of folate and vitamin B12, and children with ADHD did not have a lower dietary intake of folate and vitamin B12 than TD children.

In **chapter 4** we aim to examine whether neurocognitive profiles can be distinguished in children with ADHD and TD children. Thus far three studies applied community detection procedures to distinguish neurocognitive subgroups of individuals with ADHD, all showing distinct neurocognitive profiles (Fair, Bathula, Nikolas, & Nigg, 2012; Mostert et al., 2015; Van Hulst, De Zeeuw, & Durston, 2015). However, since all three studies used different selections of neurocognitive measures, profile characteristics differed across studies. This violation of measurement invariance, due to the selection of different neurocognitive constructs or similar constructs assessed by different instruments, limits the possibility to derive final conclusions regarding the number and type of neurocognitive profiles being core to ADHD. Another aim of the current study is to address the clinical value of neurocognitive profiling, by examining whether neurocognitive profiles are related to problems often found co-occurring in ADHD, including externalising, social and academic problems in children with ADHD. Neurocognitive data of 81 children with ADHD and 71 TD children were subjected to confirmatory factor analysis. Neurocognitive functioning was measured using the Digit Span Task, Grid Task, Flanker Task and Children's Emotion Recognition Task, targeting verbal and visuospatial memory, verbal and visuospatial working memory, interference control, processing speed, variability in responding, lapses of attention and facial emotion recognition. Factor analysis resulted in a well-fitting model that consisted of six latent factors: memory, interference control, processing speed, variability in responding, lapses of attention and emotion recognition. The resulting factors were used for community detection in the ADHD and TD group. The results showed four neurocognitive subgroups in children with ADHD, each representing a distinct neurocognitive profile, with one subgroup characterised by poor interference control, one by slow processing speed, one by poor emotion recognition, and one by increased attentional lapses and fast processing speed. Three of the neurocognitive subgroups in the ADHD group were also observed in the TD group, with children with ADHD showing generally weaker neurocognitive performance compared to TD children. More specifically, children with ADHD showed weaker neurocognitive performance than TD children on one to four factor scores within each of the profiles. In the TD group no subgroup with a profile characterised by fast processing speed and increased attentional lapses was found. Our results showed no significant differences between any of the

neurocognitive subgroups in the ADHD sample on problems often found co-occurring with ADHD, including externalising, social and academic problems.

The main goal of **chapter 5** is to gain more insight into sleep disturbances in children with ADHD, using objective measures of sleep quality and quantity. A meta-analysis of studies using subjective measures of sleep quality (questionnaires filled out by parents) shows that children with ADHD have higher bedtime resistance, more sleep onset difficulties, nocturnal awakenings, difficulties with arising in the morning and sleep–disordered breathing compared to controls, although for all results considerable heterogeneity was reported across the studies (Cortese, Faraone, Konofal, & Lecendreux, 2009). A recent meta-analysis on sleep studies using actigraphy to objectively measure sleep, showed as well that non-medicated children with ADHD have increased sleep onset latency and decreased sleep efficiency, although, again, results were inconsistent (De Crescenzo et al., 2016). The fact that evidence for sleep problems in children with ADHD is inconsistent, might be explained by confounding influences of comorbid internalising and externalising problems, and low socioeconomic status (SES) in studies performed thus far. In **chapter 5** we study sleep quality and quantity using actigraphy in 63 medication-free children with ADHD and 61 TD children, aged 6 to 13 years. Our results showed that medication-free children with ADHD did not differ from TD children in sleep quality and quantity. ADHD symptoms were not related to any of the sleep parameters within the ADHD sample. We did not find a moderating role of low SES on the association between ADHD symptoms and sleep disturbances. Moderation analyses in the ADHD group showed interaction effects between ADHD symptoms and internalising behaviour and between ADHD symptoms and externalising behaviour on total sleep time, time in bed and average sleep bout duration. However, interactions could not be easily interpreted when comparing sleep patterns in ADHD samples with and without (sub) clinical levels of comorbid psychiatric symptoms. Nevertheless, our findings indicate a complex interplay between ADHD symptoms and comorbid psychiatric symptoms, which might at least partly explain the heterogeneity within the current literature on sleep quality and quantity in ADHD.

In **chapter 6** we aim to provide paediatric reference values for blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine. Over the past decades, the use of blood spot samples to examine amino acid concentrations has increased, in particular in newborn screening (Fingerhut & Olgemöller, 2009; Zytkovicz et al., 2001).

Collecting blood spots, by means of a finger prick, is less invasive for children than taking venous blood samples, and the dried blood spot technique is sufficiently robust and stable for diagnostic purposes (Chace, Sherwin, Hillman, Lorey, & Cunningham, 1998; Kand'ár & Žáková, 2009; Rashed et al., 1997). In addition, blood spots can be assessed at home, as they can be stored at room temperature and be sent by regular mail. Analysis of amino acid concentrations in blood of children is often used in clinical practice for diagnostic purposes and to make decisions regarding treatment (Lepage et al., 2006). Adequate reference values are required to determine which amino acid concentrations should be considered abnormal, and are thereby essential for clinical decision-making. There are some reference values published on blood spot amino acid concentrations in infants, required in newborn screening (Rashed et al., 1997; Zytkovicz et al., 2001). However, to our current knowledge, there are no normative values available for blood spot concentrations of amino acids in primary school-aged children. In **chapter 6** dried blood spots were obtained in a community sample of 104 healthy children, aged 6 to 12 years (52 percent males). Blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine were determined by positive electrospray liquid chromatography–tandem mass spectrometry. We examined whether age and gender affected amino acid concentrations, to take the potential effects of age (Held, White, & Pasquali, 2011; Lepage, McDonald, Dallaire, & Lambert, 1997; Van Beynum et al., 2005; Venta, Prieto, & Alvarez, 2002) and gender (Jung & Adeli, 2009) on individual differences in amino acid metabolism into account. Neither age nor gender had an impact on amino acid blood spot concentrations in our community sample. Therefore, reference values for blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine could be presented for the whole group of primary school-aged children. Reference values were established, based on the 5^{th} and 95^{th} percentile, representing the central ninety percent of the sample distribution, which is common in a community sample (Refsum et al., 2004). Furthermore, the 10^{th} and 90^{th} percentiles were presented, which are more stable measures in a modest-sized normative sample, as used here (Lepage et al., 1997).

Finally, in **chapter 7** we aim to examine developmental effects on facial emotion recognition in primary school-aged children. The development of facial emotion recognition during childhood has received much scientific interest, although results are somewhat inconsistent on the extent and range of the change in emotion recognition ability (Herba & Phillips, 2004). A potential explanation for differences across studies

into the developmental pathway of facial emotion recognition during childhood, is that the development is emotion-dependent (Mancini, Agnoli, Baldaro, Ricci Bitti, & Surcinelli, 2013; Rodger, Vizioli, Ouyang, & Caldara, 2015). Furthermore, gender and IQ may act as moderators of emotion recognition, as there is evidence for a small female advantage in facial emotion recognition in children (McClure, 2000), and for an effect of IQ on facial emotion recognition (Buitelaar, Van der Wees, Swaab-Barneveld, & Van der Gaag, 1999). The inconsistencies across studies may also be explained by the use of different methodologies; while some paradigms used static pictures with a high expression intensity (e.g., Lawrence, Campbell, & Skuse, 2015; Mancini et al., 2013), others used morphed pictures, in which the intensity of emotional expressions was manipulated (e.g., Herba, Landau, Russell, Ecker, & Phillips, 2006; Thomas, De Bellis, Graham, & LaBar, 2007). Inconsistencies in the literature of the development of facial emotion recognition in children might also be caused by the use of pictures of adult faces versus child faces. In most studies performed thus far in children, tasks were based on pictures of adult faces, using the well-validated Ekman-Friesen Pictures of Facial Affect (e.g., Herba et al., 2006; Lawrence et al., 2015). However, children may perform better in recognising facial emotions expressed by children than in recognising those expressed by adults, due to the so-called own-age bias (Hills & Lewis, 2011; Proietti, Macchi Cassia, & Mondloch, 2015). In children, the ability to recognise emotions in other children's faces is particularly important in social interaction with peers (Nowicki & Mitchell, 1998). The aim of **chapter 7** is to examine the effects of expression intensity, emotional condition, age, gender and IQ on facial emotion recognition in primary school-aged children. For this purpose the Morphed Facial Emotion Recognition Task (MFERT) was constructed, consisting of photographs of children's faces depicting four basic emotions (anger, fear, happiness and sadness). High-intensity expressions (100 percent) were morphed with neutral expressions, resulting in 240 stimuli, varying in emotional intensity (10 to 100 percent). The MFERT was assessed in a community sample of 75 children, aged 6 to 12 years (45 percent males). Results showed that for all emotional conditions emotion intensity has a linear effect on accuracy. Furthermore, we found that accuracy is highest for happy expressions, followed by angry and frightened expressions. Accuracy is lowest for sad expressions. Age is related to emotion recognition at some intensity levels. Girls have higher accuracy at middle-intensity emotional expressions than boys, but not at low- and high-intensity expressions. The results showed no relation between IQ and facial emotion recognition.

Table 8.1. Summary of the main findings of this dissertation

Chapter	Participants	Measures	Main findings
2	83 children with ADHD 72 TD children (Study 1)	• Blood spot concentrations of tryptophan, tyrosine and phenylalanine • Parent- and teacher-rated ADHD symptoms (SWAN) • Urinary concentrations of tryptophan, tyrosine and phenylalanine (18-hour sample) • Dietary protein intake	• No group differences in blood spot concentrations of tryptophan, tyrosine and phenylalanine • AAA concentrations not related to ADHD symptoms • No group differences in protein intake and urinary AAA concentrations • Blood spot AAA concentrations not related to protein intake and urinary AAA concentrations
3	55 children with ADHD 54 TD children (Study 1)	• Blood spot concentrations of total homocysteine • Parent- and teacher-rated ADHD symptoms (SWAN) • Verbal working memory (Digit Span Task) • Visuospatial working memory (Grid Task) • Interference control, variability in responding and lapses of attention (Flanker Task) • Dietary intake of folate and vitamin B12	• No group differences in homocysteine blood spot concentrations • Homocysteine concentrations not related to ADHD symptoms or to neurocognitive functioning • No group differences in intake of folate and vitamin B12 • Intake of folate and vitamin B12 not related to homocysteine concentrations
4	81 children with ADHD 71 TD children (Study 1)	• Verbal memory and verbal working memory (Digit Span Task) • Visuospatial memory and visuospatial working memory (Grid Task) • Interference control, processing speed, variability in responding and lapses of attention (Flanker Task) • Facial emotion recognition (CERT) • Parent- and teacher-rated externalising behaviour (DBDRS) • Social acceptance and rejection (sociometric data) • Parent- and teacher-rated social problems (CBCL/TRF) • Reading comprehension, spelling and mathematics (Pupil monitoring system)	• Four distinct neurocognitive subgroups in ADHD sample; one characterised by poor interference control, one by slow processing speed, one by poor emotion recognition, and one by increased attentional lapses and fast processing speed • Three distinct neurocognitive subgroups in TD sample, closely resembling profiles of first three subgroups in ADHD group • No subgroup characterised by increased attentional lapses and fast processing speed in TD sample • Children with ADHD weaker neurocognitive performance than TD children on one to four factor scores within each profile • No differences between four neurocognitive subgroups in ADHD sample on externalising, social and academic problems

Table 8.1. *Continued*

Chapter	Participants	Measures	Main findings
5	63 children with ADHD 61 TD children (Study 1)	• Time in bed, total sleep time, nocturnal motor activity, sleep onset latency, morning arising latency, average wake bout duration and average sleep bout duration (actigraphy) • Parent- and teacher-rated internalising behaviour (CBCL/TRF) • Parent- and teacher-rated externalising behaviour (DBDRS)	• No group differences in any actigraphic measures • ADHD symptoms not related to sleep quality or quantity • Interaction effects between ADHD symptoms and internalising and externalising behaviour on time in bed, total sleep time and average sleep bout duration, which could not be easily interpreted
6	104 children from a CS (Study 1)	• Blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine	• Age not related to blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine • No gender differences in blood spot concentrations of total homocysteine, tryptophan, tyrosine and phenylalanine • Reference values provided, using the 5th, 10th, 90th and 95th percentile
7	75 children from a CS (Study 2)	• Facial emotion recognition of angry, happy, frightened and sad expressions, across 10 intensity levels (MFERT) • IQ (WISC-III)	• For all emotional conditions a positive linear effect of expression intensity on accuracy • Accuracy higher for happy expressions than for angry and frightened expressions. Recognition of sad expressions lowest • Age positively related to facial emotion recognition at some middle- and high-intensity levels • Girls higher accuracy than boys at middle-intensity levels, but not at low- and high-intensity levels • IQ not related to facial emotion recognition

Notes. AAA, aromatic amino acid; ADHD, attention-deficit/hyperactivity disorder; CBCL, Child Behavior Checklist; CERT, Children's Emotion Recognition Task; CS, community sample; DBDRS, Disruptive Behaviour Disorder Rating Scale; MFERT, Morphed Facial Emotion Recognition Task; SWAN, Strengths and Weaknesses of ADHD-symptoms and Normal Behaviour rating scale; TD, typically developing; TRF, Teacher Rating Form; WISC-III, Wechsler Intelligence Scale for Children.

General discussion

This dissertation has yielded several important insights into childhood ADHD. The main insight is that the findings described in **chapter 2 and 3** of this dissertation imply that abnormalities of tryptophan, tyrosine, phenylalanine and homocysteine in blood are not involved in the aetiology of ADHD. We therefore failed to support the idea that these amino acids may act as biomarkers for ADHD, and could not expand our understanding of the aetiology of ADHD. As was argued in **chapter 1** in this dissertation, ADHD is a clinically heterogeneous disorder. Finding a single or unitary aetiology of ADHD is therefore highly unlikely. However, we do not rule out the possibility that amino acid abnormalities contribute to the presence of ADHD symptoms in certain more homogenous subgroups of children with ADHD. For instance, it might be that only children with ADHD and severe deficiencies in executive functioning (EF) have altered tyrosine and phenylalanine concentrations, as an altered dopamine functioning in the prefrontal cortex and the striatum is thought to impair executive functions, including sustained attention and interference control in ADHD (Del Campo, Chamberlain, Sahakian, & Robbins, 2011; Oades, 2008). The suggestion of tyrosine and phenylalanine alterations in children with ADHD and severe EF deficiencies is further supported by a meta-analysis that showed evidence for the association between phenylalanine alterations (which result in tyrosine deficiencies) and EF impairment (Albrecht, Garbade, & Burgard, 2009). Likewise, it might be that only children with ADHD and comorbid autism spectrum disorder (ASD) have increased homocysteine concentrations, as previous studies showed an increased prevalence of hyperhomocysteinemia in children with ASD (Paşca et al., 2006; Puig-Alcaraz, Fuentes-Albero, Calderón, Garrote, & Cauli, 2015). This implies that amino acid alterations are not related to ADHD itself, but to associated problems often found in ADHD. Our results emphasise the complexity of exploring single aetiological risk factors in a disorder that is characterised by a large heterogeneity. Studies into aetiological risk factors for ADHD therefore require large sample sizes, enabling to perform analyses in more homogenous subgroups of ADHD.

There is growing interest in biomarkers in child psychiatry, aimed at providing a biologically guided diagnosis of psychiatric disorders and at gaining insight into biological factors that may moderate treatment response. One of the reasons underlying this interest is the criticism that current diagnostic procedures for ADHD, mainly based on subjective measures (questionnaires, interviews, observation), are insufficiently reliable and valid

(Faraone, Bonvicini, & Scassellati, 2014). Furthermore, there is hope that biomarkers could identify children at risk of developing psychiatric disorders prior to the onset of severe behavioural problems (Singh & Rose, 2009). Analysing amino acid concentrations in blood spots of children would be a relatively cheap and non-invasive addition to diagnostic procedures, in contrast to measuring biomarkers obtained through, for instance, neuroimaging techniques and electroencephalography. However, thus far, no biological markers have been found for ADHD that have been proven to be of any clinical utility, due to low sensitivity and specificity (Scassellati, Bonvicini, Faraone, & Gennarelli, 2012). Our results show that the same holds for tryptophan, tyrosine, phenylalanine and total homocysteine, implying that, based on the available evidence, these amino acids should not be considered as promising biomarkers for ADHD.

The findings that blood spot concentrations of tryptophan, tyrosine and phenylalanine are not decreased and that blood spot concentrations of homocysteine are not increased in children with ADHD, argue against certain nutritional interventions in ADHD. During the past years some studies have examined the effects of AAA supplementation in children and adults with ADHD, with inconsistent results (Nemzer, Arnold, Votolato, & McConnell, 1986; Reimherr, Wender, Wood, & Ward, 1987; Wood, Reimherr, & Wender, 1985; Zametkin, Karoum, Rapoport, Brown, & Wyatt, 1984). The apparent lack of AAA deficiencies in ADHD might explain the conflicting results of amino acid supplementation on reducing ADHD symptoms (Hurt, Arnold, & Lofthouse, 2011). Likewise, our results do not provide support for nutritional interventions with folate (Ghanizadeh, Sayyari, & Mohammadi, 2013) or vitamin B12 (Ghanizadeh et al., 2013; Patel & Curtis, 2007) in children with ADHD, as childhood ADHD is not related to increased homocysteine concentrations or decreased intake of folate and vitamin B12. This implication is in line with a growing consideration that, in case of interventions based on nutritional supplements, effects may be limited to ADHD patients with nutritional deficiencies (Hurt et al., 2011).

Another insight that is provided by this dissertation, is that associated problems in ADHD might not be related to ADHD itself, guided by our finding of no sleep disturbances in medication-free children with ADHD. Associated problems in ADHD might be epiphenomena, caused by other factors characteristic of ADHD, including stimulant medication use and comorbid psychiatric conditions. For instance, we hypothesised that stimulant medication use may mediate sleep disturbances in children with ADHD,

as previous studies have reported that methylphenidate has a (direct) negative impact on sleep quality as compared to placebo (Corkum, Panton, Ironside, MacPherson, & Williams, 2008; Schwartz et al., 2004). Therefore, children with ADHD who participated in our study into sleep disturbances (**chapter 5**), had to withdraw from stimulant medication use when sleep was monitored. This approach may have contributed to our finding that ADHD is not associated with any sleep disturbances. Our findings imply that methylphenidate use may have confounded results of earlier studies that showed an association between ADHD and sleep disturbances (Yoon, Jain, & Shapiro, 2012). Furthermore, we found interactions between ADHD symptoms and internalising and externalising problems in the association with sleep problems, indicating a moderating role of comorbid psychiatric conditions on the association between ADHD and sleep disturbances. We hypothesise that the same may hold for the association between ADHD and social problems and academic underachievement; we suggest that factors characteristic of ADHD (including comorbid psychiatric conditions) increase the risk of social problems and academic underachievement in children with ADHD. For instance, social problems in ADHD might be mediated through or moderated by comorbid ASD (Van der Meer et al., 2012) or comorbid externalising behaviour (Becker, Luebbe, & Langberg, 2012). Academic underachievement, on the other hand, might be mediated through or moderated by low SES (Sjöwall, Bohlin, Rydell, & Thorell, 2017). Our results of **chapter 4** indicate that neurocognitive profiles did not seem to mediate or moderate the association between ADHD and both social problems and academic underachievement. This lack of findings emphasises the need to explore other factors than neurocognitive deficiencies that mediate or moderate the association between ADHD and associated social and academic problems. For instance, further research might focus on other environmental factors, including family and parenting factors (Becker et al., 2012), to disentangle the true risk factors for associated social and academic problems in ADHD. This may enhance early detection of children with ADHD who are at risk of (multiple) other problems that impair daily life functioning.

Furthermore, the results of this dissertation emphasise yet again the heterogeneity of ADHD. For instance, the results described in **chapter 4** point out that there are multiple distinct neurocognitive subgroups in ADHD, each characterised by other neurocognitive strengths and weaknesses. Furthermore, the great variance found in many domains (ADHD symptoms, externalising behaviour, internalising behaviour, ASD symptoms, sleep disturbances, academic performance and social functioning) in **chapter 4 and**

5, marks the great individual differences between children with ADHD. While some children with ADHD in our sample were characterised by problems in only one domain, others were hampered by a wide range of associated problems. The great heterogeneity evokes to apply an individual approach in treatment of children with ADHD. Currently, clinical guidelines propose a standard approach to treatment of children ADHD (e.g., American Academy of Pediatrics, 2011). For instance, stimulant medication use is the first-choice treatment for children with severe ADHD symptoms. However, there is a large group of children with ADHD for whom treatment with methylphenidate is not effective (10 percent), or for whom treatment with methylphenidate is not more effective than placebo (additional 13 percent) (Greenhill et al., 2001; Vitiello et al., 2001). Moreover, there is evidence for idiosyncratic dose-response curves for methylphenidate (Greenhill et al., 2001), implying that for each individual the dose-response curve can take other forms; a higher dose does not lead to a greater reduction in symptoms in all children (Konrad, Günther, Heinzel-Gutenbrunner, & Herpertz-Dahlmann, 2005). The effects of methylphenidate may also differ across outcomes, as a particular dosage may be most effective in reducing impulsiveness, while another dosage is most effective in reducing concentration problems (Konrad et al., 2005). Taken together, pharmacological treatment of children with ADHD should be tailored and evaluated on an individual basis. For instance, it is recommended to use double-blind placebo-controlled titration when stimulant medication is advised for children with ADHD (MTA Cooperative Group, 1999). This form of titration enables detecting non-responders and placebo-responders, and may enhance prescription of optimal individual dosages.

For other interventions for ADHD an individual approach is warranted as well, and it is recommended to examine which children with ADHD benefit most from the intervention of interest. For instance, it has been shown that the presence of comorbid anxiety in children with ADHD moderates treatment response, as children with anxiety disorders showed relatively stronger response to behavioural intervention than those without anxiety disorder did (MTA Cooperative Group, 1999). Therefore, clinicians may consider a different approach for children with ADHD and anxiety, from an approach for children with ADHD and other or no comorbidities (Hinshaw, 2007). An individual approach when it comes to the selection of the most adequate treatment for children with ADHD may enhance treatment outcomes. However, currently there is insufficient scientific evidence to adjust clinical guidelines for treatment of ADHD to individual characteristics. Therefore, more research is warranted to detect which factors mediate or moderate

treatment response in children with ADHD, which may help to apply an individual approach in treatment of children with ADHD.

The heterogeneity in ADHD also evokes to expand the diagnostic procedures in children. When presented with a child with ADHD symptoms, it is recommended to perform a broad screening of associated problems during the diagnostic procedures. Using screening instruments that cover multiple psychiatric conditions, as well as functional impairments, may lead to better and earlier detection of children with ADHD who are at risk of severe problems.

Research agenda

Even though the results described in the current dissertation, being mainly null-findings, rule out some lines of research into the aetiology of ADHD and into treatment of ADHD symptoms (such as nutritional interventions targeting amino acid abnormalities), they also raise a number of research questions and themes for future research.

In **chapter 2 and 3** we explored whether amino acid abnormalities are related to childhood ADHD. One of the potential underlying mechanisms for aberrant amino acid concentrations in blood could be a decreased intake of protein, folate or vitamin B12. Our lack of finding amino acid abnormalities in ADHD can therefore partly be explained by a lack of dietary deficiencies in our childhood ADHD sample. We might question whether amino acid abnormalities would be more prevalent in malnourished children. Decreased dietary intake of protein, folate and vitamin B12 is more likely in children living in low SES environments. We suggest to further explore the risk of dietary deficiencies for ADHD in children living in low SES environments (Liu & Raine, 2006). As our ADHD sample consisted of only a small subsample of children with a low SES (*n*=14), our study was underpowered to explore the effects of low SES on dietary intake and, subsequently, on amino acid concentrations. We hypothesise that in a sample of children with ADHD and a low SES the effects of dietary deficiencies on ADHD symptoms are larger than already established for some micronutrients, including zinc (Toren et al., 1996), folate (Durá-Travé & Gallinas-Victoriano, 2014), iron (Konofal, Lecendreux, Arnulf, & Mouren, 2004), and omega-6 fatty acids (Ng, Meyer, Reece, & Sinn, 2009). In case specific deficiencies are found in children with ADHD from low SES families, the effects of nutritional interventions for these children could be explored. Indeed, beneficial effects

of multivitamin/mineral supplementation and essential fatty acids have been suggested larger in children with ADHD who have dietary deficiencies (Hurt et al., 2011).

Another avenue that could be explored in future studies, is to focus on the effects of prenatal nutritional deficiencies, as we expect that nutritional deficiencies are more detrimental during the early stage of prenatal development. For instance, a recent study showed negative effects of low prenatal folate levels on brain volume, language, learning/memory and visuospatial processing in six to eight year old children (Ars et al., 2016). This line of research may also provide more insight into the association between low SES and ADHD. Many studies have shown that low SES appears to be a risk factor for ADHD (Willcutt, 2012). However, it is not clear which factors associated with social disadvantage contribute to the onset of ADHD. It has been suggested that social disadvantage involves poor prenatal nutrition and increased pre- and post-natal toxicant exposure, which can have a detrimental impact on brain development of children (Nigg & Craver, 2014). Therefore, we suggest to further explore the effects of nutritional deficiencies in pregnant women, in relation to low SES, on later behavioural functioning of children, in a longitudinal study.

For future dietary intervention studies in ADHD, it is recommended to establish a thorough justification of the intervention of interest, by providing a sound theoretical framework on the working mechanisms underlying the supposed intervention effects. Such a theory on working mechanisms is crucial to understand why an intervention may be beneficial and to whom the intervention should be applied. For dietary interventions in ADHD, theories may be built on nutritional deficiencies in children with ADHD, in case of nutritional supplements being provided as intervention. Theories may also be built on allergic responses to nutrients occurring naturally in food (among which eggs and peanuts) or to artificial ingredients (including artificial colours) in children with ADHD, in case of elimination diets. The initial plan for the current dissertation was to focus on the treatment effect of nicotinamide (part of vitamin B3) supplementation in children with ADHD who suffer from a tryptophan deficiency. The main postulations for a randomised controlled trial with nicotinamide supplementation were that (a) a significant subgroup of children with ADHD would suffer from decreased tryptophan concentrations, and (b) nicotinamide supplementation would improve upon the uptake and transport of tryptophan, resulting in an increase of tryptophan concentrations. During the preparation of the research protocol, we realised that there was insufficient theoretical justification

for tryptophan abnormalities in children with ADHD, as well as for the beneficial effect of nicotinamide supplementation on the uptake and transport of tryptophan. Given the inconsistent evidence in the current literature for tryptophan abnormalities in children with ADHD, we decided to investigate in a carefully set-up study whether there was evidence for the postulation of tryptophan abnormalities in children with ADHD. As our results showed that this was not the case (described in **chapter 2**), further studies into nicotinamide supplementation in children with ADHD were not conducted. This dissertation illustrates that experimentally testing the postulations underlying a novel intervention, is fruitful prior to carrying out a large randomised controlled trial. Also for current nutritional interventions in ADHD, including restricted elimination diets, it would be interesting to explore the main postulations regarding the underlying working mechanisms. For instance, it is unlikely that an allergic mechanism is involved in the efficacy of restricted elimination diets (Pelsser, 2011), which invokes to explore which factors may explain beneficial effects of elimination diets. Without a sound theoretical framework, alternative explanations, including a biased perception of parents (Sonuga-Barke et al., 2013), become more plausible to explain the effects of a certain intervention that requires a large investment of participants and their parents.

A last suggestion for future research is to explore the predictive validity of neurocognitive deficiencies in children with ADHD. While our results described in **chapter 4** point out that children with ADHD generally show weaker neurocognitive performance compared to TD children, these deficiencies do not seem to mediate the association between ADHD and current externalising behaviour, academic achievement and social functioning. Furthermore, there is limited predictive validity of neurocognitive functioning for persistence of ADHD (Van Lieshout, Luman, Buitelaar, Rommelse, & Oosterlaan, 2013), and no evidence for predictive value of neurocognitive functioning for the emergence of nicotine dependence or substance use disorders later in life in individuals with ADHD (Groenman et al., 2015). One might therefore question whether it is useful to perform clinical neurocognitive assessments in children with ADHD, which may be highly time-consuming. It has been suggested that children with ADHD outgrow neurocognitive deficiencies, as EF deficiencies that were found in childhood ADHD, were not found in the follow-up of that sample in adolescence (Thissen et al., 2014). It may be, however, that a subsample of children with ADHD and neurocognitive deficiencies continues to be impaired in terms of neurocognitive functioning later in life. For instance, it was found that also in adults separate neurocognitive profiles could be detected, with individuals

with ADHD performing worse on neurocognitive measures than controls (Mostert et al., 2015). Unfortunately, it was not investigated whether the different cognitive profiles in adults with ADHD showed different functional impairments. We recommend to further explore the predictive validity of neurocognitive profiles for future functioning, using a longitudinal study design. If certain neurocognitive profiles prove to increase the risk of future academic underachievement, this may provide more insight into which deficiencies should be targeted in cognitive interventions and which children may benefit most from these interventions. Currently there seem to be no far transfer effects of cognitive interventions on academic performance in children with ADHD (Rapport, Orban, Kofler, & Friedman, 2013). However, when cognitive interventions target the neurocognitive deficiencies that are largest in some subgroups of children with ADHD, effects on academic performance may emerge. Predictive validity of neurocognitive profiles would thereby justify the role of clinical neurocognitive assessment in diagnostic procedures in children with ADHD.

References

Albrecht, J., Garbade, S.F., & Burgard, P. (2009). Neuropsychological speed tests and blood phenylalanine levels in patients with phenylketonuria: A meta-analysis. *Neuroscience & Biobehavioral Reviews, 33*(3), 414-421.

American Academy of Pediatrics. (2011). ADHD: Clinical practice guideline for the diagnosis, evaluation, and treatment of attention-deficit/hyperactivity disorder in children and adolescents. *Pediatrics, 128*(5), 1007-1022.

Ars, C., Nijs, I., Marroun, H., Muetzel, R., Schmidt, M., Steenweg-de Graaff, J., . . . Steegers, E. (2016). Prenatal folate, homocysteine and vitamin B12 levels and child brain volumes, cognitive development and psychological functioning: The Generation R Study. *British Journal of Nutrition.* doi:10.1017/S0007114515002081.

Baker, G.B., Bornstein, R.A., Rouget, A.C., Ashton, S.E., Van Muyden, J.C., & Coutts, R.T. (1991). Phenylethylaminergic mechanisms in attention-deficit disorder. *Biological Psychiatry, 29*(1), 15-22.

Becker, S.P., Luebbe, A.M., & Langberg, J.M. (2012). Co-occurring mental health problems and peer functioning among youth with attention-deficit/hyperactivity disorder: A review and recommendations for future research. *Clinical Child and Family Psychology Review, 15*(4), 279-302.

Bornstein, R.A., Baker, G.B., Carroll, A., King, G., Wong, J.T., & Douglass, A.B. (1990). Plasma amino acids in attention deficit disorder. *Psychiatry Research, 33*(3), 301-306.

Buitelaar, J.K., Van der Wees, M., Swaab-Barneveld, H., & Van der Gaag, R.J. (1999). Verbal memory and performance IQ predict theory of mind and emotion recognition ability in children with autistic spectrum disorders and in psychiatric control children. *Journal of Child Psychology and Psychiatry, 40*(6), 869-881.

Chace, D.H., Sherwin, J.E., Hillman, S.L., Lorey, F., & Cunningham, G.C. (1998). Use of phenylalanine-to-tyrosine ratio determined by tandem mass spectrometry to improve newborn screening for phenylketonuria of early discharge specimens collected in the first 24 hours. *Clinical Chemistry, 44*(12), 2405-2409.

Comings, D.E. (1990). Blood serotonin and tryptophan in Tourette syndrome. *American Journal of Medical Genetics, 36*(4), 418-430.

Corkum, P., Panton, R., Ironside, S., MacPherson, M., & Williams, T. (2008). Acute impact of immediate release methylphenidate administered three times a day on sleep in children with attention-deficit/hyperactivity disorder. *Journal of Pediatric Psychology, 33*(4), 368-379.

Cortese, S., Faraone, S.V., Konofal, E., & Lecendreux, M. (2009). Sleep in children with attention-deficit/hyperactivity disorder: Meta-analysis of subjective and objective studies. *Journal of the American Academy of Child & Adolescent Psychiatry, 48*(9), 894-908.

De Crescenzo, F., Licchelli, S., Ciabattini, M., Menghini, D., Armando, M., Alfieri, P., . . . Foti, F. (2016). The use of actigraphy in the monitoring of sleep and activity in ADHD: A meta-analysis. *Sleep Medicine Reviews, 26*, 9-20.

Del Campo, N., Chamberlain, S.R., Sahakian, B.J., & Robbins, T.W. (2011). The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. *Biological Psychiatry, 69*(12), e145-e157.

Dias, V.V., Brissos, S., Cardoso, C., Andreazza, A.C., & Kapczinski, F. (2009). Serum homocysteine levels and cognitive functioning in euthymic bipolar patients. *Journal of Affective Disorders, 113*(3), 285-290.

Durá-Travé, T., & Gallinas-Victoriano, F. (2014). Caloric and nutrient intake in children with attention deficit hyperactivity disorder treated with extended-release methylphenidate: Analysis of a cross-sectional nutrition survey. *Journal of the Royal Society of Medicine Open, 5*(2), 1-7.

Fair, D.A., Bathula, D., Nikolas, M.A., & Nigg, J.T. (2012). Distinct neuropsychological subgroups in typically developing youth inform heterogeneity in children with ADHD. *Proceedings of the National Academy of Sciences, 109*(17), 6769-6774.

Faraone, S.V., Bonvicini, C., & Scassellati, C. (2014). Biomarkers in the diagnosis of ADHD – Promising directions. *Current Psychiatry Reports, 16*(11), 1-20.

Fingerhut, R., & Olgemöller, B. (2009). Newborn screening for inborn errors of metabolism and endocrinopathies: An update. *Analytical and Bioanalytical Chemistry, 393*(5), 1481-1497.

Ford, A.H., Flicker, L., Singh, U., Hirani, V., & Almeida, O.P. (2013). Homocysteine, depression and cognitive function in older adults. *Journal of Affective Disorders, 151*(2), 646-651.

Garcia, A., & Zanibbi, K. (2004). Homocysteine and cognitive function in elderly people. *Canadian Medical Association Journal, 171*(8), 897-904.

Ghanizadeh, A., Sayyari, Z., & Mohammadi, M.R. (2013). Effect of methylphenidate and folic acid on ADHD symptoms and quality of life and aggression: A randomized double blind placebo controlled clinical trial. *Iranian Journal of Psychiatry, 8*(3), 108.

Greenhill, L.L., Swanson, J.M., Vitiello, B., Davies, M., Clevenger, W., Wu, M., . . . Conners, C.K. (2001). Impairment and deportment responses to different methylphenidate doses in children with ADHD: The MTA titration trial. *Journal of the American Academy of Child & Adolescent Psychiatry, 40*(2), 180-187.

Groenman, A.P., Oosterlaan, J., Greven, C.U., Vuijk, P.J., Rommelse, N., Franke, B., . . . Faraone, S.V. (2015). Neurocognitive predictors of substance use disorders and nicotine dependence in ADHD probands, their unaffected siblings, and controls: A 4-year prospective follow-up. *Journal of Child Psychology and Psychiatry, 56*(5), 521-529.

Held, P.K., White, L., & Pasquali, M. (2011). Quantitative urine amino acid analysis using liquid chromatography tandem mass spectrometry and aTRAQ® reagents. *Journal of Chromatography B, 879*(26), 2695-2703.

Herba, C.M., Landau, S., Russell, T., Ecker, C., & Phillips, M.L. (2006). The development of emotion-processing in children: effects of age, emotion, and intensity. *Journal of Child Psychology and Psychiatry, 47*(11), 1098-1106.

Herba, C.M., & Phillips, M. (2004). Annotation: Development of facial expression recognition from childhood to adolescence: Behavioural and neurological perspectives. *Journal of Child Psychology and Psychiatry, 45*(7), 1185-1198.

Hills, P.J., & Lewis, M.B. (2011). The own-age face recognition bias in children and adults. *The Quarterly Journal of Experimental Psychology, 64*(1), 17-23.

Hinshaw, S.P. (2007). Moderators and mediators of treatment outcome for youth with ADHD: Understanding for whom and how interventions work. *Ambulatory Pediatrics, 7*(1), 91-100.

Hoshino, Y., Ohno, Y., & Yamamoto, T. (1985). Plasma free tryptophan concentration in children with attention deficit disorder. *Folia Psychiatrica et Neurologica Japonica, 39*(4), 531-536.

Hurt, E.A., Arnold, L.E., & Lofthouse, N. (2011). Dietary and nutritional treatments for attention-deficit/hyperactivity disorder: Current research support and recommendations for practitioners. *Current Psychiatry Reports, 13*(5), 323-332.

Jung, B., & Adeli, K. (2009). Clinical laboratory reference intervals in pediatrics: The CALIPER initiative. *Clinical Biochemistry, 42*(16), 1589-1595.

Kand'ár, R., & Žáková, P. (2009). Determination of phenylalanine and tyrosine in plasma and dried blood samples using HPLC with fluorescence detection. *Journal of Chromatography B, 877*(30), 3926-3929.

Konofal, E., Lecendreux, M., Arnulf, I., & Mouren, M.-C. (2004). Iron deficiency in children with attention-deficit/hyperactivity disorder. *Archives of Pediatrics & Adolescent Medicine, 158*(12), 1113-1115.

Konrad, K., Günther, T., Heinzel-Gutenbrunner, M., & Herpertz-Dahlmann, B. (2005). Clinical evaluation of subjective and objective changes in motor activity and attention in children with attention-deficit/hyperactivity disorder in a double-blind methylphenidate trial. *Journal of Child & Adolescent Psychopharmacology, 15*(2), 180-190.

Lawrence, K., Campbell, R., & Skuse, D. (2015). Age, gender, and puberty influence the development of facial emotion recognition. *Frontiers in Psychology.* doi:10.3389/fpsyg.2015.00761.

Lepage, N., Li, D., Kavsak, P.A., Bamforth, F., Callahan, J., Dooley, K., & Potter, M. (2006). Incomplete pediatric reference intervals for the management of patients with inborn errors of metabolism. *Clinical Biochemistry, 39*(6), 595-599.

Lepage, N., McDonald, N., Dallaire, L., & Lambert, M. (1997). Age-specific distribution of plasma amino acid concentrations in a healthy pediatric population. *Clinical Chemistry, 43*(12), 2397-2402.

Liu, J., & Raine, A. (2006). The effect of childhood malnutrition on externalizing behavior. *Current Opinion in Pediatrics, 18*(5), 565-570.

Mancini, G., Agnoli, S., Baldaro, B., Ricci Bitti, P.E., & Surcinelli, P. (2013). Facial expressions of emotions: Recognition accuracy and affective reactions during late childhood. *Journal of Psychology, 147*(6), 599-617.

Mattson, M.P., & Shea, T.B. (2003). Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. *Trends in Neurosciences, 26*(3), 137-146.

McClure, E.B. (2000). A meta-analytic review of sex differences in facial expression processing and their development in infants, children, and adolescents. *Psychological Bulletin, 126*(3), 424-453.

Mostert, J.C., Hoogman, M., Onnink, A.M.H., Van Rooij, D., Von Rhein, D., Van Hulzen, K.J., . . . Norris, D.G. (2015). Similar subgroups based on cognitive performance parse heterogeneity in adults with ADHD and healthy controls. *Journal of Attention Disorders*. doi:10.1177/1087054715602332.

MTA Cooperative Group. (1999). Moderators and mediators of treatment response for children with attention-deficit/hyperactivity disorder: The Multimodal Treatment Study of children with attention-deficit/hyperactivity disorder. *Archives of General Psychiatry, 56*(12), 1088-1096.

Mullane, J.C., Corkum, P.V., Klein, R.M., & McLaughlin, E. (2009). Interference control in children with and without ADHD: A systematic review of Flanker and Simon task performance. *Child Neuropsychology, 15*(4), 321-342.

Nemzer, E.D., Arnold, L.E., Votolato, N.A., & McConnell, H. (1986). Amino acid supplementation as therapy for attention deficit disorder. *Journal of the American Academy of Child Psychiatry, 25*(4), 509-513.

Ng, K.-H., Meyer, B.J., Reece, L., & Sinn, N. (2009). Dietary PUFA intakes in children with attention-deficit/hyperactivity disorder symptoms. *British Journal of Nutrition, 102*(11), 1635-1641.

Nigg, J.T., & Craver, L. (2014). Commentary: ADHD and social disadvantage: An inconvenient truth? – A reflection on Russell et al. (2014) and Larsson et al. (2014). *Journal of Child Psychology and Psychiatry, 55*(5), 446-447.

Nowicki, S., & Mitchell, J. (1998). Accuracy in identifying affect in child and adult faces and voices and social competence in preschool children. *Genetic Social and General Psychology Monographs, 124*(1), 39-60.

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

Oades, R.D. (2010). The role of serotonin in attention-deficit hyperactivity disorder (ADHD). In C.P. Müller & B.L. Jacobs (Eds.), *Handbook of the behavioral neurobiology of serotonin* (pp. 565-584). London: Elsevier.

Oades, R.D., Dauvermann, M.R., Schimmelmann, B.G., Schwarz, M.J., & Myint, A.M. (2010). Attention-deficit hyperactivity disorder (ADHD) and glial integrity: S100B, cytokines and kynurenine metabolism - effects of medication. *Behavioral and Brain Functions, 6*(29), 1-14.

Paşca, S.P., Nemeş, B., Vlase, L., Gagyi, C.E., Dronca, E., Miu, A.C., & Dronca, M. (2006). High levels of homocysteine and low serum paraoxonase 1 arylesterase activity in children with autism. *Life Sciences, 78*(19), 2244-2248.

Patel, K., & Curtis, L.T. (2007). A comprehensive approach to treating autism and attention-deficit hyperactivity disorder: A prepilot study. *Journal of Alternative and Complementary Medicine, 13*(10), 1091-1098.

Pelsser, L.M.J. (2011). *ADHD, a food-induced hypersensitivity syndrome: In quest of a cause*. Enschede: Ipskamp Drukkers.

Proietti, V., Macchi Cassia, V., & Mondloch, C.J. (2015). The own-age face recognition bias is task dependent. *British Journal of Psychology, 106*(3), 446-467.

Puig-Alcaraz, C., Fuentes-Albero, M., Calderón, J., Garrote, D., & Cauli, O. (2015). Increased homocysteine levels correlate with the communication deficit in children with autism spectrum disorder. *Psychiatry Research, 229*(3), 1031–1037.

Rapport, M.D., Orban, S.A., Kofler, M.J., & Friedman, L.M. (2013). Do programs designed to train working memory, other executive functions, and attention benefit children with ADHD? A meta-analytic review of cognitive, academic, and behavioral outcomes. *Clinical Psychology Review, 33*(8), 1237-1252.

Rashed, M.S., Bucknall, M.P., Little, D., Awad, A., Jacob, M., Alamoudi, M., . . . Ozand, P.T. (1997). Screening blood spots for inborn errors of metabolism by electrospray tandem mass spectrometry with a microplate batch process and a computer algorithm for automated flagging of abnormal profiles. *Clinical Chemistry, 43*(7), 1129-1141.

Refsum, H., Grindflek, A.W., Ueland, P.M., Fredriksen, Å., Meyer, K., Ulvik, A., . . . Kase, B.F. (2004). Screening for serum total homocysteine in newborn children. *Clinical Chemistry, 50*(10), 1769-1784.

Reimherr, F.W., Wender, P., Wood, D., & Ward, M. (1987). An open trial of L-tyrosine in the treatment of attention deficit disorder, residual type. *American Journal of Psychiatry, 144*(8), 1071-1073.

Rodger, H., Vizioli, L., Ouyang, X., & Caldara, R. (2015). Mapping the development of facial expression recognition. *Developmental Science, 18*(6), 926-939.

Scassellati, C., Bonvicini, C., Faraone, S.V., & Gennarelli, M. (2012). Biomarkers and attention-deficit/hyperactivity disorder: A systematic review and meta-analyses. *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(10), 1003-1019.

Schwartz, G., Amor, L.B., Grizenko, N., Lageix, P., Baron, C., Boivin, D.B., & Joober, R. (2004). Actigraphic monitoring during sleep of children with ADHD on methylphenidate and placebo. *Journal of the American Academy of Child & Adolescent Psychiatry, 43*(10), 1276-1282.

Singh, I., & Rose, N. (2009). Biomarkers in psychiatry. *Nature, 460*(7252), 202-207.

Sjöwall, D., Bohlin, G., Rydell, A.-M., & Thorell, L.B. (2017). Neuropsychological deficits in preschool as predictors of ADHD symptoms and academic achievement in late adolescence. *Child Neuropsychology*, *23*(1), 111-128.

Sonuga-Barke, E.J., Brandeis, D., Cortese, S., Daley, D., Ferrin, M., Holtmann, M., . . . Döpfner, M. (2013). Nonpharmacological interventions for ADHD: systematic review and meta-analyses of randomized controlled trials of dietary and psychological treatments. *American Journal of Psychiatry, 170*(3), 275-289.

Tamm, L., Narad, M.E., Antonini, T.N., O'Brien, K.M., Hawk Jr, L.W., & Epstein, J.N. (2012). Reaction time variability in ADHD: A review. *Neurotherapeutics, 9*(3), 500-508.

Teunissen, C.E., van Boxtel, M.P., Jolles, J., de Vente, J., Vreeling, F., Verhey, F., . . . Blom, H.J. (2005). Homocysteine in relation to cognitive performance in pathological and non-pathological conditions. *Clinical Chemical Laboratory Medicine, 43*(10), 1089-1095.

Thissen, A., Rommelse, N., Hoekstra, P., Hartman, C., Heslenfeld, D., Luman, M., . . . Buitelaar, J. (2014). Attention deficit hyperactivity disorder (ADHD) and executive functioning in affected and unaffected adolescents and their parents: Challenging the endophenotype construct. *Psychological Medicine, 44*(4), 881-892.

Thomas, L.A., De Bellis, M.D., Graham, R., & LaBar, K.S. (2007). Development of emotional facial recognition in late childhood and adolescence. *Developmental Science, 10*(5), 547-558.

Toren, P., Eldar, S., Sela, B.-A., Wolmer, L., Weitz, R., Inbar, D., . . . Laor, N. (1996). Zinc deficiency in attention-deficit hyperactivity disorder. *Biological Psychiatry, 40*(12), 1308-1310.

Van Beynum, I.M., Den Heijer, M., Thomas, C.M., Afman, L., Oppenraay-van Emmerzaal, D., & Blom, H.J. (2005). Total homocysteine and its predictors in Dutch children. *American Journal of Clinical Nutrition, 81*(5), 1110-1116.

Van der Meer, J.M., Oerlemans, A.M., Van Steijn, D.J., Lappenschaar, M.G., De Sonneville, L.M., Buitelaar, J.K., & Rommelse, N.N. (2012). Are autism spectrum disorder and attention-deficit/hyperactivity disorder different manifestations of one overarching disorder? Cognitive and symptom evidence from a clinical and population-based sample. *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(11), 1160-1172.

Van Hulst, B., De Zeeuw, P., & Durston, S. (2015). Distinct neuropsychological profiles within ADHD: A latent class analysis of cognitive control, reward sensitivity and timing. *Psychological Medicine, 45*, 735-745.

Van Lieshout, M., Luman, M., Buitelaar, J., Rommelse, N., & Oosterlaan, J. (2013). Does neurocognitive functioning predict future or persistence of ADHD? A systematic review. *Clinical Psychology Review, 33*(4), 539-560.

Venta, R., Prieto, B., & Alvarez, F.V. (2002). Regression-based reference limits for urinary amino acids in a pediatric population. *Clinical Chemistry and Laboratory Medicine, 40*(4), 383-390.

Vitiello, B., Severe, J.B., Greenhill, L.L., Arnold, L.E., Abikoff, H.B., Bukstein, O.G., . . . Hinshaw, S.P. (2001). Methylphenidate dosage for children with ADHD over time under controlled conditions: Lessons from the MTA. *Journal of the American Academy of Child & Adolescent Psychiatry, 40*(2), 188-196.

Willcutt, E.G. (2012). The prevalence of DSM-IV attention-deficit/hyperactivity disorder: A meta-analytic review. *Neurotherapeutics, 9*(3), 490-499.

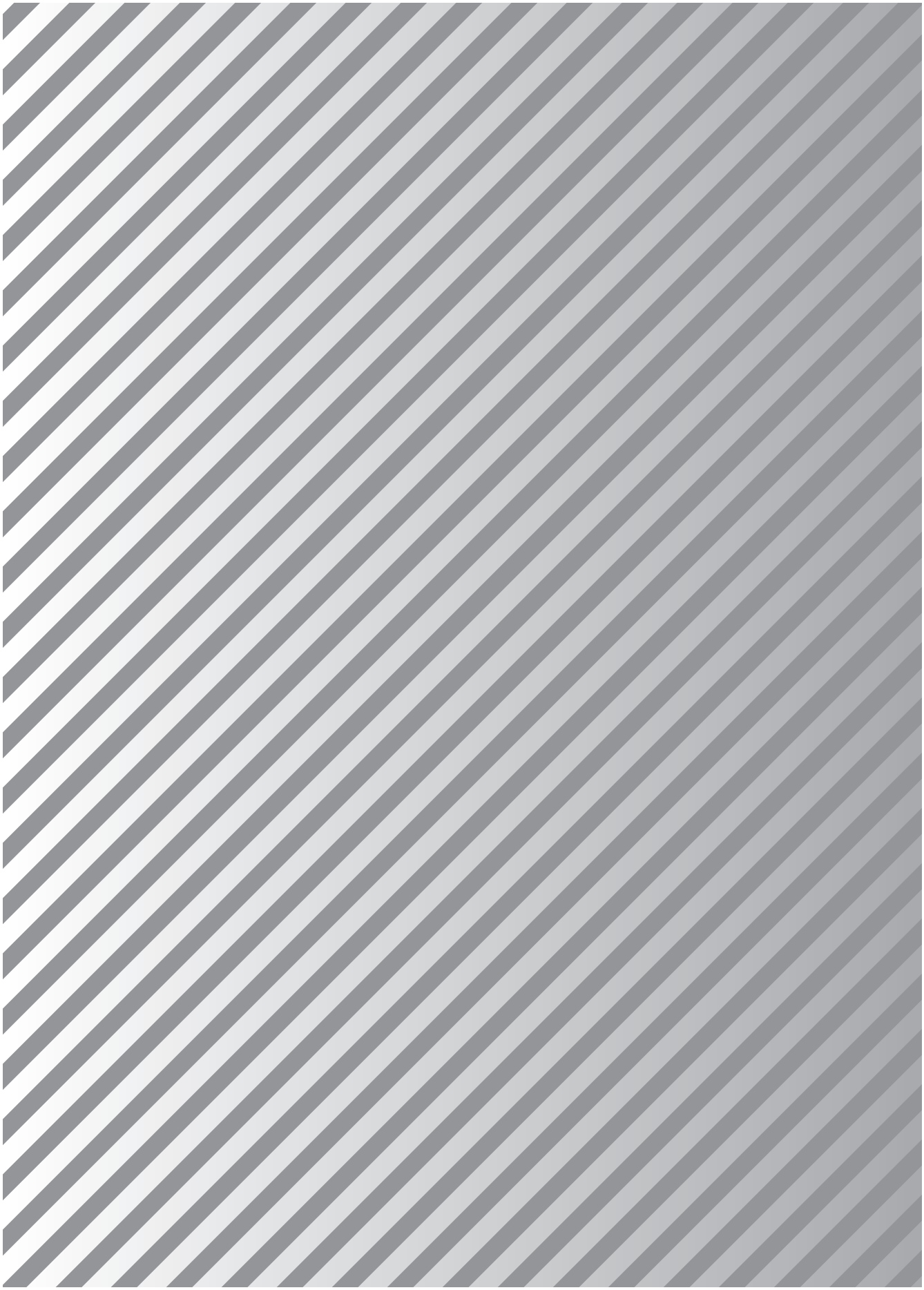
Willcutt, E.G., Doyle, A.E., Nigg, J.T., Faraone, S.V., & Pennington, B.F. (2005). Validity of the executive function theory of attention-deficit/hyperactivity disorder: A meta-analytic review. *Biological Psychiatry, 57*(11), 1336-1346.

Wood, D.R., Reimherr, F.W., & Wender, P.H. (1985). Treatment of attention deficit disorder with DL-phenylalanine. *Psychiatry Research, 16*(1), 21-26.

Yoon, S.Y.R., Jain, U., & Shapiro, C. (2012). Sleep in attention-deficit/hyperactivity disorder in children and adults: Past, present, and future. *Sleep Medicine Reviews, 16*(4), 371-388.

Zametkin, A.J., Karoum, F., Rapoport, J.L., Brown, G.L., & Wyatt, R.J. (1984). Phenylethylamine excretion in attention deficit disorder. *Journal of the American Academy of Child Psychiatry, 23*(3), 310-314.

Zytkovicz, T.H., Fitzgerald, E.F., Marsden, D., Larson, C.A., Shih, V.E., Johnson, D.M., . . . Grady, G.F. (2001). Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: A two-year summary from the New England newborn screening program. *Clinical Chemistry, 47*(11), 1945-1955.



Nederlandse samenvatting
Summary in Dutch

Achtergrond

Attention-deficit/hyperactivity disorder (ADHD), of aandachtsdeficiëntie-/hyperactiviteit-stoornis in het Nederlands, is een van de meest voorkomende psychiatrische stoornissen bij kinderen (Costello, Mustillo, Erkanli, Keeler, & Angold, 2003). ADHD is de benaming van een persistent patroon van onoplettendheid en/of hyperactiviteit-impulsiviteit, dat niet passend is bij de leeftijd. Om aan de diagnose ADHD te voldoen, moeten ten minste zes symptomen van onoplettendheid en/of ten minste zes symptomen van hyperactiviteit-impulsiviteit aanwezig zijn. Aanvullende criteria voor de diagnose zijn dat deze symptomen zich in meerdere situaties voordoen en belemmerend zijn voor het dagelijks functioneren (American Psychiatric Association, 2013). De wereldwijde prevalentie van ADHD onder kinderen wordt geschat op 5,9 procent (Willcutt, 2012). In **hoofdstuk 1** wordt uitgebreider op de prevalentie ingegaan, net zoals op de etiologie en op de beperkingen in het dagelijks functioneren die worden geassocieerd met ADHD.

Etiologie

Er zijn verschillende theorieën omtrent de oorzaken van ADHD ontwikkeld en getoetst in de afgelopen decennia. Een van de overheersende theorieën omtrent de etiologie van ADHD is gebaseerd op een model waarin (interacties tussen) meerdere genetische risicofactoren en omgevingsfactoren de gevoeligheid voor het ontwikkelen van ADHD vergroten (Faraone et al., 2015; Faraone et al., 2005). De meerderheid van de genen die aan ADHD worden gerelateerd, zijn genen die betrokken zijn bij het transport en de binding van de neurotransmitters dopamine en serotonine. Dit kan een verklaring vormen voor afwijkende dopamineconcentraties die zijn gevonden bij mensen met ADHD (Oades, 2008) en voor afwijkende serotonineconcentraties bij sommige mensen met ADHD (Oades, 2010). Genetische risicofactoren kunnen het ontstaan van ADHD echter niet volledig verklaren, wat leidt tot de veronderstelling dat de gevoeligheid voor ADHD afhankelijk is van een complex samenspel van zowel genetische als niet-genetische risicofactoren (Thapar, Cooper, Eyre, & Langley, 2013). Niet-genetische risicofactoren voor ADHD die in de afgelopen decennia zijn onderzocht, zijn onder andere afwijkingen in het metabolisme of voedingspatroon bij kinderen.

Metabole risicofactoren

Er zijn verschillende metabolieten onderzocht die mogelijk inzicht kunnen bieden in oorzaken van ADHD (Scassellati, Bonvicini, Faraone, & Gennarelli, 2012). Hierbij

zijn verschillende metabolieten in urine, serum, plasma en speeksel gevonden die in afwijkende mate aanwezig zijn bij kinderen met ADHD. De effecten van deze biomarkers zijn echter klein en het is onduidelijk in hoeverre deze afwijkende concentraties specifiek voor ADHD zijn (Scassellati et al., 2012). Een van de doelen van dit proefschrift is om nieuwe potentiële biomarkers voor ADHD te onderzoeken. Dit kan inzicht bieden in metabole risicofactoren voor ADHD. In **hoofdstuk 2 en 3** van dit proefschrift wordt onderzocht of de aminozuren tryptofaan, tyrosine, fenylalanine en homocysteïne in afwijkende concentraties aanwezig zijn bij kinderen met ADHD. Om te bepalen wanneer men kan spreken van afwijkende concentraties is in **hoofdstuk 6** een onderzoek naar normaalwaarden van deze aminozuren beschreven.

Risicofactoren op het gebied van voeding

Andere theorieën omtrent de oorzaken van ADHD zijn gericht op afwijkingen in het voedingspatroon van mensen met ADHD (Banerjee, Middleton, & Faraone, 2007). Er is enige evidentie voor tekorten aan bepaalde voedingsstoffen bij mensen met ADHD, met significante bevindingen voor zink (Toren et al., 1996), folaat (Durá-Travé & Gallinas-Victoriano, 2014), ijzer (Konofal, Lecendreux, Arnulf, & Mouren, 2004) en omega-6-vetzuren (Ng, Meyer, Reece, & Sinn, 2009). Hoewel er meer onderzoek op dit gebied nodig is, biedt deze evidentie onderbouwing voor interventies op het gebied van voeding voor ADHD (Hurt, Arnold, & Lofthouse, 2011). In **hoofdstuk 2** van dit proefschrift wordt onderzocht of kinderen met ADHD een verlaagde inname van eiwit hebben, gezien de aminozuren tryptofaan, tyrosine en fenylalanine bestanddelen zijn van eiwit in voeding. Een verlaagde inname van eiwit, in combinatie met afwijkende aminozuurconcentraties bij kinderen met ADHD, zou daarmee kunnen duiden op een risicofactor voor ADHD op het gebied van voeding. In **hoofdstuk 3** wordt onderzocht of kinderen met ADHD een verlaagde inname hebben van vitamine B12 en folaat, omdat deficiënties van vitamine B12 en folaat leiden tot verhoogde concentratie homocysteïne in het bloed. Een verlaagde inname van vitamine B12 en folaat, in combinatie met afwijkende homocysteïneconcentratie bij kinderen met ADHD, zou eveneens kunnen duiden op een risicofactor voor ADHD op het gebied van voeding.

Neurocognitieve risicofactoren

In de afgelopen jaren is getracht om neurocognitieve endofenotypes te ontdekken die mediëren tussen genetische risicofactoren bij ADHD en de symptomen van ADHD op gedragsniveau (Castellanos & Tannock, 2002; Willcutt, Doyle, Nigg, Faraone, &

Pennington, 2005). Genetische risicofactoren hebben een negatieve invloed op de structuur en het functioneren van de hersenen van kinderen met ADHD, wat vervolgens kan zorgen voor beperkingen in het neurocognitief functioneren (Faraone et al., 2015). ADHD wordt geassocieerd met verschillende neurocognitieve deficiënties, zoals beperkingen in het werkgeheugen (Willcutt et al., 2005) en de inhibitiecontrole (Barkley, 1997; Willcutt et al., 2005). Steeds meer onderzoek richt zich op neurocognitieve profielen, waarbij de focus niet ligt op één neurocognitieve factor, maar op meerdere neurocognitieve domeinen. Meerdere onderzoeken hebben aangetoond dat er verschillende neurocognitieve profielen kunnen worden gevonden binnen een ADHD-groep (Fair, Bathula, Nikolas, & Nigg, 2012; Mostert et al., 2015; Van Hulst, De Zeeuw, & Durston, 2015). Dit ondersteunt de gedachte dat ADHD etiologisch een heterogene stoornis is, waarbij meerdere routes op het gebied van neurocognitieve deficiënties de ADHD-symptomen op gedragsniveau kunnen verklaren. In **hoofdstuk 4** wordt getracht meer inzicht te verkrijgen in neurocognitieve profielen bij kinderen met ADHD.

Beperkingen in het dagelijks leven

Behalve met de last van ADHD-symptomen worden kinderen met ADHD vaak geconfronteerd met verschillende andere beperkingen in het dagelijks leven. Voorbeelden van beperkingen die met ADHD worden geassocieerd zijn onder andere gedragsproblemen (Gillberg et al., 2004; Jensen, Martin, & Cantwell, 1997), slaapproblemen (Corkum, Tannock, & Moldofsky, 1998), onderpresteren op school (Loe & Feldman, 2007), sociale problemen (McQuade & Hoza, 2008) en moeite met emotieherkenning (Shaw, Stringaris, Nigg, & Leibenluft, 2014). In **hoofdstuk 4** wordt onderzocht of bepaalde neurocognitieve profielen bij kinderen met ADHD een risico vormden voor comorbide gedragsproblemen, onderpresteren op school en sociale problemen. In **hoofdstuk 5** worden slaapproblemen bij kinderen met ADHD onderzocht. In **hoofdstuk 7** wordt de ontwikkeling van een nieuwe emotieherkenningstaak beschreven, waarin gebruik wordt gemaakt van foto's van kindergezichten die emoties in verschillende intensiteitsniveaus laten zien.

Onderzoeksontwerp

De resultaten die in dit proefschrift worden beschreven, zijn gebaseerd op twee onderzoeken die tussen februari 2013 en juli 2014 zijn uitgevoerd. Onderzoek 1 is een patiënt-controleonderzoek, waarbij 83 kinderen met ADHD in de leeftijd van 6 tot 13 jaar werden vergeleken met 72 kinderen zonder ontwikkelingsstoornis. De resultaten van

dit onderzoek zijn beschreven in **hoofdstuk 2 tot en met 5**. De groep kinderen zonder ontwikkelingsstoornis (*n*=72) werd geselecteerd uit een representatieve steekproef schoolgaande kinderen (*n*=104). Deze steekproef wordt beschreven in **hoofdstuk 6**. In **hoofdstuk 7** zijn de resultaten van een cross-sectioneel onderzoek beschreven, waarvoor een tweede representatieve steekproef schoolgaande kinderen (*n*=75) werd geworven.

Belangrijkste resultaten

Met dit proefschrift wordt getracht nieuwe inzichten in ADHD te bieden, door een breed scala aan onderwerpen te bespreken die relevant zijn rondom ADHD. Hieronder worden de belangrijkste resultaten uit **hoofdstuk 2 tot en met 7** van dit proefschrift samengevat (zie ook Tabel S1 voor een overzicht). Vervolgens worden de bevindingen kritisch geëvalueerd en worden nieuwe richtingen voor toekomstig onderzoek verkend.

De belangrijkste doelstelling van **hoofdstuk 2** is om te onderzoeken of kinderen met ADHD verlaagde concentraties aromatische aminozuren in bloeddruppels hebben. Daarnaast is een belangrijk doel om te onderzoeken of de aromatische aminozuurconcentraties in bloeddruppels verband houden met de symptomen van ADHD. Aromatische aminozuren zijn bestanddelen van eiwitten in voeding die betrokken zijn bij de biosynthese van serotonine en dopamine. Derhalve kunnen afwijkende aromatische aminozuurconcentraties bijdragen aan veranderde dopamineniveaus in ADHD (Oades, 2008) en afwijkende postsynaptische serotonineconcentraties in sommige individuen met ADHD (Oades, 2010). De hypothese voor **hoofdstuk 2** is dat verlaagde concentraties tryptofaan, tyrosine en fenylalanine in het bloed bijdragen aan de expressie van ADHD symptomen. Vanuit bestaande literatuur is er inconsistent bewijs voor de relatie tussen aromatische aminozuren en ADHD. De onderzoeken die eerder werden gedaan naar dit onderwerp zijn veelal verouderd en kennen belangrijke methodologische tekortkomingen (Baker et al., 1991; Bornstein et al., 1990; Comings, 1990; Hoshino, Ohno, & Yamamoto, 1985; Oades, Dauvermann, Schimmelmann, Schwarz, & Myint, 2010). Om meer inzicht te krijgen in de mogelijke rol van aromatische aminozuren bij ADHD wordt hier onderzoek naar gedaan in **hoofdstuk 2**. Verder wordt verkend of afwijkende concentraties tryptofaan, tyrosine en fenylalanine gerelateerd zijn aan verlaagde inname van eiwit of aan afwijkende excretie van aminozuren, wat zichtbaar zou zijn in verhoogde aminozuurconcentraties in urine. Aan het huidige onderzoek hebben 83 kinderen met ADHD (75 procent jongens) en 72 kinderen zonder ontwikkelingsstoornis (51 procent jongens) in de leeftijd van 6 tot 13 jaar deelgenomen.

Aromatische aminozuurconcentraties werden bepaald in bloeddruppels en in 18-uurs urine. De ouders van de kinderen hielden een voedingsdagboek van hun kind bij, om de inname van eiwit te bepalen. Aan de hand van ouder- en leerkrachtvragenlijsten werden ADHD-symptomen gemeten. In tegenstelling tot de hypothese en tot de resultaten uit eerder onderzoek (Baker et al., 1991; Bornstein et al., 1990; Comings, 1990), werden in het huidige onderzoek geen verschillen gevonden in de concentraties tryptofaan, tyrosine en fenylalanine in bloeddruppels tussen kinderen met ADHD en kinderen zonder ontwikkelingsstoornis. Een verlaagde concentratie (<16^{e} percentiel) van aromatische aminozuren in bloeddruppels vormde geen risicofactor voor de diagnose ADHD. In de gecombineerde groep kinderen met ADHD en kinderen zonder ontwikkelingsstoornis werden geen relaties gevonden tussen aromatische aminozuurconcentraties in bloeddruppels en ADHD-symptomen. Er was geen verschil tussen de groep kinderen met ADHD en de groep kinderen zonder ontwikkelingsstoornis op het gebied van eiwitinname of aromatische aminozuurconcentraties in urine. Ook werd er geen verband gevonden tussen aromatische aminozuurconcentraties in bloeddruppels enerzijds en eiwitinname of aromatische aminozuurconcentraties in urine anderzijds.

Het doel van **hoofdstuk 3** is om te onderzoeken of verhoogde homocysteïneconcentraties gerelateerd zijn aan ADHD bij kinderen. Hoge concentraties homocysteïne kunnen nadelig zijn voor neurocognitief functioneren, omdat hoge concentraties kunnen zorgen voor schade aan het DNA, verstoorde methylering, celdood, of een veranderde werking van glutamaatreceptoren (Mattson & Shea, 2003). Een tekort aan folaat of vitamine B12 leidt tot verhoogde concentraties homocysteïne in het bloed, omdat de conversie van homocysteïne naar methionine afhankelijk is van de cofactoren folaat en vitamine B12 (Mattson & Shea, 2003). Homocysteïne is in verband gebracht met neurocognitief functioneren bij patiënten met neurodegeneratieve stoornissen (Teunissen et al., 2005), bij gezonde ouderen (Garcia & Zanibbi, 2004) en bij psychiatrische patiënten (Dias, Brissos, Cardoso, Andreazza, & Kapczinski, 2009; Ford, Flicker, Singh, Hirani, & Almeida, 2013). Het is opvallend dat de neurocognitieve factoren die gerelateerd lijken aan homocysteïneconcentraties (werkgeheugen, interferentiecontrole en aandacht) (Dias et al., 2009; Teunissen et al., 2005) precies de neurocognitieve factoren zijn die bij (sommige) kinderen met ADHD beperkt zijn (Mullane, Corkum, Klein, & McLaughlin, 2009; Tamm et al., 2012; Willcutt et al., 2005). De rol van homocysteïneconcentraties bij neurocognitieve beperkingen werd nog niet onderzocht bij kinderen met ADHD. In **hoofdstuk 3** is de hypothese dat kinderen met ADHD

verhoogde homocysteïneconcentraties hebben in vergelijking met kinderen zonder ontwikkelingsstoornis. Onderzocht wordt of homocysteïneconcentraties bij kinderen met ADHD (a) positief gerelateerd zijn aan ADHD-symptomen, (b) negatief gerelateerd zijn aan neurocognitief functioneren, wat een verdere onderbouwing zou vormen voor de rol van homocysteïne bij ADHD en (c) negatief gerelateerd zijn aan de inname van folaat en vitamine B12, wat zou duiden op een risicofactor voor ADHD op het gebied van voeding. Homocysteïneconcentraties in bloeddruppels werden bepaald bij 55 kinderen met ADHD en 54 kinderen zonder ontwikkelingsstoornis, allen in de leeftijd van 6 tot 13 jaar. Aan de hand van ouder- en leerkrachtvragenlijsten werden ADHD-symptomen gemeten. Neurocognitief functioneren werd bepaald aan de hand van verschillende computertaken. Verbaal werkgeheugen werd gemeten met behulp van de Cijferreekstaak, visuospatieel werkgeheugen met de Rastertaak en interferentiecontrole, variabiliteit in reageren en momenten van aandachtsverlies middels de Flankertaak. Dagelijkse inname van folaat en vitamine B12 middels voeding werd bepaald met behulp van voedingsdagboeken. In tegenstelling tot de hypothese werd in het huidige onderzoek noch een verschil in homocysteïneconcentraties tussen kinderen met ADHD en kinderen zonder ontwikkelingsstoornis gevonden, noch een verband tussen homocysteïneconcentraties en ADHD-symptomen. Verder werd geen bewijs gevonden voor de bijdrage van homocysteïne aan neurocognitieve deficiënties bij kinderen met ADHD. Ook lieten de resultaten geen significante verbanden tussen homocysteïne en de inname van folaat en vitamine B12 zien, of een verlaagde inname van folaat en vitamine B12 bij kinderen met ADHD.

Hoofdstuk 4 heeft als belangrijk doel te onderzoeken welke neurocognitieve profielen kunnen worden onderscheiden bij kinderen met ADHD. Drie eerdere onderzoeken hebben *community detection*-procedures toegepast op groepen kinderen of volwassenen met ADHD en vonden daarbij meerdere aparte neurocognitieve profielen (Fair et al., 2012; Mostert et al., 2015; Van Hulst et al., 2015). De karakteristieken van de neurocognitieve profielen die werden gevonden verschilden echter tussen de drie onderzoeken, omdat bij ieder onderzoek een andere selectie van neurocognitieve maten werd gebruikt. Doordat er verschillende neurocognitieve constructen werden gemeten en er verschillende instrumenten werden gebruikt om hetzelfde neurocognitieve construct te meten, is het niet mogelijk om conclusies te trekken over het aantal en de aard van de neurocognitieve profielen die aan de basis liggen van ADHD. Een ander doel van **hoofdstuk 4** is om de klinische waarde van neurocognitieve profielen te toetsen, door te onderzoeken in

hoeverre neurocognitieve profielen in de ADHD-groep gerelateerd zijn aan problemen die vaak bij kinderen met ADHD worden gevonden, waaronder externaliserend gedrag, sociale problemen en onderpresteren op school. Voor dit onderzoek werden bij 81 kinderen met ADHD en 71 kinderen zonder ontwikkelingsstoornis neurocognitieve data verzameld. Bij deze kinderen werden de Cijferreekstaak, Rastertaak, Flankertaak en Kinderemotietaak afgenomen, om verbaal en visuospatieel geheugen, verbaal en visuospatieel werkgeheugen, interferentiecontrole, verwerkingssnelheid, variabiliteit in reageren, momenten van aandachtsverlies en emotieherkenning te meten. Een factoranalyse werd uitgevoerd, wat resulteerde in een passend model bestaande uit zes latente factoren: geheugen, interferentiecontrole, verwerkingssnelheid, variabiliteit in reageren, momenten van aandachtsverlies en emotieherkenning. Deze factoren werden gebruikt voor *community detection* bij zowel de ADHD-groep als de groep kinderen zonder ontwikkelingsstoornis. De resultaten hiervan lieten vier verschillende neurocognitieve subgroepen in ADHD-groep zien, die alle een uniek neurocognitief profiel hadden. Eén subgroep werd gekenmerkt door zwakke interferentiecontrole, één door trage verwerkingssnelheid, één door zwakke emotieherkenning en één door veel momenten van aandachtsverlies en snelle verwerkingssnelheid. Drie van deze neurocognitieve profielen werden ook gevonden in de groep kinderen zonder ontwikkelingsstoornis. Hierbij lieten de kinderen met ADHD bij alle drie de profielen over het algemeen een zwakkere neurocognitieve prestatie zien. Per profiel hadden de kinderen met ADHD een lagere score op een tot vier neurocognitieve factoren. In de controlegroep werd geen neurocognitieve subgroep met een profiel gekenmerkt door veel momenten van aandachtsverlies en snelle verwerkingssnelheid gevonden. De resultaten toonden geen verschillen tussen de vier neurocognitieve subgroepen in de ADHD-groep op maten van externaliserend, sociaal en schools functioneren.

Het belangrijkste doel van **hoofdstuk 5** is meer inzicht te verkrijgen in slaapproblemen bij kinderen met ADHD, op basis van objectieve metingen van slaapkwaliteit en -kwantiteit. Een meta-analyse van onderzoeken gebaseerd op subjectieve metingen van slaapkwaliteit (vragenlijsten ingevuld door ouders) toont aan dat kinderen met ADHD meer weerstand vertonen bij het naar bed gaan, meer moeite hebben in slaap te vallen, vaker ’s nachts wakker worden, moeite hebben met opstaan en meer ademhalingsproblemen hebben tijdens het slapen in vergelijking met kinderen zonder ontwikkelingsstoornis. Een kanttekening bij deze resultaten is de heterogeniteit in bevindingen, waarbij sommige onderzoeken uit de meta-analyse geen significante bevindingen tonen (Cortese,

Faraone, Konofal, & Lecendreux, 2009). Recent is er een meta-analyse gepubliceerd die gebaseerd is op onderzoeken bij kinderen met ADHD die geen medicatie gebruiken, waarbij actigrafie werd gebruikt om op objectieve wijze slaapproblemen te meten. Deze meta-analyse laat eveneens zien dat kinderen met ADHD meer moeite hebben in slaap te vallen en een verminderde slaapefficiëntie hebben, maar ook hierbij is er sprake van heterogeniteit in bevindingen (De Crescenzo et al., 2016). Het feit dat het bewijs voor slaapproblemen bij kinderen met ADHD inconsistent is, kan mogelijk verklaard worden door verstorende invloeden van comorbide internaliserende en externaliserende problemen en van lage socio-economische status (SES) op de resultaten van eerder onderzoek. Voor het onderzoek in **hoofdstuk 5** werden slaapkwaliteit en -kwantiteit bij 63 medicatievrije kinderen met ADHD en 61 kinderen zonder ontwikkelingsstoornis gemeten met behulp van actigrafie. De resultaten toonden dat medicatievrije kinderen met ADHD niet verschillen van kinderen zonder ontwikkelingsstoornis op het gebied van slaapkwaliteit of -kwantiteit. ADHD-symptomen waren niet gerelateerd aan enige maat van kwaliteit of kwantiteit van slaap in de ADHD-groep. Er werd geen modererende rol van lage SES op de relatie tussen ADHD-symptomen en slaapproblemen gevonden. Moderatieanalyses lieten wel interactie-effecten zien tussen ADHD-symptomen en internaliserend en externaliserend gedrag op tijd in bed, totale slaaptijd en gemiddelde duur van slaapepisodes. Deze effecten zijn niet eenvoudig te interpreteren wanneer wordt gekeken naar de slaappatronen van kinderen met ADHD en (sub)klinische niveaus van comorbide psychiatrische symptomen in vergelijking met de slaappatronen van kinderen met ADHD zonder dergelijke comorbiditeiten. De resultaten laten echter wel zien dat er een complex samenspel is van ADHD-symptomen en comorbide internaliserende en externaliserende problemen, wat mogelijk voor een deel de heterogeniteit in de literatuur op het gebied van slaapkwaliteit en -kwantiteit bij kinderen met ADHD verklaart.

Het doel van **hoofdstuk 6** is het bieden van referentiewaarden voor kinderen voor de concentraties homocysteïne, tryptofaan, tyrosine en fenylalanine in bloeddruppels. In de afgelopen decennia is het gebruik van bloeddruppels om aminozuurconcentraties te meten toegenomen, met name bij de screening van pasgeboren baby's (Fingerhut & Olgemöller, 2009; Zytkovicz et al., 2001). Het verzamelen van bloeddruppels middels een vingerprik is minder invasief voor kinderen dan bloedafname middels venapunctie. Het meten van aminozuren in bloeddruppels is voldoende robuust en stabiel voor diagnostische doeleinden (Chace, Sherwin, Hillman, Lorey, & Cunningham, 1998; Kand'ár & Žáková, 2009; Rashed et al., 1997). Een ander voordeel van de bloeddruppeltechniek is

dat de vingerprik ook in niet-klinische omgevingen (zoals thuis en op school) kan worden afgenomen, aangezien de bloeddruppel bij kamertemperatuur bewaard kan worden en via de reguliere post kan worden verzonden. Analyse van aminozuurconcentraties in het bloed van kinderen wordt regelmatig ingezet in de klinische praktijk voor diagnostische doeleinden en om beslissingen rondom behandeling te nemen (Lepage et al., 2006). Adequate referentiewaarden zijn vereist om te bepalen wanneer aminozuurconcentraties afwijkend zijn en zijn daarmee essentieel voor besluitvorming in de klinische praktijk. Er zijn publicaties van referentiewaarden voor aminozuurconcentraties in bloeddruppels bij baby's beschikbaar (Rashed et al., 1997; Zytkovicz et al., 2001). Er zijn echter geen publicaties van referentiewaarden voor aminozuurconcentraties in bloeddruppels bij schoolgaande kinderen bekend. In het onderzoek van **hoofdstuk 6** zijn daarom bloeddruppels verzameld bij een steekproef van 104 kinderen in de leeftijd van 6 tot 12 jaar (52 procent jongens). Concentraties homocysteïne, tryptofaan, tyrosine en fenylalanine in bloeddruppels werden bepaald op basis van *positive electrospray liquid chromatography–tandem mass spectrometry*. Onderzocht werd of er invloeden waren van leeftijd en geslacht op de aminozuurconcentraties, om rekening te houden met effecten van leeftijd (Held, White, & Pasquali, 2011; Lepage, McDonald, Dallaire, & Lambert, 1997; Van Beynum et al., 2005; Venta, Prieto, & Alvarez, 2002) en geslacht (Jung & Adeli, 2009) op het aminozuurmetabolisme bij kinderen. De resultaten toonden geen effecten van leeftijd of geslacht op aminozuurconcentraties. Hierdoor was het mogelijk om referentiewaarden voor concentraties homocysteïne, tryptofaan, tyrosine en fenylalanine in bloeddruppels te bepalen voor de hele groep kinderen in de leeftijd van 6 tot 12 jaar. Referentiewaarden werden vastgesteld gebaseerd op het 5^{e} en het 95^{e} percentiel, wat gebruikelijk is in een steekproef van de populatie (Refsum et al., 2004). Daarnaast werden referentiewaarden gebaseerd op het 10^{e} en het 90^{e} percentiel getoond, wat stabielere maten zijn in een normatieve steekproef met een bescheiden omvang (Lepage et al., 1997).

Tot slot is het doel van **hoofdstuk 7** om ontwikkelingseffecten bij basisschoolkinderen in gezichtsemotieherkenning te onderzoeken. De resultaten van eerder onderzoek op dit gebied zijn inconsistent voor wat betreft de mate waarin er ontwikkeling optreedt gedurende de jeugd (Herba & Phillips, 2004). Een mogelijke verklaring voor verschillen tussen onderzoeken naar de ontwikkeling van gezichtsemotieherkenning is dat deze ontwikkeling verschilt per emotie (Mancini, Agnoli, Baldaro, Ricci Bitti, & Surcinelli, 2013; Rodger, Vizioli, Ouyang, & Caldara, 2015). Verder kunnen geslacht en IQ een rol spelen bij

de ontwikkeling van emotieherkenning, gezien de evidentie voor een kleine voorsprong van meisjes in gezichtsemotieherkenning ten opzichte van jongens (McClure, 2000) en voor een effect van IQ op emotieherkenning (Buitelaar, Van der Wees, Swaab-Barneveld, & Van der Gaag, 1999). De inconsistente resultaten tussen onderzoeken kunnen verder mogelijk verklaard worden door het gebruik van verschillende onderzoeksmethoden. Terwijl sommige paradigmata statische foto's met een hoge uitdrukkingsintensiteit gebruiken (e.g., Lawrence, Campbell, & Skuse, 2015; Mancini et al., 2013), worden in andere paradigmata foto's variërend van een zeer lage uitdrukkingsintensiteit tot een zeer hoge uitdrukkingsintensiteit gebruikt (e.g., Herba, Landau, Russell, Ecker, & Phillips, 2006; Thomas, De Bellis, Graham, & LaBar, 2007). Inconsistenties in de literatuur over de ontwikkeling van gezichtsemotieherkenning zijn mogelijk ook ontstaan door verschillen in het gebruik van foto's van ofwel kinderen ofwel volwassenen. Bij de meeste onderzoeken naar emotieherkenning bij kinderen werden foto's van volwassenen gebruikt, gebaseerd op de gevalideerde fotoset Ekman-Friesen Pictures of Facial Affect (e.g., Herba et al., 2006; Lawrence et al., 2015). Kinderen zijn mogelijk beter in het herkennen van emoties in kindergezichten dan in het herkennen van emoties in gezichten van volwassenen, vanwege de zogenaamde eigen-leeftijd-*bias* (Hills & Lewis, 2011; Proietti, Macchi Cassia, & Mondloch, 2015). Bij kinderen is met name de vaardigheid in het herkennen van emoties in het gezicht van leeftijdsgenoten belangrijk voor de sociale interactie met andere kinderen (Nowicki & Mitchell, 1998). Het doel van **hoofdstuk 7** is het onderzoeken van effecten van uitdrukkingsintensiteit, emotieconditie, leeftijd, geslacht en IQ op gezichtsemotieherkenning bij basisschoolkinderen. Voor dit onderzoek is de Morphed Facial Emotion Recognition Task (MFERT) ontwikkeld, die bestaat uit foto's van kindergezichten die vier verschillende basisemoties tonen (boosheid, angst, blijheid en verdriet). Foto's met een zeer hoge uitdrukkingsintensiteit (100 procent) werden gemengd met een foto met een neutrale uitdrukking (0 procent). Door het mengen van foto's ontstonden in totaal 240 stimuli die varieerden in emotionele intensiteit (10 tot 100 procent). De MFERT werd afgenomen in een steekproef van 75 basisschoolkinderen in de leeftijd van 6 tot 12 jaar (45 procent jongens). In alle emotionele condities werd een positief lineair effect gevonden van uitdrukkingsintensiteit op accuratesse. Accuratesse was hoger voor blije dan voor boze en bange uitdrukkingen. Herkenning van verdrietige uitdrukkingen was het zwakst. De resultaten toonden dat leeftijd positief gerelateerd was aan emotieherkenning op sommige (middel)hoge intensiteitsniveaus. Meisjes hadden een hogere accuratesse dan jongens bij gemiddelde intensiteitsniveaus, maar niet bij lage en hoge intensiteitsniveaus. IQ bleek niet gerelateerd aan gezichtsemotieherkenning.

Tabel S1. Samenvatting van de belangrijkste resultaten in dit proefschrift

Hoofdstuk	Deelnemers	Maten	Belangrijkste resultaten
2	83 kinderen met ADHD 72 kinderen zonder ontwikkelingsstoornis (Onderzoek 1)	• Concentraties tryptofaan, tyrosine en fenylalanine in bloeddruppels • ADHD-symptomen, beoordeeld door ouder en leerkracht (SWAN) • Concentraties tryptofaan, tyrosine en fenylalanine in urine (18-uurs urine) • Dagelijkse inname van eiwit middels voeding	• Geen groepsverschillen in concentraties tryptofaan, tyrosine en fenylalanine in bloeddruppels • Aminozuurconcentraties in bloeddruppels niet gerelateerd aan ADHD-symptomen • Geen groepsverschillen in aminozuurconcentraties in urine en inname van eiwit • Aminozuurconcentraties in bloeddruppels niet gerelateerd aan aminozuurconcentraties in urine en inname van eiwit
3	55 kinderen met ADHD 54 kinderen zonder ontwikkelingsstoornis (Onderzoek 1)	• Concentratie homocysteïne in bloeddruppels • ADHD-symptomen, beoordeeld door ouder en leerkracht (SWAN) • Verbaal werkgeheugen (Cijferreekstaak) • Visuospatieel werkgeheugen (Rastertaak) • Interferentiecontrole, variabiliteit in reageren en momenten van aandachtsverlies (Flankertaak) • Dagelijkse inname van folaat en vitamine B12 middels voeding	• Geen groepsverschillen in concentratie homocysteïne in bloeddruppels • Homocysteïneconcentratie niet gerelateerd aan ADHD-symptomen of aan neurocognitief functioneren • Geen groepsverschillen in inname van folaat en vitamine B12 • Inname van folaat en vitamine B12 niet gerelateerd aan homocysteïneconcentratie
4	81 kinderen met ADHD 71 kinderen zonder ontwikkelingsstoornis (Onderzoek 1)	• Verbaal geheugen en verbaal werkgeheugen (Cijferreekstaak) • Visuospatieel geheugen en visuospatieel werkgeheugen (Rastertaak) • Interferentiecontrole, verwerkingssnelheid, variabiliteit in reageren en momenten van aandachtsverlies (Flankertaak) • Emotieherkenning in kindergezichten (CERT) • Externaliserend gedrag, beoordeeld door ouder en leerkracht (VvGK) • Sociale acceptatie en afwijzing (sociometrische data) • Sociale problemen, beoordeeld door ouder en leerkracht (CBCL/TRF) • Begrijpend lezen, spelling en rekenen (CITO)	• Vier verschillende neurocognitieve subgroepen in ADHD-groep; één gekenmerkt door zwakke interferentiecontrole, één door trage verwerkingssnelheid, één door zwakke emotieherkenning en één door veel momenten van aandachtsverlies en snelle verwerkingssnelheid • Drie verschillende neurocognitieve subgroepen in controlegroep, sterk lijkend op de profielen van de eerste drie subgroepen in de ADHD-groep • In de controlegroep geen subgroep gekenmerkt door veel momenten van aandachtsverlies en snelle verwerkingssnelheid • Zwakker neurocognitief functioneren in de ADHD-groep dan in de controlegroep op één tot vier factorscores per profiel • Geen verschillen tussen de vier neurocognitieve subgroepen in de ADHD-groep op maten van externaliserend, sociaal en schools functioneren

Tabel S1. *Vervolg*

Hoofdstuk	Deelnemers	Maten	Belangrijkste resultaten
5	63 kinderen met ADHD 61 kinderen zonder ontwikkelings-stoornis (Onderzoek 1)	• Tijd in bed, totale slaaptijd, nachtelijke motorische activiteit, duur van in slaap vallen, duur van opstaan in de ochtend, gemiddelde duur van waakepisodes en gemiddelde duur van slaapepisodes (actigrafie) • Internaliserend gedrag, beoordeeld door ouder en leerkracht (CBCL/TRF) • Externaliserend gedrag, beoordeeld door ouder en leerkracht (VvGK)	• Geen groepsverschillen in de actigrafische maten • ADHD-symptomen niet gerelateerd aan kwaliteit of kwantiteit van slaap • Interactie-effecten tussen ADHD-symptomen enerzijds en internaliserend en externaliserend gedrag anderzijds op tijd in bed, totale slaaptijd en gemiddelde duur van slaapepisodes. Deze effecten zijn niet eenvoudig te interpreteren
6	104 kinderen uit een steekproef van schoolgaande kinderen (Onderzoek 1)	• Concentraties homocysteïne, tryptofaan, tyrosine en fenylalanine in bloeddruppels	• Leeftijd niet gerelateerd aan concentraties homocysteïne, tryptofaan, tyrosine en fenylalanine in bloeddruppels • Geen verschil tussen jongens en meisjes in concentratie van aminozuren • Referentiewaarden bepaald, gebaseerd op het 5^{e}, 10^{e}, 90^{e} en 95^{e} percentiel
7	75 kinderen uit een steekproef van schoolgaande kinderen (Onderzoek 2)	• Herkenning van boze, blije, bange en verdrietige uitdrukkingen in kindergezichten, in 10 intensiteitsniveaus (MFERT) • IQ (WISC-III)	• In alle emotionele condities een positief lineair effect van uitdrukkingsintensiteit op accuratesse • Accuratesse hoger voor blije dan voor boze en bange uitdrukkingen. Herkenning van verdrietige uitdrukkingen is het zwakst • Leeftijd positief gerelateerd aan emotieherkenning op sommige (middel)hoge intensiteitsniveaus • Meisjes hebben hogere accuratesse dan jongens bij gemiddelde intensiteit, maar niet bij lage en hoge intensiteitsniveaus • IQ niet gerelateerd aan emotieherkenning

Afkortingen. ADHD, attention-deficit/hyperactivity disorder; CBCL, Child Behavior Checklist; CERT, Children's Emotion Recognition Task; CITO, Centraal Instituut voor Toetsontwikkeling; MFERT, Morphed Facial Emotion Recognition Task; SWAN, Strengths and Weaknesses of ADHD-symptoms and Normal Behaviour rating scale; TRF, Teacher Rating Form; VvGK, Vragenlijst voor Gedragsproblemen bij Kinderen, WISC-III, Wechsler Intelligence Scale for Children.

Algemene discussie

Dit proefschrift heeft een aantal belangrijke inzichten in ADHD bij kinderen opgeleverd. Het voornaamste inzicht is dat de resultaten die in **hoofdstuk 2 en 3** beschreven worden erop wijzen dat afwijkende concentraties tryptofaan, tyrosine, fenylalanine en homocysteïne niet betrokken zijn bij de etiologie van ADHD. Dit onderzoek kan daardoor geen ondersteuning leveren voor de gedachte dat deze aminozuren biomarkers zijn voor ADHD of voor mogelijk nieuwe etiologische paden naar ADHD. Zoals in **hoofdstuk 1** nader is uiteengezet, is ADHD een klinisch heterogene stoornis. Het is daarom onwaarschijnlijk dat er een enkele etiologische verklaring zou worden gevonden voor de gehele ADHD-populatie. Het is echter niet uitgesloten dat afwijkingen in aminozuurconcentraties bij een deel van de kinderen met ADHD een rol spelen, in sommige meer homogene subgroepen van de ADHD-populatie. Het zou bijvoorbeeld kunnen dat alleen bij kinderen met ADHD die ernstige problemen hebben met executief functioneren afwijkende concentraties tyrosine en fenylalanine aanwezig zijn. Afwijkende concentraties van deze aminozuren hebben een negatieve invloed op de beschikbaarheid van dopamine. Een afwijkend functioneren van dopamine in de prefrontale cortex en het striatum lijkt het executief functioneren, waaronder volgehouden aandacht en interferentiecontrole, te beperken bij ADHD (Del Campo, Chamberlain, Sahakian, & Robbins, 2011; Oades, 2008). Deze hypothese wordt verder ondersteund door een meta-analyse die een verband laat zien tussen afwijkende fenylalanineconcentraties (die zorgen voor een tyrosinedeficiëntie) en beperkingen in het executief functioneren (Albrecht, Garbade, & Burgard, 2009). Dezelfde wijze van redenering volgend, kan men veronderstellen dat alleen kinderen met ADHD en een comorbide autismespectrumstoornis (ASS) verhoogde homocysteïneconcentraties hebben, vanwege het bewijs voor een verhoogd risico op zeer hoge homocysteïneconcentraties bij kinderen met ASS (Paşca et al., 2006; Puig-Alcaraz, Fuentes-Albero, Calderón, Garrote, & Cauli, 2015). Dit impliceert dat afwijkende aminozuurconcentraties niet aan ADHD zelf gerelateerd zijn, maar aan problemen die vaak tegelijkertijd met ADHD voorkomen. Deze resultaten benadrukken de complexiteit van het zoeken naar separate etiologische risicofactoren voor een stoornis die gekenmerkt wordt door een grote heterogeniteit. Onderzoek naar etiologische risicofactoren voor ADHD vereist daarom grote steekproeven, zodat er binnen de groep kinderen met ADHD analyses naar meer homogene subgroepen mogelijk zijn.

Er is een groeiende interesse naar biomarkers in de kinderpsychiatrie, met als doelen dat (a) de diagnostiek van psychiatrische stoornissen gebaseerd kan worden op biologische factoren en (b) er meer inzicht ontstaat in biologische factoren die invloed hebben op behandeluitkomsten. Een van de redenen waarom dergelijk onderzoek populair is, is de kritiek op huidige diagnostische procedures voor ADHD. Op dit moment wordt de diagnostiek van ADHD gebaseerd op voornamelijk subjectieve instrumenten (zoals vragenlijsten, interviews en observaties) die onvoldoende betrouwbaar en valide zijn (Faraone, Bonvicini, & Scassellati, 2014). Biomarkers worden gezien als meer objectieve instrumenten. Verder zouden biomarkers kunnen bijdragen aan preventie van ernstige gedragsproblemen, wanneer op basis van deze biomarkers kinderen met een verhoogd risico op het ontwikkelen van psychiatrische stoornissen vroegtijdig worden geïdentificeerd (Singh & Rose, 2009). Het analyseren van aminozuurconcentraties in bloeddruppels van kinderen zou een relatief goedkope en weinig invasieve aanvulling zijn op de huidige diagnostische procedures, in tegenstelling tot het meten van biomarkers middels beeldvormende technieken (zoals MRI) en elektro-encefalografie (EEG). Tot op heden zijn er echter geen biomarkers voor ADHD gevonden die bruikbaar zijn voor de klinische praktijk, vanwege de beperkte sensitiviteit en specificiteit (Scassellati et al., 2012). Deze resultaten laten hetzelfde zien voor tryptofaan, tyrosine, fenylalanine en homocysteïne, wat suggereert dat deze aminozuren geen veelbelovende kandidaten voor nieuwe biomarkers voor ADHD zijn.

De bevinding dat de concentraties tryptofaan, tyrosine, fenylalanine en homocysteïne in bloeddruppels niet afwijkend zijn bij kinderen met ADHD, pleit tegen bepaalde voedingsinterventies voor ADHD. In eerder onderzoek werden de effecten van aromatische aminozuursupplementen op volwassenen met ADHD onderzocht, met wisselende resultaten (Nemzer, Arnold, Votolato, & McConnell, 1986; Reimherr, Wender, Wood, & Ward, 1987; Wood, Reimherr, & Wender, 1985; Zametkin, Karoum, Rapoport, Brown, & Wyatt, 1984). Nu er geen sprake lijkt van aromatische aminozuurdeficiënties bij ADHD, verklaart dit mogelijk waarom de resultaten van aminozuursupplementen op ADHD-symptomen wisselend zijn (Hurt et al., 2011). Evenzo pleiten deze resultaten niet voor een behandeling van kinderen met ADHD op basis van folaat (Ghanizadeh, Sayyari, & Mohammadi, 2013) of vitamine B12 (Ghanizadeh et al., 2013; Patel & Curtis, 2007), aangezien ADHD bij kinderen niet gerelateerd lijkt aan verhoogde homocysteïneconcentraties of aan verlaagde inname van folaat en vitamine B12. Deze implicatie is in lijn met de groeiende overtuiging dat interventies voor ADHD met

voedingssupplementen mogelijk slechts effectief zijn voor individuen met tekorten aan voedingsstoffen (Hurt et al., 2011).

Een ander inzicht dat dit proefschrift biedt is dat problemen die vaak tegelijkertijd met ADHD voorkomen mogelijk niet aan ADHD zelf gerelateerd zijn, gebaseerd op de bevinding dat er geen slaapproblemen zijn bij medicatievrije kinderen met ADHD (**hoofdstuk 5**). Problemen die bij kinderen met ADHD voorkomen zijn mogelijk epifenomenen, ofwel nevenverschijnselen, die veroorzaakt worden door andere factoren die kenmerkend zijn voor ADHD, waaronder comorbide psychiatrische stoornissen en het gebruik van stimulantia. In dit proefschrift wordt de hypothese gesteld dat stimulantia een mediërende rol spelen bij slaapproblemen bij kinderen met ADHD, omdat eerder onderzoek liet zien dat methylfenidaat een (direct) negatief effect heeft op slaapkwaliteit (Corkum, Panton, Ironside, MacPherson, & Williams, 2008; Schwartz et al., 2004). Voor deelname aan het huidige onderzoek was het daarom nodig dat kinderen met ADHD medicatievrij waren gedurende de meting van slaap. Deze aanpak heeft er mogelijk aan bijgedragen dat er in het huidige onderzoek geen slaapproblemen werden gevonden bij kinderen met ADHD. De resultaten impliceren dat het gebruik van methylfenidaat tijdens metingen van slaap in eerder onderzoek een verstorende werking kan hebben gehad, waardoor mogelijk ten onrechte werd geconcludeerd dat slaapproblemen aan ADHD gerelateerd zijn (Yoon, Jain, & Shapiro, 2012). Verder werden in het huidige onderzoek interacties gevonden tussen ADHD-symptomen enerzijds en internaliserende en externaliserende problemen anderzijds in relatie tot slaapproblemen. Dit impliceert dat comorbide psychiatrische stoornissen mogelijk de relatie tussen ADHD en slaapproblemen beïnvloeden. Vermoedelijk geldt hetzelfde voor de relatie tussen ADHD enerzijds en sociale problemen en onderpresteren op school anderzijds. Mogelijk zijn er factoren die kenmerkend zijn voor ADHD (waaronder comorbide psychiatrische stoornissen) die het risico op sociale problemen en onderpresteren op school bij kinderen met ADHD verhogen. Het zou kunnen dat sociale problemen bij ADHD worden gemedieerd of gemodereerd door comorbide ASS (Van der Meer et al., 2012) of door comorbide externaliserend gedrag (Becker, Luebbe, & Langberg, 2012). Onderpresteren op school wordt mogelijk gemedieerd of gemodereerd door een lage SES (Sjöwall, Bohlin, Rydell, & Thorell, 2015). De resultaten uit **hoofdstuk 4** laten zien dat neurocognitieve profielen geen mediërende of modererende rol lijken te spelen in de relaties tussen ADHD en sociale problemen en tussen ADHD en onderpresteren op school. Dit benadrukt dat er meer onderzoek nodig is naar andere factoren dan neurocognitieve deficiënties die de relatie tussen ADHD

en sociaal en schools functioneren verklaren. Toekomstig onderzoek kan bijvoorbeeld gericht worden op omgevingsfactoren, zoals familie- en ouderfactoren (Becker et al., 2012), waardoor mogelijk ontdekt wordt welke factoren het risico op sociale problemen en onderpresteren op school bij kinderen met ADHD verhogen. Op deze manier kan er tijdig gesignaleerd worden welke kinderen met ADHD een verhoogd risico lopen op (meerdere) andere problemen die belemmerend zijn in het dagelijks leven.

De resultaten uit dit proefschrift benadrukken verder opnieuw de heterogeniteit van ADHD. De resultaten uit **hoofdstuk 4** laten bijvoorbeeld zien dat er meerdere neurcognitieve subgroepen in de ADHD-populatie te onderscheiden zijn, waarbij elke subgroep een ander profiel van neurocognitieve sterktes en zwaktes laat zien. Verder werd in **hoofdstuk 4 en 5** grote variantie binnen de ADHD-groep gevonden op uiteenlopende domeinen, waaronder op het gebied van ADHD-symptomen, externaliserend gedrag, internaliserend gedrag, ASS-symptomen, slaapproblemen, schools functioneren en sociaal functioneren. Deze variantie benadrukt de grote individuele verschillen tussen kinderen met ADHD. Waar sommige kinderen uit de ADHD-groep slechts op een domein problemen ondervonden, hadden andere kinderen met ADHD te maken met een scala aan problemen in verschillende domeinen. De grote heterogeniteit binnen ADHD nodigt uit om bij het behandelen van kinderen met ADHD voor maatwerk te kiezen. Klinische richtlijnen stellen echter een standaard benadering voor de behandeling van kinderen met ADHD voor (e.g., American Academy of Pediatrics, 2011). Behandeling met stimulantia is bijvoorbeeld de behandeling van eerste keuze bij kinderen met ernstige ADHD-symptomen. Er is echter een grote groep kinderen met ADHD bij wie behandeling met methylfenidaat niet effectief is (10 procent), of bij wie dergelijke medicamenteuze behandeling niet effectiever dan placebo is (een aanvullende groep van 13 procent) (Greenhill et al., 2001; Vitiello et al., 2001). Bovendien is er bewijs voor idiosyncratische dosis-responscurves voor methylfenidaat (Greenhill et al., 2001), waarbij voor elk individu de dosis-responscurve andere vormen aan kan nemen; een hogere dosis leidt niet bij alle kinderen tot een grotere reductie in symptomen (Konrad, Günther, Heinzel-Gutenbrunner, & Herpertz-Dahlmann, 2005). De effecten van methylfenidaat kunnen ook verschillen tussen uitkomstdomeinen. Een bepaalde dosering is dan bijvoorbeeld het effectiefst voor het beperken van impulsiviteit, terwijl een andere dosering het effectiefst is voor het beperken van concentratieproblemen (Konrad et al., 2005). Kortom, medicamenteuze behandeling van kinderen met ADHD zou daarom op het individu moeten worden afgestemd en de effectiviteit zou per kind

moeten worden gemeten. Het is bijvoorbeeld aan te raden om gebruik te maken van dubbelblinde placebogecontroleerde titratie wanneer behandeling met stimulantia wordt voorgeschreven voor kinderen met ADHD (MTA Cooperative Group, 1999). Deze vorm van titratie maakt het mogelijk om te ontdekken bij welke kinderen methylfenidaat niet werkt of niet beter werkt dan placebo. Het maakt het tevens mogelijk om op objectieve wijze de optimale dosering per kind te bepalen.

Voor andere behandelmethodes voor ADHD is maatwerk eveneens geboden, waarbij het raadzaam is om verder te onderzoeken welke kinderen met ADHD het meest gebaat zijn bij een bepaalde interventie. Onderzoek heeft bijvoorbeeld aangetoond dat comorbide angstproblemen bij kinderen met ADHD de behandeluitkomst beïnvloeden, waarbij kinderen met angststoornissen relatief beter reageerden op gedragstherapie dan kinderen zonder angststoornis (MTA Cooperative Group, 1999). Behandelaren zouden daarom een andere benadering kunnen kiezen voor kinderen met ADHD en angstproblematiek dan voor kinderen met ADHD zonder angststoornis (Hinshaw, 2007). Maatwerk op het gebied van behandeling van kinderen met ADHD kan de behandeluitkomsten verbeteren. Op dit moment is er echter te weinig wetenschappelijk bewijs aanwezig om klinische richtlijnen voor de behandeling van ADHD te specificeren op basis van individuele kenmerken van kinderen. Er is daarom meer onderzoek nodig om te bepalen welke factoren een mediërend of modererend effect hebben op behandeluitkomsten bij kinderen met ADHD, wat nodig is om meer zicht te krijgen op wanneer maatwerk wenselijk is.

De heterogeniteit in ADHD roept er daarnaast toe op om de diagnostische procedures bij kinderen uit te breiden. Het is raadzaam om tijdens de diagnostiek een brede screening van bijkomende problemen te verrichten wanneer er een vermoeden is van ADHD bij kinderen. Het gebruik van screeningsinstrumenten waarmee meerdere psychiatrische stoornissen en beperkingen in het dagelijks leven worden uitgevraagd, kan ertoe leiden dat eerder wordt gesignaleerd of een kind met ADHD een verhoogd risico heeft op het ontwikkelen van ernstige problemen.

Onderzoeksagenda

De resultaten van dit proefschrift zijn voornamelijk nulbevindingen en sluiten daarmee sommige onderzoeksrichtingen op het gebied van de etiologie van ADHD en de behandeling van ADHD-symptomen uit. Tegelijkertijd roepen de resultaten nieuwe onderzoeksvragen op en wijzen ze op het belang van verder onderzoek.

In **hoofdstuk 2 en 3** wordt getoetst of aminozuurafwijkingen gerelateerd zijn aan ADHD bij kinderen. Een van de mogelijke oorzaken van afwijkende aminozuurconcentraties in het bloed kan een verlaagde inname van eiwit, folaat of vitamine B12 zijn. Het feit dat de ADHD-groep geen afwijkende aminozuurconcentraties heeft, kan daarom deels verklaard worden doordat er geen voedingstekorten zijn in de ADHD-groep. Dit roept de vraag op of afwijkende aminozuurconcentraties wel zouden voorkomen bij ondervoede kinderen met ADHD. Verlaagde inname van eiwit, folaat en vitamine B12 is aannemelijker bij kinderen met een lage SES. Het is daarom aan te raden om verder onderzoek te doen naar het effect van voedingstekorten op ADHD voor kinderen met een lage SES (Liu & Raine, 2006). Aangezien de huidige steekproef slechts een kleine subgroep kinderen met een lage SES bevatte (*n*=14), was het statistisch gezien niet verantwoord om de effecten van een lage SES op voeding en op aminozuurconcentraties te onderzoeken. Vermoedelijk zijn in een steekproef van kinderen met ADHD en een lage SES de effecten van voedingstekorten op ADHD groter dan de effecten die reeds gevonden zijn voor sommige voedingsstoffen zoals zink (Toren et al., 1996), folaat (Durá-Travé & Gallinas-Victoriano, 2014), ijzer (Konofal et al., 2004) en omega-6-vetzuren (Ng et al., 2009). Wanneer er specifieke tekorten aan voedingsstoffen worden gevonden bij kinderen met ADHD en een lage SES, biedt dit aanleiding om de effecten van voedingsinterventies bij deze kinderen te onderzoeken. Mogelijk is behandeling met voedingssupplementen op basis van multivitaminen, mineralen of vetzuren effectiever voor kinderen met ADHD die tekorten aan voedingsstoffen hebben (Hurt et al., 2011).

Een andere richting voor nieuw onderzoek is de focus op effecten van prenatale voedingstekorten, omdat voedingstekorten naar verwachting een sterker nadelig effect hebben in een vroeg stadium van de prenatale ontwikkeling. In een recent onderzoek werden bijvoorbeeld de negatieve effecten getoond van lage prenatale folaatconcentraties op hersenvolume, taal, leren/geheugen en visuospatiële verwerking bij kinderen zes tot acht jaar later (Ars et al., 2016). Een dergelijke onderzoekslijn kan

ook meer inzicht bieden in de relatie tussen lage SES en ADHD. Veel onderzoeken hebben aangetoond dat lage SES een risicofactor is voor ADHD (Willcutt, 2012). Het is echter onduidelijk welke factoren die gerelateerd zijn aan een lage positie op de maatschappelijke ladder bijdragen aan de ontwikkeling van ADHD. Er wordt gesuggereerd dat een lage SES samenhangt met beperkte prenatale voeding en een verhoogde pre- en postnatale blootstelling aan giftige stoffen, wat een negatief effect kan hebben op de hersenontwikkeling van kinderen (Nigg & Craver, 2014). Het is daarom aan te raden om een longitudinaal onderzoek te verrichten naar de effecten van voedingstekorten bij zwangere vrouwen, in samenhang met lage SES, op het latere gedragsmatige functioneren van kinderen.

Voor toekomstig onderzoek naar voedingsinterventies voor ADHD is het raadzaam om uit te gaan van een degelijke onderbouwing voor de desbetreffende interventie, waarbij een gedegen theoretisch kader wordt gehanteerd ten aanzien van de werkingsmechanismen die ten grondslag liggen aan de veronderstelde effecten van de interventie. Een dergelijke theorie ten aanzien van het werkingsmechanisme is cruciaal om te begrijpen waarom een interventie een positieve werking heeft en voor wie de behandeling het meest passend is. Bij voedingsinterventies voor ADHD kunnen zulke theorieën worden gebaseerd op voedingstekorten bij kinderen met ADHD, wanneer de interventie bestaat uit voedingssupplementen. Theorieën kunnen ook gebaseerd zijn op allergische reacties van kinderen met ADHD op bepaalde voedingstoffen die voorkomen in natuurlijke producten (zoals eieren of noten) of op kunstmatige voedingsstoffen (waaronder voedingskleurstoffen), wanneer de interventie bestaat uit een dieet. Het oorspronkelijke plan voor dit proefschrift was om de focus te leggen op de behandeleffecten van nicotinamide (deel van vitamine B3) voor kinderen met ADHD die een tryptofaandeficiëntie hebben. De belangrijkste assumpties voor een gerandomiseerd onderzoek met nicotinamidesupplementen zijn dat (a) een significante subgroep kinderen met ADHD verlaagde tryptofaanconcentraties heeft en (b) nicotinamidesupplementen een positief effect hebben op de opname en het transport van tryptofaan, wat kan resulteren in een verhoging van tryptofaanconcentraties in het bloed. Gedurende de voorbereiding van het onderzoeksprotocol werd duidelijk dat er onvoldoende theoretische onderbouwing was voor zowel een tryptofaandeficiëntie bij kinderen met ADHD, als voor het effect van nicotinamidesupplementen op de opname en het transport van tryptofaan. Aangezien er in de bestaande literatuur inconsistent bewijs is voor een tryptofaandeficiëntie bij kinderen met ADHD, werd besloten om in een zorgvuldig

opgezet onderzoek de assumptie van afwijkingen in tryptofaanconcentraties in het bloed van kinderen met ADHD te toetsen. Toen de resultaten aantoonden dat hier geen sprake van was (zie **hoofdstuk 2**), werd er geen verder onderzoek verricht naar de effectiviteit van nicotinamidesupplementen. Dit proefschrift illustreert hiermee dat het waardevol is om de assumpties die onderliggend zijn aan een innovatief interventieonderzoek te toetsen, voordat er een groot gerandomiseerd onderzoek plaatsvindt. Ook voor huidige voedingsinterventies voor ADHD, waaronder eliminatiediëten, zou het interessant zijn om de assumpties die onderliggend zijn aan de werkingsmechanismen te toetsen. Het is bijvoorbeeld niet waarschijnlijk dat allergische reacties een verklaring vormen voor de effectiviteit van eliminatiediëten (Pelsser, 2011), wat de vraag oproept welke factoren de effectiviteit wel kunnen verklaren. Zonder een gedegen theoretisch kader worden alternatieve verklaringen voor positieve effecten meer plausibel, waaronder een gekleurde perceptie van ouders (Sonuga-Barke et al., 2013). Het is aannemelijk dat ouders de effecten van een interventie overschatten wanneer zij deelnemen aan een interventie die een grote investering van deelnemers en hun ouders vraagt.

Een laatste suggestie voor vervolgonderzoek is om de predictieve validiteit van neurocognitieve deficiënties te toetsen. Hoewel de resultaten uit **hoofdstuk 4** laten zien dat kinderen met ADHD over het algemeen een zwakker neurocognitief functioneren vertonen dan kinderen zonder ontwikkelingsstoornis, lijkt het er niet op dat deze deficiënties een mediërende rol spelen in de relatie tussen ADHD en huidige problematiek op het gebied van externaliserend gedrag, schools presteren en sociaal functioneren. Daarnaast biedt neurocognitief functioneren slechts in beperkte mate predictieve validiteit voor persistentie van ADHD (Van Lieshout, Luman, Buitelaar, Rommelse, & Oosterlaan, 2013) en is er geen bewijs voor de predictieve validiteit van neurocognitief functioneren voor het ontstaan van rookverslaving of middelenmisbruik later in het leven van individuen met ADHD (Groenman et al., 2015). Het is daarom de vraag of het zinvol is om in de klinische praktijk neurocognitieve testen af te nemen, wat veel tijd in beslag kan nemen. Er wordt gesteld dat kinderen met ADHD over hun neurocognitieve beperkingen heen groeien, omdat problemen in het executief functioneren die bij hen werden gevonden, later niet meer zichtbaar waren toen diezelfde kinderen adolescenten waren (Thissen et al., 2014). Het is mogelijk dat er een subgroep kinderen met ADHD is die de neurcognitieve deficiënties die zij gedurende de kindertijd hebben ervaren ook later in het leven blijven ervaren. In een recent onderzoek werd getoond dat ook bij volwassenen verschillende neurocognitieve profielen

kunnen worden onderscheiden, waarbij de personen met ADHD per profiel slechter presteren op sommige neurocognitieve maten dan personen zonder ADHD (Mostert et al., 2015). Helaas werd in het desbetreffende onderzoek niet bekeken in hoeverre de verschillende neurocognitieve profielen bij volwassenen met ADHD gerelateerd waren aan beperkingen in het dagelijks leven. Het is aan te raden om de predictieve validiteit van neurocognitieve profielen verder te onderzoeken met een longitudinaal onderzoek. Indien blijkt dat bepaalde neurocognitieve profielen het risico op toekomstig onderpresteren op school verhogen, kan dit meer inzicht bieden in welke specifieke neurocognitieve zwaktes aangepakt zouden kunnen worden bij cognitieve interventies en welke kinderen het meeste baat hebben bij dergelijke interventies. Tot op heden lijken interventies gericht op cognitieve functies niet te resulteren in effecten op schools functioneren bij kinderen met ADHD (Rapport, Orban, Kofler, & Friedman, 2013). Het is mogelijk dat wanneer de cognitieve interventies gericht zijn op de neurocognitieve domeinen waarin sommige subgroepen van kinderen met ADHD het zwakst presteren, de effecten op het neurocognitief functioneren groter zijn en dat de effecten ook zichtbaar worden in het schools presteren. Op die manier zou de predictieve validiteit van neurocognitieve profielen de rol van neurocognitieve testen bij de diagnostiek van kinderen met ADHD rechtvaardigen.

Referenties

Albrecht, J., Garbade, S.F., & Burgard, P. (2009). Neuropsychological speed tests and blood phenylalanine levels in patients with phenylketonuria: A meta-analysis. *Neuroscience & Biobehavioral Reviews, 33*(3), 414-421.

American Academy of Pediatrics. (2011). ADHD: Clinical practice guideline for the diagnosis, evaluation, and treatment of attention-deficit/hyperactivity disorder in children and adolescents. *Pediatrics, 128*(5), 1007-1022.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders, fifth edition*. Arlington: American Psychiatric Association.

Ars, C., Nijs, I., Marroun, H., Muetzel, R., Schmidt, M., Steenweg-de Graaff, J., . . . Steegers, E. (2016). Prenatal folate, homocysteine and vitamin B12 levels and child brain volumes, cognitive development and psychological functioning: The Generation R Study. *British Journal of Nutrition.* doi:10.1017/S0007114515002081.

Baker, G.B., Bornstein, R.A., Rouget, A.C., Ashton, S.E., Van Muyden, J.C., & Coutts, R.T. (1991). Phenylethylaminergic mechanisms in attention-deficit disorder. *Biological Psychiatry, 29*(1), 15-22.

Banerjee, T.D., Middleton, F., & Faraone, S.V. (2007). Environmental risk factors for attention-deficit hyperactivity disorder. *Acta Paediatrica, 96*(9), 1269-1274.

Barkley, R.A. (1997). *ADHD and the nature of self-control*. New York: The Guilford Press.

Becker, S.P., Luebbe, A.M., & Langberg, J.M. (2012). Co-occurring mental health problems and peer functioning among youth with attention-deficit/hyperactivity disorder: A review and recommendations for future research. *Clinical Child and Family Psychology Review, 15*(4), 279-302.

Bornstein, R.A., Baker, G.B., Carroll, A., King, G., Wong, J.T., & Douglass, A.B. (1990). Plasma amino acids in attention deficit disorder. *Psychiatry Research, 33*(3), 301-306.

Buitelaar, J.K., Van der Wees, M., Swaab-Barneveld, H., & Van der Gaag, R.J. (1999). Verbal memory and performance IQ predict theory of mind and emotion recognition ability in children with autistic spectrum disorders and in psychiatric control children. *Journal of Child Psychology and Psychiatry, 40*(6), 869-881.

Castellanos, F.X., & Tannock, R. (2002). Neuroscience of attention-deficit/hyperactivity disorder: The search for endophenotypes. *Nature Reviews Neuroscience, 3*(8), 617-628.

Chace, D.H., Sherwin, J.E., Hillman, S.L., Lorey, F., & Cunningham, G.C. (1998). Use of phenylalanine-to-tyrosine ratio determined by tandem mass spectrometry to improve newborn screening for phenylketonuria of early discharge specimens collected in the first 24 hours. *Clinical Chemistry, 44*(12), 2405-2409.

Comings, D.E. (1990). Blood serotonin and tryptophan in Tourette syndrome. *American Journal of Medical Genetics, 36*(4), 418-430.

Corkum, P., Panton, R., Ironside, S., MacPherson, M., & Williams, T. (2008). Acute impact of immediate release methylphenidate administered three times a day on sleep in children with attention-deficit/hyperactivity disorder. *Journal of Pediatric Psychology, 33*(4), 368-379.

Corkum, P., Tannock, R., & Moldofsky, H. (1998). Sleep disturbances in children with attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child & Adolescent Psychiatry, 37*(6), 637-646.

Cortese, S., Faraone, S.V., Konofal, E., & Lecendreux, M. (2009). Sleep in children with attention-deficit/hyperactivity disorder: Meta-analysis of subjective and objective studies. *Journal of the American Academy of Child & Adolescent Psychiatry, 48*(9), 894-908.

Costello, E.J., Mustillo, S., Erkanli, A., Keeler, G., & Angold, A. (2003). Prevalence and development of psychiatric disorders in childhood and adolescence. *Archives of General Psychiatry, 60*(8), 837-844.

De Crescenzo, F., Licchelli, S., Ciabattini, M., Menghini, D., Armando, M., Alfieri, P., . . . Foti, F. (2016). The use of actigraphy in the monitoring of sleep and activity in ADHD: A meta-analysis. *Sleep Medicine Reviews, 26*, 9-20.

Del Campo, N., Chamberlain, S.R., Sahakian, B.J., & Robbins, T.W. (2011). The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. *Biological Psychiatry, 69*(12), e145-e157.

Dias, V.V., Brissos, S., Cardoso, C., Andreazza, A.C., & Kapczinski, F. (2009). Serum homocysteine levels and cognitive functioning in euthymic bipolar patients. *Journal of Affective Disorders, 113*(3), 285-290.

Durá-Travé, T., & Gallinas-Victoriano, F. (2014). Caloric and nutrient intake in children with attention deficit hyperactivity disorder treated with extended-release methylphenidate: Analysis of a cross-sectional nutrition survey. *Journal of the Royal Society of Medicine Open, 5*(2), 1-7.

Fair, D.A., Bathula, D., Nikolas, M.A., & Nigg, J.T. (2012). Distinct neuropsychological subgroups in typically developing youth inform heterogeneity in children with ADHD. *Proceedings of the National Academy of Sciences, 109*(17), 6769-6774.

Faraone, S.V., Asherson, P., Banaschewski, T., Biederman, J., Buitelaar, J. K., Ramos-Quiroga, J.A., . . . Franke, B. (2015). Attention-deficit/hyperactivity disorder. *Nature Reviews Disease Primers, 1*, 1-23.

Faraone, S.V., Bonvicini, C., & Scassellati, C. (2014). Biomarkers in the diagnosis of ADHD – Promising directions. *Current Psychiatry Reports, 16*(11), 1-20.

Faraone, S.V., Perlis, R.H., Doyle, A.E., Smoller, J.W., Goralnick, J.J., Holmgren, M.A., & Sklar, P. (2005). Molecular genetics of attention-deficit/hyperactivity disorder. *Biological Psychiatry, 57*(11), 1313-1323.

Fingerhut, R., & Olgemöller, B. (2009). Newborn screening for inborn errors of metabolism and endocrinopathies: An update. *Analytical and Bioanalytical Chemistry, 393*(5), 1481-1497.

Ford, A.H., Flicker, L., Singh, U., Hirani, V., & Almeida, O.P. (2013). Homocysteine, depression and cognitive function in older adults. *Journal of Affective Disorders, 151*(2), 646-651.

Garcia, A., & Zanibbi, K. (2004). Homocysteine and cognitive function in elderly people. *Canadian Medical Association Journal, 171*(8), 897-904.

Ghanizadeh, A., Sayyari, Z., & Mohammadi, M.R. (2013). Effect of methylphenidate and folic acid on ADHD symptoms and quality of life and aggression: A randomized double blind placebo controlled clinical trial. *Iranian Journal of Psychiatry, 8*(3), 108.

Gillberg, C., Gillberg, I.C., Rasmussen, P., Kadesjö, B., Söderström, H., Råstam, M., . . . Niklasson, L. (2004). Co–existing disorders in ADHD–implications for diagnosis and intervention. *European Child and Adolescent Psychiatry, 13*(1), i80-i92.

Greenhill, L.L., Swanson, J.M., Vitiello, B., Davies, M., Clevenger, W., Wu, M., . . . Conners, C.K. (2001). Impairment and deportment responses to different methylphenidate doses in children with ADHD: The MTA titration trial. *Journal of the American Academy of Child & Adolescent Psychiatry, 40*(2), 180-187.

Groenman, A.P., Oosterlaan, J., Greven, C.U., Vuijk, P.J., Rommelse, N., Franke, B., . . . Faraone, S.V. (2015). Neurocognitive predictors of substance use disorders and nicotine dependence in ADHD probands, their unaffected siblings, and controls: A 4-year prospective follow-up. *Journal of Child Psychology and Psychiatry, 56*(5), 521-529.

Held, P.K., White, L., & Pasquali, M. (2011). Quantitative urine amino acid analysis using liquid chromatography tandem mass spectrometry and aTRAQ® reagents. *Journal of Chromatography B, 879*(26), 2695-2703.

Herba, C.M., Landau, S., Russell, T., Ecker, C., & Phillips, M.L. (2006). The development of emotion-processing in children: Effects of age, emotion, and intensity. *Journal of Child Psychology and Psychiatry, 47*(11), 1098-1106.

Herba, C.M., & Phillips, M. (2004). Annotation: Development of facial expression recognition from childhood to adolescence: Behavioural and neurological perspectives. *Journal of Child Psychology and Psychiatry, 45*(7), 1185-1198.

Hills, P.J., & Lewis, M.B. (2011). The own-age face recognition bias in children and adults. *The Quarterly Journal of Experimental Psychology, 64*(1), 17-23.

Hinshaw, S.P. (2007). Moderators and mediators of treatment outcome for youth with ADHD: Understanding for whom and how interventions work. *Ambulatory Pediatrics, 7*(1), 91-100.

Hoshino, Y., Ohno, Y., & Yamamoto, T. (1985). Plasma free tryptophan concentration in children with attention deficit disorder. *Folia Psychiatrica et Neurologica Japonica, 39*(4), 531-536.

Hurt, E.A., Arnold, L.E., & Lofthouse, N. (2011). Dietary and nutritional treatments for attention-deficit/hyperactivity disorder: Current research support and recommendations for practitioners. *Current Psychiatry Reports, 13*(5), 323-332.

Jensen, P.S., Martin, D., & Cantwell, D.P. (1997). Comorbidity in ADHD: Implications for research, practice, and DSM-V. *Journal of the American Academy of Child & Adolescent Psychiatry, 36*(8), 1065-1079.

Jung, B., & Adeli, K. (2009). Clinical laboratory reference intervals in pediatrics: The CALIPER initiative. *Clinical Biochemistry, 42*(16), 1589-1595.

Kand'ár, R., & Žáková, P. (2009). Determination of phenylalanine and tyrosine in plasma and dried blood samples using HPLC with fluorescence detection. *Journal of Chromatography B, 877*(30), 3926-3929.

Konofal, E., Lecendreux, M., Arnulf, I., & Mouren, M.-C. (2004). Iron deficiency in children with attention-deficit/hyperactivity disorder. *Archives of Pediatrics & Adolescent Medicine, 158*(12), 1113-1115.

Konrad, K., Günther, T., Heinzel-Gutenbrunner, M., & Herpertz-Dahlmann, B. (2005). Clinical evaluation of subjective and objective changes in motor activity and attention in children with attention-deficit/hyperactivity disorder in a double-blind methylphenidate trial. *Journal of Child & Adolescent Psychopharmacology, 15*(2), 180-190.

Lawrence, K., Campbell, R., & Skuse, D. (2015). Age, gender, and puberty influence the development of facial emotion recognition. *Frontiers in Psychology.* doi:10.3389/fpsyg.2015.00761.

Lepage, N., Li, D., Kavsak, P.A., Bamforth, F., Callahan, J., Dooley, K., & Potter, M. (2006). Incomplete pediatric reference intervals for the management of patients with inborn errors of metabolism. *Clinical Biochemistry, 39*(6), 595-599.

Lepage, N., McDonald, N., Dallaire, L., & Lambert, M. (1997). Age-specific distribution of plasma amino acid concentrations in a healthy pediatric population. *Clinical Chemistry, 43*(12), 2397-2402.

Liu, J., & Raine, A. (2006). The effect of childhood malnutrition on externalizing behavior. *Current Opinion in Pediatrics, 18*(5), 565-570.

Loe, I.M., & Feldman, H.M. (2007). Academic and educational outcomes of children with ADHD. *Journal of Pediatric Psychology, 32*(6), 643-654.

Mancini, G., Agnoli, S., Baldaro, B., Ricci Bitti, P.E., & Surcinelli, P. (2013). Facial expressions of emotions: Recognition accuracy and affective reactions during late childhood. *Journal of Psychology, 147*(6), 599-617.

Mattson, M.P., & Shea, T.B. (2003). Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. *Trends in Neurosciences, 26*(3), 137-146.

McClure, E.B. (2000). A meta-analytic review of sex differences in facial expression processing and their development in infants, children, and adolescents. *Psychological Bulletin, 126*(3), 424-453.

McQuade, J.D., & Hoza, B. (2008). Peer problems in attention deficit hyperactivity disorder: Current status and future directions. *Developmental Disabilities Research Reviews, 14*(4), 320-324.

Mostert, J.C., Hoogman, M., Onnink, A.M.H., Van Rooij, D., Von Rhein, D., Van Hulzen, K.J., . . . Norris, D.G. (2015). Similar subgroups based on cognitive performance parse heterogeneity in adults with ADHD and healthy controls. *Journal of Attention Disorders*. doi:10.1177/1087054715602332.

MTA Cooperative Group. (1999). Moderators and mediators of treatment response for children with attention-deficit/hyperactivity disorder: The Multimodal Treatment Study of children with attention-deficit/hyperactivity disorder. *Archives of General Psychiatry, 56*(12), 1088-1096.

Mullane, J.C., Corkum, P.V., Klein, R.M., & McLaughlin, E. (2009). Interference control in children with and without ADHD: A systematic review of Flanker and Simon task performance. *Child Neuropsychology, 15*(4), 321-342.

Nemzer, E.D., Arnold, L.E., Votolato, N.A., & McConnell, H. (1986). Amino acid supplementation as therapy for attention deficit disorder. *Journal of the American Academy of Child Psychiatry, 25*(4), 509-513.

Ng, K.-H., Meyer, B.J., Reece, L., & Sinn, N. (2009). Dietary PUFA intakes in children with attention-deficit/hyperactivity disorder symptoms. *British Journal of Nutrition, 102*(11), 1635-1641.

Nigg, J.T., & Craver, L. (2014). Commentary: ADHD and social disadvantage: An inconvenient truth? – A reflection on Russell et al. (2014) and Larsson et al. (2014). *Journal of Child Psychology and Psychiatry, 55*(5), 446-447.

Nowicki, S., & Mitchell, J. (1998). Accuracy in identifying affect in child and adult faces and voices and social competence in preschool children. *Genetic, Social, and General Psychology Monographs, 124*(1), 39-60.

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

Oades, R.D. (2010). The role of serotonin in attention-deficit hyperactivity disorder (ADHD). In C.P. Müller & B.L. Jacobs (Eds.), *Handbook of the behavioral neurobiology of serotonin* (pp. 565-584). London: Elsevier.

Oades, R.D., Dauvermann, M.R., Schimmelmann, B.G., Schwarz, M.J., & Myint, A.M. (2010). Attention-deficit hyperactivity disorder (ADHD) and glial integrity: S100B, cytokines and kynurenine metabolism - effects of medication. *Behavioral and Brain Functions, 6*(29), 1-14.

Paşca, S.P., Nemeş, B., Vlase, L., Gagyi, C.E., Dronca, E., Miu, A.C., & Dronca, M. (2006). High levels of homocysteine and low serum paraoxonase 1 arylesterase activity in children with autism. *Life Sciences, 78*(19), 2244-2248.

Patel, K., & Curtis, L.T. (2007). A comprehensive approach to treating autism and attention-deficit hyperactivity disorder: A prepilot study. *Journal of Alternative and Complementary Medicine, 13*(10), 1091-1098.

Pelsser, L.M.J. (2011). *ADHD, a food-induced hypersensitivity syndrome: In quest of a cause*. Enschede: Ipskamp Drukkers.

Proietti, V., Macchi Cassia, V., & Mondloch, C.J. (2015). The own-age face recognition bias is task dependent. *British Journal of Psychology, 106*(3), 446-467.

Puig-Alcaraz, C., Fuentes-Albero, M., Calderón, J., Garrote, D., & Cauli, O. (2015). Increased homocysteine levels correlate with the communication deficit in children with autism spectrum disorder. *Psychiatry Research, 229*(3), 1031–1037.

Rapport, M.D., Orban, S.A., Kofler, M.J., & Friedman, L.M. (2013). Do programs designed to train working memory, other executive functions, and attention benefit children with ADHD? A meta-analytic review of cognitive, academic, and behavioral outcomes. *Clinical Psychology Review, 33*(8), 1237-1252.

Rashed, M.S., Bucknall, M.P., Little, D., Awad, A., Jacob, M., Alamoudi, M., . . . Ozand, P.T. (1997). Screening blood spots for inborn errors of metabolism by electrospray tandem mass spectrometry with a microplate batch process and a computer algorithm for automated flagging of abnormal profiles. *Clinical Chemistry, 43*(7), 1129-1141.

Refsum, H., Grindflek, A.W., Ueland, P.M., Fredriksen, Å., Meyer, K., Ulvik, A., . . . Kase, B.F. (2004). Screening for serum total homocysteine in newborn children. *Clinical Chemistry, 50*(10), 1769-1784.

Reimherr, F.W., Wender, P., Wood, D., & Ward, M. (1987). An open trial of L-tyrosine in the treatment of attention deficit disorder, residual type. *American Journal of Psychiatry, 144*(8), 1071-1073.

Rodger, H., Vizioli, L., Ouyang, X., & Caldara, R. (2015). Mapping the development of facial expression recognition. *Developmental Science, 18*(6), 926-939.

Scassellati, C., Bonvicini, C., Faraone, S.V., & Gennarelli, M. (2012). Biomarkers and attention-deficit/hyperactivity disorder: A systematic review and meta-analyses. *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(10), 1003-1019.

Schwartz, G., Amor, L.B., Grizenko, N., Lageix, P., Baron, C., Boivin, D.B., & Joober, R. (2004). Actigraphic monitoring during sleep of children with ADHD on methylphenidate and placebo. *Journal of the American Academy of Child & Adolescent Psychiatry, 43*(10), 1276-1282.

Shaw, P., Stringaris, A., Nigg, J., & Leibenluft, E. (2014). Emotion dysregulation in attention deficit hyperactivity disorder. *American Journal of Psychiatry, 171*(3), 276-293.

Singh, I., & Rose, N. (2009). Biomarkers in psychiatry. *Nature, 460*(7252), 202-207.

Sjöwall, D., Bohlin, G., Rydell, A.-M., & Thorell, L.B. (2015). Neuropsychological deficits in preschool as predictors of ADHD symptoms and academic achievement in late adolescence. *Child Neuropsychology*, *23*(1), 111-128.

Sonuga-Barke, E.J., Brandeis, D., Cortese, S., Daley, D., Ferrin, M., Holtmann, M., . . . Döpfner, M. (2013). Nonpharmacological interventions for ADHD: systematic review and meta-analyses of randomized controlled trials of dietary and psychological treatments. *American Journal of Psychiatry, 170*(3), 275-289.

Tamm, L., Narad, M.E., Antonini, T.N., O'Brien, K.M., Hawk Jr, L.W., & Epstein, J.N. (2012). Reaction time variability in ADHD: A review. *Neurotherapeutics, 9*(3), 500-508.

Teunissen, C.E., van Boxtel, M.P., Jolles, J., de Vente, J., Vreeling, F., Verhey, F., . . . Blom, H.J. (2005). Homocysteine in relation to cognitive performance in pathological and non-pathological conditions. *Clinical Chemical Laboratory Medicine, 43*(10), 1089-1095.

Thapar, A., Cooper, M., Eyre, O., & Langley, K. (2013). Practitioner review: What have we learnt about the causes of ADHD? *Journal of Child Psychology and Psychiatry, 54*(1), 3-16.

Thissen, A., Rommelse, N., Hoekstra, P., Hartman, C., Heslenfeld, D., Luman, M., . . . Buitelaar, J. (2014). Attention deficit hyperactivity disorder (ADHD) and executive functioning in affected and unaffected adolescents and their parents: Challenging the endophenotype construct. *Psychological Medicine, 44*(4), 881-892.

Thomas, L.A., De Bellis, M.D., Graham, R., & LaBar, K.S. (2007). Development of emotional facial recognition in late childhood and adolescence. *Developmental Science, 10*(5), 547-558.

Toren, P., Eldar, S., Sela, B.-A., Wolmer, L., Weitz, R., Inbar, D., . . . Laor, N. (1996). Zinc deficiency in attention-deficit hyperactivity disorder. *Biological Psychiatry, 40*(12), 1308-1310.

Van Beynum, I.M., Den Heijer, M., Thomas, C.M., Afman, L., Oppenraay-van Emmerzaal, D., & Blom, H.J. (2005). Total homocysteine and its predictors in Dutch children. *American journal of Clinical Nutrition, 81*(5), 1110-1116.

Van der Meer, J.M., Oerlemans, A.M., Van Steijn, D.J., Lappenschaar, M.G., De Sonneville, L.M., Buitelaar, J.K., & Rommelse, N.N. (2012). Are autism spectrum disorder and attention-deficit/hyperactivity disorder different manifestations of one overarching disorder? Cognitive and symptom evidence from a clinical and population-based sample. *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(11), 1160-1172.

Van Hulst, B., De Zeeuw, P., & Durston, S. (2015). Distinct neuropsychological profiles within ADHD: A latent class analysis of cognitive control, reward sensitivity and timing. *Psychological Medicine, 45*, 735-745.

Van Lieshout, M., Luman, M., Buitelaar, J., Rommelse, N., & Oosterlaan, J. (2013). Does neurocognitive functioning predict future or persistence of ADHD? A systematic review. *Clinical Psychology Review, 33*(4), 539-560.

Venta, R., Prieto, B., & Alvarez, F.V. (2002). Regression-based reference limits for urinary amino acids in a pediatric population. *Clinical Chemistry and Laboratory Medicine, 40*(4), 383-390.

Vitiello, B., Severe, J.B., Greenhill, L.L., Arnold, L.E., Abikoff, H.B., Bukstein, O.G., . . . Hinshaw, S.P. (2001). Methylphenidate dosage for children with ADHD over time under controlled conditions: Lessons from the MTA. *Journal of the American Academy of Child & Adolescent Psychiatry, 40*(2), 188-196.

Willcutt, E.G. (2012). The prevalence of DSM-IV attention-deficit/hyperactivity disorder: A meta-analytic review. *Neurotherapeutics, 9*(3), 490-499.

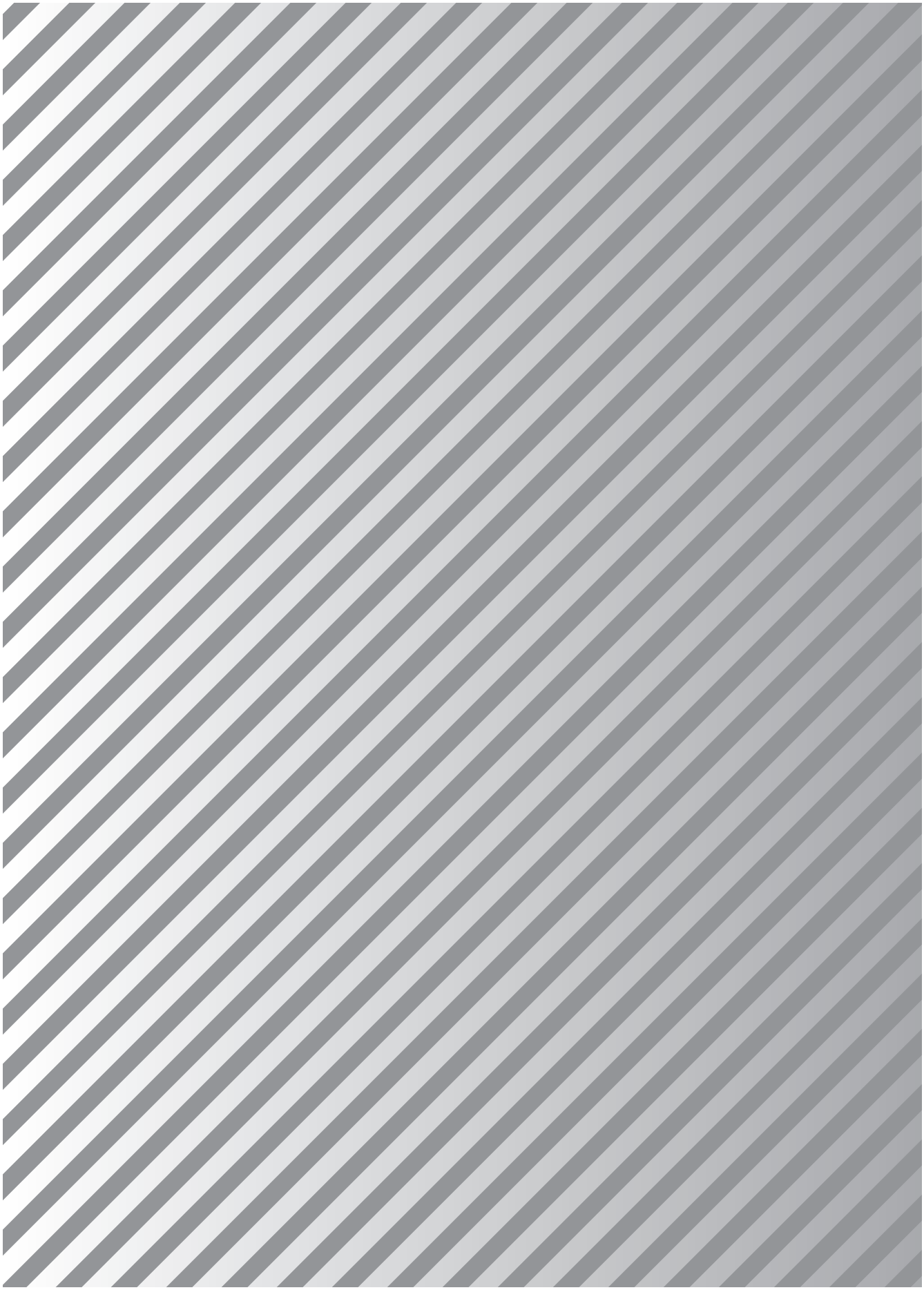
Willcutt, E.G., Doyle, A.E., Nigg, J.T., Faraone, S.V., & Pennington, B.F. (2005). Validity of the executive function theory of attention-deficit/hyperactivity disorder: A meta-analytic review. *Biological Psychiatry, 57*(11), 1336-1346.

Wood, D.R., Reimherr, F.W., & Wender, P.H. (1985). Treatment of attention deficit disorder with DL-phenylalanine. *Psychiatry Research, 16*(1), 21-26.

Yoon, S.Y.R., Jain, U., & Shapiro, C. (2012). Sleep in attention-deficit/hyperactivity disorder in children and adults: Past, present, and future. *Sleep Medicine Reviews, 16*(4), 371-388.

Zametkin, A.J., Karoum, F., Rapoport, J.L., Brown, G.L., & Wyatt, R.J. (1984). Phenylethylamine excretion in attention deficit disorder. *Journal of the American Academy of Child Psychiatry, 23*(3), 310-314.

Zytkovicz, T.H., Fitzgerald, E.F., Marsden, D., Larson, C.A., Shih, V.E., Johnson, D.M., . . . Grady, G.F. (2001). Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: A two-year summary from the New England newborn screening program. *Clinical Chemistry, 47*(11), 1945-1955.



Dankwoord
Acknowledgements

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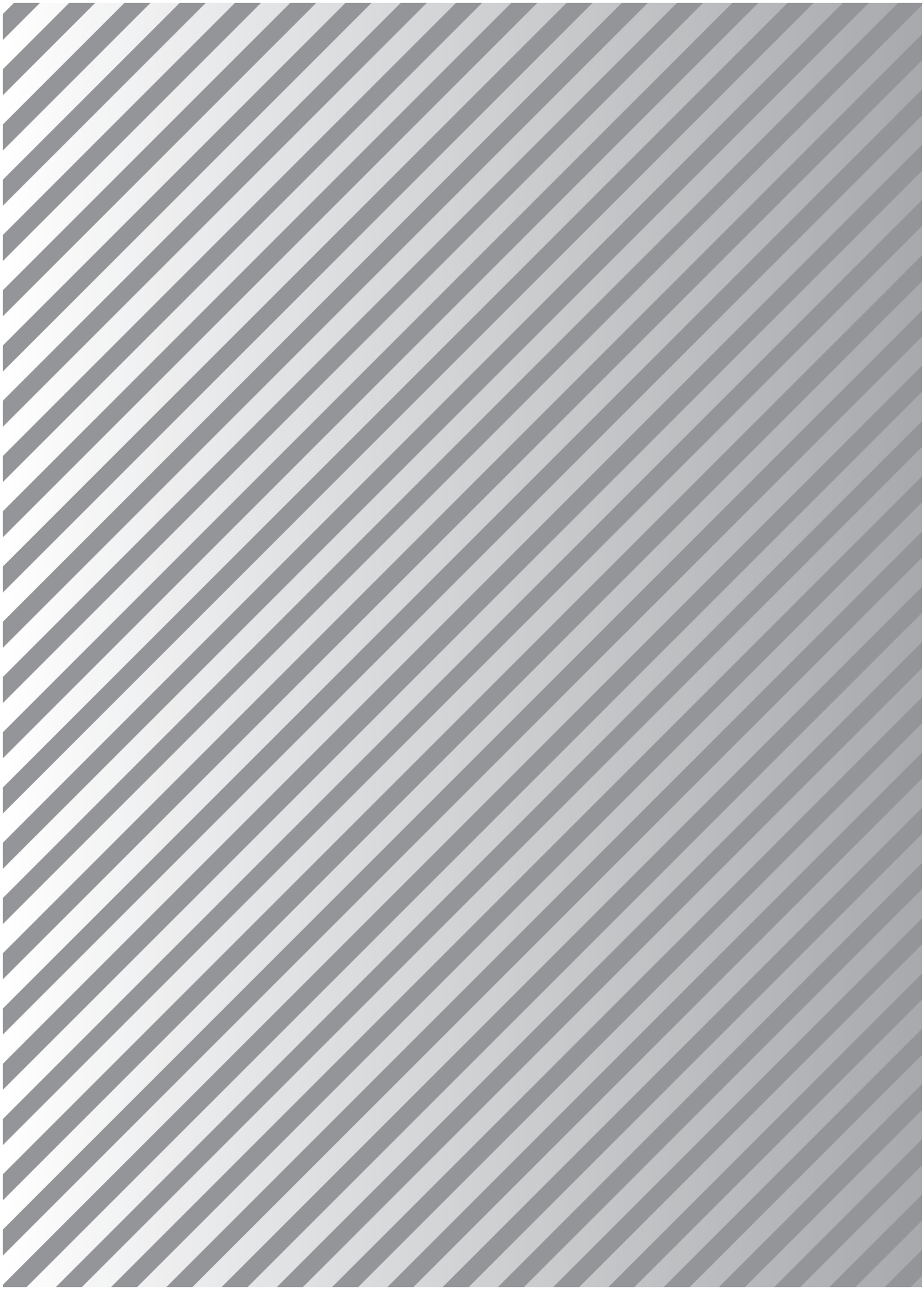

About the author

List of publications

Published

Bergwerff, C.E., Luman, M., Weeda, W.D., & Oosterlaan, J. (in press). Neurocognitive profiles in children with attention-deficit/hyperactivity disorder and their predictive value for functional outcomes. *Journal of Attention Disorders.*

Bergwerff, C.E., Luman, M., & Oosterlaan, J. (2016). No objectively measured sleep disturbances in children with attention-deficit/hyperactivity disorder. *Journal of Sleep Research*, *25*(5), 534–540.

Bergwerff, C.E., Luman, M., Blom, H.J., & Oosterlaan, J. (2016). No tryptophan, tyrosine and phenylalanine abnormalities in children with attention-deficit/hyperactivity disorder. *PLOS ONE.* doi: 10.1371/journal.pone.0151100.

Oosterlaan, J., Hoekstra, P.J., Van den Hoofdakker, B.J., Van der Oord, S., Van Nieuwenhuizen, C., Bink. M., & **Bergwerff, C.E.** (2015). *ADHD, een update. Actuele stand van wetenschappelijke inzichten rond diagnostiek en behandeling van ADHD.* Den Haag: De Zagerij.

Stevenson, C.E., **Bergwerff, C.E.**, Heiser, W.J., & Resing, W. (2014). Working memory and dynamic measures of analogical reasoning as predictors of children's math and reading achievement. *Infant and Child Development*, *23*(1), 51-66.

Submitted

Bergwerff, C.E., Luman, M., Blom, H.J., & Oosterlaan, J. (*under review*). Homocysteine concentrations and neurocognitive functioning in children with attention-deficit/hyperactivity disorder.

Bergwerff, C.E., Luman, M., Blom, H.J., & Oosterlaan, J. (*revision*). Paediatric reference values for total homocysteine, tryptophan, tyrosine and phenylalanine in blood spots.

Bergwerff, C.E., Luman, M., Meffert, H., Blair, R.J.R., & Oosterlaan, J. (*under review*). Measuring social cognition in school-aged children using a morphed facial emotion recognition task.

Curriculum Vitae

Carlijn Bergwerff was born on 14 February 1986 in Gouda, the Netherlands. In 2004 Carlijn completed her secondary education at the Gereformeerde Scholengemeenschap Randstad in Rotterdam. She decided to move to Milan afterwards, to take care of two Italian children and to learn Italian. In 2005 Carlijn returned to the Netherlands and started studying Facility Management at The Hague University of Applied Sciences. She obtained a bachelor's degree in Business Administration in 2008. Driven by her interest in the development of children and adolescents, Carlijn chose to pursue a degree in Child and Adolescent Psychology. As part of the master's programme, Carlijn performed a clinical internship at a private mental health care practice (BTSW). In 2012 Carlijn graduated as child and adolescent psychologist and started as PhD student within the Child Study Group of the Clinical Neuropsychology section at Vrije Universiteit Amsterdam. Her research focused on several aspects of attention-deficit/hyperactivity disorder in children, resulting in the present dissertation. Carlijn was also involved in some other projects, including coordinating a project that resulted in a guide for practitioners on recent scientific insights into diagnosis and treatment of ADHD. Further, she coordinated a grant proposal on research into placebo-controlled titration of methylphenidate in children and adolescents with ADHD, for which 250.000 euro has been awarded. After finishing her dissertation in 2016, Carlijn started working at the HR department of Careyn, a health care organization. In her spare time, Carlijn is involved in local politics in Leiden. She is spokesperson for a Christian democratic political party (ChristenUnie) on the topics of youth, education, health care, wellbeing and sports.