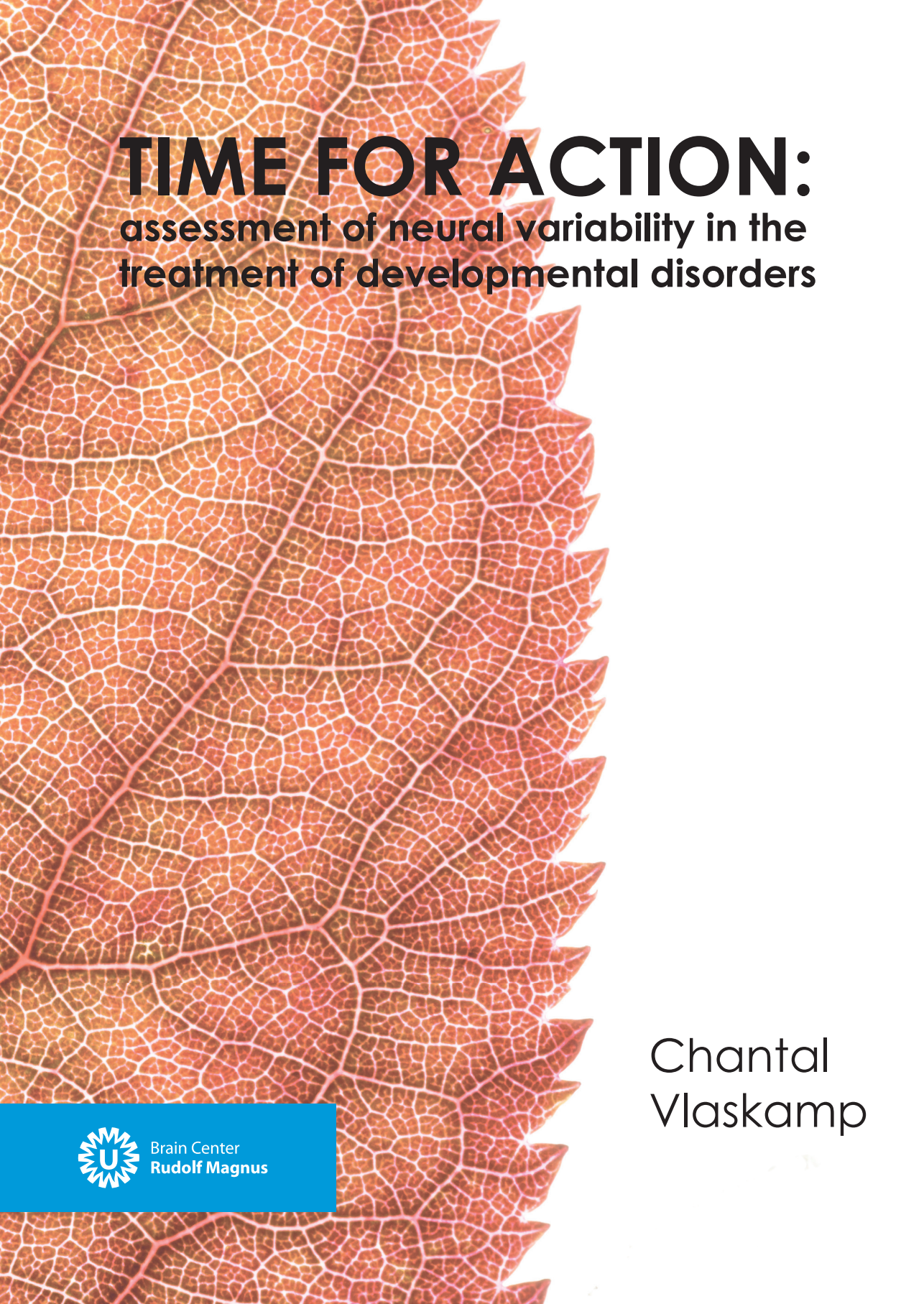

TIME FOR ACTION:

assessment of neural variability in the treatment of developmental disorders

Chantal Vlaskamp

Brain Center
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TIME FOR ACTION:

assessment of neural variability in the treatment of developmental disorders

TIJD VOOR ACTIE:

het meten van neurale variabiliteit in de behandeling van ontwikkelingsstoornissen

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. H.R.B.M. Kummeling, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op woensdag 31 oktober 2018 des middags te 4.15 uur

door

Chantal Vlaskamp

geboren op 15 juli 1989 te 't Harde

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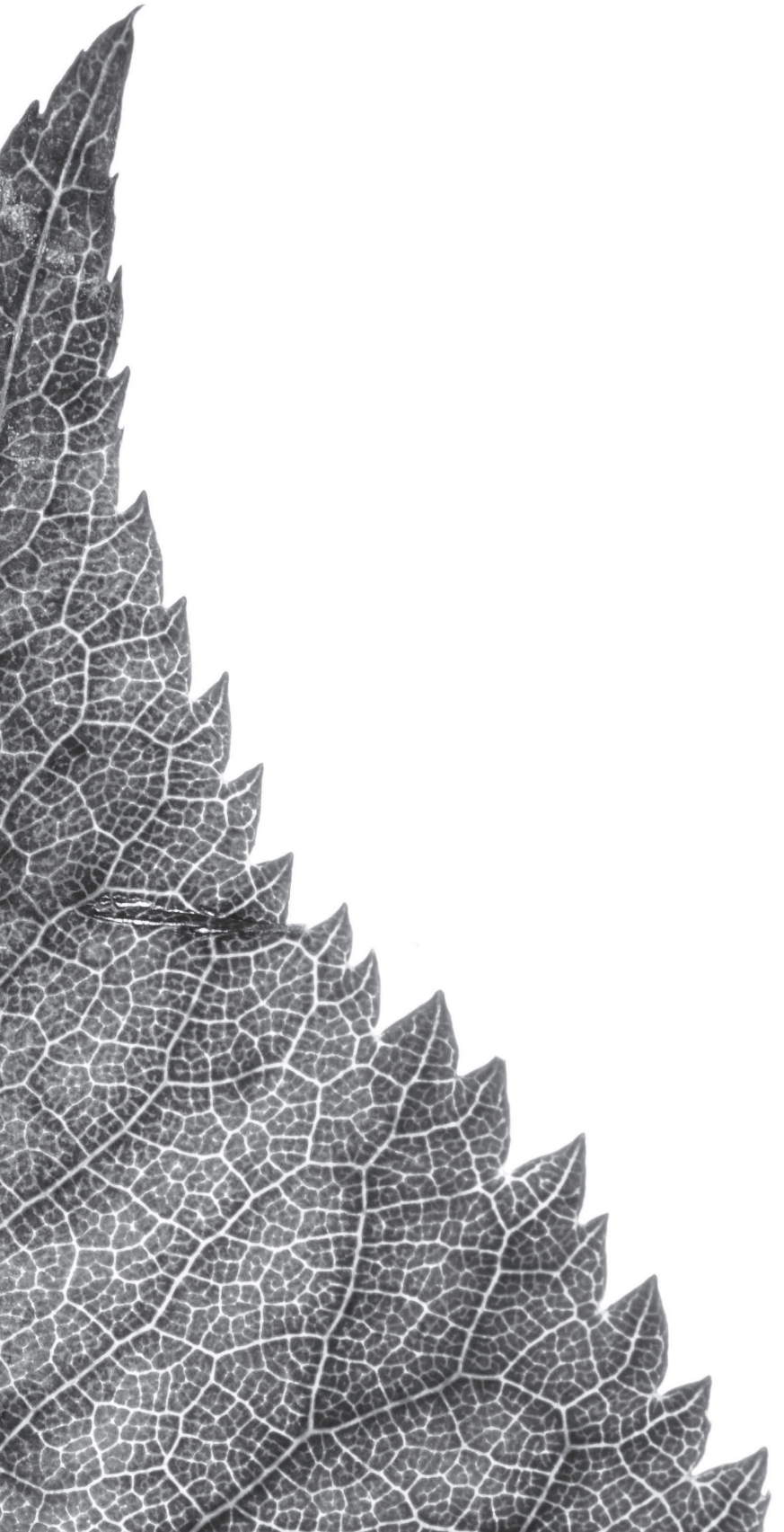


1

INTRODUCTION

CHAPTER 1: INTRODUCTION

The prevalence of developmental disorders has substantially increased in the last decades. These disorders create a huge burden for the children and adults who suffer from them, as well as for their family members and others surrounding them. Developmental disorders are defined and diagnosed on behavioral symptoms and to date have not been linked to uniform biological mechanisms. In addition, these clinical behavioral definitions are broad and encompass broad diagnostic categories, such as autism spectrum disorder and ADHD. As a consequence, individuals with many different symptom constellations are grouped under the same diagnostic label. Together, the lack of clear biological mechanisms and the clinical heterogeneity obstruct progress in developing better treatments for individuals with these disorders.

In this thesis, we aim to assess this neural variability in developmental disorders and explore its relation to treatment. We focus on autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) with steps out to epilepsy and tuberous sclerosis complex (TSC).

General research questions to be addressed in this thesis:

1. Are physiological correlates of auditory sensory processing different in children with autism spectrum disorder compared to typically developing children and are these related to behavior or subtypes? (**Chapter 2 & 3**)

2. Can we find neural correlates of the variability in methylphenidate response in children with ADHD? (**Chapter 4**)

3. Is it possible to use more neurophysiological-based treatment to treat neuropsychiatric problems in a genetic subtype of ASD (tuberous sclerosis complex (TSC)) (**chapter 5**)?

4. Overall, what is the potential of assessing neural variability in predicting or finding treatments for developmental disorders?

Developmental disorders

Developmental disorders are mental disorders with an onset during development, and are characterized by neurocognitive, psychosocial and other deficits giving rise to impairments in daily life. Affected domains range from emotional, behavioral, social, learning, communication, motor and physical health. Typically, these disorders manifest during childhood, before

children reach school age (APA, 2013). The diagnostic and statistic manual of mental disorders (DSM) 5th edition classifies the following as developmental disorders: intellectual disability, communication disorders, ASD, ADHD, motor disorders, and learning disorders (APA, 2013).

Diagnosis of a developmental disorder is currently based on criteria of the DSM: a man-made construct of standardization originally intended to create relatively homogeneous diagnostic categories. However, developmental disorders present with highly heterogeneous phenotypes, meaning that many different behavioral phenotypes are grouped under the same diagnostic umbrella. Also, co-occurrence between developmental disorders is very high, as is the risk of developing other (develop-)mental disorders later in life, making developmental disorders even more complex traits to study – and treat (Costello *et al*, 2003). In general, more boys than girls are affected by developmental disorders, with ADHD and ASD the most common (APA, 2013). The incidence of developmental disorders, especially ASD and ADHD, has progressively increased in the last decades (Baio *et al*, 2018; Danielson *et al*, 2018). Partially, this can be explained by better and earlier recognition of these disorders, more (scientific) knowledge and more accurate diagnostic tools (King and Bearman, 2009), although epidemiological studies have indicated that half of the rise is unexplained and possibly due to yet unidentified environmental risk factors. These facts underline the tremendous societal burden and the urgent need for optimizing treatments for individuals suffering from these disorders (Weintraub, 2011).

ASD

Autism spectrum disorder is a disorder characterized by persistent deficits in social communication and interaction, and restrictive and repetitive patterns of behavior (APA, 2013). Although on a continuum ranging from mild to severe, typical symptoms of ASD include deficits in social-emotional reciprocity, deficits in non-verbal communicative behaviors and problems with developing and maintaining relationships. In addition, individuals with ASD often present with stereotyped or repetitive speech or behavior, adherence to routine (difficulties with adaptive functioning), restricted interests and hyper- or hyporeactivity to sensory input. Difficulties in sensory processing were only added to the criteria for the disorder in the 5th edition of the DSM in 2013, but have long been acknowledged to represent an enormous and prevalent burden in up to 96% of individuals with autism (Kern *et al*, 2006). For example, sensory hyperreactivity can result in distress or an exaggerated negative response to sensory input, often leading to avoidance

and hypervigilance related to the stimulus. Hyporesponsivity can result in an unawareness of, or slow responding to a stimulus that would normally be expected to elicit a response. Also, individuals may present with sensory seeking behavior: for example, an unusual craving for, or preoccupation with certain sensory experiences (APA, 2013; Leekam *et al*, 2007). ASD has a great impact on functioning in daily life, academic performance and social relationships, and forms a burden both for individuals with ASD themselves and their caregivers (Volkmar *et al*, 2014). Although ASD is conceptualized as one diagnostic category, albeit on a spectrum, there is large heterogeneity within ASD. Many different behavioral phenotypes exist, as well as possible biological mechanisms thought to be associated with ASD (Auerbach *et al*, 2011). As mentioned above, the prevalence of ASD has exponentially increased over the past decades: nowadays, the Centers for Disease Control and prevention (CDC) report estimates of 1 in 59 individuals for the United States (Baio *et al*, 2018), and other studies report similar prevalence estimates ranging from 1-1.5% worldwide (Volkmar *et al*, 2014). ASD affects more boys than girls, with a ratio of 4:1. ASD persists into adulthood, and can give rise to challenges when leaving high school. It is estimated that 50% of individuals with ASD do not start follow-up education or are unemployed, after leaving high school (Shattuck *et al*, 2012).

ADHD

ADHD is the most prevalent developmental disorder in children (Feldman and Reiff, 2014) with current estimates varying from 5% to 9.4% worldwide (Costello *et al*, 2003; Danielson *et al*, 2018), affecting more boys than girls (ratio 3:1). It is characterized by persistent patterns of age inappropriate inattention and/or hyperactivity-impulsivity that interfere with daily life (APA, 2013). For example, individuals with ADHD often experience difficulties in focusing attention, are easily distracted, have difficulties organizing their lives and can be forgetful, Also, they can have difficulties sitting still or engaging in leisure activities quietly, or find it difficult to await their turn or blurt out answers before questions are finished. The DSM-5 identifies three different subtypes: the inattentive subtype (the individual meets criteria of inattention only), the hyperactive-impulsive subtype (the individual meets criteria of hyperactivity-impulsivity only), and – most common- the combined subtype (the individual meets criteria of both inattention and hyperactivity-impulsivity). Even within these more 'homogeneous' subgroups, patients with ADHD still differ from each other substantially, similar to ASD (Castellanos *et al*, 2006; Nigg *et al*, 2005). Also similar to ASD, the prevalence of ADHD has increased over the past decades. The burden of ADHD is high, as it affects academic performance,

as well as social relationships and impairs daily functioning (Brod *et al*, 2012). Although ADHD is frequently conceptualized as a childhood disorder, more than two third of individuals continue to experience debilitating symptoms or meet criteria for diagnosis in adulthood (Faraone *et al*, 2015), and ADHD is often diagnosed in adulthood. In adult ADHD, inattention problems often outweigh hyperactivity symptoms, leading to impairments of social, academic and occupational functioning (Volkow and Swanson, 2013). In addition, symptoms often present differently. For example, in the transition from childhood to adulthood, the diminishing symptoms of hyperactivity may manifest as restlessness (Faraone *et al*, 2015; Wender, 1998).

- *Autism and epilepsy*

It is estimated that 6 to 46% of children with ASD have comorbid epilepsy or experience seizures at some point in their life (Trauner, 2015). Epilepsy is a serious neurological disorder involving spontaneous and uncontrolled brain activity (i.e. seizures). Seizures can either be focal (i.e. localized and limited to one hemisphere) or generalized (i.e. widespread in the brain and bilateral), and can often be observed (e.g. as spikes) on an EEG (electroencephalogram) (Rosenow *et al*, 2015). Focal background and epileptiform abnormalities are frequently found in EEGs of children with ASD, suggesting possible commonalities in the biology of these disorders (Gilby and O'Brien, 2013; Levisohn, 2007). In addition, excessive response to sensory stimuli (hyperreactivity), as is often observed in ASD, can facilitate the occurrence of seizures (Kasteleijn-Nolst Trenite, 1989; Symonds, 1959) and vice versa, children with epilepsy often display increased prevalence of sensory issues (van Campen *et al*, 2015).

- *TSC*

One natural, biological model for both autism and epilepsy is tuberous sclerosis complex (TSC): a multisystem genetic developmental disorder that is often accompanied by seizures and behavioral problems, such as intellectual disability and autism (Bolton *et al*, 2015; Curatolo *et al*, 2015; Curatolo *et al*, 2002). Similar to ASD, irritability, sensory arousal, repetitive behaviors, sleeping problems and deficits in social communication are very common in patients with TSC and these so-called tuberous sclerosis-associated neuropsychiatric disorders (TAND) are often extremely debilitating (de Vries *et al*, 2015). TSC is a rare genetic disorder, with a prevalence of 1 in 6000 and explains approximately 1% of all ASD cases (Curatolo and Maria, 2013; Curatolo *et al*, 2004; Guo *et al*, 2012). Vice versa, estimates of clinical ASD diagnoses in TSC are reported up to 61% (Jeste *et al*, 2008; Vignoli *et al*, 2015; Wiznitzer, 2004),

although symptoms of TAND (also including ASD symptoms, but without full blown diagnosis) are present in up to 90% of individuals with TSC (de Vries *et al*, 2015).

Co-occurrence of developmental disorders

Boundaries between complex developmental disorders can be blurry: for example, symptoms of ADHD and ASD are often overlapping, complicating suitable diagnostics, as well as treatments. Indeed, children with (any) developmental disorder are 3.7 times more likely to be diagnosed with an additional disorder than children without a developmental disorder (Costello *et al*, 2003). For instance, in ASD, children not only often have co-morbid anxiety disorders (~29%) or ADHD (~30%) (Simonoff *et al*, 2008), but also have a larger risk to develop schizophrenia (Chisholm *et al*, 2015). In ADHD, children often have comorbid oppositional defiant disorder (ODD) (30-60 %) as well as ASD (~30%) (Reimherr *et al*, 2013; Simonoff *et al*, 2008). Children with ADHD also have a higher risk to develop substance abuse disorders and addiction in adulthood (Levy *et al*, 2014). In addition, medical disorders such as gastrointestinal problems, neurological disorders (e.g. epilepsy, encephalopathy) and sleep disorders have also been reported more often in developmental disorders (Jeste, 2015; Lai *et al*, 2014).

As mentioned above, diagnoses of developmental disorders are based on behavioral criteria, resulting in great biological heterogeneity and a lack of treatments based on biological mechanisms. The development and prescription of effective treatments may benefit from being biologically, in addition to behaviorally, informed.

Neurobiology of developmental disorders

It is widely accepted that both ASD and ADHD have neurobiological underpinnings, and the last few decades have seen an explosion of advanced neuroimaging techniques and the amount of studies using these to investigate the neurobiological mechanisms of developmental disorders. Studies investigating genetics, neuroimaging and cognitive aspects have contributed to our mechanistic understanding of developmental disorders (Jeste, 2015; Lai *et al*, 2014; Spencer *et al*, 2007). In spite of the heterogeneity described above, the estimated heritability of developmental disorders is high, up to 80% (Posthuma and Polderman, 2013). Most developmental disorders are therefore believed to be related to variation within multiple genes and

their interaction with behavioral and environmental factors (Vorstman and Ophoff, 2013). In addition, different types of developmental disorders appear to share common genetic changes, making it even more difficult – if not impossible - to associate specific genetic variation with specific disorders. Therefore, it is believed that certain gene variants may increase susceptibility for developing a developmental disorder, but that they are not causal in isolation. Rather, they are likely related to normal variation of behavior in the typically developing population (Geschwind, 2011).

At a different level, neuroimaging is frequently used to investigate the neurobiology of psychiatric disorders (Mayberg, 2014). Neuroimaging techniques such as structural and functional magnetic resonance imaging (MRI), and electroencephalography (EEG) can teach us about the structure and function of the brain in a non-invasive manner. Structural MRI can be used to identify structural and volumetric differences between or within groups, whereas functional MRI indirectly measures neural activity, e.g. when doing a task or when subjects are at rest. MRI is a technique with high spatial resolution and is therefore suitable for investigating neural activity in regions of interest. EEG on the other hand, has high temporal resolution and is therefore suitable to investigate neural processes over time. EEG can be used to investigate brain activity at rest for example, or to study brain connectivity. In addition, event related potentials (ERPs) can be studied: ERPs represent the averaged brain response triggered by sensory stimuli, the characteristics of which depend on the paradigms used (Luck, 2012). ERPs are therefore typically used to measure information processing in the brain, ranging from early (preconscious) to late (conscious) sensory processing. These basic, low-level brain processes as can be measured with early ERPs are thought to constitute building blocks for more higher-order processes (Baum *et al*, 2015). Differences in cognitive ability are part of the phenotype of developmental disorders, and are frequently researched. Besides the implicated domains of attention, memory, executive functioning and social cognition (Bishop, 2009; de Vries, 2010; Dickinson *et al*, 2007; Jeste, 2015; van Hulst *et al*, 2015), general cognitive ability as measured with IQ is also often found to be lower in individuals with developmental disorders.

In addition to the above described genetic vulnerability, brain structure and function, cognition and behavior, environment also plays a large mediating role in the development of any mental disorder (Bishop, 2009; Geschwind, 2011). Pre- and postnatal development constitute the most vulnerable and plastic periods in life, and thus can be affected by many factors, both positive and negative (Dahl, 2004; Dennis *et al*, 2014).

ASD

The estimated heritability of ASD is high, up to 80% (Lichtenstein *et al*, 2010). However, genetic etiologies, e.g. highly penetrant mutations or copy number variations, can so far only be identified for only 10 to 20% of ASD cases, and many individuals with these genetic etiologies do not develop ASD (Curatolo *et al*, 2004). Single gene disorders in which ASD is common, such as Fragile X syndrome, Rett syndrome and TSC do however underline the relevance of genetics to ASD. Besides genetics, many studies have investigated the neurobiology of ASD using neuroimaging techniques. However, in studies comparing brain structure of ASD individuals with that of typically developing peers, large variability exist. The most consistent finding in ASD is that of a cortical overgrowth in the first 2-4 years of life (Courchesne, 2002), measured with structural MRI (sMRI), although the course of brain development after these early years is less clear (Courchesne *et al*, 2011). In addition, studies have often reported reduced area of the corpus callosum, a structure important for interhemispheric connections (Hardan *et al*, 2000; Stanfield *et al*, 2008). Additionally, studies investigating connectivity have suggested that ASD is associated with developmental under-connection in a widely distributed set of networks (Geschwind and Levitt, 2007). Further, although results are variable, functional magnetic resonance imaging (fMRI) studies have identified changes in neural activity during tasks involving social and affective judgments and differences in the processing of facial and non-facial stimuli (Volkmar *et al*, 2014). Similar to these (f)MRI studies, reports on auditory processing in ASD using event related potentials (ERPs) are also inconsistent. Several (auditory) ERPs are also investigated throughout this thesis and will therefore be described in somewhat more detail. P50 suppression is an early measure of sensory gating and represents a preconscious filtering mechanism in the brain. Studies have reported normal sensory gating in ASD (Kemner *et al*, 2002; Madsen *et al*, 2015), whereas others found decreased suppression (i.e. less 'filtering') in young children with ASD (Orekhova *et al*, 2008). Mismatch negativity (MMN) is an automatic orienting reflex registering deviancy in the environment, and can be elicited by different types of deviancy, e.g. in pitch (frequency) and duration of stimuli or a combination of both (Naatanen and Alho, 1995). Studies of MMN in ASD compared to TD children are largely inconsistent, with studies showing normal, increased as well as decreased MMN in children, using different types of deviants and paradigms (Dunn *et al*, 2008; Ferri *et al*, 2003; Weismuller *et al*, 2015). The P300 is a later ERP component and reflects (pre)conscious perception, dependent on paradigm and stimuli characteristics. The P300 is often separated into a

P3a and P3b component, where P3a represents a preconscious and P3b a more conscious measure of attentional orienting (Polich, 2004). In ASD, P3a and P3b are often reported to be either unaffected (Lepisto *et al*, 2006) or decreased (Donkers *et al*, 2015; Ferri *et al*, 2003). It has been suggested that P3a and P3b amplitude are related to cognitive abilities such as working memory and attention (Jeste and Nelson, 2009; Polich, 2004). Indeed, neuropsychological studies investigating measures of cognition often report differences in ASD, including in working memory, attention and executive functioning, in addition to deficits in theory-of-mind (taking the perspective of another person) (Jeste, 2015). The only known environmental risk factor for ASD that may act on these different levels of neurobiology is advanced parental age (Lampi *et al*, 2013), although studies have also suggested that preterm birth or birth complications may form a risk factor (Schendel and Bhasin, 2008; Zwaigenbaum *et al*, 2002).

- *Imbalance in excitatory and inhibitory processes in ASD and related disorders?:*

A popular theory on the biology of ASD is that it may reflect an imbalance between excitation and inhibition (E/I imbalance) in the brain, in particular in circuits governing sensory processes, memory, and social and emotional behaviors (Rubenstein and Merzenich, 2003). Indeed, studies in animal models as well as experimental neurophysiological studies have suggested that such an E/I imbalance may lead to disturbed information processing and functional brain development (Baum *et al*, 2015). An elevated E/I balance or hyperexcitability is also suggested by the frequent concurrence of epilepsy or seizures in children with ASD and the strong aversive (hypersensitive) reactions of children with ASD to sensory stimuli (Kern *et al*, 2006; Trauner, 2015; Tuchman and Cuccaro, 2011). However, not all forms of ASD need to be related to elevated E/I imbalances, and it has been suggested that the reverse, excessive inhibition, may also occur (Baroncelli *et al*, 2011).

ADHD

The estimated heritability of ADHD is also high, with estimates of 70 - 80% (Lichtenstein *et al*, 2010) indicating an important genetic component in its pathogenesis. Indeed, genes coding for the dopamine receptor, dopamine transporter and serotonin receptor have been identified as risk-genes, of which the dopamine receptor D4 (DRD4) has been the most commonly reported and most strongly implicated (Faraone *et al*, 2005; Vorstman *et al*, 2013). At the level of neuroimaging, structural MRI studies comparing individuals with ADHD with their typically developing peers have shown

mean reductions in total brain volume and gray matter volume (Castellanos *et al*, 2002). In particular, the prefrontal cortex, basal ganglia and cerebellum have been found to be reduced in volume- at the group level (Faraone *et al*, 2015; Greven *et al*, 2015). From a developmental perspective, studies suggest that children with ADHD may show a delay in brain development (Shaw *et al*, 2007). Functional MRI studies have mainly shown decreases in activity in areas of interest during task-performance. For example, hypo-activity has often been reported in frontoparietal executive networks, the inferior frontal gyrus as well as in the ventral attention network (Rubia *et al*, 1999; Rubia *et al*, 2005), in tasks to assess brain activity related to behavioral control. The ability to exert control over ones behavior has been suggested to be among the core problems in ADHD (Barkley, 1997), and can be quantitatively assessed using neuropsychological tasks such as a stop signal task (Logan *et al*, 1984). The stop signal task is a continuous performance task in which subjects occasionally have to stop their (motor-) response when instructed to do so (see also chapter 4, in which a stop task in combination with fMRI is used). Problems in behavioral control can involve both proactive (persistent anticipation of the occasional need to interrupt the on-going task) and reactive mechanisms (responding to the external interrupt signal only after it has occurred) (Aron, 2011). In addition to problems with behavioral control, individuals with ADHD often have difficulties with reward processing and timing (Durston *et al*, 2011; van Hulst *et al*, 2015). Known environmental risk factors for the development of ADHD include alcohol- and cigarette use of the mother during pregnancy, low birth weight of the child, and preterm birth (the previous examples possibly being related) (Banerjee *et al*, 2007).

- *TSC*

Tuberous sclerosis complex differs from idiopathic ASD in that it has a known cause, although its phenotypical presentation remains heterogeneous (Curatolo *et al*, 2013; Harrison and Bolton, 1997; Northrup *et al*, 1993). TSC is caused by a mutation in either the TSC1 or TSC2 gene (tumor suppressor genes), leading to dysregulation of the mTOR pathway (mammalian target of rapamycin: important for cell proliferation and growth) and subsequently abnormal cell proliferation and tumor growth in the brain and many other organs (Curatolo *et al*, 2015; Northrup *et al*, 1993). TSC can be studied through animal models, and as the occurrence of ASD symptoms in TSC is significant, this can possibly yield information about ASD pathophysiology as well. in structural magnetic resonance imaging studies brain lesions have been reported in TSC, including tubers and white matter abnormalities. Studies investigating the neurobiology of TSC have mainly focused on epilepsy and

brain lesions, and less on the relationship with neuropsychiatric difficulties. Also, as will be further described in chapter 4, chloride homeostasis is suggested to be disrupted in and around tubers in TSC. Hence, this could possibly lead to (focal) depolarizing GABA activity, causing GABA to act excitatory as opposed to what is typically the case, inhibitory. Consequently, this could cause an altered balance between excitation and inhibition (E/I) in these regions (Talos *et al*, 2012). This further suggests that there may be similarities in the biology of TSC and ASD.

In sum, the biology of developmental disorders is as complex as the phenotypes themselves. Several decades of biological research has greatly expanded our knowledge, yet treatments are still largely not informed by knowledge of biological changes at an individual level.

Available treatments for developmental disorders

ASD

At present, no medication exists to treat the core symptoms of ASD. All medication prescribed to individuals with ASD targets comorbid symptoms, such as anxiety, hyperactivity, aggression or epilepsy. These medications include selective serotonin reuptake inhibitors (SSRIs) for anxiety, antipsychotics for irritability, stimulant medication (e.g. methylphenidate) for symptoms of hyperactivity and anti-epileptic drugs (AEDs) for comorbid epilepsy (Lai *et al*, 2014). The only FDA approved medications currently available for ASD are the atypical antipsychotics, risperidone and aripiprazole: both can be prescribed to treat irritability (McCracken *et al*, 2002; Owen *et al*, 2009). Although these treatments can definitely improve the quality of life for sufferers, they do not target the core symptoms of ASD, such as problems related to social communication or sensory issues. Furthermore, stimulants and antipsychotics might lower seizure threshold in individuals (Pisani *et al*, 2002), which can sometimes be a contra-indication for prescription. In addition to pharmacological treatments, also nonpharmacological options may aid in the improvement of daily life functioning. Early life behavioral intervention therapies are currently thought to be most promising treatment strategy in ASD. However, they are very intensive and expensive, and treatment outcomes vary greatly between individuals (Lai *et al*, 2014; Peters-Scheffer *et al*, 2012). Other nonpharmacological treatments include specific trainings to improve social skills or sensory integration therapies, although results regarding effectiveness are mixed (Watling and Hauer, 2015; Williams White *et al*, 2007).

ADHD

The main treatment of ADHD is through the stimulant methylphenidate (MPH) that improves symptoms in up to 80% of individuals (Spencer *et al*, 1996). However, 20-30% of children with ADHD do not or only partially respond to MPH. The working mechanism of MPH is thought to rely on inhibiting reuptake of dopamine through blockage of the dopamine transporter (DAT), as well as through acting on norepinephrine and serotonin, thereby improving catecholaminergic neurotransmission (Frolich *et al*, 2014). Besides MPH, the stimulant dexamphetamine can also be effective in ADHD, which has a similar mechanism of action (Solanto, 1998). Other pharmacological options include norepinephrine reuptake inhibitors (atomoxetine) and a2-adrenergic agonists (guanfacine and clonidine), although effect sizes are lower (Newcorn *et al*, 2008; Pliszka, 2007; Sallee *et al*, 2009). Besides, or in addition to pharmacological interventions, behavioral therapies can also improve quality of life for affected individuals, especially in academic performance and conduct problems (Faraone *et al*, 2006; MTA, 1999). In addition, it has been suggested that supplementation with Omega 3 fatty acid (add-on to MPH) may also improve symptoms of inattention in ADHD (Bos *et al*, 2015).

For individuals with TSC, as well as for patients with epilepsy, the focus of treatment is mostly on seizure control, meaning that behavioral problems often remain untreated (de Vries *et al*, 2015). However, co-morbid neuropsychiatric problems are very common and debilitating, and call for treatment (Curatolo *et al*, 2015). As TSC is a genetic disorder caused by mutation in either the TSC1 or TSC2 gene, it has a clearer neurobiology, and studies have been set up of more neurobiology-based treatments. For example, rapamycin is an mTOR pathway inhibitor, and is currently being tested as a possible treatment for a wide variety of neurological sequelae of TSC (de Vries, 2010). Although promising for seizure reduction, no clear effects on behavioral outcome have yet been reported. In ASD as well as TSC, the E/I balance is thought to be elevated, offering a possible treatment target for the chloride lowering diuretic bumetanide. Bumetanide is a selective chloride transporter (NKCC1) antagonist that may strengthen neural inhibition. Studies are still ongoing, but there is some evidence that bumetanide might lower hyperexcitability in patients and reduce behavioral symptoms related to sensory responsivity, one of the most debilitating symptoms in ASD as well as in TSC (Lemonnier *et al*, 2012; Lemonnier *et al*, 2017; Talos *et al*, 2012). See also chapter 5 of this thesis.

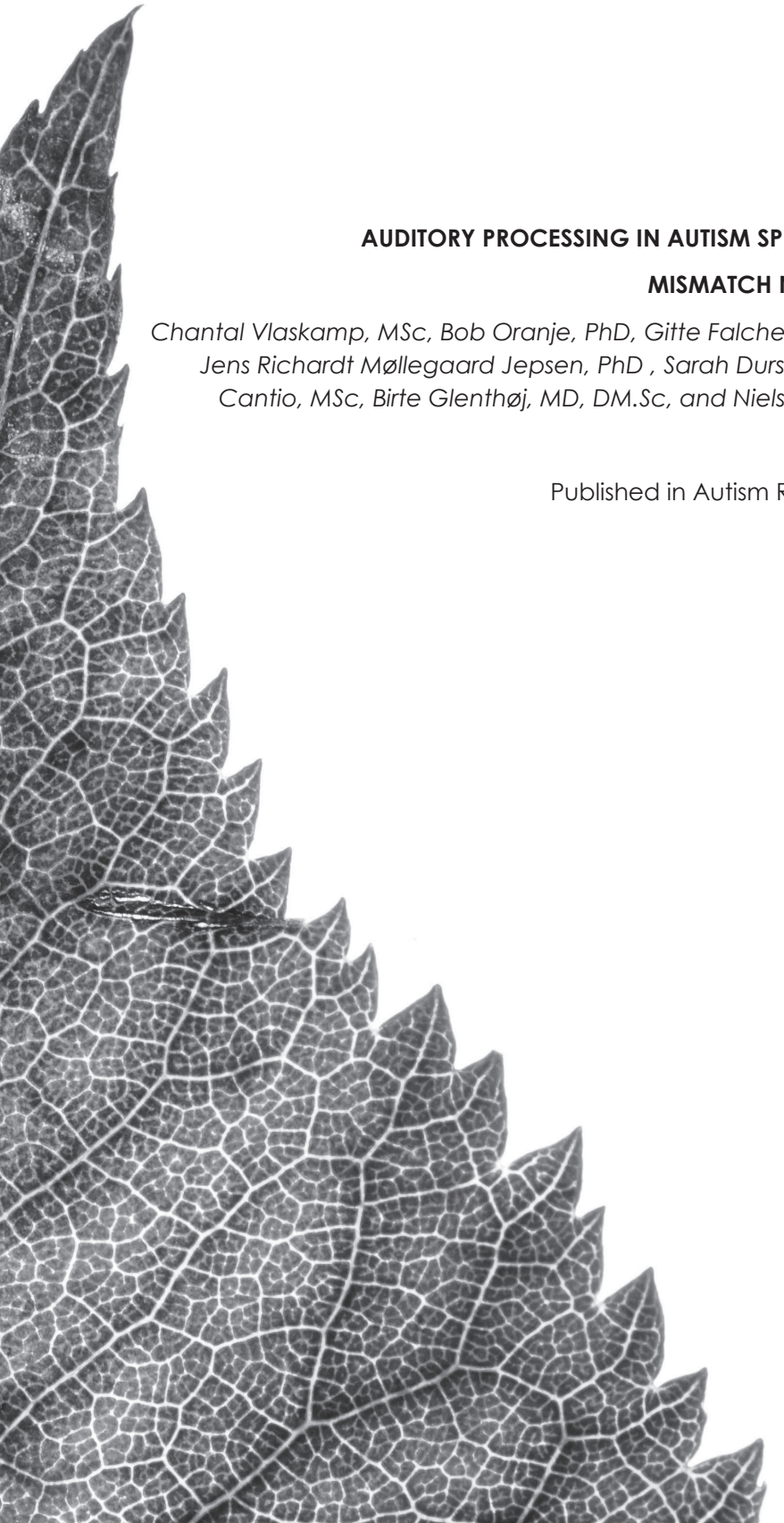
Outline of this thesis

Developmental disorders are diagnosed based on behavioral symptoms only. As such, individuals with ASD or ADHD are currently included under one diagnostic umbrella, even though the changes in neurobiology associated with their symptoms may vary widely. Therefore pharmacological treatments may be most effective if they are more targeted at the neurobiology associated with symptoms within a particular individual.

In this thesis we explore the variability in neurobiology in autism spectrum disorder, epilepsy and TSC, and ADHD. We use several methodologies, such as electrophysiology (i.e. EEG/ERPs), fMRI and measures of cognition and behavior, to investigate the relation between neurobiological heterogeneity and aim to tie it to treatment options.

Auditory sensory processing has been suggested to be different in children with ASD, as evidenced by behavior as well as electrophysiology. **In chapter 2,** we investigate (automatic) auditory processing in a group of children with and without ASD, using mismatch negativity (MMN) with three types of deviants. **In chapter 3,** we further explore auditory processing in ASD. Here we use a large electrophysiological test battery including multiple ERPs, and we split our ASD cohort based on comorbid epilepsy. In addition, we focus on the relationship with sensory issues.

In chapter 4 we explore the neural correlates of methylphenidate response in a sample of children with ADHD. In this within-subject design, we link fMRI and ERP measures to behavioral strategy to investigate variability in methylphenidate response, given that MPH is usually ineffective in approximately 20% of cases. **Chapter 5** describes a case study, where a young woman with tuberous sclerosis complex (TSC) and behavioral problems was treated with the diuretic bumetanide, and effects were assessed at the behavioral and electrophysiological level. Finally, in the summary and general discussion (**chapter 6**) we summarize and discuss the main findings in this thesis, as well as the relevance and potential clinical utility of our approaches.



2

AUDITORY PROCESSING IN AUTISM SPECTRUM DISORDERS: MISMATCH NEGATIVITY DEFICITS

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AUDITORY PROCESSING IN AUTISM SPECTRUM DISORDERS: MISMATCH NEGATIVITY DEFICITS

Abstract

Background. Children with autism spectrum disorders (ASD) often show changes in (automatic) auditory processing. Electrophysiology provides a method to study auditory processing, by investigating event-related potentials (ERPs) such as mismatch negativity (MMN) and P3a amplitude. However, findings on MMN in autism are highly inconsistent, partly due to small sample sizes in the studies and differences in MMN paradigms. Therefore, in the current study, MMN and P3a amplitude were assessed in a relatively large sample of children with ASD, using a more extensive MMN paradigm and compared with that of typically developing children (TDC).

Methods. Thirty-five children (aged 8-12 y) with ASD and 38 age and gender matched typically developing children (TDC) were assessed with a MMN paradigm with three types of deviants, i.e. frequency, duration and a combination of these two.

Results. MMN elicited by duration and frequency-duration deviants was significantly reduced in the ASD group. P3a amplitude elicited by duration deviants was significantly increased in the ASD group.

Conclusions. Differences in MMN were found in children with ASD. This suggests that children with ASD may be less responsive to environmentally deviant stimuli at an early (sensory) level. P3a amplitude was increased in ASD, implying a hyper-responsivity at the attentional level. In addition, as similar MMN deficits are found in schizophrenia, these results may explain some of the frequently reported increased risk of children with ASD to develop schizophrenia later in life.

Introduction

Autism spectrum disorders (ASD) are neurodevelopmental disorders mainly characterized by deficits in social interaction and communication, repetitive behaviour, restricted interests and deficits in adaptive functioning (American Psychiatric Association, 2000). Besides, there is increasing evidence suggesting that children – as well as adults – with autism have changes in sensory processing, especially in the auditory domain (Dunn, Gomes, & Gravel, 2008). Individuals with ASD tend to show atypical behaviour in response to auditory stimuli, e.g. a preoccupation with background noise and hypo- or hypersensitivity to certain sounds (Ludlow et al., 2014). However, the exact mechanism of this deviant auditory processing in autism is still unclear. One possibility to study auditory processing, or rather the preconscious automatic auditory processing, is by means of electrophysiology, e.g. by using a mismatch negativity (MMN) paradigm (Naatanen, 1995; Naatanen & Alho, 1995).

Mismatch negativity (MMN) is generally believed to reflect an automatic orienting reflex triggered in the brain by its detection of disturbances in an individual's direct environment. MMN is usually quantified with electroencephalography (EEG) in a so-called auditory oddball paradigm, where an occasional deviant sound (the oddball) is presented in a sequence of standard sounds. This deviant stimulus then elicits a negative deflection in the EEG that is known as MMN. Depending on the characteristics of the paradigm, MMN usually appears between 100 – 200ms following stimulus presentation, and often reaches maximum amplitude at fronto-central sites when assessed on the scalp (Naatanen, Paavilainen, Rinne, & Alho, 2007).

Most studies have investigated MMN in adults, in particular in schizophrenia: subjects with schizophrenia consistently show reductions in MMN amplitude compared to healthy controls (Perez et al., 2014; Todd et al., 2008a; Witten et al., 2014; Rydkjaer et al., 2017). However, there are also studies showing that MMN can be elicited and reliably assessed in children with autism spectrum disorder (Cheour, Korpilahti, Martynova, & Lang, 2001; Dunn et al., 2008; Uwer & von, 2000; Gomot, Giard, Roux, Barthelemy, & Bruneau, 2000). This is interesting, as many studies report a clinical and biological overlap between autism and schizophrenia, such as deficits in social communication, withdrawn behaviour, and reduced performance on theory of mind tasks (Rapoport, Chavez, Greenstein, Addington, & Gogtay, 2009; King & Lord, 2011; Unenge, Lugnegard, & Gillberg, 2012). Also, children with ASD are known for having higher risks of developing schizophrenia later in life (Chisholm, Lin, Abu-Akel, &

Wood, 2015). Findings on MMN in children with autism are highly inconsistent (Dunn et al., 2008; Naatanen et al., 2007). Some studies report larger MMN and/or shorter latencies in children with autism (Ferri et al., 2003; Kujala et al., 2007), whereas other studies report smaller or even normal MMN and/or longer latencies in children with ASD compared to age-matched typically developing children (TDC) (Dunn et al., 2008; Jansson-Verkasalo et al., 2003; Roberts et al., 2011; Seri, Cerquiglini, Pisani, & Curatolo, 1999). In part, these inconsistencies can be explained by differences in methodology: some studies used visual MMN paradigms, other studies used (auditory) verbal standard and deviant stimuli, whereas yet other studies used either pitch, frequency or duration deviants (Kemner, Verbaten, Cuperus, Camfferman, & van, 1994). In addition to these methodological differences, the heterogeneity of autism spectrum disorders has most likely also contributed to these inconsistencies (Sinclair, Oranje, Razak, Siegel, & Schmid, 2016), especially in studies using smaller sample sizes.

Another measure of attention that is often assessed simultaneously with MMN nowadays is the P3a amplitude, a positive deflection in the EEG succeeding MMN, following presentation of a deviant stimulus. P3a amplitude is thought to reflect an involuntary attention switch. Similar to MMN, the P3a amplitude has frequently been found to be altered in individuals with autism (Ferri et al., 2003; Lepisto et al., 2006), as well as in schizophrenia (Jahshan et al., 2012; Kaur et al., 2011). However, results concerning P3a findings in these populations remain inconsistent.

In this study, we investigated differences in MMN and P3a amplitude between children with ASD and typically developing children (TDC). In addition, we explored the relation between ERP data and symptomatology. Our MMN paradigm contained three types of deviants: a frequency deviant, a duration deviant and a combination of the two. Since there is evidence that frequency and duration based MMN are differentially affected in other disorders, such as schizophrenia, we used them both in our paradigm, as well as the combination to increase the discriminative power and to obtain more insight in sensory processing (Todd et al., 2008b). Paradigms using such a combination of deviants have mostly been used with adult clinical populations (Oranje, Jensen, Wienberg, & Glenthoj, 2008a) and have not yet been used with children. Given the before mentioned overlap between schizophrenia and autism, we expected to find similar changes in MMN for our sample of ASD children as are typically found in individuals with schizophrenia.

Methods

The study was approved by the Ethical Committee of the Region of Southern Denmark (S-20090071). Parents of the participants were informed orally and in writing before signing a written informed consent form.

Participants

A total of 35 patients with ASD (autistic disorder, N=11; Asperger's syndrome, N=7; PDD NOS, N= 17) and 40 typically developing children aged 8 to 12 were included in the study. All subjects were matched for age, gender, IQ and parental socioeconomic status.

Children with ASD were recruited from two child and adolescent mental health services and schools with special education classes for children with ASD in the region of Southern Denmark. ASD diagnoses were confirmed according to the DSM-IV-TR criteria by means of the Autism Diagnostic Observation Schedule (ADOS), the Autism Diagnostic Interview-Revised (ADI-R), and information from the children's hospital files. The intelligence level of all (TDC and ASD) children was assessed using the Wechsler Intelligence Scale for Children, Third edition, Danish edition (2004).

Six ASD children (all boys) were medicated. Two were medicated with neuroleptics, three with central stimulants, and one with both neuroleptics and a selective serotonin reuptake inhibitor (SSRI). Medicated ASD children had a washout period of 24 hours before electrophysiological testing.

TDC were recruited from four state schools through the schools' websites. Both patients and controls were screened for general psychopathology and autistic features, using three parent-report questionnaires: the Child Behaviour Checklist (CBCL), the Social Communication Questionnaire (SCQ), and the Social Responsiveness Scale (SRS). In addition, teachers of the participants were also asked to fill in the SCQ and SRS. TDC were excluded if they had a history of psychiatric illness, SCQ or SRS scores above cut-off for ASD screening (SRS total *T*-score >59 or SCQ total score >15), or a CBCL subscore >1 SD above mean score of the general population (as an indication of possible psychiatric morbidity). Exclusion criteria for both groups included a history of organic brain disorder (e.g. epilepsy), significant head injury, history of schizophrenia in first-degree relatives, puberty, and impaired hearing. Prepubertal status was confirmed by information from parents and children regarding pubic and axillary hair growth, and for girls also menarche and breast development. Before EEG assessment, subjects were tested for hearing deficits at 500, 1000, and 6000Hz (40dB). All participants had good

language comprehension and no problems in understanding the instructions for the test. See Table 1 for the participants' characteristics. (Copied and adjusted from Madsen et al, 2014, with approval)

MMN paradigm

All participants were assessed in the same physiological test battery, the CPTB (Copenhagen psychophysiological test battery), which among other paradigms, includes a paradigm to assess MMN. To keep a focus, the current paper will only present the data on MMN, the other CPTB data will be, or already has been, published elsewhere (Madsen, Bilenberg, Cantio, & Oranje, 2014; Madsen et al., 2015a).

The procedures concerning MMN assessment as used in the current study were identical to the ones previously described by During et al (2014) (During, Glenthoj, Andersen, & Oranje, 2014). In short, subjects were seated in a comfortable armchair in a sound-insulated room (sound reduction level of 40dB) situated next to the control room. They were instructed to sit still, watch a movie (muted documentary on animals) and to ignore the sounds they were presented.

The MMN paradigm consisted of 1800 stimuli. All stimuli were presented binaurally through tubal insert ear phones (EARtone®, Etymotic Research), by a computer running Presentation® software (Neurobehavioral Systems Inc.). The paradigm consisted of 4 stimuli: In 83% of the cases, a standard tone with a frequency of 1000Hz, intensity of 75dB and duration of 50ms was presented. Within this sequence of standard stimuli, three types of deviants were presented, each with a probability of 6% and intensity of 75dB: Frequency deviants of 1200Hz and 50ms, duration deviants of 1000Hz and 100ms and frequency-duration deviants of 1200Hz and 100ms. The interstimulus interval (ISI) was randomized between 300 and 500ms. The total duration of the MMN task was approximately 15 minutes.

Signal recording and processing

The EEG signal was recorded using a BioSemi® system with 64 active-two electrodes, according to the 10-20 system. In addition to these 64 electrodes, electrodes were placed on both mastoids for reference purposes.

Analysis and processing of the EEG signal was carried out using Brain Electrical Source Analysis (BESA) software (version 5.2.4, MEGIS Software GmbH, Gräfelfing, Germany). Only data from relevant electrodes were processed and analysed; i.e. where MMN was expected to reach maximum amplitude: the frontal/midline electrodes Fz, FCz and Cz (van et al., 2010; Wienberg,

Glenthoj, Jensen, & Oranje, 2010; Oranje, Jensen, Wienberg, & Glenthoj, 2008b).

Processing of the data started with resampling from the original 4kHz to 250Hz, to allow easier file handling. Second, the data were corrected for eye-artefacts by using the adaptive method of BESA. Third, the data were epoched (from 100ms prestimulus to 900ms poststimulus) and corrected for movement (or other paradigm unrelated) artefacts, by removing those epochs from the database that contained amplitude differences between maximum and minimum exceeding 75μV, in the for MMN and P3a relevant scoring windows (see below). Subsequently, the data were band-pass filtered (high-pass: 0.5Hz, low-pass: 40Hz), after which MMN for each of the three deviant types was expressed as the average ERP to the relevant deviant stimuli, subtracted with the average ERP to standard stimuli for each subject separately. Linked mastoids were used as a reference. Finally, MMN amplitudes were scored as the minimum amplitude within a window between 75 and 300ms. P3a amplitudes were scored as the maximum amplitude in a window between 175 and 300ms. In addition, to consider possible contributions of ERP components of standard and deviant stimuli on MMN morphology, N2 and P2 ERPs on standard and deviant stimuli were also analysed, in a window between 200 - 300ms and 20ms - 180ms respectively.

Statistical analyses

All statistical analyses were carried out using SPSS Statistics version 20.0 (SPSS Inc., USA). All MMN amplitude data were normally distributed (Kolmogorov-Smirnov test). Initially, the data were analysed by a repeated measures analysis of variance and multiple analysis of variance (General linear model: MANOVA), with between factor "group" (TDC or patients) and within factor "deviant-type" (frequency, duration or frequency-duration deviant). Identical analyses were performed for the P2, N2 and P3a amplitude data, which were also normally distributed. Latency data of MMN and P3a were non-normally distributed and thus non-parametrically analysed (Mann Whitney U tests). Last, bivariate correlation analyses between MMN, P3a and symptomatology were carried out in the ASD group only.

Results

Participants

A total of 35 ASD patients and 40 TDC were assessed in the CPTB. However, two TDC did not complete the MMN paradigm and were therefore excluded from the analyses. All of the children in the ASD group completed the MMN paradigm. Consequently, 35 ASD subjects and 38 TDC were included for statistical analysis (see Table 1 for demographics).

Table 1. Demographic data of patients and controls recruited and analysed

	ASD patients	TDC	
	Recruited and analysed (MMN paradigm)	Recruited	Analysed (MMN paradigm)
Number of subjects (N)	35	40	38
Boys	28	28	27
Girls	7	12	11
Mean age (years (SD))			
All	11.1 (1.4)	10.8 (1.3)	10.9 (1.3)
Boys	11.1 (1.3	11.1 (1.2)	11.1 (1.2)
Girls	10.7 (1.6)	10.2 (1.5)	10.3 (1.5)
Total IQ (SD) (WISC-III)	98.5 (22.8)	107.5 (18.3)	107.6 (17.4)
Verbal IQ (SD)	97.5 (21.5)	108.6 (18.2)	108.8 (17.9)
Performance IQ (SD)	99.6 (20.0)	102.6 (16.3)	102.7 (15.5)
SCQ (Parents mean total score (SD))	14.8 (6.4)*	2.6 (2.1)*	2.7 (2.0)*
SCQ (School mean total score (SD))	13.4 (5.8)	6.3 (3.4)	6.3 (3.4)
SRS (Parents mean total score (SD))	88.9 (33.4)*	16.5 (13.0)*	16.8 (13.2)*
SRS (School mean total score (SD))	71.1 (27.8)	16.8 (13.1)	16.8 (13.1)
CBCL (mean total score (SD))	61.3 (25.6)*	13.9 (11.1)*	14.3 (11.2)*
ADI-R (mean total score (SD))	32.1 (9.7)	-	-

*ASD= autism spectrum disorder, TDC= typically developing children, MMN= mismatch negativity, SCQ= social communication questionnaire, SRS= social responsiveness scale, CBCL= child's behaviour checklist, ADI-R= Autism diagnostic interview- revised. *Significant difference between ASD patients and TDC (p<.001)*

MMN data

All of the participants showed clear mismatch negativity, as was indicated by all amplitude data being significantly different from the intercept.

Repeated measures analyses revealed a main effect of group [$F(1,71)= 7.16$, $p= .009$, $\eta^2= .092$] and a main effect of electrode (i.e. Fz, FCz, Cz): [$F(2,142)= 5.21$, $p= .007$, $\eta^2= .068$]. In addition, a first order interaction was found between type of deviant and group [$F(2,142)= 3.79$, $p= .025$, $\eta^2= .051$]. Further, a second order interaction was found between type of deviant, electrode and group that reached trend level significance [$F(4,284)= 2.15$, $p= .075$, $\eta^2= .029$]. Next, we split the data by type of deviant, i.e. frequency (FreqMMN), duration (DurMMN), and frequency-duration (FreqDurMMN). As MMN amplitude reached maximum for all deviant types at electrode FCz, we performed all further analyses on FCz data only (see also Figure 1).

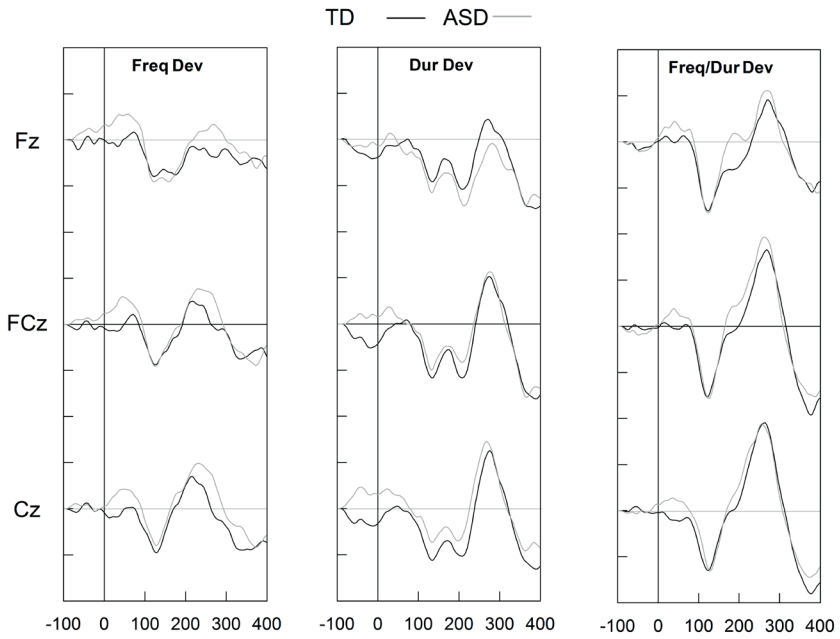

Figure 1: Grand averages for both patients and TDC, specified for each electrode (Fz, FCz and Cz) and for each deviant (frequency, duration and their combination). The individually scored data showed significantly reduced DurMMN and FreqDurMMN amplitude in patients, compared to the controls. Vertical spacing indicates steps of 1μV, positivity is illustrated upwards.

Multiple analyses of variance revealed no significant effect of group (TDC vs. ASD) on FreqMMN. However, analyses of DurMMN as well as FreqDurMMN showed a main effect of group, [F(1,71)= 9.01, *p*=.004, *d*= .704, η^2=.113] and [*F*(1,71)= 7.29, *p*=.009, *d*= .631, η^2=.009] respectively, indicating less MMN to these deviants in the ASD children than in the TDC (see also Figure 2).

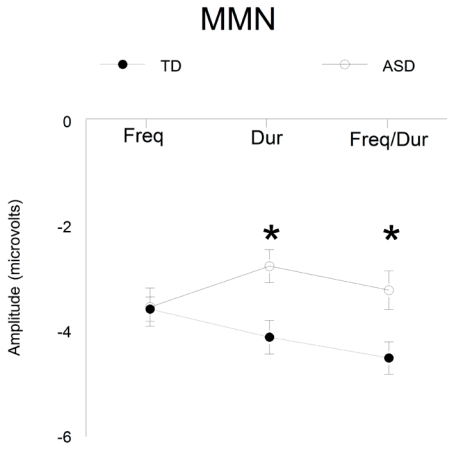

Figure 2: MMN visualized per deviant and group, illustrating significant differences between TDC and ASD. Vertical bars depict ±SEM

Non-parametric analysis of MMN latencies revealed no significant differences between ASD and TD control subjects (*p*>.05).

ERP components (N2 and P2), measured at FCz, on standard stimuli did not significantly differ between groups, whereas N2 amplitudes to Duration and Frequency-duration deviant stimuli did ([F(1,70)= 9.438, *p*= .003, η^2= .119] and [F(1,70)= 5.926, *p*= .017, η^2= .078], respectively).

P3a data

Similar as for MMN, P3a amplitude reached maximum amplitude at the frontocentral electrode (FCz) in both groups, further P3a analyses were therefore performed on FCz data only. Repeated measures analyses showed a main effect of type of deviant [F(2, 142)= 20.05, *p*= .000, □^{2}= .220]. Multiple analyses of variance showed an effect of group (TDC vs ASD) on P3a for

duration deviants (DurP3a) only [F(1, 70)= 7.85, *p*= .007, *d*= .656, η^2= .100], indicating larger P3a in the ASD group, but not for frequency (FreqP3a) or frequency-duration (FreqdurP3a) deviants (See Table 2).

Non parametric analysis of P3a latency revealed a significant group difference on FreqDurP3a only (U= 487.5, *p*= .05, *d*= .367).

Table 2. Mean MMN and P3a latencies and amplitudes specified per group at electrode FCz

	Group			
	TDC (N=38)		ASD (N= 35)	
Deviant	Mean latency ms (SD)	Mean amplitude µV (SD)	Mean latency ms (SD)	Mean amplitude µV (SD)
FreqMMN	159.79 (47.88)	-3.60 (1.45)	160.69 (50.33)	-3.56 (2.15)
DurMMN	169.68 (45.78)	-4.13 (1.98)	159.66 (55.19)	-2.78 (1.86)*
FreqDurMMN	155.16 (38.29)	-4.53 (1.91)	158.29 (47.64)	-3.24 (2.18)*
FreqP3a	247.47 (39.63)	2.25 (2.26)	245.60 (39.04)	2.63 (1.81)
DurP3a	266.32 (33.50)	2.16 (1.87)	258.40 (37.71)	3.40 (1.91)*
FreqDurP3a	257.37 (34.84)	2.64 (2.38)	245.26 (31.03)*	3.95(2.40)

**= significantly different from TDC (P<.05)*

For illustrative purposes we have computed combinations of MMN and P3a amplitude in our ASD sample in pie charts, also to partly display the heterogeneity of the patient population. See supplementary figure 1.

Correlations

First, as studies report MMN and P3a to reflect either dependent (Naatanen, Kujala, & Winkler, 2011) or independent (Van der Molen et al., 2012) processes, correlation analyses were carried out between these two ERP components. In the whole group (ASD +TDC), a positive correlation between Frequency MMN and P3a and Duration MMN and P3a was found (r= .253, N= 73, *p*= .031 and r= .355, N= 73, *p*= .002, respectively). This correlation was not present for the groups separately.

Associations between psychophysiological measures and symptomatology were only explored in the ASD group. Bivariate correlations showed a

significant negative correlation between the SRS School total score, SRS school mannerism and BRIEF school initiation and DurMMN amplitude (r= -.362, n=35, *p*= .035, r= -.438, n=34, *p*= .010 and r= -.353, n=35, *p*= .037 respectively), a negative correlation (point-biserial) between positive scores on the thought problem scale of the CBCL (dichotomized: cut-off 10 points) and DurMMN amplitude (r_s=-.338, n=35, *p*=.047) and a negative correlation between ADI social interaction, SCQ Parents stereotyped behaviour, SRS School social motivation, SRS school mannerism and FreqdurMMN (r= -.366, n=35, *p*= .031, r=-.370, n=35, *p*= .029, r=-.354, n=34, *p*= .04, r=-.416, n=34, *p*=.015, respectively). In contrast, a positive correlation between BRIEF School initiation and FreqMMN was found (r= .356, n=35, *p*= .036)

In addition, FreqP3a negatively correlated with the BRIEF School flexibility score (r= -.338, n=35, *p*= .049). Last, FreqdurP3a correlated positively with the SCQ School communication and total score (r= .514, n=30, *p*= .004 and r= .410, n= 30, *p*= .027, respectively).

For a summarizing correlation matrix, see supplementary table 1.

Discussion

Processing of sensory stimuli is frequently reported to be atypical in autism spectrum disorders (ASD). In the current study we investigated auditory mismatch negativity (MMN) and P3a amplitude in a cohort of children with ASD and typically developing children (TDC). Using a MMN paradigm with multiple types of deviants, both in frequency, duration and their combination, the discriminative power of this study was expected to be higher than studies using only one type of deviant.

Mismatch negativity reached maximum amplitude in the frontocentral region of the brain (at electrode FCz). We found smaller MMN amplitudes triggered by duration (DurMMN) and frequency-duration deviants (FreqDurMMN) in our children with ASD compared to the TDC. Groups did not significantly differ in ERP responses on standard stimuli, indicating true hypo-responsiveness and not a side-effect caused by general amplitude reduction. Smaller MMN amplitude in ASD children indicates a less accurate automatic orienting reflex to deviancy compared to TDC, a finding that is consistent with earlier literature (Dunn et al., 2008; Ludlow et al., 2014). Surprisingly, we found no group differences triggered by frequency deviants (FreqMMN), whereas other studies did (Ferri et al., 2003; Kujala et al., 2010; Lepisto et al., 2005). Smaller MMN amplitudes may indicate a hyporesponsivity to auditory stimuli.

As Guiraud and colleagues (2011) and Baranek and colleagues (2013) have proposed, hyporesponsiveness to (social/non-social) sensory stimuli might be more characteristic for ASD than hyperresponsiveness (Baranek et al., 2013; Guiraud et al., 2011). Furthermore, hyporesponsiveness has been suggested to be associated with lower levels of social communication, a trait frequently reported in ASD (Baranek et al., 2013; Liss, Saulnier, Fein, & Kinsbourne, 2006), as well as in schizophrenia (Wible, 2012; Dickinson, Bellack, & Gold, 2007).

In addition, the fact that we found smaller MMN amplitudes in the duration and frequency-duration deviant trials but not in the frequency deviant trials in our ASD patients may indicate a deficit in encoding temporal (i.e. in time) auditory processing or temporal discrimination (Todd et al., 2008a). Indeed, data of studies investigating auditory processing in children with autism support abnormal (diminished) temporal processing (Szelag, Kowalska, Galkowski, & Poppel, 2004; Maister & Plaisted-Grant, 2011). Interestingly, temporal processing deficits are also found in adult populations of individuals with schizophrenia (Todd, Michie, & Jablensky, 2003; Michie, 2001; Michie et al., 2000a). Also similar to our current findings, patients with schizophrenia most consistently show MMN deficits, in particular with duration and frequency-duration type of deviants (Michie et al., 2000b; Todd et al., 2008a). This further supports the theory that children with ASD may be susceptible for developing schizophrenia later in life. Follow up on these children would therefore be very interesting.

P3a amplitude data showed significant between-group differences in the duration deviant (DurP3a), demonstrating larger DurP3a amplitudes in ASD compared to TDC. In contrast to our data, other reports show decreased P3a amplitude in subjects with ASD (Ceponiene et al., 2003; Donkers et al., 2015; Dunn et al., 2008; Lepisto et al., 2005; Marco, Hinkley, Hill, & Nagarajan, 2011). However, Lepisto and colleagues (2005) did not find an attenuated P3a amplitude when triggered by duration deviants in ASD (Lepisto et al., 2005) and Kujala and colleagues (2007) did not find any group effects on P3a amplitude at all (Kujala et al., 2007). These inconsistencies in the literature might be due to differences in sample characteristics, such as age and sub-diagnoses. Since P3a amplitude is believed to reflect involuntary orienting or attention to stimuli, our data might suggest that children with ASD also have some issues at the attentional level, in addition to the deficits at the sensory (MMN) level. The counterintuitive reduction of MMN amplitude in combination with an increased P3a amplitude in our ASD group might be explained by a compensatory mechanism which decreases MMN. Yet even despite this lowered automatic discrimination (MMN), subjects still show a

hyper-responsivity to deviant sounds, possibly also making these children more distractible in daily life. Furthermore, the reduction of MMN amplitude in combination with an increased P3a amplitude in our ASD group suggests that MMN and P3a are partly independent processes, consistent with the earlier suggestions of van der Molen et al (2012). We found negative correlations between DurMMN amplitude and scores on social responsiveness (SRS total and mannerism score), initiation (BRIEF) and thought problems (CBCL), and between FreqDurMMN and the social interaction score of the ADI-R, social motivation and mannerism of the SRS and stereotyped behaviour measured by SCQ. In contrast, FreqMMN positively correlated with initiation (BRIEF). Similarly, Todd and colleagues (2008) reported an association between symptom severity of schizophrenia and MMN amplitude, further supporting the usefulness of investigating MMN in relation to symptom severity. However, correlations between symptom severity and MMN amplitude mainly revealed that the presence of more severe symptoms correlated with larger MMN amplitudes in our ASD group. One possible explanation could be that ASD children who show larger (meaning less from TD children deviating) MMN amplitude, have more sensitive automatic discrimination and therefore show hyper-responsiveness to deviant sounds. For example, the ASD children who experienced more (social) problems could also have had more difficulties with ignoring the presented sounds in spite of the fact that a video was presented as distraction, and therefore responded more to the stimuli from the paradigm than children with less problems.

Lastly, our analyses showed a positive correlation between the SCQ school communication and SCQ school total scale and FreqdurP3a amplitude in our ASD participants, indicating larger P3a amplitudes when more (social communication) problems are present. A possible explanation for this association could be that children with higher scores on the SCQ are also more hyper-responsive and distractible, similar to the findings regarding MMN amplitude and symptom severity. In contrast, FreqP3a correlated negatively with scores of the BRIEF school flexibility scale, implying that lower scores and thus better flexibility as measured by the BRIEF, is associated with a higher FreqP3a amplitude. This might suggest an increased distractibility in children with ASD that are more flexible.

A strength of our study was the relatively large number of phenotypically well described subjects. A further strength was our MMN paradigm, where the multiple types of deviants allowed a more precise indication of the processes behind the observed deficits. Previously, we reported increased sensitization in this same ASD cohort (Madsen et al., 2014), as well as attenuated P50

amplitude in the children with Asperger's syndrome (Madsen et al., 2015b). Furthermore, other studies from our laboratory have indicated the usefulness of our CPTB battery for studying schizophrenia, by showing differences between antipsychotic naïve, first-episode patients with this disorder and healthy controls (During et al., 2014; Oranje & Glenthoj, 2014).

In conclusion, we found reduced MMN amplitude in children with ASD compared to TDC. In addition, we found an association between MMN amplitude and severity of behavioural problems in our ASD subjects. Also, we found an increase in P3a amplitude for duration deviants in children with ASD. Therefore, we conclude that children with ASD might be less responsive to environmentally deviant stimuli on an (early) sensory level, but also show issues on attentional levels. In addition, as similar deficient MMN is also frequently reported in schizophrenia, our results may explain some of the increased risk of children with ASD to develop schizophrenia later in life.

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Conflict of interest

The authors declare that they have no (financial) conflict of interest.

Ethical standards

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

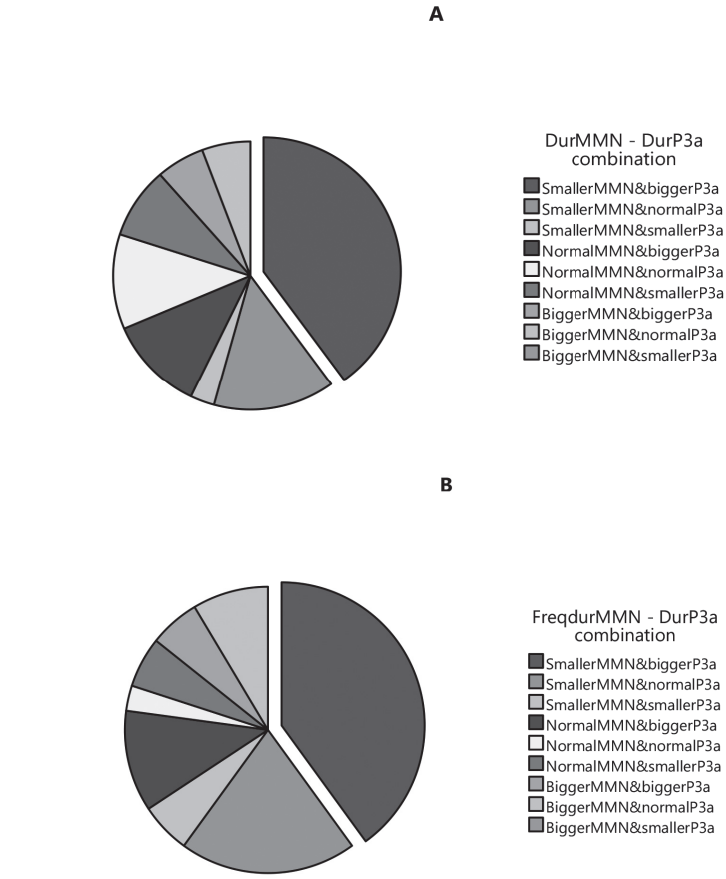
Supplementary material – chapter 2

Supplementary table 1. Correlation matrix of ERP data with behavioural data

	FreqMMN	DurMMN	FreqDur-MMN	FreqP3a	DurP3a	FreqDurP3a
Thought problems CBCL	-.008	-.338*	-.218	.315	.008	.158
ADI social interaction	.181	-.172	-.366*	.108	-.069	.153
SCQ Stereotyped behaviour (Parents)	-.087	-.317	-.370*	.126	-.118	-.064
SCQ Communication (School)	-.024	.131	-.140	.302	.262	.514**
SCQ Total (School)	-.058	-.128	-.314	.277	.131	.410*
SRS Social motivation (School)	-.232	-.332	-.354*	-.024	-.132	.183
SRS Mannerism (School)	-.280	-.438**	-.416*	-.103	-.228	-.015
SRS Total score (School)	-.279	-.362*	-.334	-.087	-.188	.171
BRIEF Planning (Parents)	.356*	-.021	.073	.035	.051	-.198
BRIEF Flexibility (School)	-.114	-.094	.191	-.338*	.190	-.280
BRIEF Initiation (School)	-.183	-.353*	-.167	-.205	-.051	-.144

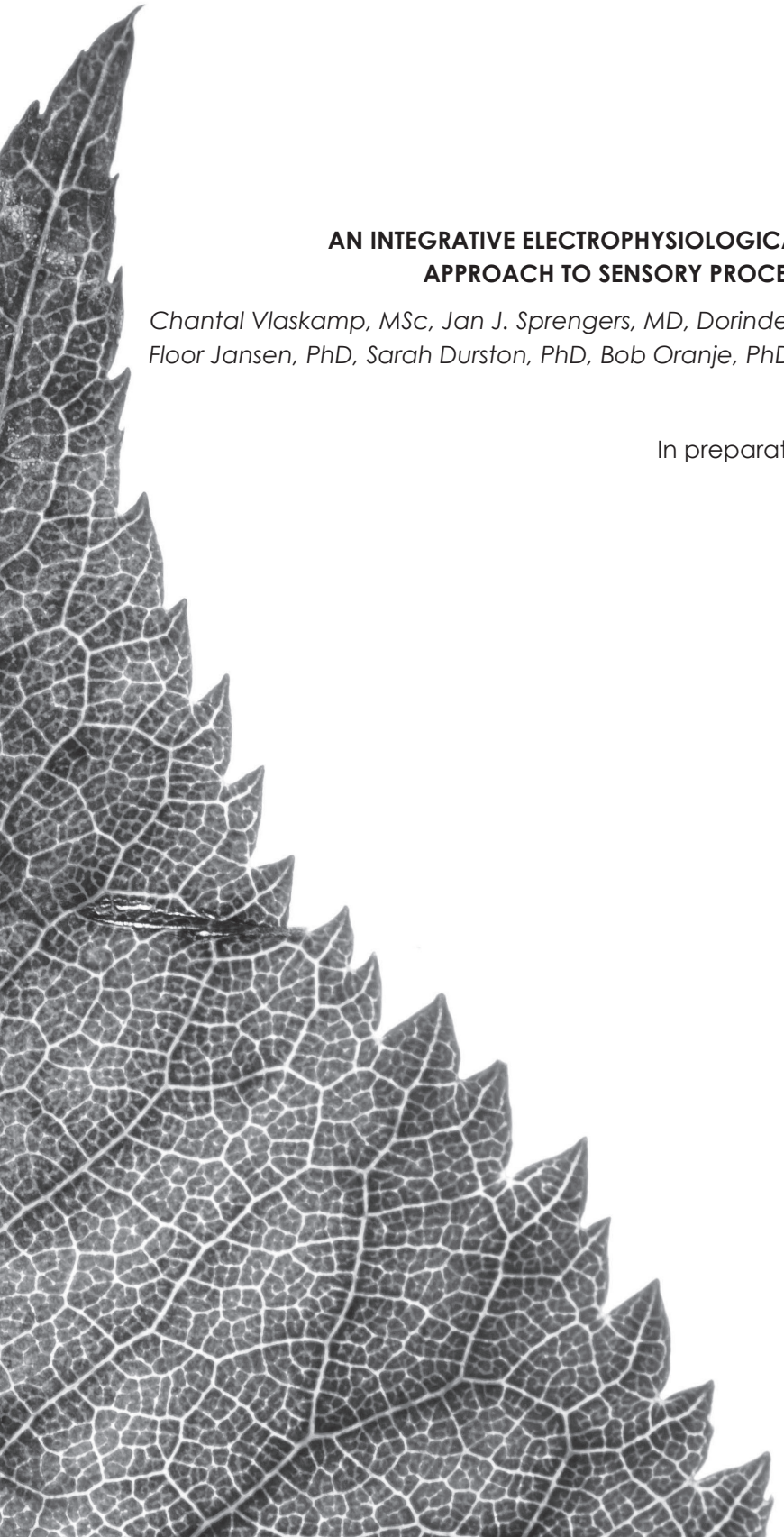
**= significantly different p<.05, **= significantly different p=.001*

Supplementary figure 1



Pie charts illustrating the distribution of combinations of MMN and P3a directions (i.e. smaller, normal and bigger than typically developing controls), hereby illustrating the heterogeneity of the ASD sample. 'Normal' is defined as a Z-value between -.5 and .5.

A: Combinations of duration MMN and duration P3a. The chart illustrates that 40% of all ASD patients had lower DurMMN and higher DurP3a than TD controls. **B:** Combinations of Frequencyduration MMN and duration P3a. The chart illustrates that 40% of all ASD patients had lower FreqDurMMN and higher DurP3a than TD controls.



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AN INTEGRATIVE ELECTROPHYSIOLOGICAL AND BEHAVIORAL APPROACH TO SENSORY PROCESSING ISSUES IN ASD

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Abstract

Issues with sensory processing are a core problem for many individuals with autism spectrum disorder (ASD), as evidenced by behavioral sensory sensitivities and neurological problems such as comorbid epilepsy. However, to date studies have struggled to relate behavioral symptoms to neurological correlates of sensory processing. Here, we undertook a comprehensive approach to overcome this struggle by investigating associations between sensory problems and a broad range of electrophysiological parameters of basic sensory processing in a population of children with ASD with sensory issues, with and without epilepsy.

We assessed children with ASD with and without epilepsy (n=20 and n=57 respectively, age range 7-15 years), as well as 36 age matched typically developing controls using a comprehensive battery of electrophysiological tests and behavioral measures.

The combined ASD sample had reduced P3a and P3b amplitude and less accurate deviancy detection in the selective attention paradigm. Individuals with ASD and epilepsy had enhanced frequency-based MMN amplitude compared to individuals with ASD without epilepsy and typically developing controls. In addition, multiple regression analyses suggested a possible segregation between ASD subgroups with and without epilepsy based on combined frequency MMN and P3a amplitude. Last, we found several associations between electrophysiological measures and sensory issues.

Our results suggest an effect of comorbid epilepsy on early sensory processing in ASD. These findings may be useful for detecting seizure susceptibility in individuals with ASD who have not yet presented with overt signs of epilepsy. We demonstrate that by using an integrative approach, where multiple electrophysiological measures are combined with behavioral measures, associations between physiology and behavior in the autism spectrum can be detected.

Introduction

There is a broad consensus that autism spectrum disorder (ASD) has neurodevelopmental underpinnings, yet the disorder is clinically diagnosed based on a broad range of behavioral symptoms. As a consequence, many different behavioral phenotypes are grouped under the same diagnostic umbrella (Geschwind, 2009). Conversely, it has been shown that similar phenotypes can be related to different aetiologies (Auerbach *et al*, 2011). Indeed, it has been argued that a clinical framework based solely on behavioral manifestation has limited promise for biological stratification and has created a deadlock in efforts to develop more individualized treatments (Waterhouse and Gillberg, 2014).

Ever since Kanner's initial report on autism in 1943 (Kanner, 1943), evidence has been accumulating of neurological involvement in ASD, including reports of seizures and problems with sensory processing. Focal background and epileptiform abnormalities are frequently found in electroencephalograms (EEGs) of children with ASD, suggesting changes in brain excitability in ASD. In addition, epilepsy and/or seizures are commonly reported in children with ASD, with an estimated 6-46% experiencing clinical seizures at some time in their lives (Cuccaro *et al*, 2012; Lee *et al*, 2015; Levisohn, 2007; Tuchman *et al*, 2011). Thus, this suggests there may be some shared pathophysiology between epilepsy and ASD (Gilby *et al*, 2013). At the same time, evidence is increasing that changes in sensory processing may be associated with neurobiological changes associated with ASD (Baum et al, 2015). Sensory processing is an umbrella term that refers to the entire process of the brain registering sensory input from the outside world to the individual generating a response based on that input. Clinically, atypical sensory processing may manifest as inappropriate responses to sensory input that involve emotional and behavioral disruptions, and interfere with an individual's daily functioning. It has been suggested that up to 96% of children with ASD experience sensory problems. Indeed, they were added as a core symptom to the description of ASD in the fifth edition of the diagnostic and statistical manual of mental disorders (DSM-5) in 2013 (Association, 2013). Furthermore, the characteristic aversive reactions of children with ASD to sensory stimuli suggest that cortical networks do not process relatively neutral stimuli in an adequate manner (Kern *et al*, 2006). Also, twin studies support a genetic overlap between ASD and sensory reactivity (Taylor *et al*, 2017). Excessive response to sensory stimuli can facilitate seizures (Kasteleijn-Nolst Trenite, 1989; Symonds, 1959) and, vice versa, children with epilepsy display increased prevalence of sensory sensitivity (van Campen *et al*, 2015). These notions suggest an association

between seizure susceptibility and changes in sensory processing in ASD. Taken together, the overlap between epileptiform and sensory dysfunctions in ASD may offer a neurophysiological framework to gain more understanding of its clinical behavioral manifestations.

Sensory processing also seems to hold promise for including more objective functional brain measures in the clinical triage of ASD. Notably, event related potentials (ERPs) can be used to measure early temporal correlates of sensory information processing from basic orienting reflexes to conscious perception. Although many previous studies have investigated ERPs in ASD, most tested ERP paradigms in isolation, and as such have yielded inconsistent results (Baum *et al*, 2015). Furthermore, several reviews have shown that these inconsistent results might also be due to differences between samples included in studies using classic patient control designs (Baum *et al*, 2015; O'Connor, 2012). A more integrative approach including combinations of classic ERP paradigms such as P50 suppression, mismatch negativity and selective attention (Luck, 2012; Naatanen, 1992) may allow for more dynamic insights in the temporal, (pre)conscious processing of sensory stimuli and provide a more detailed perspective on the relationship between physiology and behavior in sensory processing issues in the autism spectrum.

The study was part of the Sensory Processing Program that focusses on children with ASD with clinical signs of sensory processing problems. We used an integrative approach combining a comprehensive battery of electrophysiological paradigms with behavioral assessment to test children with ASD with and without epilepsy for sensory issues. We hypothesized that comorbid epilepsy would be reflected in electrophysiological parameters of auditory sensory processing. Furthermore, we aimed to identify markers of sensory problems that, in turn, may provide opportunities for future electrophysiological clinically relevant stratification of ASD cohorts .

Material and methods

Study design & participants

The present study was part of the Sensory Processing Program (SPP) at the Department of Psychiatry, University Medical Center Utrecht (UMCU): an academic outpatient care program developing EEG assisted classification and treatment of sensory processing problems in children with ASD, receiving nation-wide referrals of children with ASD and sensory issues in daily functioning. In addition to routine enrollment of patients, recruitment was

supported by patient advocacy group website advertisements. Typically developing children (TD) were recruited through schools in (proximity to) Utrecht and website advertisements. The study was approved by the UMCU medical ethical committee, and met ethical standards according to the declaration of Helsinki (as revised in 2008). All subjects and their parent(s) were informed verbally and in writing before participation, and written consent was obtained from all subjects; for children younger than 12 years, both parents provided consent; children aged 12 years or older provided written consent themselves as well. All suitable participants enrolled in the SPP that completed study procedures between July 2015 and December 2017 were included in this study.

Inclusion criteria of the three study populations consisted of: 1) ASD without epilepsy (ASD non-epi) group: children with an expert diagnosis of ASD (based on the DSM- IV or DSM V), and/or a total score of ≥ 7 on the Autism Diagnostic Observation Scale (ADOS), and/or a T-score of minimal 60 points on the Social Responsiveness Scale (SRS, i.e. subclinical threshold for ASD) and no history of seizures. ASD diagnosis was retrospectively confirmed by an ASD expert child psychiatrist (HB). 2) ASD with epilepsy (ASD epi) group: children with an expert diagnosis of epilepsy, history of epilepsy diagnosis, or who had expert confirmed seizures in the past. The validity of epilepsy diagnosis was retrospectively confirmed by our in-house pediatric neurologist (FJ), through retrospective chart analyses and visual inspection of EEGs (when available). In addition to an epilepsy diagnosis, these children had an expert diagnosis of ASD (confirmed by HB) and a total score of ≥ 7 on the ADOS, and/or a T-score of ≥ 60 on the SRS. Of the ASD-epi children, 7 out of 20 had had seizures in the last year (13 achieved seizure control). 3) typically developing (TD) group: children had no psychiatric diagnosis and did not have (a history of) behavior or learning problems or seizures.

In addition, for ASD subjects (i.e. groups 1 and 2) comorbid ADHD diagnosis was allowed, defined by expert DSM-IV or DSM V diagnosis. Further, given the naturalistic approach of our study, ASD groups were allowed to use medication (i.e. psychostimulants, antipsychotics, antiepileptic drugs (AED) or a combination). For demographic details, see Table S1. Additional inclusion criteria of all ASD and TD subjects consisted of ability to comply with study procedures (IQ> 55).

The sample consisted of 113 boys and girls aged 7-15: 57 individuals with ASD without epilepsy (ASD non-epi), 20 individuals with ASD and epilepsy (ASD epi) and 36 age-matched TD children.

Procedure & study measures

All patients participating in the SPP receive a standard phenotyping battery including behavioral, and neurophysiological (EEG) tests. On the first test day, an abbreviated form of the Wechsler intelligence scale for children (WISC)-III was used for IQ estimation (consisting of subtasks Similarities, Vocabulary, Block Design and Figure Assembly), if not administered within the previous 2 years. Patients were assessed with the ADOS-2 and parents filled out the SRS to screen for study eligibility. On a different test day, usually within 2 weeks, subjects were assessed with a neurophysiological test battery (see below) to measure early correlates of auditory sensory processing.

Questionnaires

Children's parent(s) completed the Sensory Profile, Dutch ed. (SP-NL) (Dunn and Brown, 1997), addressing sensory behavior. In addition, the aberrant behavior checklist (ABC) (Aman *et al*, 1985) and the social responsiveness scale (SRS) (Constantino *et al*, 2003) were used to assess externalizing behavior and social behavior, respectively. We developed sensory sensitivity indexes for hypo- and hyper-sensitivity (SSI hypo and SSI hyper, respectively), based on items of the SP-NL reflecting a direct response to sensory stimuli. For details about questionnaires and the SSIs, see supplementary material.

ERP measures

All participants participated in an auditory electrophysiological test battery including paradigms to assess P50 suppression, mismatch negativity (MMN) and selective attention (SA). The procedures concerning P50 suppression, MMN and SA assessment were identical to the ones previously described in (During *et al*, 2014; Oranje *et al*, 2008; Vlaskamp *et al*, 2017). Participants were tested in the morning, and assessments took up to 2.5 hours, including set up. During assessment, subjects were seated in a comfortable armchair and were requested to sit still. All stimuli were presented through tubal insert ear phones (EARtone®, Etymotic Research), by a computer running Presentation® software (Neurobehavioral Systems Inc.). For details about the ERP paradigms, acquisition and processing, see supplementary material.

Statistical analyses

All statistical analyses were carried out in SPSS statistics version 22.0. All questionnaire and ERP amplitude data were normally distributed, except for P50 suppression data (ratios). Latencies were non-normally distributed. The majority (>90%) of possible outliers (>3SD) were observed in the ASD groups and not in TD and were therefore regarded as actual, meaningful results

and included in the analyses. Independent T-tests and multivariate analyses of variances (MANOVAs) were used to investigate group differences in behavioral and ERP amplitude measures (i.e. combined ASD versus TD, as well ASD with and without comorbid ADHD). For P50 suppression ratios and latency data, nonparametric Mann-Whitney U tests and Kruskal-Wallis tests were used. For diagnostic group comparisons (ASD epi/ ASD non-epi/ TD), *p* values of < .017 were considered significant to correct for three-group comparisons. Depending on the distribution of the data, either Pearson or Spearman correlation analyses were run to investigate the relationship of ERP measures with symptom scales. Here, $p < .006$ was deemed significant, correcting for comparisons with eight symptom scales. Last, to explore the relationship between multiple ERP measures, their interactions and behavior, multiple backward linear regression analyses (MRAs) were run, including all dependent variables and centering them to control for possible multicollinearity.

Results

Sensory problems

There were no differences in age between the (combined) ASD and TD group. Average IQ was lower for the ASD than the TD group (TIQ; $t(105) = 5.609$, $p = .000$, PIQ; $t(102) = 2.452$, $p = .016$, VIQ: $t(103) = 4.552$, $p = .000$), and boys were overrepresented in ASD but not in TD ($t(61.549)$, $p = .047$). The combined ASD group had more (sensory) problems than their TD peers, as indicated by scores on SRS total, SP quadrants and the ABC irritability scale. There was no difference in sensory problems between ASD non-epi and ASD epi groups. (See Table S2). Direct responses of sensory over and under responsivity were measured with SSI indexes derived from the SP-NL (see supplementary methods). These indexes confirmed an abundance of sensory issues in both ASD non-epi and ASD epi groups (Figure 1) in line with the focus of the Sensory Processing Program. Additionally, both ASD groups showed larger variability in all behavioral measures *(Levene's statistics $p < .001$)* compared to the TD, except for IQ.

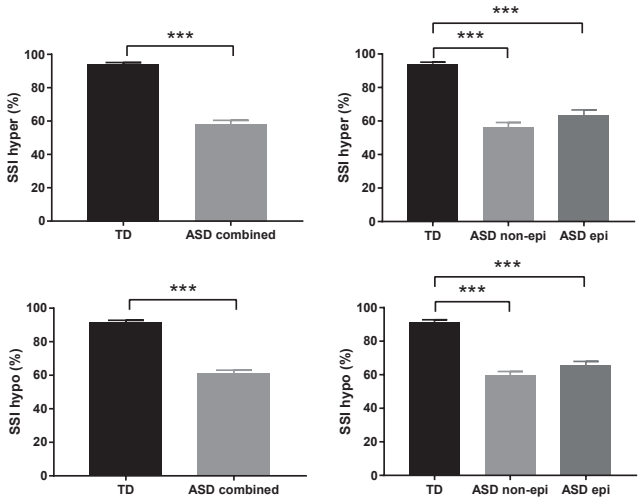

Figure 1: Sensory reactivity is enhanced in ASD with and without epilepsy versus TD. *Sensory sensitivity indexes for hyper- and hypo sensitivity in whole group ASD versus TD (left panels) and ASD with and without epilepsy versus TD (right panels). Lower percentages indicate more impaired sensory behavior ***= p< .001 Error bars display +/- standard error of mean*

In addition to comorbid epilepsy, we tested for effects of comorbid ADHD on sensory outcome. Whereas comorbid epilepsy did not affect sensory problems, children with comorbid ADHD had more problems than ASD children without ADHD in SSI-hyper (t(72)= 2.792, *p*= .007), SP quadrants 2 (sensory seeking) and 3 (sensory sensitivity) (t(72)= 3.788, *p*= .000, t(72)= 2.568, *p*= .012, respectively) and ABC irritability (t(59.850)= 3.420, *p*= .001), regardless of their epilepsy status. Further analyses of medication status and type, epilepsy type (i.e. focal vs generalized) and seizure control had no effect on sensory problems.

Auditory processing (ERP)

First, we tested for differences in individual ERP components between combined ASD and TD groups. The combined ASD group showed decreased orienting to, and (pre)conscious attentional processing of, auditory deviancy compared to TD, as reflected by smaller P3a and P3b amplitudes (t(45.473)= 2.179, *p*= .035 and t(103)= 2.279, *p*= .025, respectively). No further differences were found between these two groups, i.e. no differences in sensory gating as reflected in the P50 ratio T/C of the P50 suppression paradigm (although trend

level of significance, Z= -1.696, *p*= .09) or any of the types of MMN measures (i.e. frequency MMN, duration MMN, frequency duration MMN nor the MMN of the selective attention task (MMN(SA)), or N1 and N2 amplitudes), see also Table S2. However, variability was higher in the ASD group for frequency duration MMN (Levene's statistic= 5.049, *p*= .027) and higher in the TD group for P3a amplitudes (Levene's statistic= 9.638, *p*= .002).

Next, we found a main effect of diagnosis (ASD non-epi/ASD epi/TD) on frequency MMN (freqMMN) (F(2,99)= 6.474, *p*= .002), and a nominally significant effect on P3a amplitude (F(2,99)= 3.351, *p*= .039). Further exploration showed that this effect on freqMMN was due to enhanced automatic discrimination in ASD epi (i.e. a more negative freqMMN amplitude) compared to TD (t(53)= 3.775, *p*= .000) and ASD non-epi (t(72)= 3.476, *p*= .001). Further exploration of the group effect on P3a showed that it was related to lower P3a amplitudes for the ASD epi group compared to TD (t(51)= 2.148, *p*= .037). For an overview of physiological profiles per group, see Figure S1.

Comorbidity for ADHD did not affect any ERP components, in contrast to the effects on sensory problems mentioned above. Neither the effect on freqMMN nor P3a and P3b amplitudes was affected by possible confounding factors, such as epilepsy type (i.e. focal versus generalized), seizure control or medication status and type.

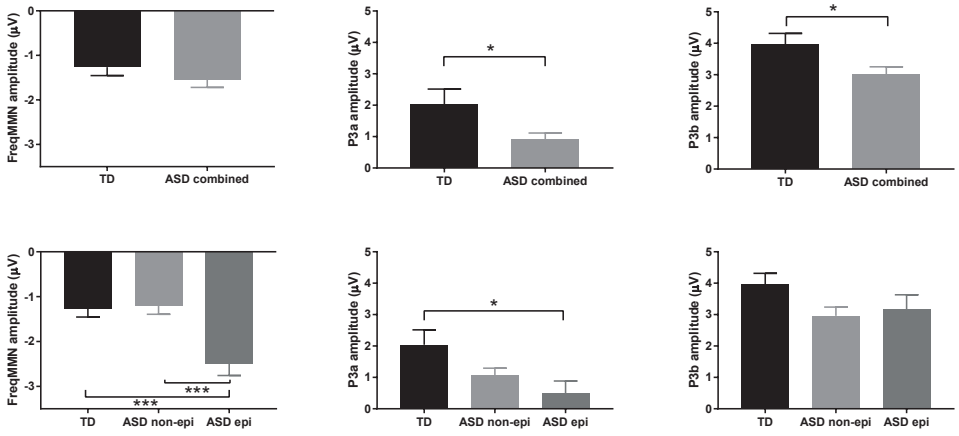

Figure 2: Differences in ERP between TD and ASD groups. *ERP amplitudes (µV) in combined ASD versus TD (upper panels) and ASD with and without epilepsy versus TD (lower panels). For an overview of all ERP measures per group, see Table S2. *= p< .05, ***=p< .001, Error bars display +/- standard error of mean.*

ERP task performance (selective attention)

Children in the combined ASD group were as fast as their TD peers on the selective attention task, as assessed by reaction time (RT) to attended deviants. However, they had less accurate deviancy detection as reflected by a lower percentage of hits to target stimuli (t(87)= 2.870, *p*=.005) and made more false alarms (t(79.129)= 2.957, *p*= .004). The number of false alarms correlated negatively with P3b amplitude in ASD subjects only (R -.409, n=63, *p*=.001), confirming less accurate deviancy recognition. The group difference in the percentage of hits was driven by the performance of the ASD non-epi group (t(70)= 3.065, *p*= .003). Post-hoc analyses showed that performance on the task was affected by medication use, where children using (any) medication performed worse, i.e. had lower accuracy (t(87)= 3.311, *p*= .001) and made more false alarms (t(45.549)=2.875, *p*= .006).

Correlations between ERPs and sensory problems

First we carried out simple correlations. In the combined ASD group, N1 amplitude correlated positively with SSI hypo and SP quadrant 2 (sensory seeking) (R= .429, n= 67, *p*= .000 and R= .400, n=67, *p*= .001, respectively) and duration MMN correlated negatively with SRS scores (nominally significant: R= -.322, n= 67, *p*= .006).

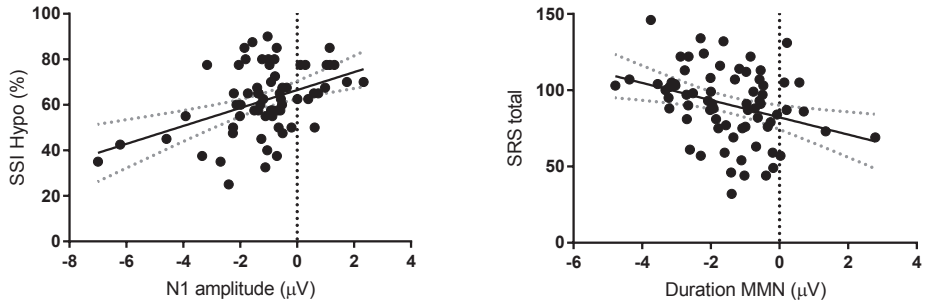

Figure 3: Correlations between ERPs and sensory problems. *Correlation of N1 amplitude with sensory hyposensitivity (SSI hypo %, left panel) and correlation of duration MMN with scores on SRS (right panel).*

Next, we used backwards multiple regression analyses to integrate multiple ERP and behavioral measures. First we explored if it would be possible to predict group membership (ASD non-epi /ASD epi/TD) based on all ERP data. A combination of freqMMN and P3a amplitude was found to be the best

(partial) predictor of group membership (F (2,90)= 6.246, *p*= .003, R= .349). Further multiple regression analyses in the combined ASD group showed that freqMMN could be partially predicted by a model comprising ABC irritability and quadrant 2 of the SP-NL (sensory seeking), F (2,65)= 4.402, *p*= .016, R= .345 and duration MMN could be partially predicted by a model including SRS, ABC irritability, and quadrant 3 of the SP-NL (sensory sensitivity) F (3,64)= 6.835, *p*= .000, R= .493.

Discussion

In this study, we combined electrophysiological and behavioral measures to relate auditory sensory processing to sensory problems in children with ASD and sensory issues, with and without comorbid epilepsy. We hypothesized that there would be differences in event related potentials (ERP) between typically developing (TD) and ASD children, and that this would also relate to comorbid epilepsy. We found that children with ASD and comorbid epilepsy (ASD epi) had enhanced automatic discrimination of frequency deviants (more pronounced freqMMN amplitude), which was unrelated to confounding factors, such as epilepsy type (focal versus generalized), seizure control, and medication use.

Our results show that including non-prototypical ASD subjects with comorbid epilepsy, often excluded in clinical studies, may aid to stratify broad ASD into meaningful clinical subcategories. Indeed, the results of previous studies exploring MMN in ASD children are highly inconsistent, partly due to differences in the paradigms used and the heterogeneity within and between ASD cohorts (Dunn *et al*, 2008; Sinclair *et al*, 2017; Vlaskamp *et al*, 2017; Weismuller *et al*, 2015) Here, we suggest that epilepsy or seizure susceptibility may be an important factor in explaining variability in MMN in ASD samples. Indeed, we could separate ASD with epilepsy from ASD without epilepsy using frequency MMN and P3a measures in our sample: individuals with comorbid epilepsy had more negative frequency MMN and lower P3a amplitudes, regardless of medication status. Such analyses might prove to be clinically meaningful for detecting seizure susceptibility in children with ASD who have not yet presented with overt seizure symptoms. It has been suggested that many non-epileptic ASD patients have seizures (Boutros *et al*, 2015), and segregating these patients might give new insights and opportunities for more effective individual treatment. Furthermore, seizure susceptibility forms a possible contra-indication for some of the medications prescribed in ASD as they can lower seizure threshold (e.g. stimulants and antipsychotics).

In the combined ASD sample, we found further differences in ERP irrespective of epilepsy comorbidity, with reduced late auditory processing in the combined ASD group. This was indicated by lower P3a and P3b amplitude, suggesting reduced orienting to and (pre)conscious processing of auditory deviant stimuli. Children with ASD also made more errors and false alarms, confirming less accurate deviancy discrimination. Our data is consistent with previous studies reporting P3a and P3b amplitude to be decreased in ASD (Donkers *et al*, 2015; Ferri *et al*, 2003) and suggesting that decreased (pre) attentional, top-down processing of deviancy may form a mechanism for sensory issues in ASD.

In addition to these differences in group averages, our analysis of the dimensional relation between ERP and behavior showed associations that may be related to the sensory problems these children experience. We found an association between N1 amplitude, a measure of stimulus detection, and sensory problems as measured with our sensory sensitivity indexes (SSI) and SP-NL quadrants. Also, duration MMN showed a nominally significant correlation with social behavior as measured with the social responsiveness scale (SRS), similar to our previous study *(Vlaskamp et al, 2017)*. These associations might indicate the presence of neurophysiological dysfunction partly specific for the sensory and social behavioral domain. Previous studies in ASD have struggled to find correlations between ERPs and behavior (Schauder and Bennetto, 2016). Indeed, apart from the above mentioned association with N1 amplitude, also we found no further straightforward correlations with sensory behavior. Therefore, we carried out the more complex analyses (integrative multiple regression analyses) to see if combinations of ERPs or behavioral measures could predict each other, where we found correlations between MMN measures and sensory issues. We first explored whether (sensory) behaviors could be linked to specific ERPs and found that frequency MMN and duration MMN could both be partly 'predicted' using a combination of questionnaire data, and treatments targeting these specific ERPs may therefore prove useful in reducing sensory issues in ASD. For example, studies have suggested that NMDA receptor antagonists might lower MMN amplitude (Umbricht *et al*, 2002; Umbricht *et al*, 2000), whereas escitalopram, a selective serotonin reuptake inhibitor, might increase MMN (Oranje *et al*, 2008). Also, dopamine antagonists (e.g. sulpride) or NMDA receptor antagonists can alter P3 amplitudes (Oranje *et al*, 2000; Takeshita and Ogura, 1994). Gaining more insights in physiological profiles and underlying mechanisms might likewise aid in the application of early intervention therapies, as EEG measurement is non-invasive, low-cost and feasible in young individuals.

Our ASD cohort was enriched for sensory problems and included non-prototypical subjects with comorbid epilepsy, typically excluded in studies of ASD. We show that including comorbid epilepsy can enhance physiological subtyping. Similar research approaches using more sophisticated analyses (e.g. machine learning) and including comorbidities and vulnerabilities underlying ASD heterogeneity may further enhance the contrasts indicated here.

In conclusion, we show differences in the processing of auditory input in individuals with ASD, with and without epilepsy, that seem to involve both bottom-up and top-down processes. It seems that a comprehensive approach involving multiple electrophysiological measures is needed to differentiate the diverse sensory phenotypes observed among individuals with ASD.

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Disclosures

The authors declare that they do not have any competing (financial) interests

Supplementary material- chapter 3

1. Supplementary methods

Questionnaires

- Sensory Profile – NL

The Sensory Profile, developed by Dunn, 1997 is a caregiver questionnaire that measures a child's sensory processing abilities and their impact on daily functioning. It consists of a 125-item assessment on which parents report the frequency their child responds to items in eight categories: Auditory, Visual, Taste/Smell, Movement, Body Position, Touch, Activity Level, and Emotional/ Social. The Sensory Profile has been translated into Dutch and has been validated with norm-scores for Dutch typically developing children of different age ranges. The Sensory Profile can further be used to classify children into one of the four general sensory processing quadrants: Low registration, Sensation seeking, Sensory sensitivity and Sensation avoiding.

In this study, we also developed new sensory sensitivity indexes (SSI, hypo- and hyper-), as the quadrants are less sensitive to hyper- or hyposensitive behavior. We used items reflecting a direct response to sensory stimuli to establish behavioral scales of hyper- or hyporesponsivity to sensory stimuli. Items that reflected hypersensitive behavior to sensory stimuli (i.e. items 1-5, 10, 14, 15 29, 30, 32-34, 36, 54, 55, 57) constitute a hypersensitivity index. Items that reflected hyposensitive behavior to sensory stimuli (i.e. items 6-8, 40, 41, 45-47, 53 and 59) constitute the hyposensitivity index. The index score was then calculated as a percentage, i.e. the sum score for the items divided by the maximum score minus the minimal score for all items. In line with the SP-NL 100% reflects a normal score, whereas scores towards 0% reflect increasing abnormalities.

- Aberrant behavior checklist

The aberrant behavior checklist (ABC) is a caregiver questionnaire that measures the presence and severity of psychiatric symptoms and behavioral disturbances. It is a 58-item questionnaire that derives 5 subscales: irritability, lethargy, stereotypy, hyperactivity and inappropriate speech. For this study, we used the irritability subscale.

- Social responsiveness scale

The Social Responsiveness Scale (SRS) is a caregiver questionnaire aimed at distinguishing autism spectrum disorder from other child psychiatric disorders by identifying presence and severity of autistic social impairment. This 65-

item rating scale measures the severity of autism spectrum symptoms as they occur in natural social settings. The SRS provides a clear picture of a child's social impairments, assessing social awareness, social information processing, capacity for reciprocal social communication, social anxiety/avoidance, and autistic preoccupations and traits.

ERP paradigms

- P50 suppression paradigm

The P50 paradigm consisted of 3 identical blocks of 40 paired clicks presented binaurally. Subjects were instructed to count the number of clicks and to stay awake. All clicks had a duration of 1.5ms and intensity of 80 dB. Interstimulus interval (ISI) was consistently 500ms and click pairs were separated by 10s. The total duration of the P50 suppression task was approximately 21 minutes.

- Mismatch negativity (MMN) paradigm

The MMN paradigm consisted of 1800 trials and 4 types of stimuli: In 83% of the cases, a standard tone with a frequency of 1000Hz, intensity of 75dB and duration of 50ms was presented. Within this sequence of standard stimuli, three types of deviants were presented, each with a probability of 6% and intensity of 75dB: Frequency deviants of 1200Hz and 50ms, duration deviants of 1000Hz and 100ms and frequency-duration deviants of 1200Hz and 100ms. The interstimulus interval (ISI) was randomized between 300 and 500ms. Subjects were asked to ignore the stimuli and watched a muted documentary on a screen in front of them. The total duration of the MMN task was approximately 15 minutes.

- Selective attention (SA) paradigm

The auditory selective attention task consisted of 400 stimuli, presented randomly in either the right or the left ear. Two types of stimuli were presented: standard tones, which appeared in 80% of the cases, and deviant tones, which appeared in the remaining 20% of the cases. The stimuli were evenly presented to the left or right ear (attended deviants were never presented immediately following each other). The subject was instructed to push a button as fast as possible if the deviant tone occurred in the previously designated ear. Ear designation was balanced randomly across the subjects. After this initial task the subjects were presented the next auditory selective attention task in which they had to monitor the other ear for deviant stimuli. Standard and deviant stimuli differed in frequency only (either 1000 and 1200

Hz respectively). The intensity of each stimulus was 75 dB and was 50 ms in duration, presented with an ISI between 700 and 900 ms. The total of the two tasks was approximately 11 minutes. The selective attention task provides data on N1 an N2 amplitude, mismatch negativity (MMN(SA)), P3a and P3b amplitude, as well as behavioral data (correct responses/false alarms and reaction time (RT)).

EEG acquisition and processing

The EEG signal was recorded using a BioSemi® system with 64 active-two electrodes, according to the 10-20 system. Analysis and processing of the EEG signal was carried out using Brain Electrical Source Analysis (BESA) software (version 6.0, MEGIS Software GmbH, Gräfelfing, Germany). Preprocessing of the data started with resampling from the original 4kHz to 250Hz for MMN and SA, and to 500Hz for P50, to allow easier file handling. Second, the data were corrected for eye-artefacts by using the adaptive method of BESA. Third, the data were epoched (from 100ms prestimulus to 900ms poststimulus) and corrected for movement (or other paradigm unrelated) artefacts, by removing those epochs from the database that contained amplitude differences between maximum and minimum exceeding 75μV, in the for P50, MMN and SA relevant scoring windows (see below).

Only data from the electrodes relevant for this study were analyzed (i.e. where the maximum activity for the ERPs was found): the midline electrodes Cz (for P50, P3a(SA), N1(SA)), Fz (for MMN(SA)), FCz (for freqMMN, durMMN, freqdurMMN, N2(SA)) and Pz (for P3b(SA)).

All data were band-pass filtered (0.5Hz- 70Hz for P50 suppression data, 0.5Hz-40Hz for MMN and SA data), and average reference was used as a reference.

For P50, the amplitude was defined as the largest trough to peak amplitude within an interval of 40–90ms following the first (conditioning or "C") stimulus in each paired click. The P50 amplitude following the second (testing or "T") stimulus was identified as the largest trough to peak amplitude within an interval of 10 ms of the latency of the maximum P50 amplitude to the C-stimulus. P50 suppression was expressed as the ratio "T/C."

For MMN, amplitude was calculated for each of the three deviant types, expressed as the average ERP to the relevant deviant stimuli, subtracted with the average ERP to standard stimuli for each subject separately. MMN amplitudes were then scored as the minimum amplitude in a window between 90 and 170 ms for frequency and frequency-duration, and in a window between 150-240 ms for duration deviants.

For SA, the MMN component was calculated as the average ERP to the non-attended deviant stimulus subtracted with the average ERP to the non-attended standard stimulus. MMN was scored in a window between 120 and 230 ms. N1 and N2 components were scored as the minimum amplitude in windows of 80-130 ms and 210-290 ms, respectively, in the attended deviant condition. P3a and P3b were scored as the maximum amplitude in windows of 130-210 ms and 400- 700 ms, respectively. Here, P3a was scored in the non-attended deviant condition, P3b in the attended deviant condition.

2. Supplementary material

Table S1. Demographics

Mean (SD)	ASD combined	ASD non-epi	ASD epi	TD controls
N:boys/girls	77:56/21*	57:43/14*	20:13/7	36:19/17
Age in years	10.18 (1.74)	10.11 (1.64)	10.39 (2.04)	10.27 (1.79)
Total IQ	97 (19)***	99 (19)*	92 (19)*	118 (16)
Verbal IQ	102 (17)***	103 (17)***	99 (18)***	119 (20)
Performal IQ	91 (21)*	92 (22)*	89 (19)*	102 (17)
ADOS total score	10.07 (3.74)	10.29 (3.74)	9.45 (3.78)	NA
SRS total	90.90 (23.56)***	93.25 (23.22)***	84.70 (23.94)***	19.38 (10.31)
SSI hyper	58.11 (18.37)***	56.15 (19.34)***	63.38 (14.57)***	94.21 (5.12)
SSI hypo	61.45 (14.61)***	59.95 (15.60)***	65.50 (10.81)***	91.53 (7.24)
SPQ1: low registration	52.41 (11.58)***	53.63 (10.75)***	49.10 (13.33)***	71.74 (3.18)
SPQ2: sensory seeking	97.91 (14.24)***	96.07 (15.24)***	102.85 (9.78)***†	124.16 (4.78)
SPQ3: sensitivity	70.20 (11.93)***	69.00 (12.59)***	73.45 (9.47)***	94.23 (5.01)
SPQ4: avoiding	97.88 (15.11)***	96.91 (15.22)***	100.50 (13.97)***	132.61 (8.22)
ABC irritability	16.56 (8.67)***	17.23 (8.34)***	14.68 (9.53)***	1.19 (1.8)
ADHD comorbidity	24/77	21/57	3/20	none
Medication	none 38, AP 7, STM 7, AED 14, combi 11	none 35, AP 7, STM 7, combi 8	None 3, AED 14, combi 3	none
Epilepsy details				
1. Focal/ generalized			14/6	
2. Genetic/ structural/ unknown			9/7/4	
3. Active/ non-active			7/13	

ADOS= autism diagnostic observation scale, SRS= social responsiveness scale, SSI= sensory sensitivity index, SPQ= sensory profile quadrant, ABC= aberrant behavior checklist. Medication: AP= antipsychotics, STM= stimulants, AED= antiepileptic drugs, combi= combination of STM and AP (in ASD non-epi) or AED and AP (in ASD epi) ***= p<.05 significant difference compared to TD,*** ***= p< .01, ***= p< .001, †= p< .05 significant difference ASD only and epilepsy (††=p< .01, †††= p< .001).*

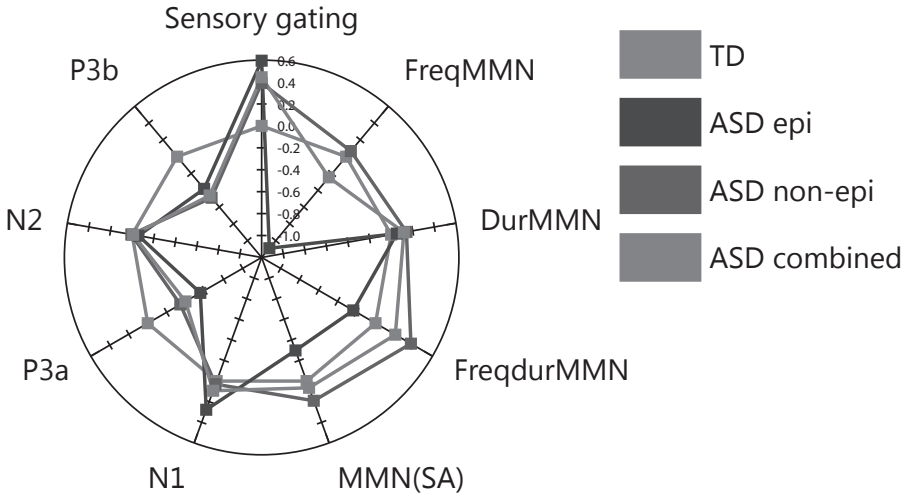
3. Supplementary results

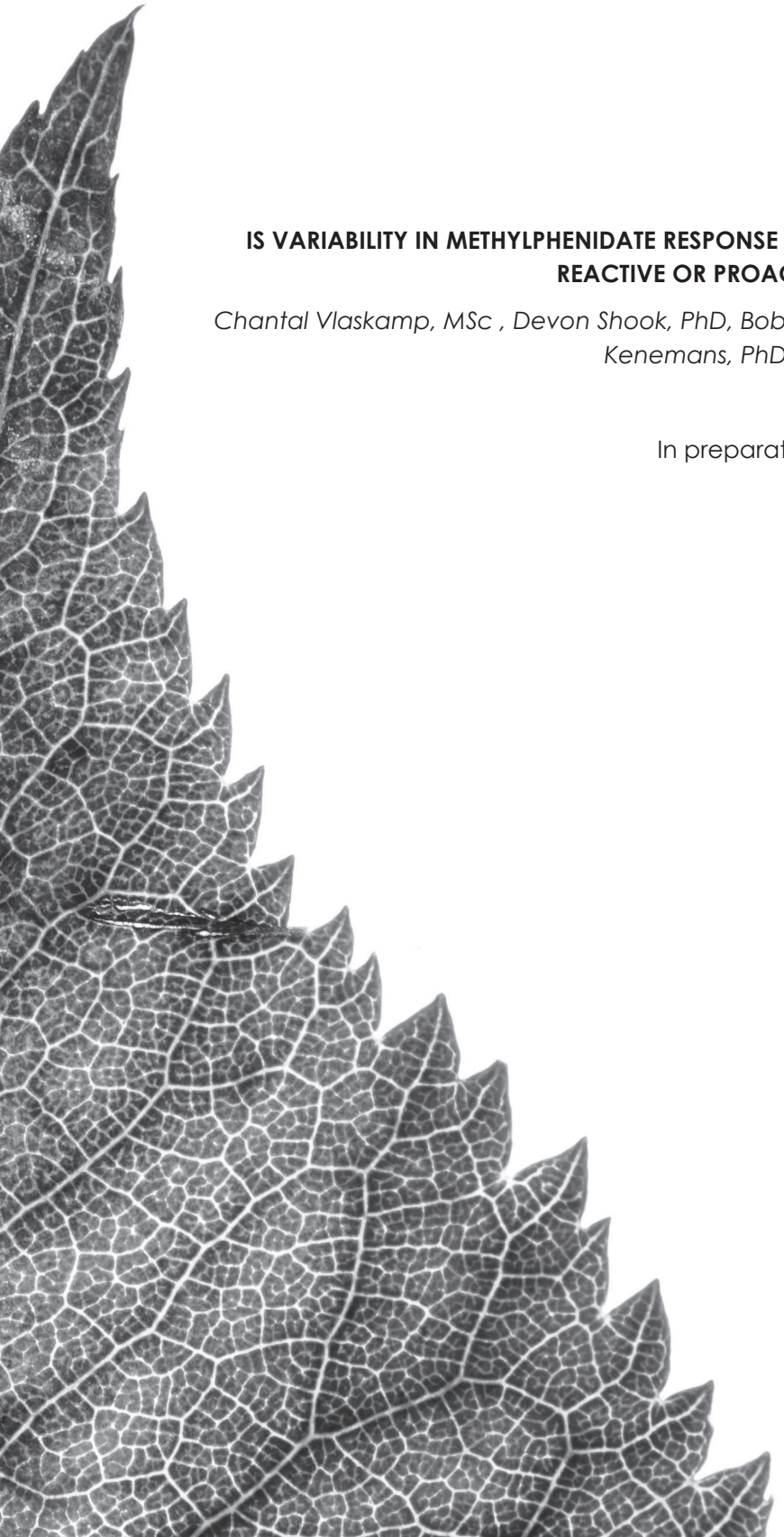
Table S2. ERP data

Means (SD) *Latencies in ms, amplitudes in µV*	**ASD combined**	**ASD non-epi**	**ASD epi**	**TD controls**
P50 suppression paradigm				
P50 TC ratio	.77 (1.04)	.73 (1.09)	.86 (.91)	0.48 (0.64)
MMN paradigm				
Latency FreqMMN	128.97 (26.90)	128.96 (28.13)	129.00 (23.92)	138.29 (27.48)
Amplitude FreqMMN	-1.54 (1.53)	-1.19 (1.49)	-2.49 (1.22)*** †††	-1.26 (1.12)
Latency DurMMN	198.38 (27.89)	198.52 (26.46)	198.00 (32.20)	198.74 (30.23)
Amplitude DurMMN	-1.47 (1.38)	-1.43 (1.52)	-1.56 (.91)	-1.63 (1.41)
Latency FreqdurMMN	133.46 (24.66)	134.30 (24.86)	131.20 (24.61)	126.17 (23.21)
Amplitude FreqdurMMN	-1.82 (1.67)	-1.62 (1.61)	-2.33 (1.77)	-2.06 (1.17)
SA paradigm				
Latency P3a (ND)	174.69 (27.95)	177.31 (26.24)	167.11 (31.99)	169.49 (28.98)
Amplitude P3a (ND)	.92 (1.61)*	1.08 (1.56)	.49 (1.70)*	2.04 (2.81)
Latency P3b (AD)	546.23 (80.63)	541.62 (80.75)	559.56 (81.04)	525.14 (82.23)
Amplitude P3b (AD)	3.02 (1.99)*	2.96 (2.03)*	3.18 (1.94)	3.97 (2.06)
Latency MMN (SA)	174.06 (36.97)	176.23 (37.12)	167.78 (36.88)	175.66 (36.06)
Amplitude MMN (SA)	-1.70 (1.60)	-1.54 (1.40)	-2.13 (2.07)	-1.77 (1.20)
Latency N1	100.91 (19.19)	102.69 (19.45)	95.78 (17.97)	96.91 (14.33)
Amplitude N1	-1.08 (1.60)	-1.19 (1.74)	-.78 (1.05)	-1.24 (1.65)
Latency N2	250.06 (28.62)	250.46 (28.11)	248.89 (30.88)	248.23 (33.47)
Amplitude N2	-1.09 (2.04)	-1.06 (1.96)	-1.20 (2.29)	-1.08 (2.17)
SA performance				
RT SA (ms)	565.53 (61.66)	568.52 (59.70)	558.15 (67.57)	562.79 (65.45)
Percentage corrects	53.33 (26.17)**	51.15 (26.50)**	59.24 (25.05)	70.45 (24.09)
False alarms	61.04 (8.90)**	60.01 (11.11)*	63.82 (14.03)*	26.19 (7.73)

*MMN= mismatch negativity, SA= selective attention, FreqMMN=Frequency MMN, DurMMN= Duration MMN, FreqdurMMN= Frequency-duration MMN *= p<.05 significant difference compared to TD, **= p< .01, ***= p< .001, †= p< .05 significant difference ASD only and epilepsy (††=p< .01, †††= p< .001).*

Figure S1: physiological integration of ERP results





4

IS VARIABILITY IN METHYLPHENIDATE RESPONSE IN ADHD RELATED TO REACTIVE OR PROACTIVE MECHANISMS?

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In preparation to be submitted.

IS VARIABILITY IN METHYLPHENIDATE RESPONSE IN ADHD RELATED TO REACTIVE OR PROACTIVE MECHANISMS?

Abstract

The stimulant methylphenidate (MPH) is beneficial for many but not all children with attention deficit hyperactivity disorder (ADHD). The neurobiology associated with this variability in treatment response is not understood. Improvement in symptoms with medication may be related to improvements in children's strategies to control their behavior, e.g. through better reactive stopping or more proactive slowing. To address variability in MPH response, we included 19 children with ADHD (aged 8-13 years) in a single dose within-subject study where they performed a stop signal anticipation task (SSAT) to assess the effect of MPH on reactive stopping (SSRT) and proactive slowing. We assessed clinical improvement with the Swanson, Noland and Pelham Questionnaire (SNAP), and possible neurobiological mechanisms at baseline with functional magnetic imaging (fMRI) and EEG. On average, MPH treatment was associated with shorter stop signal reaction time (SSRT) and fewer SNAP symptoms. However, there was great variability in treatment response, with almost all children showing improvements in either proactive slowing or SSRT. Improvements in symptoms were related to improvements in reactive stopping only (SSRT). We found only modest correlations between baseline measures of neural activity and improvements with MPH. In conclusion, this study suggests that reactive and proactive mechanisms both contribute to inter-individual variability in MPH response and may perhaps do so differentially, with reactive mechanisms more closely related to clinical improvement.

Introduction

Methylphenidate (MPH) improves symptoms in 70-80% of children with attention deficit hyperactivity disorder (ADHD) (Spencer *et al*, 1996). However, 20-30% of children with ADHD experiences little to no clinical benefit from MPH. It could be clinically relevant to understand the basis of this inter-individual variability in order to predict who is likely to benefit from treatment with MPH and who is not. Yet, little is known about associated differences in behavioral strategy or neurobiology.

Behavioral control is the ability to flexibly adjust behavior in response to shifting environmental demands (van Belle *et al*, 2014). At least two strategies for behavioral control can be distinguished: a reactive and a proactive strategy (Aron, 2011). Reactive strategies involve responding to environmental demands as they arise, such as stopping in response to a stop signal in a continuous performance task. In contrast, proactive strategies rely on the anticipation of future events and may involve slowing down in expectation of such a stop signal. Reactive stopping can be assessed using a so-called stop task (Logan *et al*, 1984): a choice-reaction time task where go stimuli are occasionally followed by a stop stimulus that instructs participants to withhold their response. Reactive stopping is then operationalized as the stop signal reaction time (SSRT), the speed of the stopping process. At the group level, children and adults with ADHD have been shown to perform more poorly on stop tasks, with slower reaction times (RT), lower stop accuracy, and longer SSRT than their typically developing peers (Alderson *et al*, 2007). However, there are large differences between individuals with ADHD and it has been estimated that as many as 50 to 70% of affected individuals do not have a true deficit in reactive stopping (Nigg *et al*, 2005). Traditional stop tasks do not assess proactive mechanisms, and proactive slowing has hardly been investigated in ADHD. We recently used an adapted stop-task to assess both reactive stopping and proactive slowing simultaneously in children with ADHD, and found that at the group level, they had poorer performance than their peers for reactive stopping, but not proactive slowing (van Hulst *et al*, 2018).

Reactive and proactive regulation strategies may rely on partially distinct neural systems. Reactive stopping has been associated with fronto-striatal networks, including striatum, inferior frontal gyrus (IFG), superior frontal gyrus (SFG) and supplementary motor area (SMA) (Aron *et al*, 2004; van Belle *et al*, 2014; Zandbelt and Vink, 2010). Proactive mechanisms rely on IFG and its interactions with basal ganglia (Aron, 2011; Majid *et al*, 2013). Studies

using electroencephalography (EEG) have shown stop-signal elicited event-related potentials, including a right frontal stop N2 component and a frontal stop P3 (Bekker *et al*, 2005a; de Jong *et al*, 1990). Both stop N2 and stop P3 reflect by definition reactive mechanisms, thought to be generated in inferior frontal gyrus (IFG) (Schmajuk *et al*, 2006) and in superior frontal gyrus (SFG), respectively (Kenemans, 2015).

Studies of ADHD have reported that, on average, children with ADHD show decreased activation in inferior frontal lobe and caudate nucleus during stopping (Rubia *et al*, 1999; Rubia *et al*, 2005). In addition, stop N2 has been reported to be reduced (Dimoska *et al*, 2003) or absent (Liotti *et al*, 2010) in ADHD, as has stop P3 (Bekker *et al*, 2005b; Senderecka *et al*, 2012). However, these studies could not differentiate between proactive and reactive strategies. Overall, MPH appears to have a positive effect on stop task performance in children and adults with ADHD, as indicated by improvements in accuracy and SSRT (Aron *et al*, 2003). It has been shown to upregulate activity in a network of regions associated with stopping, including medial frontal gyrus, IFG, parietal regions, as well as basal ganglia (Rubia *et al*, 2011) and to restore the (reduced) stop N2 in ADHD (Broyd *et al*, 2005), but not the stop P3 (Overtoom *et al*, 2009). Taken together, these results suggest that MPH may affect both control strategies, with a possible larger impact on proactive mechanisms (Kenemans, 2015).

In this study, we set out to investigate whether differences in behavioral strategy and associated neural mechanisms might be associated with variability in MPH response. We used the stop signal anticipation task (SSAT) to differentiate between proactive and reactive mechanisms of behavioral regulation. We hypothesized that improvements in proactive slowing would be related to lower activity in dorsal IFG at baseline (with more room for improvement) and reduced associated N2, whereas improvements in reactive stopping would be related to lower activity in SFG and lower associated P3 at baseline.

Methods

Participants

Nineteen participants (11 boys; aged 8 to 13 years; Table 1) with a diagnosis of ADHD (DSM-IV TR) were included in the study. Participants were either medication naïve (n=3) or on immediate release methylphenidate (e.g., Ritalin®) and discontinued MPH for 48 hours before testing. Subjects were free of other psychoactive medication. Additional inclusion criteria were

a minimum full-scale IQ of 75 and no psychiatric comorbidity, except for oppositional defiant disorder (ODD). Originally, 21 participants were recruited,, but two participants were excluded due to low IQ or psychiatric comorbidity (assessed during psychiatric interview). Participants were recruited through our outpatient clinic for developmental disorders, (special education) schools in the Utrecht area and website advertising. The study was approved by the University Medical Centre ethics committee (METC), in accordance with the declaration of Helsinki. All subjects and their parents were informed orally and in writing before signing informed consent.

Procedure

The study design was a single dose repeated measure within-subject design to assess cognitive and clinical response to methylphenidate, with baseline off-medication EEG and fMRI assessments.

Study participation included two test days: on the first test day, participants were assessed using the abbreviated version of the Wechsler intelligence scale for children (WISC)-III (i.e. Vocabulary, Similarities, Block Design and Object Assembly) to estimate IQ. Meanwhile, parent(s) participated in the diagnostic interview schedule for children (DISC)-IV interview to confirm ADHD diagnosis and exclude psychiatric comorbidity. Parents were also asked to fill in the Swanson and Noland and Pelham Questionnaire (SNAP), rating their child's behavior without medication (i.e. baseline condition).

For the second test day, subjects discontinued medication for 48h. In the morning, subjects performed a stop task twice, once during an fMRI and once during an EEG session. The order of fMRI and EEG was counterbalanced across subjects. After both sessions, subjects had a single dose of MPH (either 10mg or their usual dose) and performed the stop task again after ~60 min (based on T-max) to assess the acute effect of MPH on task performance (without fMRI or EEG).

Stop tasks

The primary task used to assess MPH effects on task performance was the stop signal anticipation task (SSAT). This task was performed during the fMRI session at baseline and again at follow-up. During the EEG session, subjects performed a standard stop signal task. Details of both tasks are available as Supplemental Material.

Reactive stopping (SSRT) on the SSAT was calculated by subtracting mean stop signal delay (SSD) on stop trials from median reaction time on go trials, weighted for all probability conditions. The mean difference between

weighted RT on go trials where a stop was possible and RT on safe go trials where no stop was possible was calculated as the measure of proactive slowing.

SNAP questionnaire

To assess ADHD symptoms at baseline (off MPH) and clinical improvement on MPH, the Swanson and Noland and Pelham Questionnaire (SNAP) was rated by the parent(s), both at baseline and on MPH (~6 weeks after participation). The SNAP questionnaire is a 26-item rating scale that assesses symptoms of attention, hyperactivity and impulsivity. Scores are based on the first 18 items.

fMRI procedure and data processing

Before MRI scanning, children participated in a mock MRI practice session to reduce possible anxiety and motion artifacts. They practiced the SSAT to become acquainted with the task and the MRI environment.

Procedures for fMRI data acquisition and data processing were similar to previous described methods in Zandbelt & Vink (2010) (Zandbelt *et al*, 2010) and are described in detail in the supplementary material.

ERP procedure and data processing

Procedures for ERP acquisition and data processing were similar to that of Logemann et al (Logemann *et al*, 2013), and are described in detail in the supplementary material.

Statistical analyses

All statistical analyses were performed using SPSS 22.0. The SSRT and SNAP data were normally distributed. Yet all analyses were carried out non-parametrically in consideration of our modest sample size. We assessed the effect of MPH on reactive stopping, proactive slowing and clinical measures by subtracting the baseline condition (off MPH) from the response condition (on MPH), yielding difference scores. We reversed the sign of the effect where necessary so that positive scores corresponded to improvement. Spearman correlations were calculated to investigate hypothesized relationships between MPH response and measures of brain activity.

Results

1. *Baseline SSAT performance*

At baseline, participants had a mean SSRT of 293 ms (comparable to the SSRTs in children with ADHD we have reported before (i.e. 298 ms (van Hulst *et al*, 2018)). Average response time on safe go trials (i.e., go trials with 0% stopping probability) was close to the target response time of 800ms (818 ms) with a small standard deviation (31 ms), indicating that all subjects were able to perform the task. Wilcoxon signed-rank tests showed no difference in response time on safe go trials compared to those where stop signals could occur for the whole group (Z= -.316, *p*=.717). Similarly, Friedman's test of differences among repeated measures showed no significant effect of higher probability on go response times ($\chi 2(4) = 6.430$, $p = 0.169$), suggesting that not all children showed clear proactive slowing at baseline. Indeed, 11 of 19 children had shorter or the same average RT on trials where a stop could occur compared to safe trials. The only effect of age was on baseline SSRT and SNAP scores, where younger children had longer SSRTs and higher symptom scores. See Table 1 for demographics and baseline performance.

Table 1. Demographics

General	
Age (years) (SD)	9.88 (1.16)
Gender (boys/girls)	11/8
Total IQ (SD)	108 (16)
MPH dose (mg) (SD)	16.84 (14.96)
MPH duration (months) (SD)	14.47 (17.75)
Diagnose (C/ I/ H-I)	9/9/1
Cognitive measures (SSAT)	
Go RT safe trials (ms) (SD)	818 (31)
Go RT trials >0% (ms) (SD)	820 (28)
SSRT (ms) (SD)	293 (25)
Proactive slowing (RT difference (ms) (SD)	2 (12)
Behavioral scores	
SNAP Parents Total (SD)	32.06 (9.71)

*MPH= methylphenidate, Diagnose (subtypes): C= combined; I= inattentive; H-I= hyperactive-impulsive, Go RT= go response time, SSRT = stop signal reaction time, SNAP= Swanson Noland and Pelham questionnaire; *p< .05, **p<.01. Demographics and SSAT, N=19. SNAP baseline n=17*

2. *Effect of methylphenidate on symptoms and performance*

Wilcoxon signed-rank tests showed that methylphenidate had a positive effect on reactive stopping (SSRT) and on ADHD symptoms (SNAP). See Table 2. Furthermore, we found evidence of proactive slowing at the group level at follow-up (on medication) whereas it was not evident at baseline: at follow-up, RT on safe go trials (where no stop signal could occur) was faster than on go trials where stop signals could occur (Z=-2.093, *p*=.036). 13 of 19 children showed proactive slowing at follow-up (compared to 8 at baseline) and Friedman's test of differences among repeated measures showed significant proactive slowing ($\chi2(4) = 12.229$, $p = 0.016$). However, the improvement in proactive slowing in response to MPH failed to reach statistical significance at the group level. See also Figure 1.

Table 2. Effect of MPH on stop task performance and behavior

	OFF MPH	**ON MPH**	***Improvement***	***Difference OFF-ON MPH***
Cognitive measures (SSAT)				
Go RT safe trials (ms) (SD)	818 (31)	813 (15)	4.47 (24.56)	Z= -0.97, p= .334
Go RT trials >0% (ms) (SD)	820 (28)	820 (16)	0 (23)	Z= -0.362, p= .717
SSRT (ms) (SD)	293 (25)	269 (24)	24 (35)	**Z= -2.58, *p*= .010***
Proactive slowing (RT difference (ms) (SD)	2 (12)	7 (13)	5 (19)	Z= -1.127, p= .260
Behavioral scores				
SNAP Parents Total (SD)	32.06 (9.71)	15.93 (10.00)	17.43 (12.03)	**Z= -3.172, *p*= .002****

*MPH= methylphenidate, Go RT= go response time, SSRT = stop signal reaction time, SNAP= Swanson Noland and Pelham questionnaire; *p< .05, **p<.01. SSAT N=19. SNAP baseline n=17, SNAP follow-up n=14.*

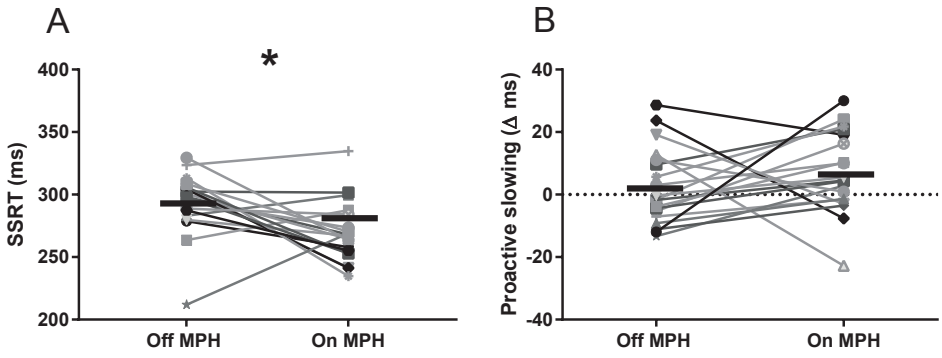

Fig 1. Individual change in SSAT performance in response to MPH. A) Individual SSRTs at baseline and in response to MPH. Children with ADHD showed a positive response to MPH, but we found marked inter-individual variability in response, with some individuals showing worse SSRT after MPH *(n=4)*. **B)** Individual proactive slowing at baseline and in response to MPH, again showing inter-individual variability in response. At baseline, proactive slowing could not be reliably detected at the group level, whereas at follow-up it was evident. *Horizontal grey lines represent mean values.*

2.1 Correlations between improvements in task performance and symptoms

Correlations between improvements in SSRT and SNAP did not exceed trend level (R_s= .465, n=14, *p*= .094). When we only included subjects who showed improvement on SSRT, this correlation met the nominally significant level (R_s= .610, n=11, *p*=.046). Interestingly, improvement in proactive slowing correlated negatively with improvement on SNAP, i.e. children who showed more proactive improvement benefited less from MPH clinically (R_s= -.564, n=14, *p*= .036).

Children who showed no improvement in SSRT *(n=4)* did show improvement in proactive slowing and vice versa *(n=5)*, suggesting that, for all individuals, at least one strategy for behavioral control improved with MPH. We found no correlations between improvements in reactive and proactive strategies.

Baseline SSRT correlated positively with SSRT improvement when controlled for age (R= .679, *p*= .002), and baseline proactive slowing correlated negatively with proactive improvement (R_s= -.654, N= 19, *p*= .002), both indicating more improvement in children with poorer baseline performance. Furthermore, proactive slowing at baseline correlated with improvement in SSRT (R_s= .644, N=19, *p*= .003), where children who showed more proactive slowing at baseline responded better to MPH (in terms of their SSRT).

3. *fMRI analyses*

After motion correction, 6 of 19 subjects had to be excluded from the fMRI analyses leaving 13 fMRI-datasets available for analysis.

First, we ran a whole-brain one-sample F-test to determine if the data were sufficient to detect neural signal associated with the task. We found activation in right inferior frontal gyrus (IFG) during successful stopping (cluster-P < 0.05, FWE- corrected) showing that the SSAT could successfully elicit stop-related neural activity in this modest sample of children with ADHD (Figure 2). In addition, we found activity in right insula during successful go trials with stop probability > 0% (cluster-P < 0.05, FWE- corrected).

A **B**

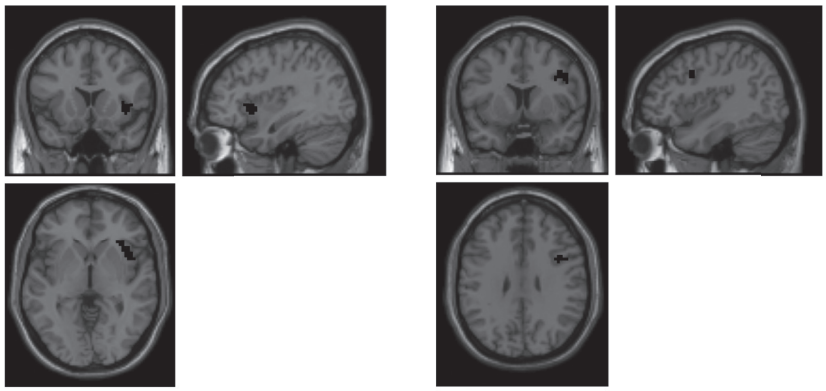

Fig. 2: Activity in right IFG and insula during SSAT performance A) Activity in right inferior frontal gyrus for correct stops (fMRI contrast 2). **B)** Activity in right insula for correct go's (fMRI contrast 3), both cluster-P < 0.05, FWE-corrected.

4. *ERP analyses*

To further support our baseline neural hypotheses, we collected an ERP version of the stop signal task (SST), with the aim of investigating stop N2 and stop P3. We used a modified version of the SST, more suited to the temporal sensitivity of ERP. See Supplemental Materials. This version of the task was harder for the children with ADHD to perform and we found high inter-individual variability in response times and SSRT (Mean SSRT: 445 ms, SD 200 ms). Three subjects were excluded from further analyses because of poor performance on the SST (*n*=2, <10% correct inhibitions) or poor EEG data quality (*n*= 1), leaving a

total of 16 ERP-datasets for analysis. Given the modest N, we limited ourselves to hypothesis-driven analyses.

First, we confirmed that the task worked as expected for the remaining 16 subjects: we could detect the frontocentral P3 associated with stopping, and found the expected negative correlation between P3 and SSRT (from the SST) and positive correlation between P3 and percentage successful inhibitions (R_s -.538, n=16, *p*=.031 and R_s= .526, n=16, *p*=.036, respectively).

Stop N2 was negatively correlated with SSRT from the ERP assessment, where large (greater negativity) N2 was associated with longer SSRT (R_s= -.568, n=16, *p*= .022).

5. *Correlation analyses of brain activity and improvements in task performance and symptoms*

Given the modest N, we focused on our hypothesis-driven analyses of whether activity at baseline in the hypothesized ROIs (bilateral IFG and SFG) predicted improvement in task performance (both proactive slowing and SSRT) and symptoms.

For the fMRI correlation analyses, we used ROIs from an earlier study using this task (Zandbelt *et al*, 2010). Given the number of regions under consideration (5) and the number of contrasts (4), only *p*-levels of <.0025 were considered truly significant (i.e., after correction for multiple comparisons), whereas *p* <.05 was considered nominally significant and taken as suggestive. For the EEG-analyses, *p* <.025 was considered significant.

5.1 Correlations between brain activity and improvements in task performance

There was a nominally significant negative correlation between baseline activity in right dorsal inferior frontal gyrus (right dIFG) associated with the parametric modulation of stop probability (fMRI contrast 4) and improvements in reactive inhibition (SSRT; R_s= -.626, n=13, *p*= .022), where children who showed less effect of stop signal probability on activation in this area had a more beneficial response to MPH. See Fig. 3. In addition, there was a nominally significant negative correlation with baseline activity in right SFG to correct stops (fMRI contrast 2) and improvement in proactive slowing (R_s= -.582, n=13, *p*= .037), where children who had less activity associated with correct stops in this area showed less proactive improvement. There were no correlations between ERP measures (stop N2 and P3) and improvements in task performance.

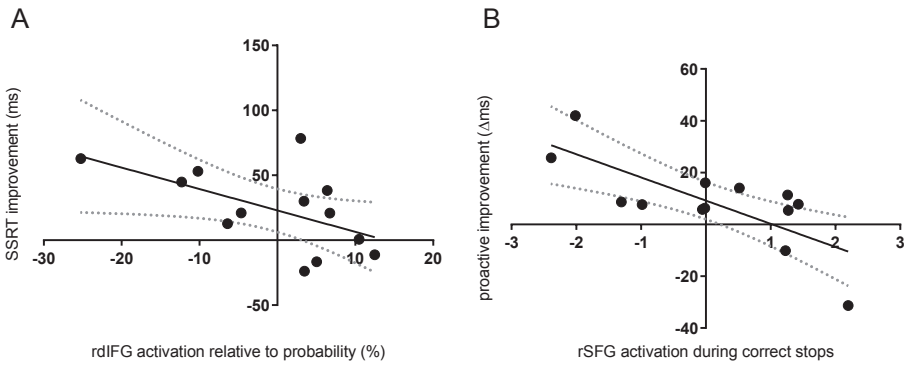

Fig 3. Activity in IFG and SFG correlate with pro- and reactive improvement. A) Less effect of stop probability on activity in right dorsal IFG correlates with greater improvements in reactive inhibition (SSRT) after MPH administration. **B)** Activity in right SFG during correct stopping (correct stops vs 0% baseline) negatively correlated with proactive slowing improvement. *rdIFG= right dorsal inferior frontal gyrus, rSFG= right superior frontal gyrus.*

5.2 Correlations between brain activity and improvements in symptoms

Baseline activity in right SFG to correct stops at baseline correlated with improvements in ADHD symptoms on SNAP, but only at a nominally significant level (R=.782, n=9, *p*=.013). There were no correlations between ERP measures (stop N2 and P3) and improvements in symptoms.

Discussion

This study aimed to disentangle the differential contribution of reactive and proactive strategies and their neural correlates to variability in methylphenidate (MPH) response in children with ADHD. We found that reactive and proactive mechanisms were both related to variability in MPH response, with reactive mechanisms possibly more related to clinical improvement.

All children were able to perform the stop signal anticipation task (SSAT) successfully. However, on average subjects showed no clear proactive slowing at baseline. This suggests that not all children employed a proactive strategy, and indeed proactive slowing was only evident for 8 out of 19 children with ADHD. On average, MPH was beneficial to subjects in terms of their ADHD symptoms and task performance, as previously reported (Tannock *et al*, 1989): we found a positive effect of MPH on reactive stopping, with a decrease in mean stop signal reaction time (SSRT), and a greater number of individuals

showed proactive slowing at follow-up. However, there was marked inter-individual variability in the methylphenidate response: interestingly, children who did not improve in one strategy (proactive or reactive), did improve in the other. Furthermore, there was a positive relationship between proactive slowing at baseline and improvements in reactive stopping. Although this did not generalize to the other direction, it could suggest that children benefit most for the strategy where there is most room for improvement.

Clinical improvement with MPH was related to improvements in reactive stopping for those children who also showed improvements in that measure, but this was not the case for children who improved in proactive slowing: they benefitted less clinically. This may suggest that clinical benefits from MPH are more related to reactive than proactive strategies.

We aimed to address the neural correlates of both reactive and proactive strategies using fMRI and EEG at baseline. We were able to successfully collect neural data for only a subset of our sample (n=13 for fMRI; n=16 for EEG). Therefore, we chose to run only hypothesis-driven neural analyses, to make best use of our limited power. Both neural assessments elicited reliable neural activity, as shown by activation in right inferior frontal gyrus related to successful stopping for fMRI (consistent with (Aron *et al*, 2004)) and correlations between stop P3 and successful stops and SSRT for EEG (consistent with (Bekker *et al*, 2005a; Overtoom *et al*, 2002)).

We investigated correlations between MPH response on measures of symptom severity, proactive slowing and reactive stopping with activity at baseline in five key regions (in bilateral IFG and SFG) for fMRI and two ERPs (stop N2 and P3). Less increase in activity in right dorsal inferior frontal gyrus as a function of stop probability nominally correlated with greater improvement in reactive stopping. This could be related to lower sensitivity of IFG to anticipatory mechanisms in our ADHD sample. Less activity in the right SFG during correct stops correlated with better improvement in proactive slowing, perhaps similarly suggesting less sensitivity of this region to proactive mechanisms, and possibly more room for improvement. Furthermore, greater activity in right SFG during correct stopping correlated with better clinical response to MPH. This may indicate that greater activity related to reactive stopping at baseline leaves more room for improvement in proactive than reactive strategies; a similar tradeoff was suggested by the correlation between proficient proactive slowing at baseline and MPH-induced improvement specifically for reactive SSRT. Stop N2 and P3 did not correlate with improvements either clinically, or in terms of task performance. In all, our correlations between our

measures of brain activity at baseline and improvements with MPH should be considered preliminary, given the modest N and the fact that they did not exceed nominal significance. However, they could be taken to suggest that neural circuits associated with these two strategies for behavioral control are not as clearly differentiated as has been previously suggested, given that the correlations we report were not specific to hypothesized systems.

The greatest limitation of this study is its modest sample size, which reduced our power to detect neural changes associated with variability in MPH response. However, as our sample showed variability in MPH response, we were able to relate response magnitude to measures of symptoms, behavioral control and its neural correlates. Furthermore, we used a within-subject design, meaning a smaller sample was sufficient to investigate within-subject effects. In addition, although stop tasks have been shown to be quite robust to test-retest effect (Soreni *et al*, 2009), our subjects did perform the SSAT twice on the same day, of which once in an MRI scanner. The retest effect plus different environment (MRI scanner versus test room) might have affected the results.

Overall, we set out to investigate whether differences in strategy for exerting behavioral control and associated neural mechanisms might be associated with variability in MPH response in children with ADHD. We used a stop signal anticipation task (SSAT) to differentiate between proactive and reactive mechanisms of behavioral regulation. We found that MPH improved task performance, where children displayed either improvements in proactive slowing or in reactive stopping, or both. Clinical improvements were more related to improvements in reactive stopping than proactive slowing. Our power to detect differences in neural circuits was limited, but our findings do not fully support hypotheses of distinct neural circuits for these strategies.

Funding and disclosures

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Acknowledgements

The authors would like to thank the participating children and their parent(s). The authors also would like to recognize the work of Janna van Belle, PhD for conception and design of the study, and Bram Zandbelt, PhD and Matthijs Vink, PhD for introducing the stop signal anticipation task (SSAT).

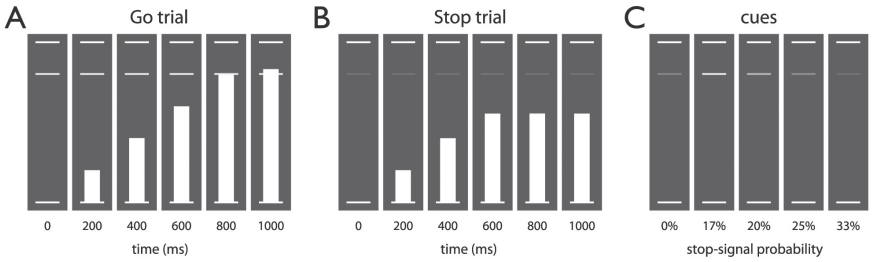
Supplementary material – chapter 4

Stop tasks

For fMRI, we used the SSAT developed by Zandbelt & Vink (2010), but shortened the practice session from 15min to 5 min – validated in a pilot study *(unpublished, 2012)*. Briefly, three horizontal lines were displayed one above the other on the screen (Supplemental Figure 1). On each trial, a bar moved at a constant speed from the lower line towards the upper line, crossing the middle line at 800ms. On go trials, the instruction was to stop the bar as close as possible to the middle line, by pressing a button. On stop trials, the bar stopped before reaching the middle line, and participants were instructed to withhold their responses. Anticipation of stopping was manipulated using a visual cue (color of the middle line) that indicated stop signal probability (i.e. green, 0%; yellow, 17%; amber, 20%; orange, 25% and red, 33%). A staircase procedure, i.e. adjusting the stop signal delay based on performance, ensured a roughly equal number of successful and unsuccessful trials, necessary to calculate inhibition performance. The stop-signal onset time was initially set to 550 ms (250 ms before the target response time) for all stop-signal probability levels, and was adjusted in steps of 25 ms depending on stopping performance and for each stop-signal probability level separately.

The task consisted of three experimental blocks, following a practice session. The total task duration was 16min 36s. Trials were presented in trial blocks consisting of 12 to 15 trials, with either baseline (go-trials with stop-signal probability of 0%; green cues) or experimental (go- and stop-trials with stop signal probability of > 0%; yellow, amber, orange or red cues) blocks with an intertrial interval of 1000 ms. In total, 234 go trials with stop-signal probability of 0%, 180 go trials with stop-signal probability >0% (yellow, n=30; amber, n=48; orange, n=54; red, n=48), and 60 stop trials (yellow, n=6; amber, n=12; orange, n=18; red, n=24) were presented.

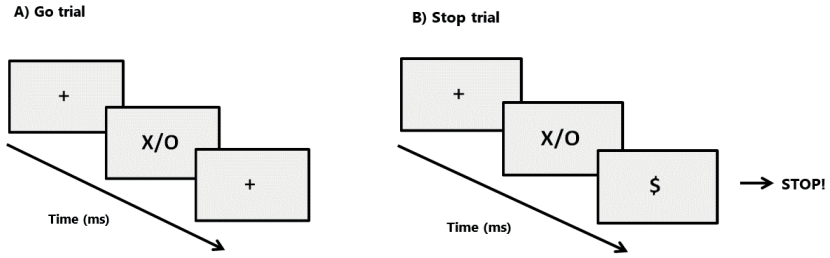
The task was presented in a storyline format, with Buzz Lightyear controlling a spaceship. Participants were instructed to land the spaceship on the moon, by stopping the bar as close as possible to the middle line (go trials). However, when circumstances became too dangerous, the automatic pilot would stop the spaceship from landing (stop trials), and participants had to withhold their response. Participants were told that the colors of the bar indicated probability of stopping: green representing 'never', yellow 'occasionally', red 'quite often', and amber and orange 'intermediately often'. Participants were instructed that it was equally important to get as close to the middle line as possible as it was to withhold response in case of automatic stopping.



Supplementary Fig. 1: The stop signal anticipation task (SSAT). A) On each trial, a bar moved at constant speed from bottom to top, reaching the colored middle line at 800ms (the target time). The task was to stop the bar as close as possible to the middle line, by pressing a button. If subjects exceeded the upper line of 1000ms, the trial was defined as inaccurate. **B)** On stop trials, the bar stopped moving automatically before the middle line and participants had to withhold their response by not pressing the button. If they did press the button, the stop trial was defined as inaccurate. **C)** The probability of a trial being a stop trial was indicated using different colors for the middle line. *Figure reprinted with approval from Zandbelt & Vink 2010, 'on the role of the striatum in response inhibition'*

To measure inhibition related ERP components, we used the visual stop signal task (SST), identical to Logemann *et al,* 2013 (Logemann *et al*, 2013), originally from Schmajuk *et al*, 2006 (Schmajuk *et al*, 2006). Participants were instructed to respond to go stimuli as quickly and accurately as possible, and to withhold their response if a stop signal occurred (See Supplementary Figure 2). Go stimuli were presented randomly and sequentially and consisted of two letters, an X and an O. In response to these letters a differential response was required (e.g. left button press after X, right button press after O). Letters were presented for 150 ms and trials varied between 1.5 and 1.8 second duration. In 25% of trials, the go stimulus was followed by a stop signal: the "$" sign, indicating the subject was to withhold the response.

Subjects practiced the task before EEG recording started, and the left-right condition was reversed half way, yielding three left X blocks and three right X blocks. The order was counterbalanced across subjects. Blocks consisted of 128 trials, and total duration of the task was 27 minutes. In the first stop signal block, stop signal onset asynchrony (SOA) was set at 250 ms, after each block the SOA was dynamically adjusted in a staircase procedure based on performance, to ensure a roughly equal number of successful and unsuccessful trials. For all stop signal blocks, the trials were randomized with the exception that no more than three stop signal trials would occur in succession.



Supplementary Fig. 2: the stop signal task (SST). A) During go trials, participants were instructed to press the button corresponding to the X or O as fast as possible. **B)** During stop trials, the go stimulus was followed by a stop signal ($) instructing subjects to withhold their response. Participants were instructed that it was equally important to react as fast as possible as it was to withhold their response on a stop trial.

fMRI procedure and data processing

Functional MRI-scans were acquired on a standard Philips Allegra 3.0 Tesla scanner at the Radiology Department of the UMC Utrecht. Scanning sessions included three (2D) echo planar image (EPI)- SENSE pulse sequences with blood oxygen level dependent (BOLD) contrast covering the whole brain and a T1-weighted scan for within-subject localization purposes. EPI scans contained 208 volumes per session, 30 slices per volume, interleaved acquisition; TR= 1.6s, TE= 23ms, field of view (FOV)= 208x256, flip angle= 72.5°, voxel size= 4x4x4mm. T1-weighted image contained 200 slices, repetition time 10.0ms, echo-time= 4.6ms, flip angle= 8°; FOV= 240x240, voxel size= .75x.8x.75mm isotropic.

Preprocessing and first-level statistical analyses were performed following the procedure described in Zandbelt & Vink (2010), using Statistical and Parametric Mapping 12.0 (SPM12; Wellcome Trust Center for Neuroimaging) in Matlab 2014b (Mathworks, Inc., Natick, MA, USA). All images were realigned to the first volume using rigid body transformations. Frame-to-frame movement was assessed using ArtRepair (Hoeft *et al*, 2007). Scans with more than1.0-mm frame-to-frame movement or where the average signal deviated more than 1.5% from the average global signal over scans, were replaced using a linear interpolation of the values of neighboring scans. Six participants with more than 20% of their scans replaced where excluded from further fMRI analyses. The anatomical image was co-registered to the first fMRI image and subsequently normalized to Montreal Neurological Institute (MNI) space

using unified segmentation. Next, functional images were normalized using the parameters generated in the previous step. Finally, the fMRI images were spatially smoothed using a Gaussian kernel with a full width at half maximum (FWHM) of 8 mm.

First-level statistical analysis followed Zandbelt & Vink(2010). Briefly, this involved modeling of successful stop trials, failed stops and correct go trials with stop-signal probability > 0% for each subject within the framework of the general linear model. Two parametric regressors modeling response time and stop-signal probability level of go trials were also included. Go trials with stop-signal probability of 0% were not modelled and constituted an implicit baseline. We accounted for head motion by including the motion parameters from realignment into the model. Contrast images were generated for (1) successful stops versus failed stops, (2) successful stops vs baseline, (3) correct go responses with stop probability > 0% vs baseline, (4) parametric modulation of probability.

First-level contrasts were analyzed in a second-level random-effects analysis using one-sample F-tests and tested for significance at voxel-wise P<0.001 and cluster-wise P<0.05 family-wise error corrected for multiple comparisons to check whether the SSAT was effective in eliciting neural signal associated with stopping. A region-of-interest approach was used to extract parameter estimates for all first-level contrasts from bilateral inferior frontal gyrus (IFG) and superior frontal gyrus (SFG) using ROIs previously created by Zandbelt & Vink (2010). Signal was extracted from five ROIs in left and right SFG, left IFG and right ventral and dorsal IFG (coordinates x, y, z: left IFG: -56,8,20; right ventral IFG: 44, 16, 4; right dorsal IFG: 48, 12, 32; left SFG: -20, 0, 68; right SFG: 24, -4, 60).

EEG procedure and data processing

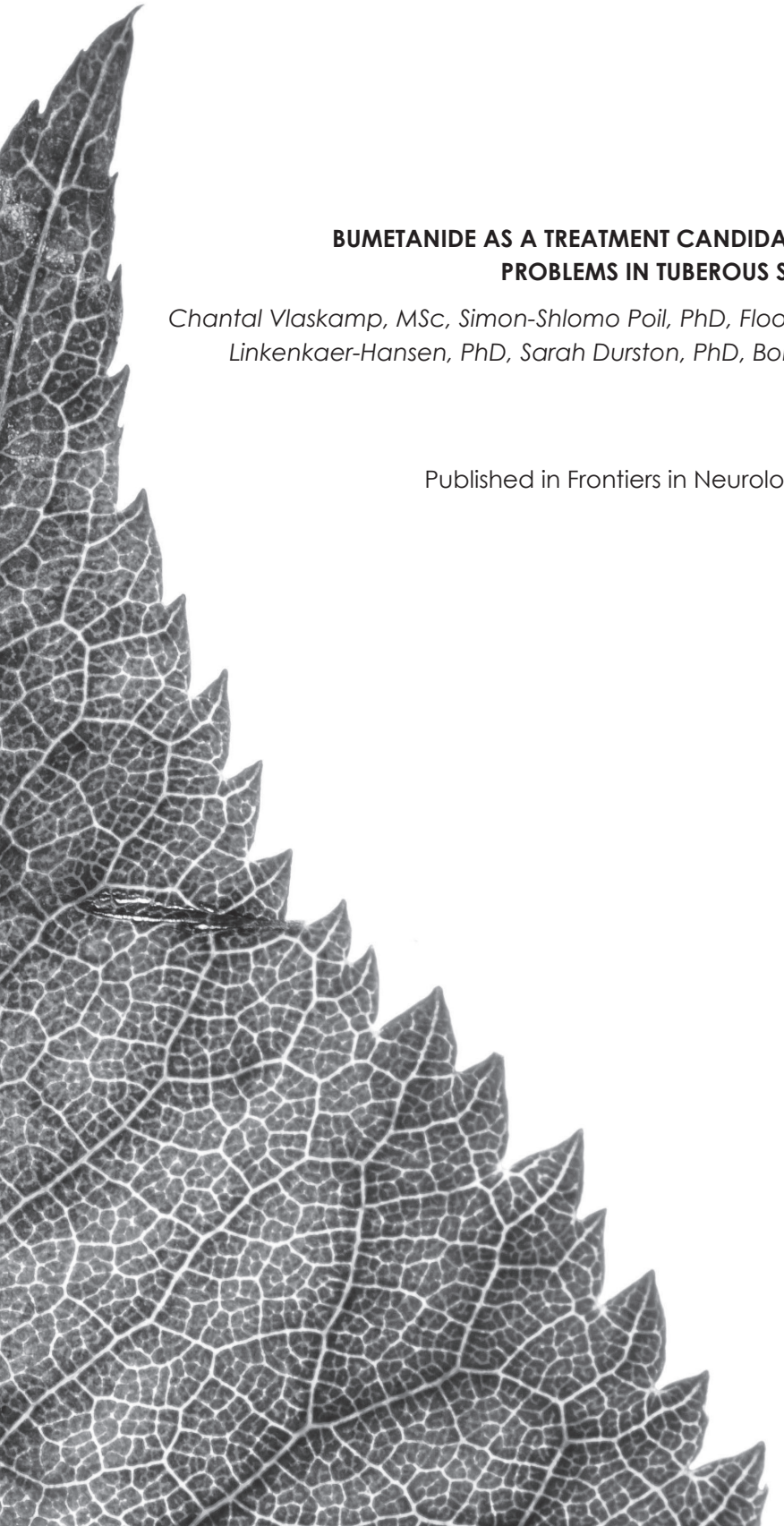
EEG was recorded using a BioSemi system with 64 active-two electrodes, according to the 10-20 system with CMS-DRL reference set up. Additionally, external electrodes were placed on both mastoids for reference purposes and above, below and at both sides of the eyes (vertical and horizontal electrooculogram (VEOG and HEOG)). ERPs were recorded while participants performed the stop signal task (SST) presented by a computer running Presentation® software in a sound reducing room.

ERPs were analyzed using BrainVision Analyzer (v1.05). Data processing began by resampling the original data from 2048 Hz to 250 Hz, preceded by standard anti-aliasing filtering. A 2Hz high pass filter and a 30Hz low pass filter

were used offline. Left and right blocks were appended – after an initial check - and the data was re-referenced to the mastoids. Epochs were corrected for ocular activity using the Gratton and Coles algorithm (VEOG) (Gratton *et al*, 1983). Next, artifacts in epochs were removed using a difference criterion of 100 μV based on the most relevant electrodes: FC4 and FCz (for N2 and P3, respectively). For each subject, epochs containing failed stops, and (separately) successful stops were averaged, time-locked to the go stimulus and to the stop stimulus.

Second-level processing included the Adjacent Response (ADJAR) procedure to isolate processes related to stopping by removing overlap distortion associated with the go process (Woldorff, 1993). Due to technical difficulties, the ADJAR procedure could not be run in four subjects, whose data were therefore included without ADJAR correction. After the ADJAR procedure, a 0-50ms post-stimulus baseline correction was applied to correct for residual go-related baseline activity (Lansbergen *et al*, 2007).

After visual inspection of the grand average and difference wave of successful – failed stops, the window for stop P3 was defined between 300 and 360 ms, analyzed as the mean area at FCz (where maximum amplitude was expected) of the difference wave. Although stop N2 was hard to detect in the grand average, N2 was analyzed as the mean area at right frontal electrode FC4 for all participants, in a window between 170 and 210 ms, based on visual inspection of the difference wave and the window used by Pliszka *et al* (2007).



5

BUMETANIDE AS A TREATMENT CANDIDATE FOR BEHAVIORAL PROBLEMS IN TUBEROUS SCLEROSIS COMPLEX

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BUMETANIDE AS A TREATMENT CANDIDATE FOR BEHAVIORAL PROBLEMS IN TUBEROUS SCLEROSIS COMPLEX

Abstract

Background. Recent studies indicate excitatory GABA action in and around tubers in patients with Tuberous Sclerosis Complex (TSC). This may contribute to recurrent seizures and behavioral problems that may be treated by agents that enhance GABAergic transmission by influencing chloride regulation.

Case presentation. Here, we used the chloride transporter antagonist bumetanide to treat a female adolescent TSC patient with refractory seizures, sensory hyper-reactivity and a variety of repetitive and compulsive behaviors.

Methods. To evaluate the effect of bumetanide on behavior, auditory sensory processing and hyperexcitability, we obtained questionnaire data, event-related potentials (ERP) and resting state EEG at baseline, after three and six months of treatment and after one month washout period.

Discussion. Six months of treatment resulted in a marked improvement in all relevant behavioral domains, as was substantiated by the parent questionnaires. In addition, resting-state electroencephalography and event-related potentials suggested a favorable effect of bumetanide on hyperexcitability and sensory processing. These findings encourage further studies of bumetanide on neuropsychiatric outcome in TSC.

Introduction

This case report describes a 20-year old female patient with Tuberous Sclerosis Complex (TSC) characterized by a mild mental retardation, autism spectrum disorder, focal epileptic seizures and a wide range of disabling behavioral problems. The behavioral problems became unmanageable and she presented to our outpatient clinic for treatment possibilities. The wide range of refractory behavioral problems and previous paradoxical response to valproic acid led to the suspicion of depolarizing GABA activity.

Background

Treatment in Tuberous Sclerosis Complex (TSC) is mostly aimed at controlling seizures, whereas there are few treatment options for the accompanying behavioral and neuropsychiatric problems at present. Irritability, sensory arousal, repetitive behaviors, sleeping problems and deficits in social communication are very common in patients with TSC (Curatolo *et al*, 2015) and these so-called tuberous sclerosis-associated neuropsychiatric disorders (TAND) are often extremely debilitating, although rarely treated successfully. Pharmacotherapy to reduce TAND is mostly attempted with psychostimulants such as methylphenidate or antipsychotic treatments, both of which can have serious side-effects, such as lowering the seizure threshold (Asato and Hardan, 2004). Treatment of TAND is presumably complicated by the variability and heterogeneity of TAND (de Vries *et al*, 2015). However, syndrome specific mechanisms such as mTOR dysregulation may be a general target to reduce TAND across the TSC population.

Indeed, mTOR inhibitors are being tested as possible treatment for a wide variety of neurological sequelae of TSC. Although promising for seizure reduction no clear effects on behavioral outcome have yet been established (de Vries, 2010). Recent studies have suggested another avenue for treatment of neurological problems. It has been shown that chloride homeostasis may be disrupted in and around tuber tissue in TSC as indicated by altered expression levels of the chloride cotransporters NKCC1 and KCC2 (Na–K–2Cl cotransporter isoform 1 and K–Cl cotransporter isoform 2, respectively). These disturbances could be leading to (focal) depolarizing GABA activity and, consequently, altered balance between excitation and inhibition (E/I) in these regions (Ruffolo *et al*, 2016; Talos *et al*, 2012). These findings suggest that the NKCC1 chloride transporter antagonist bumetanide might enhance synaptic transmission by strengthening inhibition in TSC. Indeed, Talos et al.

(2012) showed that excitatory GABA activity in slice preparations of human tuber tissue was effectively attenuated by bumetanide *in vitro* (Talos *et al*, 2012).

Clinical benefit of bumetanide therapy has not yet been demonstrated in TSC, but has been shown to reduce core behavioral problems in autism spectrum disorder (ASD) (Grandgeorge *et al*, 2014; Lemonnier *et al*, 2012). The strong association of TSC with ASD further suggests that bumetanide might be able to improve behavioral problems in TSC. Additionally, we have previously suggested that (history of) a paradoxical reaction to GABAergic drugs may be a prognostic marker of depolarizing GABA activity, suggesting bumetanide may be efficacious (Bruining *et al*, 2015). In the current study, bumetanide was administered to a 20-year old female with TSC and a history of paradoxical reaction to valproic acid. Effects on behavior and sensory processing were assessed using questionnaires and a neurophysiological test battery.

Case presentation

A 20-year old female patient with TSC presented to our clinic with a long history of neurodevelopmental problems characterized by moderate mental retardation (Total IQ ~50,), autism spectrum disorder (pervasive developmental disorder not otherwise specified (PDD-NOS) according to the DSM-IV TR (diagnostic and statistical manual of mental disorders, fourth edition, text-revision) and focal epileptic seizures. The manifestation of TAND in this patient was characterized by intellectual disability, sensory hyper-reactivity, irritability, obsessive and compulsive behaviors, behavioral rigidity and inflexibility, accompanied by sleeping problems.

Family history showed 2nd-degree history of ASD. Birth was complicated by meconium aspiration that resulted in several days of assisted ventilation. The first established developmental problems were motor- and speech delay at toddler age. TSC was suspected because of focal seizures from the age of 9 months, skin pigment stains and structural anomalies on brain imaging. Genetic investigation confirmed a spontaneous deleterious mutation in the TSC1 gene. Subsequent MRI scans showed subcortical tubers and subependymal noduli in both hemispheres. Since childhood, the patient experienced focal to bilateral tonic-clonic seizures 1–2 times a year and focal seizures with behavioral arrest and automatisms 1–5 times daily. Localization of interictal epileptiform discharges was left frontally. Previous treatment

with various antiepileptic drugs (AED) did not sufficiently control seizures. The patient had experienced a paradoxical response (evident increase instead of decrease of irritability and anxiety) to valproic acid (VPA). AED regime at time of the study consisted of oxcarbazepine monotherapy.

The previous paradoxical response to VPA and daily burden of behavioral problems and seizures led us to suspect depolarizing GABA activity. Therefore, we treated the patient with 0.5 mg bumetanide twice daily (according to Lemonnier et al, 2012) for a 6-month trial followed by a 1-month washout period to substantiate efficacy. Parents of the patient gave consent for off-label treatment. Monitoring of treatment effect included an extensive evaluation of behavioral questionnaires and EEG/ERP measurements. Blood tests and physical examinations were carried out as described previously (Bruining *et al*, 2015). Transient hypokalemia was successfully treated with potassium supplements. Treatment did not cause other disturbances or discomfort due to diuretic effects. The patient received no concurrent therapies or interventions during the course of treatment, other than continuation of oxcarbazepine (a sodium channel inhibitor).

Methods

Questionnaires

Clinical Global improvement (CGI) was assessed by the treating psychiatrist. Questionnaires included the Social Responsiveness Scale (SRS) (Constantino *et al*, 2003), the Sensory Profile (SP-NL) (Tomchek and Dunn, 2007), the Repetitive Behavior Scale- Revised (RBS-R) (Lam and Aman, 2007), the Aberrant Behavior Checklist (ABC) (Rojahn *et al*, 2003) and the Behavior Rating Inventory of Executive Function (BRIEF) (Huizinga and Smidts, 2011) and were filled in by both parents and tutor.

ERP measurements

Auditory sensory processing was assessed using a P50 suppression task to measure sensory gating and a passive oddball paradigm to measure mismatch negativity (MMN). Both tasks originate from the Copenhagen Psychophysiological test battery (Madsen *et al*, 2015; Oranje B, 2017), which additionally consisted of a startle paradigm (PPI) and a selective attention task. All testing was performed with a 64 electrode BioSemi set-up according to the 10-20 system. Sampling started as soon as the paradigms were started, and lasted to the end of it (continuous recording). The sample frequency was 2048 Hz, and a low-pass setting of 1/5 of the AD rate. All stimuli were

presented binaurally through tubal insert ear phones (EARtone®, Etymotic Research), by a computer running Presentation® software (Neurobehavioral Systems Inc.).

P50 paradigm: The P50 paradigm consisted of three experimental blocks, each consisting of 40 pairs of identical bursts of (1.5 ms and 80 dB) white noise, with an instantaneous rise time, an inter-stimulus interval of 500 ms, and a fixed inter-trial interval of 10 sec. The subject was asked to count the stimuli she was presented and to stay awake. The total duration of the P50 task was approximately 21 minutes.

MMN paradigm: The MMN paradigm consisted of 1800 stimuli. The paradigm consisted of 4 types of stimuli: In 82% of the cases, a standard tone with a frequency of 1000 Hz, intensity of 75 dB and duration of 50 ms was presented. Within this sequence of standard stimuli, three types of deviants were presented, each with a probability of 6% and intensity of 75 dB: Frequency deviants of 1200 Hz and 50 ms, duration deviants of 1000 Hz and 100 ms and frequency-duration deviants of 1200 Hz and 100 ms. The inter-stimulus interval (ISI) was randomized between 300 and 500 ms. The subject was asked to ignore the stimuli while watching a silent movie. The total duration of the MMN task was approximately 15 minutes.

ERP analysis: Analysis and processing of the EEG signal was carried out using Brain Electrical Source Analysis (BESA) software (version 5.2.4, MEGIS Software GmbH, Gräfelfing, Germany). Only data from relevant electrodes were processed and analyzed; i.e. Cz for P50 and FCz for MMN. Processing of the data started with re-sampling from the original 2048 Hz to 250 Hz to allow easier file handling. Second, the data were corrected for eye-artefacts by using the adaptive method of BESA. Third, the data were epoched (from 100 ms prestimulus to 400 ms post-stimulus for P50, and 900 ms post-stimulus for MMN), and corrected for movement (or other paradigm unrelated) artefacts by removing epochs that contained amplitude differences between maximum and minimum exceeding 75 μV in the relevant scoring windows. Subsequently, the data were band-pass filtered (high-pass: 1 Hz, low-pass: 70 Hz for P50, 40 Hz for MMN).For MMN, each of the three deviant types was expressed as the average ERP to the relevant deviant stimuli, subtracted with the average ERP to standard stimuli for each subject separately. Linked mastoids were used as a reference. Finally, MMN amplitudes were scored as the minimum amplitude within a window between 100 and 230 ms.

For P50 analysis we used an average reference. P50 amplitude was defined as the largest trough to peak amplitude within an interval of 40–90ms following

the first (conditioning or "C") stimulus in each paired click. The P50 amplitude following the second (testing or "T") stimulus was identified as the largest trough to peak amplitude within an interval of 10 ms of the latency of the maximum P50 amplitude to the C-stimulus. P50 suppression was expressed as the ratio "T/C."

Resting-state EEG measurements

Resting-state EEG was measured during 3 minutes eyes-closed rest using BioSemi with a sampling frequency of 2048 Hz (resampled offline to 512 Hz for analysis) with no hardware filters. Analysis was performed using the Neurophysiological Biomarker Toolbox (Hardstone et al, 2012). The signals were reviewed and transient artifacts were cut out. As a measure of long-range temporal correlations, we applied detrended fluctuation analysis (see Linkenkaer-Hansen et al., 2001 (Linkenkaer-Hansen *et al*, 2001)) to the amplitude envelop of band-pass filtered signal (FIR filter with a Blackman window, transition bandwidth 1 Hz) in the bands Alpha 8–13 Hz and 6–13 Hz. A DFA exponent of 0.5 implies that oscillations amplitudes fluctuate in an uncorrelated fashion over time. When exponents increase from 0.5 towards 1.0, they signify increasing strength of long-range temporal correlations. Power spectral density was calculated using Welch's method with 8 second hamming windows. Relative power was calculated as the bandwidth power normalized by the power in the 1–45 Hz band.

Results

No significant change in seizure frequency or severity was observed. From approximately 1 month of treatment, marked improvements in social responsiveness, attention, reduced irritability and restlessness were noted by both parents. In addition, sleep efficiency was improved. Parental observations were substantiated through questionnaires showing marked improvement in behavior following bumetanide treatment.

The greatest improvements (> 50% from baseline) after 3 and 6 months of treatment were seen on (subscales of) the Sensory Profile (SP-NL), Aberrant Behavior Checklist (ABC), Social Responsiveness Scale (SRS) and the Repetitive Behavior Scale (RBS-R). In addition, improvements in behavior were also seen on the Behavior Rating Inventory Executive Function questionnaire (BRIEF) rated by the tutor of the patient. Figure 1 illustrates the most pronounced behavioral improvements, Table 1 shows the measured scores on all behavioral questionnaires at baseline, during treatment and at washout, and

percentage of improvement after treatment.

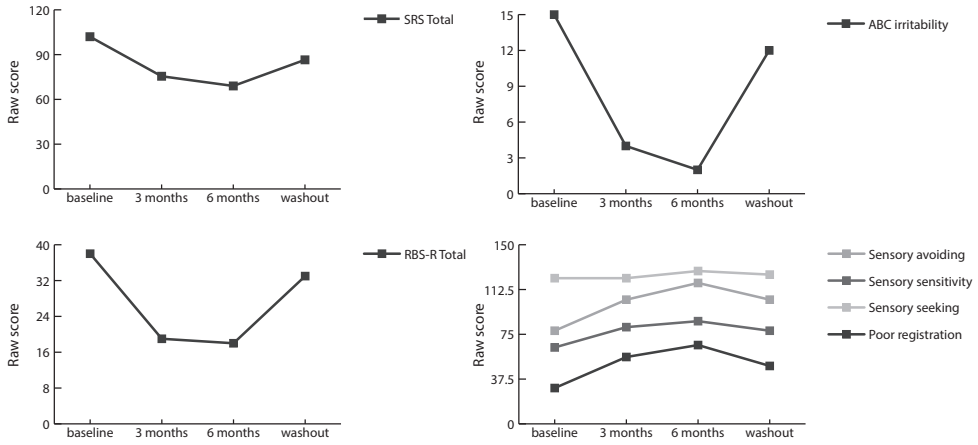


Figure 1: Effect of bumetanide on behavior. (A) Total score of the social responsiveness scale is decreased by treatment with bumetanide, the effect is removed at washout **(B)** Irritability, as measured by the abberant behavior checklist subscale, is markedly decreased by bumetanide treatment. At washout, irritability increases again. **(C)** Repetitive behavior is decreased by bumetanide treatment, as indicated by total score on the repetitive behavior checklist (RBS-R). Washout again removes this effect. **(D)** Similarly, sensory behavior as measured by Sensory Profile subscales improves during three and six months of bumetanide. Again, washout reverses the effect.

Table 1. Questionnaire scores (Raw scores) *-continues on next page*

Questionnaires - Raw scores	baseline	3 months	6 months	Washout	Improvement 3 months (%)	Improvement 6 months (%)
CGI	6	4	3	3	33,33	50,00
SP-NL Poor registration*	30	56	66	48,5	86,66	120,00
*SP-NL Sensory seeking**	122	122	128	125	0	2,46
SP-NL Sensory sensitivity*	64	81	86	78	26,56	21,88
SP-NL Sensory avoiding*	78	104	118	104	33,33	51,28
SRS total	129	79	66	100	38,76	48,84
SRS social awareness	17	10,5	9	10,5	38,24	47,06
SRS social cognition	29	17	14	22,5	41,38	51,72
SRS social communication	48	29	22	36	39,58	54,17
SRS social motivation	11	8	7	12	27,27	36,36
SRS restricted interests	24	14,5	14	19	39,58	41,67
ABC total	71	17,5	16	45	75,35	77,46
ABC irritability	15	4	2	12	73,33	86,67
ABC lethargy	19	6	4	12,5	68,42	78,95
ABC stereotypy	6	1,5	2	1,5	75,00	66,67
ABC hyperactivity	21	4	5	13	80,95	76,19
ABC inappropriate speech	10	2	3	6	80,00	70,00
RBS-R total	36	19	18	33	47,22	50,00
RBS-R stereotyped behavior	0	0	0	0,5	0,00	0,00
RBS-R self-injurious behavior	*1*	*1*	*3*	*2*	*0,00*	*-200,00*
RBS-R compulsive behavior	10	3,5	2	5	65,00	80,00
RBS-R routine behavior	9	3,5	3	9	61,11	66,67
RBS-R sameness behavior	13	8,5	7	12,5	34,62	46,15
BRIEF total *parents (P)*	178	143,5	129	149	19,38	27,53
BRIEF inhibition *P*	26	22	19	21,5	15,38	26,92
BRIEF shift *P*	22	16,5	16	18	25,00	27,27
BRIEF emotional control *P*	27	18,5	17	21	31,48	37,04
BRIEF initiate *P*	16	15,5	13	15,5	3,13	18,75
BRIEF working memory *P*	25	19	18	20,5	24,00	28,00
BRIEF plan/organize *P*	25	20	17	20	20,00	32,00
BRIEF organization materials *P*	16	13	11	14	18,75	31,25

BRIEF monitor *P*	21	19	18	18,5	9,52	14,29
BRIEF total *school (S)*	191	163	151	179	14,66	20,94
BRIEF inhibition *S*	30	26	24	28	13,33	20,00
BRIEF shift *S*	29	22	20	25	24,14	31,03
BRIEF emotional control *S*	24	21	17	24	12,50	29,17
BRIEF initiate *S*	18	17	15	14	5,56	16,67
BRIEF working memory *S*	26	22	22	25	15,38	15,38
BRIEF plan/organize *S*	20	15	16	20	25,00	20,00
BRIEF organization materials *S*	19	15	16	17	21,05	15,79
BRIEF monitor *S*	25	25	21	26	0,00	16,00

Colored rows indicate greatest improvement after 3-6 months (i.e. 50%+) *Sensory profile subscales: higher scores are more towards normality (except for sensory seeking) *CGI= clinical global improvement, SP-NL= sensory profile, Dutch edition, SRS= social responsiveness scale, ABC= aberrant behavior checklist, RBS-R= repetitive behavior scale – revised, BRIEF= behavior rating inventory executive function*

To investigate physiological effects of treatment, we measured event-related potentials (ERP) using standard EEG paradigms of P50 suppression to assess sensory gating and a passive auditory oddball paradigm to assess mismatch negativity (MMN). In addition, we recorded resting-state EEG before and after treatment and washout. Sensory gating increased with bumetanide, followed by a decrease after washout (See Fig. 2A). MMN amplitude to frequency deviants slightly increased after treatment, whereas the amplitude of duration and frequency-duration MMN slightly decreased. Again, washout appeared to reverse these effects (See Fig. 2C). It should be noted that sensory gating as well as MMN were within normal ranges at baseline and during treatment (Rydkjaer *et al*, 2017).

Power spectrum analysis of resting-state EEG showed that the peak frequency of alpha activity was around 7 Hz, which is lower than in the normal population (8–13 Hz). No pronounced effects of bumetanide on peak or alpha power were observed, although (extended) alpha power was highest after six months of treatment and decreased again at washout. Detrended fluctuation analysis (DFA) measures the strength of long-range temporal correlations (Hardstone *et al*, 2012) and has previously been coupled to changes in the excitatory/inhibitory balance (Poil *et al*, 2012; Poil *et al*, 2011). We applied this analysis as a possible proxy of changes in overall E/I imbalances of GABAergic enhancement through bumetanide. DFA in extended alpha band (6–13 Hz)

showed an increase of 0.04 (median across channels) in the DFA exponent after six months of bumetanide treatment, and a decrease at washout (See Fig. 2B). This increase in the DFA exponent is on the order of magnitude of the effects seen in other brain disorders, such as Alzheimer's disease (Montez *et al*, 2009) and schizophrenia (Nikulin *et al*, 2012).

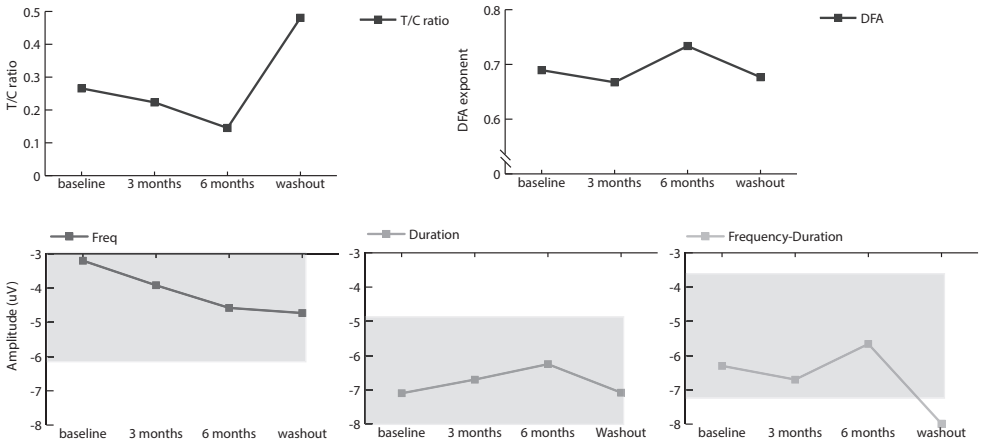


Figure 2: Effect of bumetanide on ERP and resting-state EEG. (A) T/C ratio illustrating sensory gating (as measured by the P50 suppression paradigm) is increased (lower T/C) after three and six months of bumetanide treatment. Washout decreases sensory gating again. (**B)** Average DFA exponents at different measurement time points (median across all channels) in the extended 6–13 Hz Long-range temporal correlations (DFA exponents) are strengthened after six months bumetanide treatment. (**C)** Mismatch negativity for frequency (C1), duration (C2) and frequency-duration (C3) deviant. Frequency MMN amplitude increases after six months treatment, duration and frequency-duration MMN amplitude show a decrease after six months' treatment. Washout removes this effect. Grey areas represent normal range, +/- 1 SD from healthy population as in (Rydkjaer *et al*, 2017). Note: For duration MMN, normal range goes up to -11.98 μV.

Discussion

This case describes beneficial effects of treatment with bumetanide on behavior in a 20-year-old female diagnosed with Tuberous Sclerosis Complex (TSC). This provides clinical support of preclinical studies that suggested that neuronal disinhibition (immature GABA activity) may be a mechanism

contributing to TSC pathogenesis. Various domains of behavioral functioning improved during 6 months of bumetanide treatment, as indicated by both parents and scores on behavioral questionnaires rated by parents as well as the tutor of the patient.

Although this is merely a case report, some of the neurophysiological measurements appear to support a positive effect of bumetanide treatment on neuronal inhibition. Most notably, the strength of long-range temporal correlations (DFA measurement) was normalized during treatment, an effect that disappeared during the wash-out period. This finding is in line with suggestions of an effect of bumetanide on the excitatory/inhibitory balance, as modeling and clinical studies have shown less strong long-range temporal correlations when the E/I balance is impaired (Hardstone *et al*, 2012; Montez *et al*, 2009; Poil *et al*, 2012). Sensory gating and MMN also demonstrated a possible positive effect of bumetanide, although this should be interpreted with caution due to lack of experience with individual ERP assessment.

Washout measurements suggest that bumetanide did not result in continued therapeutic effects when administration ceased in this patient, as behavior and DFA (and possibly ERPs) deteriorated after discontinuation of bumetanide.

Our group previously reported effectiveness of bumetanide in a child with ASD and epilepsy with a previous paradoxical reaction to GABAergic drugs. This girl also suffered from a structural brain lesion in the temporal lobe (Bruining *et al*, 2015). Indeed, temporal lobe epilepsy has been linked to elevated expression of the chloride importer NKCC1, the specific target of bumetanide (Huberfeld *et al*, 2007). In addition, increased NKCC1 expression has been found in cortical dysplasia specimens from humans with focal epilepsy related to those found in TSC (Talos *et al*, 2012). The current study therefore provides further preliminary evidence that neuronal disinhibition constitutes a treatment target in to cortical dysgenesis related neurodevelopmental disorders, especially when paradoxical responses to GABAergic drugs have been noted.

Concluding remarks

This case report confirms that bumetanide may be effective in reducing the high burden of behavioral problems in TSC, at least for some patients. More extensive trials are required to confirm the efficacy of bumetanide in TSC.

Acknowledgements

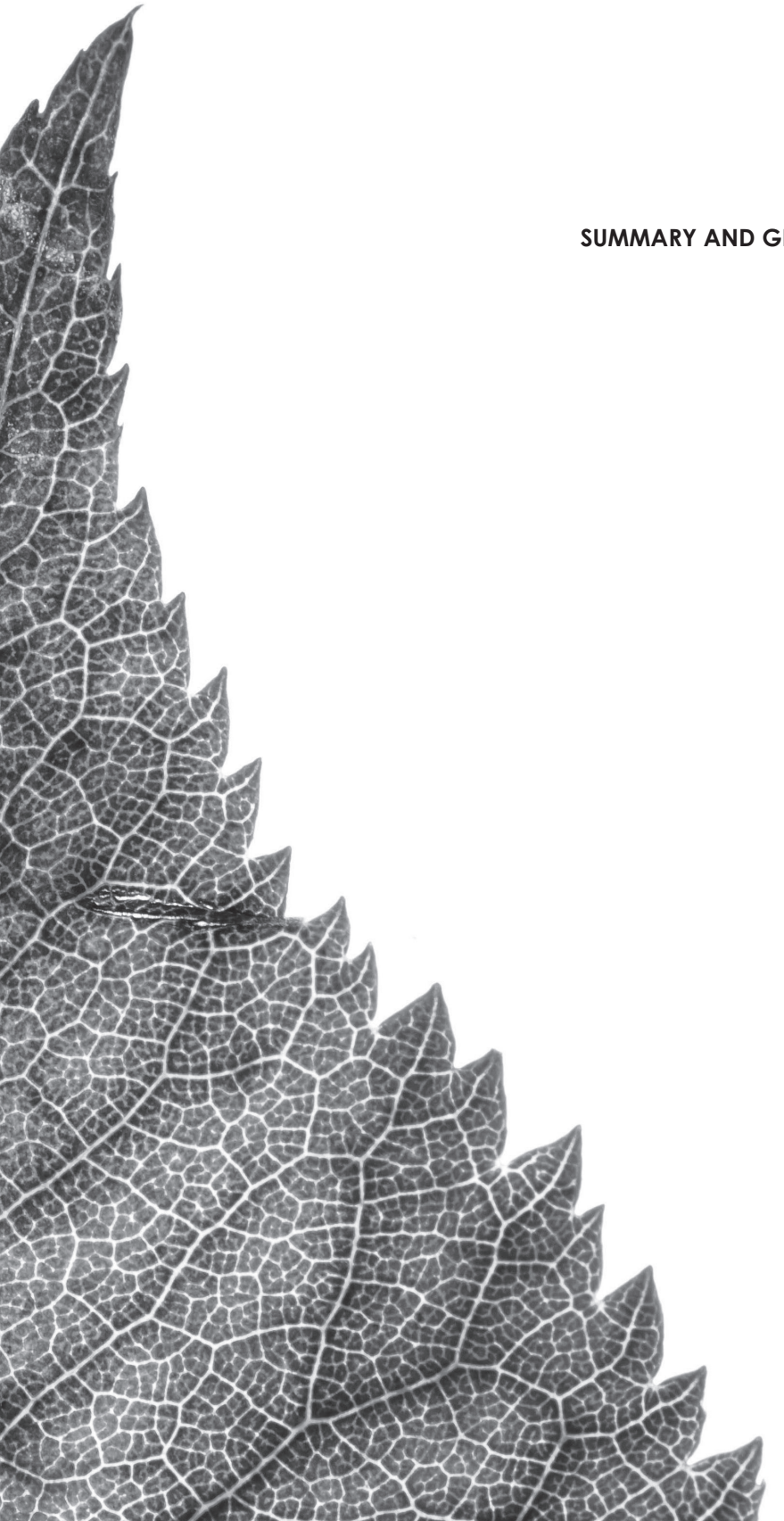
The authors thank the patient and her parents for the compliance and detailed evaluation.

Patient consent

The patient and her parents provided verbal and written consent for publication of this case report.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



6

SUMMARY AND GENERAL DISCUSSION

CHAPTER 6: SUMMARY AND GENERAL DISCUSSION

Developmental disorders such as autism spectrum disorder and attention deficit hyperactivity disorder are extremely debilitating. In addition, their prevalence has risen exponentially over the last decades. Classified through behavioral observations, according to the diagnostic statistical manual of mental disorders (DSM-5), these constructs are heterogeneous by clinical definition. In this thesis, we investigated a range of neural measures to dissect the heterogeneity (interindividual variability) that characterizes developmental disorders and explore its relation with treatment.

Summary of main findings

In **chapter 2**, we investigated auditory processing using an auditory oddball paradigm in a Danish sample of children with autism spectrum disorder and their typically developing peers. We found differences in mismatch negativity (MMN) elicited by duration and frequency duration deviants, indicating ASD children were less responsive to environmentally deviant stimuli at an early- sensory- level than their TD peers, specifically for temporal (i.e. in time) encoding stimuli. In addition, an increase in *(MMN-paradigm derived)* duration related P3a amplitude suggested difficulties at the attentional level. Also, correlations were found between these ERPs and behavior, e.g. between duration MMN and scores on the social responsiveness scale (SRS), indicating a relationship between early auditory processes and behavior. In **chapter 3**, we went a step further and investigated a large number of additional, consecutive temporal ERP components not only addressing mismatch negativity, but also sensory gating and selective attention, in a large group of children with ASD and additional sensory issues, compared to TD children. Furthermore, we included non-prototypical ASD children with comorbid epilepsy, enabling us to investigate whether this would represent sensory subtypes in ASD. Where in chapter 2 we found possible hypo-responsivity to auditory stimuli in ASD, we did not find such MMN differences in chapter 3. One possible explanation might be differences between the samples: whereas the Danish sample in chapter 2 included more prototypical ASD subjects (e.g. presented with more social communication problems), the ASD sample in chapter 3 included subjects recruited via the sensory processing program (SPP), who therefore presented with more sensory issues. Indeed, the finding of decreased duration and frequency-duration MMN in the Danish sample suggests a deficit in temporal (i.e. in time) processing of auditory stimuli (Maister and Plaisted-Grant, 2011). Temporal processing is important

for the development of language, and might therefore be more related to problems in social communication (Basu *et al*, 2010; Tallal, 1980), which were possibly more present in the ASD sample of chapter 2. Baranek and colleagues have suggested that hyporesponsiveness might be associated with low levels of social communication (Baranek *et al*, 2013). Accordingly, problems in social communication problems and decreased duration and frequency-duration MMN have frequently been found in adult populations with schizophrenia (Michie, 2001; Michie *et al*, 2000; Todd *et al*, 2003; Todd *et al*, 2008). Interestingly, subdividing the combined ASD group in chapter 3 showed increased frequency MMN amplitude in children with comorbid epilepsy. This finding was not detectable in the combined ASD group, nor were there differences in behavior between ASD children with and without epilepsy. This MMN difference may thus reflect hyperexcitability or seizure vulnerability, as these differences were not present in behavioral measures. Further, in chapter 3 we found a decrease in P3a and P3b amplitudes in the combined ASD group, suggestive of less, or different, attentional processing compared to TD children. In contrast, we observed an increase in duration P3a amplitude in chapter 2. The P3a used in chapter 2 is derived from the MMN paradigm, and therefore represents a difference wave to duration deviants, thus does not represent a 'true P3a'and does not compare well to the P3a (and P3b) in chapter 3, which is derived from the ERPs elicited by our selective attention paradigm, based on deviation in frequency. In addition, in our selective attention paradigm participants are exposed to distracting stimuli presented to the other ear while, on top of that, these (other) stimuli needed to be attended. These differences make it difficult to compare and interpret the differences in findings between both studies. Nevertheless, the increase in MMN-derived P3a in our Danish sample was related to duration deviancy, and this points towards a difference in temporal processing. Alternatively, the increase in MMN-derived P3a may also be caused by the reduced MMN, lifting the ERP waveform, as MMN and derived P3a may not be completely independent processes. Earlier studies have quite frequently reported a decrease in (true) P3a and P3b as found in chapter 3 (Donkers *et al*, 2015; Ferri *et al*, 2003), suggesting that children with ASD may invest fewer attentional resources to the further cognitive processing of auditory stimuli. In addition, and similar to our findings in chapter 2, we found several associations between behavior and ERPs in chapter 3. Using simple correlations, we found an association between N1 amplitude (stimulus detection) and sensory issues, as well as a correlation between duration MMN and social problems as measured with the SRS, similar to the association in chapter 2. These

correlations suggest that ERPs may have clinical utility, and that certain ERP components might be differentially related to sensory or social behavior. In addition, we investigated the potential of ERPs to predict behavior and vice versa by integrating multiple variables in regression analyses. These results indicated that the ASD and epilepsy group could be partially predicted by a combination of frequency MMN and P3a amplitude. This is interesting, as studies have reported the occurrence of seizures and estimates of epileptiform discharges e.g. during sleep (and thus usually only rarely noticed) to be up to 60% in ASD (Trauner, 2015). This specific physiological profile might therefore be associated with seizure susceptibility. The results from both chapters support the growing literature of changes in auditory sensory processing in ASD, and the possible clinical relevance of studying this with ERPs.

Where chapters 2 and 3 addressed the possible clinical relevance of using ERPs in studying ASD, we used a similar approach to explore neural correlates for predicting ADHD treatment response. In **chapter 4**, we investigated stopping-related ERPs and fMRI measures, in addition to measures of cognitive control strategy (i.e. reactive stopping and proactive slowing), to address variability in methylphenidate response in a sample of children with ADHD. We found that on average children responded well to MPH, indicated by a decrease in stop signal reaction time (SSRT) and behavioral symptoms, as measured with the Swanson, Noland and Pelham Questionnaire (SNAP). However, we found large interindividual variability in treatment response. Improvement in behavioral symptoms was related to improvements in reactive stopping, but not proactive slowing. This was the only evident correlation with MPH response in our study. Neural activity in inferior frontal gyrus (IFG) as well as superior frontal gyrus (SFG) showed only few nominally significant correlations with MPH response, whereas stop ERPs (i.e. N2 and P3) did not show any associations with behavioral or cognitive outcome. The modest sample (n=13 for fMRI, n= 16 for ERPs) of this study hinders interpretation of these negative finding. Nevertheless, the results in chapter 4 suggest that reactive as well as proactive strategies might be (differentially) associated with inter-individual variability in MPH response.

Similar to chapter 4, we explored associations between neural correlates and treatment in **chapter 5**. In that chapter, we explored the effect of the diuretic bumetanide (a GABAergic inhibition enforcing agent) on behavioral problems and auditory sensory processing in a female patient with tuberous sclerosis complex. We investigated the effect on behavioral measures, ERP components (sensory gating and MMN), as well as resting state EEG and found that six months of treatment with bumetanide resulted in marked

improvement in behavior of the young woman. In addition, we found an improvement of long-range temporal correlations measured with resting state EEG, suggestive of a normalization of the E/I balance (Hardstone *et al*, 2012; Montez *et al*, 2009; Poil *et al*, 2012). In addition, sensory gating and MMN appeared to improve with bumetanide. However, as usual with case reports, it is difficult to extrapolate these findings to the general population of TSC patients, but these first results are promising for an effective role of bumetanide in the treatment of TSC, possibly supported by physiological correlates.

Summary of main findings

- As a group, children with autism spectrum disorder show changes in auditory sensory processing, from early and preconscious – to later and conscious - levels of processing.

- Children with ASD and comorbid epilepsy diagnosis show enhanced frequency-based mismatch negativity compared to TD and ASD without epilepsy, suggesting enhanced automatic discrimination. This contrast was not found in the combined ASD group, nor was a difference observed in behavioral measures

- Children with ADHD show inter-individual differences in methylphenidate response, with improvements in reactive stopping possibly more related to clinical (behavioral) MPH response

- The diuretic bumetanide was found to ameliorate behavioral problems in tuberous sclerosis complex in a case study of a young woman with TSC.

General discussion

In this thesis we studied samples of children with ASD with and without epilepsy, children with ADHD and a young woman with tuberous sclerosis complex. Our overall aim was to use neural measures to dissect the interindividual variability that characterizes developmental disorders in order to better predict treatment response. We used varying samples and research designs to achieve this aim.

Differences in sensory processing have been associated with ASD, albeit from different sources ranging from the behavioral to the neural level (Kern *et al*,

2006; Schauder *et al*, 2016; Sinclair *et al*, 2017). There has been an increase in studies focusing on sensory processing in ASD and it is hypothesized to be a factor in more higher-order deficits (Baum *et al*, 2015). In addition to ASD, sensory processing deficits are frequently reported in other (developmental) disorders, such as epilepsy, schizophrenia and ADHD (Fiedler *et al*, 2006; Gene-Cos *et al*, 2005; Little *et al*, 2018; Oranje *et al*, 2013; Rydkjaer *et al*, 2017; van Campen *et al*, 2015; Witten *et al*, 2014). Although the findings of differences in sensory processing remain highly variable and come from different sources (Donkers *et al*, 2015; Dunn *et al*, 2008; Kemner *et al*, 2002; Kern *et al*, 2006; Liss *et al*, 2006; Madsen *et al*, 2014; Marco *et al*, 2011; Orekhova *et al*, 2008; Sinclair *et al*, 2017; Tomchek *et al*, 2018), it does suggest that sensory processing may be an electrophysiological stepping stone to study in order to predict treatment response in these disorders. In this thesis we confirm that different sensory processing alterations are present in ASD (chapter 2 & 3) and TSC, a disorder related to ASD (chapter 5). This is further supported by several associations between electrophysiological correlates and behavior that we found in chapters 2 and 3, e.g. duration MMN associated with scores on the SRS and N1 amplitude was associated with sensory behavioral problems. In addition to showing that neural correlates of sensory processing are related to sensory issues at the behavioral level, we also addressed a possible target for treatment. As we show in chapter 5, bumetanide proved promising for not only treating behavioral symptoms of TSC but also for improving sensory processing. In addition, bumetanide has been shown to reduce core behavioral problems in ASD. The results of our studies in chapters 2, 3 and 5 show overlapping sensory processing deficits in ASD and TSC and may provide entries to predict bumetanide's efficacy, i.e. by improving sensory processing and therewith reducing symptomatology. In addition, in chapter 4 we showed that improvement of measures of reactive stopping (SSRT) were associated with behavioral improvement through methylphenidate in ADHD. These associations suggest that the assessment of underlying neural variability in developmental disorders may be valuable for predicting of treatment outcomes.

Methodological considerations

Techniques

The main method used throughout this thesis is EEG, in particular event related potentials (ERPs). ERP components are well-researched and some of them appear to have equivalents in animal species, e.g. rodents, pigs or primates

(Broberg *et al*, 2010; Modi and Sahin, 2017; Witten *et al*, 2016; Witten *et al*, 2014). Because of the precise temporal resolution of ERPs, they are particularly valuable for measuring sensory and perceptual processing. However, studying ERPs also comes with limitations. Although ERPs have a high intra-individual stability, inter-individual variability is usually high (Luck, 2005). EEG measures brain activity at the scalp, and although its temporal resolution is high, its spatial resolution is rather low. One possible confounding factor and influence on interindividual differences in ERP morphology is differences in cortical folding of participants' brains. In addition, skull thickness and conductivity affect ERP morphology (Hoekema *et al*, 2003). Further, despite the relatively good within-subject stability, ERPs are known to be affected by many factors, such as caffeine intake, medication use, sleep deprivation, smoking (Dimpfel *et al*, 1993; Knott *et al*, 2010), but also mood and hormonal levels in women (Bazanova *et al*, 2014). In research settings, we try to control these factors as much as we can, by for instance requesting subjects not to smoke or drink caffeine before assessments, but there are some factors we cannot control for. For example, what is the effect of stress on ERPs? Some children with ASD might be more anxious when they are presented with auditory stimuli than others. Further, the morphology of ERP components is dependent on what paradigms are used (Luck, 2012; Polich, 2004; Squires *et al*, 1977). We can control what paradigms we use, but we cannot control whether subjects follow our instructions. For example, when subjects are asked to 'relax, watch the muted documentary and try to ignore the sounds presented', children with ASD may have more difficulties following these instructions than typically developing children. Similar to EEG, functional magnetic resonance imaging (fMRI), as used in chapter 4, comes with limitations. fMRI measurements are susceptible to movement and our studied population (i.e. unmedicated children with ADHD) often have difficulties lying still. Dropout rates due to movement are up to 30% in children with ADHD compared to only 10-20% in TD children (Yerys *et al*, 2009). Last, conceptualization or quantification of behavior using questionnaires is common in research. However, (sub)scores of questionnaires are often based on questions addressing different areas of certain behavior. For example, the sensory profile (SP-NL) addresses questions related to hypo- en hypersensitivity in the same quadrant scores. In addition, some questions are based on the response to a stimulus (e.g. covering one's ears when hearing the sound of a blender), whereas some questions are based on perception (the ability to detect or discriminate stimuli), which are two different things with possibly different underlying neural mechanisms (Schauder *et al*, 2016). Furthermore, sensory modalities are often taken

together in subcategories of these questionnaires. In the case of ASD we know that hypo- and hypersensitivity to stimuli can co-occur within an individual, and that sensory sensitivity can differ across modalities (APA, 2013; Kern *et al*, 2006; Marco *et al*, 2011). In chapter 3 we therefore attempted to create more specific behavioral measures addressing sensory responsivity, based on items of the SP-NL addressing hypo- and hyper responsive behavior.

Statistics and interpretations

Interpretations should always be done with careful consideration and common sense: a correlation does not imply causality. Besides, mediating factors should also be considered: in our associations between behavioral measures and neural correlates, factors such as cognitive abilities or other problems could play a mediating role and thus should be accounted for. Further, what can group-obtained results (differences as well as correlations) tell us about the individual patient visiting our clinic? We should be cautious to generalize to the individual level (Fisher *et al*, 2018), especially when researching such heterogeneous samples as the ones in this thesis. In addition, sample size is also very important when interpreting statistical results. For example in chapter 4, we investigated a small sample of children with ADHD, and in chapter 5 we explored treatment in one individual only. Therefore, larger samples are needed for more generalizable interpretations.

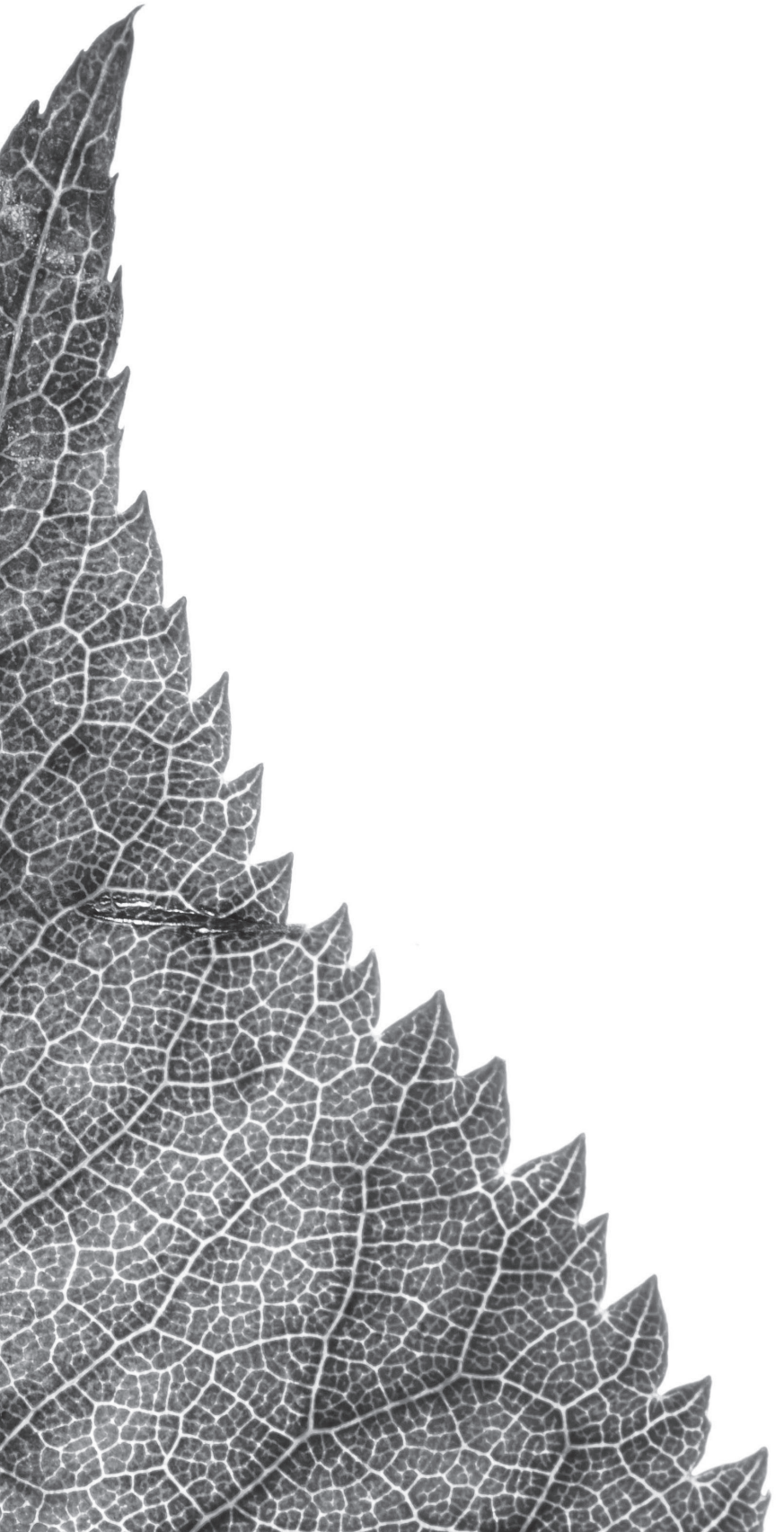
Relevance to clinical practice and future directions

Every researcher in psychiatry is well aware that developmental disorders are extremely heterogeneous. Yet, we try to exclude as many non-prototypical factors in study samples as possible, e.g. comorbidities, use of medication, low IQs etcetera to create more homogenous samples. However, the status quo in outpatient clinics is that individuals often present with multiple diagnoses and difficulties. As stated in an opinion paper by Thapar in Lancet, 2017: "Complexity is the nature of clinical problems, so perhaps it is better to acknowledge this complexity and to incorporate this into research designs if the gaps between neuroscience, mental health research and real life clinical practice are ever to be bridged" The current clinical status is that clinicians often focus on one diagnosis in an individual, while there are more problems present. One example is conduct problems in ADHD; they are often not considered if the threshold for diagnosis is not reached, but they can still form a considerable part of the symptoms for which the family is seeking help. Also, mood and irritability used to be considered integral

to ADHD, but are now seen as related to co-occurring disorders. It would therefore be valuable to embrace complexity rather than try to reduce it to co-morbid diagnoses. Indeed, there appears to be a shift taking place towards more dimensional rather than categorical research: more and more researchers acknowledge the heterogeneous nature and possible common etiologies of developmental disorders, and the need for more targeted and individualized approaches (Insel and Landis, 2013; Thapar *et al*, 2017). More integrative approaches combining multiple levels of analysis- ranging from genetics, neural correlates, cognition and behavior - might help to create more complete pictures: as an individual's need is not best captured by diagnosis alone, there is a need for more interdisciplinary approaches (Joyce *et al*, 2017; Volkmar *et al*, 2014).

Conclusions

In this thesis we have emphasized the heterogeneity of developmental disorders, the importance of neural correlates and the need for bridging the gap between research and the clinic. The studies in this thesis provide evidence that studying neural variability in developmental disorders may indeed be useful for both pathophysiological understanding and the prediction and assessment of possible treatment targets. In addition, our results underscore the need for more complex and integrative, multidimensional approaches.



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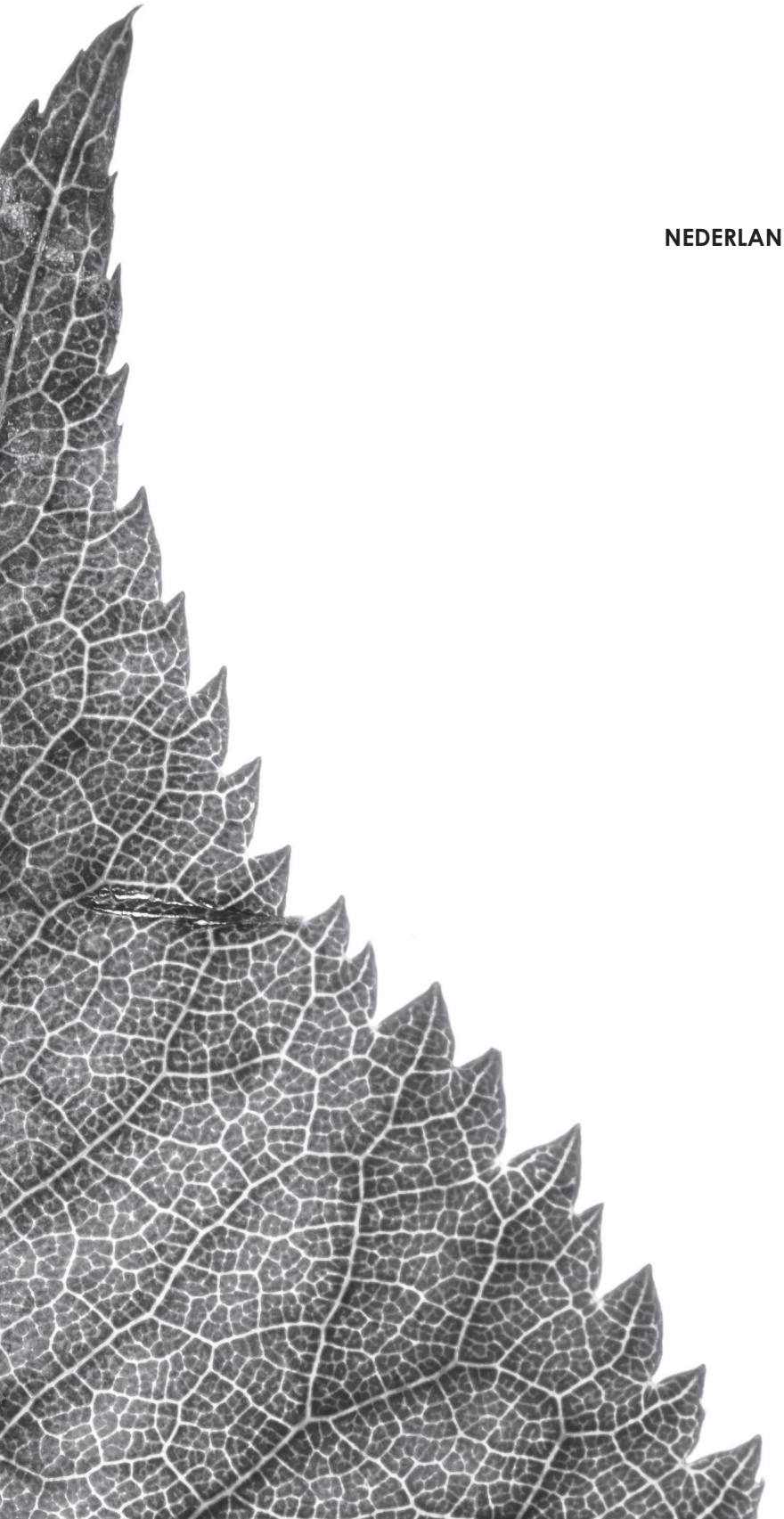

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NEDERLANDSE SAMENVATTING

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Ontwikkelingsstoornissen zoals autismespectrumstoornis (ASS) en aandachtstekortstoornis met hyperactiviteit (ADHD) zijn extreem belemmerend voor individuen en worden de laatste jaren steeds vaker gediagnosticeerd (Baio *et al*, 2018; Danielson *et al*, 2018). Classificatie en diagnostisering van een ontwikkelingsstoornis wordt gedaan op basis van gedragsobservatie volgens het handboek van de psychiatrie: de DSM (diagnostic and statistic manual of mental disorders), met inmiddels zijn vijfde editie (APA, 2013). Deze diagnostische constructen zijn heterogeen en zijn niet gelinkt aan uniforme biologische mechanismen. Als gevolg hiervan worden individuen met veel van elkaar verschillende symptoomprofielen gegroepeerd onder hetzelfde diagnostische label. Het gebrek aan duidelijke biologische mechanismen en de klinische heterogeniteit belemmeren samen de vooruitgang in het ontwikkelen van betere behandelingen voor personen met deze aandoeningen. In dit proefschrift hebben we verschillende neurale maten geanalyseerd om zo de heterogeniteit (interindividuele variabiliteit) van ontwikkelingsstoornissen te ontleden en om de relatie met behandeling te onderzoeken. We richten ons hierbij op autismespectrumstoornissen (ASS) en aandachtstekortstoornis met hyperactiviteit (ADHD), met een zijstap naar epilepsie en tubereuze sclerose complex (TSC).

Achtergrond

Ontwikkelingsstoornissen zijn stoornissen die ontstaan tijdens de ontwikkeling en worden gekarakteriseerd door neurocognitieve, psychosociale en andere problemen die het dagelijks functioneren belemmeren. Vaak manifesteren deze stoornissen zich rond de kindertijd, nog voor de basisschool leeftijd. De DSM-5 kent de volgende ontwikkelingsstoornissen: verstandelijke beperkingen, communicatiestoornissen, ASS, ADHD, motorische stoornissen en leerstoornissen (APA, 2013). De grenzen tussen verschillende complexe ontwikkelingsstoornissen kunnen echter vaag zijn: symptomen van ADHD en ASS overlappen bijvoorbeeld vaak, wat het moeilijker maakt om goede diagnoses te stellen en geschikte behandelingen te vinden. Kinderen met een ontwikkelingsstoornis hebben 3.7 keer zo vaak gelijktijdig een andere stoornis ten opzichte van kinderen zonder ontwikkelingsstoornis (Costello *et al*, 2003). Zo hebben ~30% van de kinderen met ASS bijvoorbeeld comorbide (gelijktijdig) ADHD (Simonoff *et al*, 2008). Ook hebben kinderen met een ontwikkelingsstoornis een groter risico op het ontwikkelen van een andere stoornis op latere leeftijd (bijvoorbeeld schizofrenie bij ASS, en middelenmisbruik in ADHD).

Autismespectrumstoornis (ASS)

ASS is een stoornis die gekenmerkt wordt door problemen in sociale communicatie en interactie, en door stereotiepe en repetitieve gedragingen en interesses. Sinds de komst van de DSM-5 wordt de diagnose gezien als een spectrum, variërend van milde tot ernstige problematiek. Ook is er meer aandacht voor problemen in de prikkelverwerking, die voor tot wel 96% van de personen met ASS een ernstige belemmering vormen (Kern *et al*, 2006). ASS is een heterogene stoornis, hetgeen betekent dat er veel verschillen tussen en binnen individuen met ASS aanwezig zijn, zowel in gedrag als biologische mechanismen die er mogelijk aan ten grondslag liggen. Naar schatting komt ASS wereldwijd bij 1-1.5% van de bevolking voor (Baio *et al*, 2018; Volkmar *et al*, 2014). ASS komt vaker voor bij jongens dan bij meisjes (ratio 4:1) en heeft een grote impact op het dagelijks functioneren, school prestaties en het maken en in stand houden van sociale relaties. Momenteel bestaan er geen geneesmiddelen om de kernsymptomen van ASS te behandelen. Alle medicatie die wordt voorgeschreven aan personen met ASS richt zich op comorbide symptomen, zoals angst, hyperactiviteit, agressie of epilepsie (Lai *et al*, 2014).

ADHD

ADHD is de bekendste en meest voorkomende ontwikkelingsstoornis bij kinderen (Feldman *et al*, 2014), met een geschatte prevalentie van 5-9.4% wereldwijd (Costello *et al*, 2003; Danielson *et al*, 2018). ADHD komt vaker voor bij jongens dan bij meisjes (ratio 3:1). ADHD wordt gekenmerkt door problemen met aandacht en hyperactiviteit en/of impulsiviteit, die niet passend zijn voor de leeftijd. De DSM-5 classificeert 3 subtypes ADHD: het onoplettende type, het hyperactief-impulsieve type en het gecombineerde type (APA, 2013). Desalniettemin is ADHD, net als ASS, extreem heterogeen en verschillen individuen binnen de diagnose en zelfs binnen een subtype substantieel van elkaar (Castellanos *et al*, 2006; Nigg *et al*, 2005). Het hebben van ADHD heeft een grote impact op het functioneren, zowel thuis als op school. Behandeling van ADHD gebeurt doorgaans met methylfenidaat (MPH) dat bij 70-80% van de kinderen succesvol is en de gedragssymptomen verbetert (Spencer *et al*, 1996).

- *ASS en epilepsie*

Naar schatting heeft 6-46% van alle kinderen met ASS gelijktijdig epilepsie, of epileptische aanvallen gehad op enig moment in hun leven (Trauner, 2015). Epilepsie is een ernstige neurologische aandoening gekarakteriseerd

door hyperexcitabiliteit, met spontane en ongecontroleerde hersenactiviteit (d.w.z. aanvallen). Focale (gelokaliseerde) en epileptiforme afwijkingen worden vaak gevonden in het electroencephalogram (EEG) van kinderen met ASS (Rosenow *et al*, 2015), hetgeen mogelijke overeenkomsten in de biologie van deze aandoeningen suggereert (Gilby *et al*, 2013; Levisohn, 2007).

- *Tubereuze sclerose complex*

Een biologisch model voor zowel ASS als epilepsie is tubereuze sclerose complex (TSC): een erfelijke ontwikkelingsstoornis veroorzaakt door een mutatie in het TSC1 of TSC2 gen, waardoor op verschillende plaatsen in het lichaam goedaardige gezwellen ontstaan (Curatolo *et al*, 2015; Northrup *et al*, 1993). TSC gaat vrijwel altijd gepaard met epileptische aanvallen en gedragsproblemen, zoals verstandelijke beperking en ASS (Bolton *et al*, 2015; Curatolo *et al*, 2015; Curatolo *et al*, 2002). De gedragsproblemen, zoals irritabiliteit, prikkelgevoeligheid en problemen met sociale communicatie zijn, net als in ASS, extreem belemmerend (de Vries *et al*, 2015). TSC is zeldzaam en komt voor in 1 op de 6000 mensen en is verantwoordelijk voor een geschatte 1% van alle ASS gevallen (Curatolo *et al*, 2013; Curatolo *et al*, 2004; Guo *et al*, 2012). Behandeling van TSC is voornamelijk gericht op de structurele afwijkingen en controle van epileptische aanvallen, waardoor gedragsproblemen vaak onderbelicht en onbehandeld blijven (de Vries *et al*, 2015).

Neurobiologie van ontwikkelingsstoornissen

Het is algemeen aanvaard dat zowel ASD en ADHD een neurobiologische basis hebben, en de laatste decennia zijn er steeds meer geavanceerde neuroimaging technieken en studies om de neurobiologische mechanismen van ontwikkelingsstoornissen te onderzoeken. Studies naar genetica, neuroimaging en cognitie hebben bijgedragen aan ons begrip van de pathofysiologie van ontwikkelingsstoornissen (Jeste, 2015; Lai *et al*, 2014; Spencer *et al*, 2007). Structurele en functionele magnetische resonantie beeldvorming (MRI) en elektro-encefalografie (EEG) kan ons leren over de structuur en functie van de hersenen op een niet-invasieve manier. Structurele MRI kan worden gebruikt om structurele en volumetrische verschillen in de hersenen tussen of binnen groepen te identificeren, terwijl functionele MRI indirect hersenactiviteit meet, bijvoorbeeld bij het uitvoeren van een taak of wanneer personen in rust zijn. MRI is een techniek met een hoge

spatiële (ruimtelijke) resolutie en is daarom geschikt voor het onderzoeken van neurale activiteit in specifieke hersengebieden. EEG daarentegen heeft een hoge temporele resolutie en is daarom uitermate geschikt om neurale processen in tijd te onderzoeken. EEG kan worden gebruikt om hersenactiviteit te onderzoeken in rust of om de connectiviteit tussen verschillende structuren in de hersenen te bestuderen. Daarnaast kunnen event related potentials (ERPs) worden bestudeerd: ERPs zijn de gemiddelde respons van de hersenen op zintuiglijke prikkels, waarvan de kenmerken afhankelijk zijn van de gebruikte testen (Luck, 2012). ERPs worden daarom meestal gebruikt om informatieverwerking in de hersenen te meten, variërend van vroege (onbewuste) tot late (bewuste) sensorische informatieverwerking (prikkelverwerking). Deze basisprocessen van laag niveau in de hersenen, zoals die met vroege ERP kunnen worden gemeten, worden beschouwd als bouwstenen voor meer hogere cognitieve processen.

Samenvatting van de bevindingen in het proefschrift

In **hoofdstuk 2** hebben we gekeken naar vroege auditieve informatie-verwerking bij een groep Deense kinderen met autismespectrumstoornis en dit vergeleken met typisch ontwikkelende kinderen. Hier hebben we vooral mismatch negativity (MMN) bestudeerd: een negatieve ERP component die optreedt als reactie op een (infrequente) afwijking in de omgeving. In een zogenoemde oddball taak krijgen kinderen een serie dezelfde (standaard) tonen te horen, waarvan er af en toe één afwijkt: dit veroorzaakt een grotere reactie in de hersenen, en het verschil in reactie op de afwijkende toon (de deviant) en de standaard noemen we de MMN. Tijdens deze taak worden de deelnemers afgeleid met een documentaire, en er wordt gevraagd om de tonen te negeren. Automatisch wordt de deviant echter wel opgemerkt. MMN kan worden getriggerd door een afwijking in toonhoogte (frequentie MMN), duur/lengte van de toon (duur MMN) of een combinatie van beide (frequentie-duur MMN). In de ERP component van de oddball taak kunnen we ook naar een latere component kijken, namelijk de P3a: in dit hoofdstuk is de P3a een positieve ERP component, afgeleid van het verschil in ERP tussen de deviant en de standaard.

Hoofdstuk 2 laat zien dat MMN veroorzaakt door de duur en de frequentie -duur combinatie deviant gemiddeld kleiner is bij kinderen met ASS dan bij kinderen zonder ASS. Dit suggereert dat kinderen met ASS minder goed reageren op afwijkende geluiden in de omgeving en dat al in een vroeg, automatisch stadium, en dan vooral als het gaat om afwijkingen in

temporele eigenschappen (d.w.z. in tijd). Ook vonden we een verhoogde P3a amplitude in de duur-deviant, wat tevens problemen in aandacht suggereert. Naast groepsverschillen vonden we ook relaties tussen ERP maten en gedragsmaten, zoals tussen duur MMN en scores op de SRS (social responsiveness scale), hetgeen een relatie suggereert tussen vroege informatieverwerkingsprocessen en sociaal gedrag.

In **hoofdstuk 3** zijn we een stapje verder gegaan en hebben we een aantal opeenvolgende ERP maten onderzocht, van vroege tot late auditieve informatieverwerkingsprocessen. Hier hebben we naast MMN gekeken naar sensorische filteringsmechanismen en selectieve aandacht bij kinderen met ASS met gelijktijdig vermoedelijke problemen in de prikkelverwerking en deze vergeleken met typisch ontwikkelende kinderen. Selectieve aandacht wordt hier onderzocht met een soortgelijke taak als de oddball taak in hoofdstuk 2, maar hier moeten kinderen soms wel reageren door op een knop te drukken als ze een afwijkende toon horen. Dit maakt het mogelijk om zowel naar bewuste aandacht als onbewuste aandacht te kijken *(respectievelijk wanneer wel en niet op een knop gedrukt moet worden)*. Bovendien hebben we in dit hoofdstuk ook niet-prototypische kinderen met ASS geïncludeerd, namelijk kinderen met comorbide epilepsie, om te kijken of we subtypes in ASS kunnen identificeren. Zoals genoemd vonden we in hoofdstuk 2 mogelijke auditieve ondergevoeligheid in ASS, maar dit vonden we niet in hoofdstuk 3. Een mogelijke verklaring voor deze discrepantie zou de verschillen tussen de groepen kunnnen zijn: de Deense groep kinderen in hoofdstuk 2 had mogelijk meer prototypische ASS kinderen (met bijvoorbeeld meer problemen in sociale communicatie), terwijl de ASS groep van hoofdstuk 3 geworven is via het zorgprogramma prikkelverwerking (ZPPV) van het UMC Utrecht, en daardoor meer problemen in prikkelverwerking hadden. De bevindingen van verminderde duur en frequentie-duur MMN in hoofdstuk 2 suggereren een probleem in temporele informatieverwerking (d.w.z. in tijd) (Maister *et al*, 2011), en inderdaad: temporele informatieverwerking is belangrijk voor de taalontwikkeling en zou daardoor meer gerelateerd kunnen zijn aan problemen in de sociale communicatie (Basu *et al*, 2010; Tallal, 1980). Ook wordt gesuggereerd dat hyporesponsiviteit, ofwel ondergevoeligheid, meer gerelateerd is aan lage sociale communicatie (Baranek *et al*, 2013). Zowel lage niveaus van sociale communicatie en een ondergevoeligheid geïmpliceerd door duur en frequentie-duur MMN worden ook vaak gevonden in volwassenen met schizofrenie (Michie, 2001; Michie *et al*, 2000; Todd *et al*, 2003; Todd *et al*, 2008).

Het onderverdelen van de kinderen met ASS in hoofdstuk 3 in groepen met

en zonder epilepsie liet zien dat kinderen met comorbide epilepsie een versterkte respons hebben op afwijkingen in frequentie, zoals aangetoond met een versterkte frequentie MMN. Dit vonden we niet terug in de gecombineerde ASS groep, en we vonden ook geen verschillen in gedrag tussen ASS kinderen met en zonder epilepsie. Dit verschil in MMN kan dus mogelijk gerelateerd zijn aan hyperexcitabiliteit of kwetsbaarheid voor epilepsie, omdat we deze verschillen wel op ERP maar niet op gedragsniveau vonden. Ook zien we in hoofdstuk 3 een verminderde P3a en P3b amplitude in de gecombineerde ASS groep, hetgeen impliceert dat er minder, of in elk geval andere, verwerking van aandacht plaatsvindt in deze kinderen. Daarentegen vonden we in hoofdstuk 2 juist een verhoogde P3a. De P3a die we in hoofdstuk 2 hebben bestudeerd is echter een afgeleide van de MMN component en is dus gebaseerd op een verschil in ERP van duur devianten en is dus niet representatief voor een 'echte' P3a, en kan daarom niet goed vergeleken worden met de resultaten van P3a (en P3b) in hoofdstuk 3. De P3a en P3b uit hoofdstuk 3 komen namelijk van de ERP componenten veroorzaakt door de selectieve aandachtstaak gebaseerd op afwijkingen in frequentie. Daarnaast is het ook zo dat bij de selectieve aandachtstaak kinderen worden blootgesteld aan afleidende stimuli in het andere oor terwijl ommige stimuli juist wel opgemerkt moeten worden. Deze verschillen in taak en het soort deviant maken het lastig om de resultaten uit de hoofdstukken te vergelijken en de verschillen te interpreteren. Desalniettemin, de afwijkingen in MMN-gerelateerde P3a in de Deense groep had betrekking tot afwijkingen in duur, en suggereren daarom vooral een afwijking in temporele informatieverwerking. Eerdere studies rapporteren vaak een verminderde (*echte*) P3a en/of P3b in ASS, zoals in hoofdstuk 3 (Donkers *et al*, 2015; Ferri *et al*, 2003). Dit impliceert dat kinderen met ASS mogelijk minder aandacht investeren in de verdere cognitieve informatieverwerking van auditieve stimuli.

Zowel in hoofdstuk 2 als hoofdstuk 3 vonden we verschillende associaties tussen ERPs en gedrag. Simpele correlatie analyses lieten een associatie tussen de N1 amplitude (ERP component gerelateerd aan de detectie van een toon) en problemen in de prikkelverwerking zien. Ook vonden we een relatie tussen duur MMN en sociaal gedrag, gemeten met de SRS. Deze correlaties suggereren dat ERPs mogelijk klinisch waardevol zijn en dat bepaalde ERP componenten mogelijk specifiek gerelateerd zijn aan bepaald sensorisch of sociaal gedrag. Bovendien hebben we gekeken naar de potentie van ERPs in relatie tot gedrag door meerdere variabelen in multiple regressie analyses te integreren: kunnen we bijvoorbeeld diagnose of bepaald gedrag voorspellen

op basis van informatie uit het EEG? Met deze analyses vonden we dat de ASS met epilepsie groep redelijk succesvol gesegregeerd kon worden door een combinatie van frequentie MMN en P3a amplitude. Dit is interessant, omdat geschat wordt dat epileptische aanvallen of epileptiforme activiteit, bijvoorbeeld tijdens slaap, in tot wel 60% van de kinderen met ASS kan voorkomen (Trauner, 2015). Het specifieke ERP profiel van versterkte frequentie MMN en verminderde P3a amplitude zou dus geassocieerd kunnen zijn met een bepaalde kwetsbaarheid voor epilepsie. De resultaten van zowel hoofdstuk 2 als 3 sluiten aan bij de groeiende hoeveelheid literatuur waarin veranderingen in auditieve informatieverwerking in ASS gevonden wordt, en laten zien dat dit mogelijk klinisch relevant is om dit met ERPs te onderzoeken.

Waar we in hoofdstuk 2 en 3 ingaan op de mogelijke klinische relevantie van ERPs binnen ASS, hebben we in **hoofdstuk 4** een soortgelijke aanpak gebruikt om neurale correlaten te onderzoeken om methylfenidaat respons in kinderen met ADHD te voorspellen. Hier hebben we ERP en fMRI maten onderzocht in relatie tot cognitieve controle (het controleren van eigen gedrag) om zo verschillen in methylfenidaat (MPH) respons te bekijken in een kleine groep kinderen met ADHD. De mogelijkheid om eigen gedrag te remmen kan worden gemeten met een stoptaak: een reactietijd taak waar deelnemers hun (motor-) reactie moeten remmen bij een infrequent optredend stopsignaal. Problemen met cognitieve controle kunnen gerelateerd zijn aan zowel reactieve en proactieve mechanismen, waar reactief stoppen een directe respons is na een stopsignaal en proactief stoppen gebruik maakt van de anticipatie op een mogelijk stop signaal. De meeste kinderen in hoofdstuk 4 reageerden goed op MPH en verbeterden in reactief stoppen: ze lieten een kortere stop-tijd (stop signal reaction time (SSRT)) zien en hadden minder gedragsproblemen, gemeten door de SNAP (Swanson, Noland and Pelham Questionnaire, een ADHD lijst) na MPH inname. Echter vonden we ook een grote interindividuele variabiliteit in medicatie respons. Verbetering in gedragssymptomen was geassocieerd met verbetering in reactief stoppen (SSRT), maar niet met proactief stoppen. Dit was de enige correlatie met MPH verbetering in deze studie. De neurale correlaten laten zien dat activiteit in de inferieure frontale gyrus (IFG) en in de superieure frontale gyrus (SFG) alleen nominaal correleerden met MPH respons, en dat stop ERPs (N2 en P3) helemaal geen correlaties met MPH respons laten zien, op gedrag of cognitief niveau. Omdat we in deze studie maar een kleine groep kinderen hebben onderzocht (n= 13 voor fMRI, n=16 voor ERPs), kunnen we deze negatieve bevindingen niet verder interpreteren. Desalniettemin suggereren de resultaten in hoofdstuk 4 dat strategieën

voor reactief en proactief stoppen beide mogelijk geassocieerd zijn met interindividuele variabiliteit in MPH respons.

In **hoofdstuk 5** bestudeerden we ook associaties tussen neurale correlaten en medicamenteuze behandeling. Hier hebben we het effect van bumetanide (een plaspil) op gedragsproblemen en auditieve informatieverwerking bij een jonge vrouw met tubereuze sclerose complex (TSC) getest. Bumetanide is een middel dat GABAerge inhibitie versterkt en dus mogelijk zou kunnen werken bij stoornissen met veronderstelde hyperexcitabiliteit, zoals TSC. We hebben het effect van bumetanide behandeling getest op gedrag en eerder genoemde ERP maten (sensorisch filter en MMN). Daarnaast hebben we ook gekeken naar het effect op de balans tussen excitatie en inhibitie (de E/I balans) door middel van EEG in rust. Hoofdstuk 5 laat zien dat 6 maanden bumetanide behandeling resulteerde in enorme verbetering op meerdere gedragsmaten. Ook vonden we een verbetering in correlaties tussen activiteit in hersengebieden (long range temporal correlations) in het EEG, wat suggestief is voor een verbeterde E/I balans, ofwel verminderde hyperexcitabiliteit (Hardstone *et al*, 2012; Montez *et al*, 2009; Poil *et al*, 2012). Het sensorisch filteren van informatie en MMN leek ook te verbeteren na behandeling met bumetanide. Het is moeilijk om resultaten van deze case studie te extrapoleren naar de gehele TSC populatie, al zijn deze resultaten veelbelovend wat betreft een mogelijke rol voor bumetanide in de behandeling van TSC, mogelijk ondersteund door fysiologische correlaten.

De resultaten van dit proefschrift onderschrijven de heterogeniteit van ontwikkelingsstoornissen, het belang van neurale correlaten en de behoefte om de brug tussen wetenschap en kliniek dicht te slaan. De verschillende studies in dit proefschrift geven enige aanwijzing dat het onderzoeken van neurale variabiliteit in ontwikkelingsstoornissen nuttig kan zijn voor zowel het verkrijgen van pathofysiologisch inzicht als het voorspellen en meten van mogelijke ingangen voor behandeling. De resultaten van dit proefschrift laten ook zien dat er meer behoefte is aan complexe, integratieve en multidimensionale benaderingen.

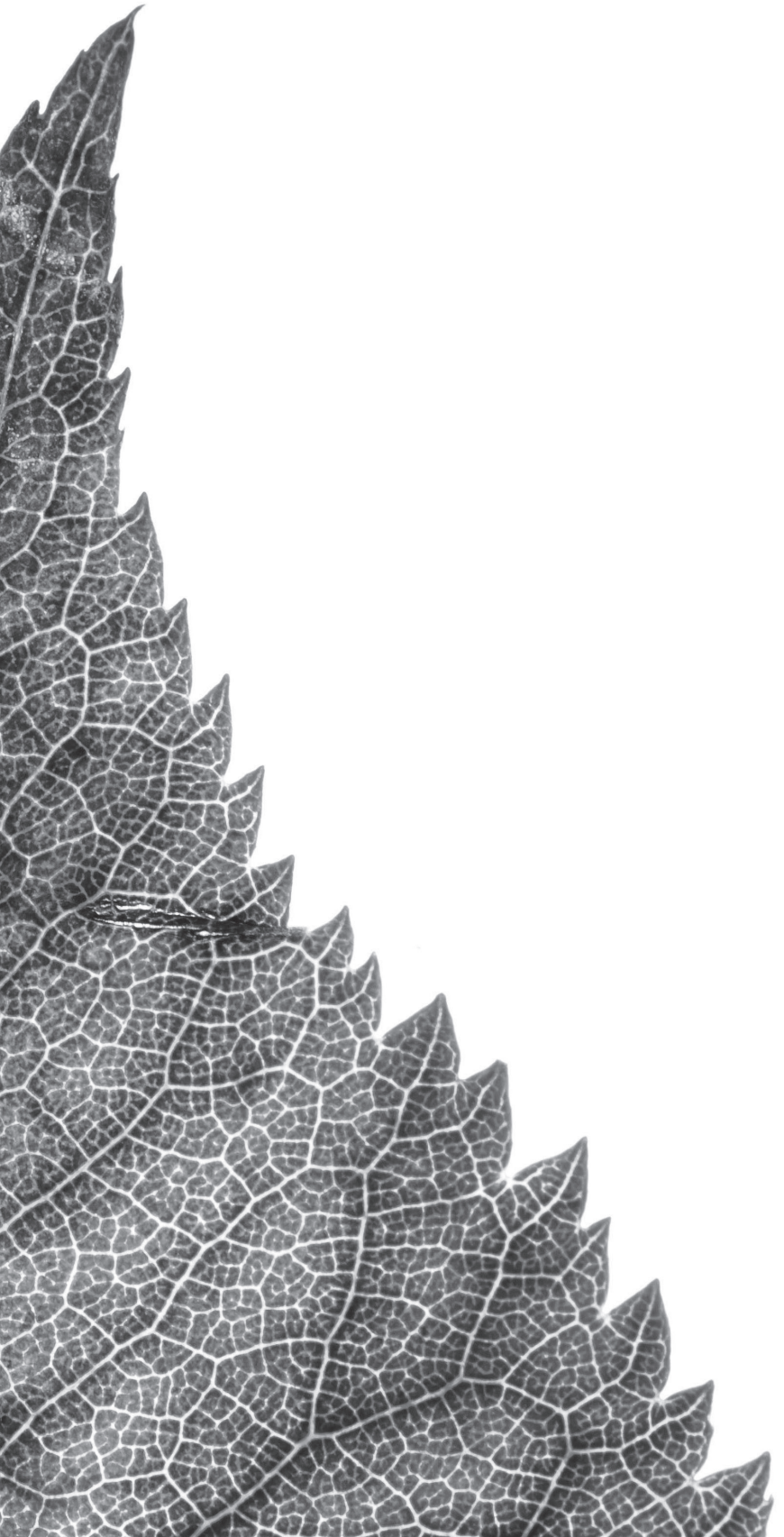
Conclusies

- Op groepsniveau laten kinderen met ASS andere auditieve informatieverwerking zien, van een vroeg en onbewust niveau tot latere en bewuste niveaus van verwerking

- Kinderen met ASS en comorbide epilepsie laten versterkte frequentie MMN zien ten opzichte van typisch ontwikkelende kinderen, hetgeen een versterkte automatische discriminatie van geluiden in de omgeving suggereert. Dit werd niet gevonden in de gecombineerde ASS groep, alsook niet in maten van gedrag.

- Binnen kinderen met ADHD vonden we interindividuele verschillen in methylfenidaat respons, waar verbetering in reactief stoppen mogelijk gerelateerd is aan klinische (gedrags-) respons.

- De plaspil bumetanide vermindert gedragsproblemen in een jonge vrouw met tubereuze sclerose complex in een case studie, en verbetert mogelijk ook enkele EEG en ERP correlaten.



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En dan is het nu tijd om iedereen te bedanken, voor bijdragen aan dit proefschrift, maar vooral voor de fijne tijd die ik heb gehad tijdens het maken er van.

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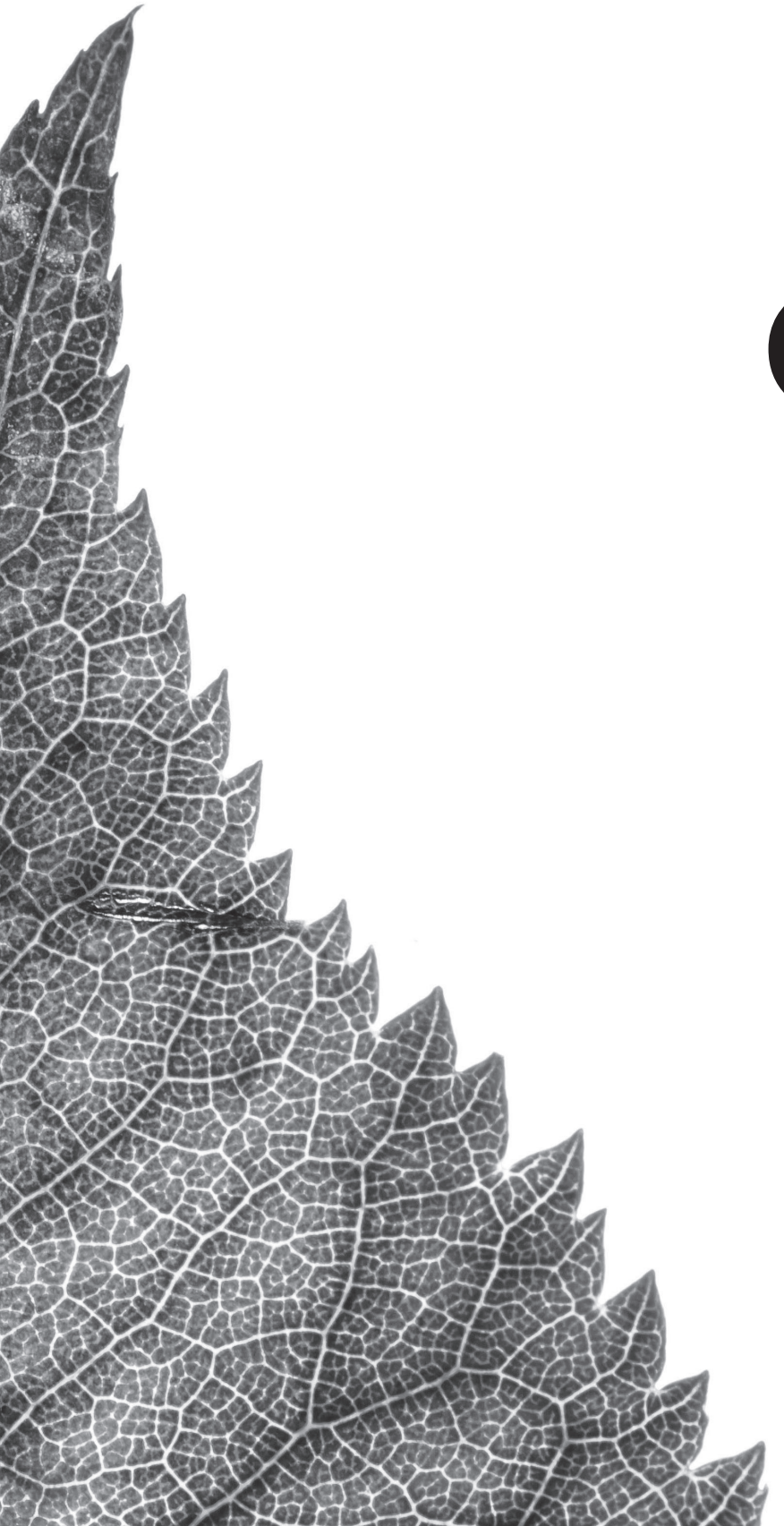

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Published

Vlaskamp, C., Oranje, B., Madsen, G. F., Møllegaard Jepsen, J. R., Durston, S., Cantio, C., Glenthøj B., & Bilenberg, N. (2017). Auditory processing in autism spectrum disorder: Mismatch negativity deficits. Autism Research, 1857-1865.

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In preparation to be submitted

Vlaskamp C., Sprengers J.J., van Andel D.M., Jansen F., Durston S., Oranje B., Bruining H. An integrative electrophysiological and behavioral approach to sensory processing issues in ASD.

Vlaskamp C., Shook D., Oranje B., Kenemans J.L., Durston S. Is variability in methylphenidate response in ADHD related to reactive or proactive mechanisms?

CURRICULUM VITAE

Chantal was born on July 15th, 1989 in 't Harde, the Netherlands. After she finished high school in 2006, she did a bachelor of applied sciences at the Saxion in Deventer (Biology and medical laboratory research) that she finished in 2010. During internships she has worked with nematodes, mice and postmortem human material. Fascinated by the brain, she next did a master Neurosciences at the VU Amsterdam, and graduated in 2012. Her final master thesis was about inhibitory control in children with ADHD, which she did at the NICHE lab, department of Psychiatry, UMC Utrecht. After her internship she started as a research assistant, also at the NICHE lab, working on several different projects. October 2013 her PhD project started, which ultimately resulted in this thesis. During her PhD she also undertook several teaching activities, and at the moment her career interests lie in a combination of research and teaching.

Chantal werd geboren op 15 juli 1989 op 't Harde. Na het behalen van haar middelbare schooldiploma in 2006, heeft ze de bachelor biologie en medisch laboratoriumonderzoek gedaan aan het Saxion in Deventer, die ze in 2010 afmaakte. Tijdens stages heeft ze gewerkt met nematoden, muizen en postmortem humaan materiaal. Gefascineerd door het brein deed ze vervolgens een master Neurowetenschappen aan de VU Amsterdam en studeerde af in 2012. Haar laatste masterscriptie betrof inhibitie onderzoek bij kinderen met ADHD, op het NICHE lab, afdeling Psychiatrie, UMC Utrecht. Na haar stage is ze als onderzoeksassistente begonnen, ook bij het NICHE lab, waar ze werkte aan verschillende projecten. Vanaf oktober 2013 is ze gestart met haar PhD project, wat uiteindelijk heeft geresulteerd in dit proefschrift. Tijdens haar promotie heeft ze ook veel onderwijs gegeven, en haar carrière ambities liggen momenteel in een combinatie van onderzoek en onderwijs.

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