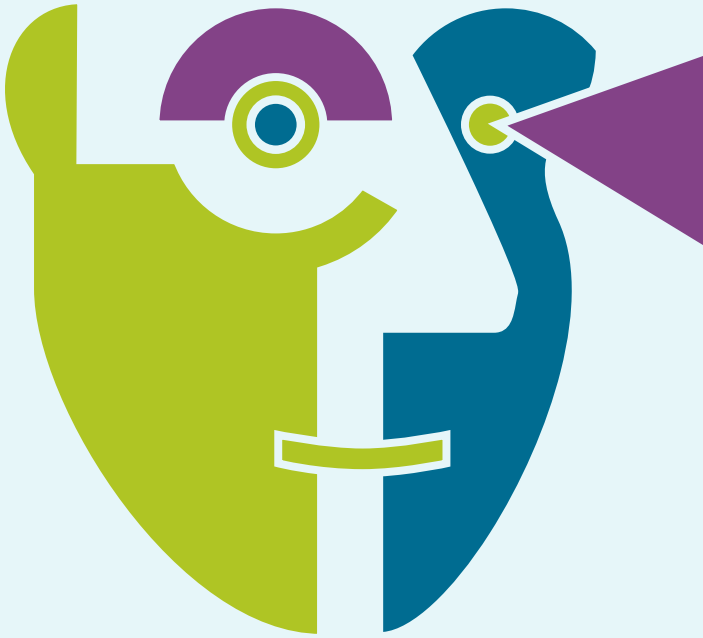
WHAT YOU SEE IS WHAT YOU GET



Autism spectrum disorder:

Outcome in adolescence
& perception of and responses
to social stimuli

ANNEKE LOUWERSE

WHAT YOU SEE IS WHAT YOU GET

Autism spectrum disorder:
Outcome in adolescence
& perception of and responses
to social stimuli

ANNEKE LOUWERSE

What You See is What You Get
Autism spectrum disorder: Outcome in adolescence and perception of & responses to social stimuli

ISBN: 978-94-6169-535-2

Thesis Erasmus MC, Department of Child and Adolescent Psychiatry/psychology.

Cover picture: istockphoto
Lay-out and printing: Optima Grafische Communicatie, Rotterdam

This study was financially supported by a grant from the Sophia Foundation for Scientific Research (SSWO; grant 586) and a grant from the Nuts Ohra Foundation (grant 0803-53).

What You See is What You Get

Autism spectrum disorder:
Outcome in adolescence
and
perception of & responses to social stimuli

Wat je ziet is wat je krijgt
Een stoornis binnen het autisme spectrum:
uitkomsten in de adolescentie
en
waarneming van & reacties op sociale stimuli

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus
prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op
vrijdag 10 oktober 2014 om 11:30

Suzanne Christine Louwerse
geboren te Middelburg

PROMOTIECOMMISSIE:

Promotor:	Prof. dr. F. C. Verhulst
Overige leden:	Prof. dr. I.H. Franken
	Dr. C.A. Hartman
	Dr. J.H.M. Tulen
Co-promotor:	Dr. K. Greaves-Lord

"And above all, watch with glittering eyes the whole world around you because the greatest secrets are always hidden in the most unlikely places."

Roald Dahl

CONTENTS

CHAPTER 1

General introduction

INTRODUCTION

The present thesis aims to contribute to the autism literature by examining the behavioural outcome and underlying mechanisms of autism spectrum disorder (ASD). The results of two lines of investigation are described. The first line of investigation (part 1) was performed with the aim to provide information about the outcome in adolescence of clinically referred individuals with symptoms of ASD in childhood using a seven-year follow up study. The second line of investigation (part 2) was performed with the aim to get a better understanding of the mechanisms that may underlie problems in social functioning in individuals with ASD, by evaluating *perception of* and *responses to* social stimuli in individuals with ASD and typically developing controls during a lab session.

In this introduction, firstly, background information will be provided on the conceptualisation of autism and ASD, the outcome in adolescence of children with ASD, and the perception of and responses to social stimuli in individuals with ASD. Based on this background information, the research questions of the current thesis will be provided. Lastly, the samples included in the studies in the current thesis will be described, and the outline of this thesis will be given.

The conceptualisation and operationalization of autism (historical perspective)

The conceptualisation and the operationalization of autism are based on its behavioural properties, namely problems in social interaction and communication, and restricted, repetitive behaviours and interests (e.g. APA, 2013). These behavioural properties, have been operationalized as diagnostic criteria and described in the Diagnostic and Statistical Manual of Mental Disorders (DSM). The diagnostic classification in this handbook together with the more general conceptualisation of autism have varied largely over the past decades (e.g. Volkmar, et al., 2013). To give some historical background on the concept of autism, Bleuler (1911) was the first to use the term 'autismus' (German) to refer to the mental states of human beings based on 'autos' (Greek; self), i.e. to lose or minimalize contacts or relationships with the external world and withdraw into one's own world. Bleuler used this term in research reports on dementia and schizophrenia, referring to mental states of adolescents and adults. The word autism was used for the first time to explain a distinct clinical condition in children in the two classical case reports of autistic disorder (Kanner, 1943) and Asperger's disorder (Asperger, 1944). The formal operationalization of autism as a childhood disorder did not yet happen until later, as it was not yet included as a diagnostic category in the first two versions of the DSM (APA, 1952, 1968). In the DSM-III (APA, 1980), autism was formally introduced as a diagnostic category, by including 'infantile autism' as one single classification. Over the years, the conceptualisation of autism broadened (e.g. Wing, 1988), which was reflected in its operationalization in the DSM-IV and DSM-IV-TR (APA, 1994, 2000). Here the overarching category of Pervasive Developmental Disorder (PDD) was introduced, which included mul-

tiple classifications, ranging from the classification of Autistic Disorder (AD) with rather strict criteria of several symptoms on three symptom domains, to categories with more lenient criteria, i.e. less symptoms on the domains, such as Asperger's Disorder (AS) and Pervasive Developmental Disorder – Not Otherwise Specified (PDD-NOS). In the latest edition of the DSM, the DSM-5 (APA, 2013), the category PDD got replaced by the category of ASD, which has no sub-classifications and is defined as persistent deficits on two domains: 'social communication & social interaction' and 'restricted, repetitive patterns of behaviour, interests, or activities'. To classify ASD, the ASD symptoms have to limit and impair everyday functioning. The symptoms of ASD should be present in early childhood, but they may have not become fully manifested until social demands exceed limited capacities (APA, 2013). Although the two domains of impairment seem clear-cut, ASD is nevertheless a heterogeneous category including a range of clinical manifestations and large variation in functioning in everyday life (Kamp-Becker, et al., 2010). Nowadays, approximately one out of 100 individuals meets criteria for ASD (Maenner, Rice, Arneson, & et al., 2014).

The majority of research in the last decades included individuals with a clinical diagnosis, i.e. a DSM classification fitting the criteria of the specific time period of the study. However, such criteria for a clinical diagnosis are restrained to the clinical 'state-of-the-art' during that particular period in time (i.e. DSM version). To enhance research, i.e. by enabling international researchers to effectively communicate findings about similar groups, it is important to use diagnostic schemes that can consistently be applied across patients, settings and time (Volkmar, et al., 2013, Lord, et al., 2012). Moreover, in addition to categorical approaches to diagnosis (i.e. presence versus absence of a disorder), dimensional approaches can contribute valuable information on the level of severity. Several studies have indicated that autistic traits are continuously distributed across the general population (Constantino & Todd, 2003; Skuse, et al., 2009), ranging from no or few traits in typically developing (TD) individuals to many traits in individuals with a clinical diagnosis (Figure 1). As autistic traits are continuously distributed, the cut-off between a classification and no classification remains a topic of debate (e.g. Constantino, 2011). Therefore, research should not only focus on ASD as one separate diagnostic classification, but should also investigate the broader autism phenotype (BAP, i.e. individuals displaying a certain degree of autistic symptoms, but who do not necessarily pass the clinical threshold for a classification) and categorical as well as continuous measures.

Since the conceptualisation and operationalization of autism remains shifting - especially in current times due to the recent introduction of the DSM-5 - in the following paragraph, the terminology and operationalization's used in the current thesis are described (Figure 1). Throughout the current thesis, we chose to use the term ASD when we a) describe findings from previous research (usually on the former DSM-IV category of PDD, i.e. the classifications AD, AS and PDD-NOS), and b) refer to a classification of ASD in individuals that took part in the current research projects. Please note that individuals with an ASD classification in the current studies (Chapter 2-6) exceeded the cut-off on a continuous screening measure for

Figure 1. Dimensional conceptualisation of the autism spectrum and its operationalization in the current thesis.
TD = individuals from the general population who do not have a level of autistic traits that is up or above the clinical threshold of an ASD screening measure (i.e. in our studies; the CSBQ). BAP = clinically referred individuals that did not meet criteria for a classification of ASD (i.e. in our studies; the ADOS) but who do have a level of autistic traits that is up or above the clinical threshold of an ASD screening measure (i.e. in our studies; CSBQ). ASD = individuals who meet criteria for a classification of ASD (in most previous studies: a clinical diagnosis of PDD and/or a classification on the ADOS and/or the ADI-R, in our follow-up studies (Chapter 2 and 3); a classification on the ADOS, and in the experimental studies (Chapter 4-6) also a classification on the ADI-R). AD = Autistic Disorder, this classification is part of the ASD spectrum and is often referred to as most severe classification within the ASD spectrum.

ASD (i.e. (Children's Social Behavior Questionnaire; CSBQ, Hartman, Luteijn, Serra, & Minderaa, 2006) and a diagnostic assessment measure (i.e. an ASD classification on the Autism Diagnostic Observation Schedule; ADOS, Lord, Rutter, DiLavore, & Risi, 1999). In the studies of part 2 (Chapter 4, 5 & 6, describing the results from the lab sessions), individuals with an ASD classification also exceeded the cut-off for ASD on the Autism Diagnostic Interview – Revised (ADI-R, Bildt, et al., 2013; Rutter, Le Couteur, & Lord, 2003; Sung, et al., 2005). The term BAP is used in the current thesis (part 1: Chapter 2 and 3) when we refer to clinically referred individuals that had a level of autistic traits exceeding the clinical threshold of the CSBQ, but who did not meet criteria for a classification of the ASD on the ADOS. The studies in part 2 (Chapter 4, 5 & 6) also included a TD control group. To operationalize a 'typical development' for individuals from the general population, we only included individuals with a level of autistic traits that was below the clinical threshold of the CSBQ. We chose to use these operationalizations based on a research perspective: the current operationalizations facilitate scientific communication, since they can be clearly translated to operationalizations/measures used in many other previous and current international studies, and can be replicated in future studies. They were used regardless from clinical judgment of DSM diagnostic criteria, that is known to differ among clinicians and settings (Lord et al., 2012).

The aetiology of ASD is not understood at all, although previous studies have indicated that the vulnerability to develop ASD is largely genetically determined (Rutter, 2000; Silverman, et al., 2002). Neurobiological studies have suggested structural and functional brain abnormalities in individuals with ASD (DiCicco-Bloom, et al., 2006; Stigler & McDougle, 2013). Evidence has been found for increased total brain volume, differences in grey and white matter volume, atypical trajectory of neurodevelopment in children with ASD, and abnormalities in cortical activation and connectivity during experimental tasks (for a review, see: Stigler & McDougle, 2013). It can be hypothesized that ASD results from atypical early brain de-

velopment caused by genetic influences; however, more research is needed to substantiate this hypothesis. In the current absence of unequivocal biological markers, the diagnosis of ASD is presently based on behavioural assessments. Information about the behaviour of the individual is gathered by trained clinicians to complete a picture of the skills, abilities and impairments of the individual, focusing on the symptoms domains 'social communication & social interactions' and 'restricted, repetitive patterns of behaviour, interests, or activities'. This information is preferably retrieved from multiple informants; the individual, his/her close family members (parents/partner) and broader social environment (teachers/colleagues) with the use of standardized assessment tools; questionnaires, interviews and observations.

Outcome in adolescence of children with symptoms of ASD

ASD is generally considered a lifelong disorder (Gezondheidsraad, 2009; Knapp, Romeo, & Beecham, 2009). Previous studies reported a high stability of autism (i.e. around 95%, Billstedt, Gillberg, & Gillberg, 2005; Sigman & McGovern, 2005). The majority of studies concerning the outcome in adolescence/adulthood of individuals diagnosed with autism in childhood is based on studies including individuals diagnosed with 'infantile autism' that date from before 1990 (DSM-III), and were usually cognitively impaired (i.e. an IQ below 70). Since the introduction of the DSM-IV (APA, 1994), the diagnostic criteria became more lenient, influencing rates of prevalence and stability. Fombonne et al. (2009) reported that prevalence rate of PDD-NOS is 1.5 times higher than prevalence rates of AD. Also, more than sixty percent of individuals diagnosed with Autism *Spectrum* Disorder (ASD) have an IQ above 70 (CDC, 2012; Fombonne, 2009). The existing literature about the long-term outcome of individuals with autism may thus not be representative of the population diagnosed with ASD today (Henninger & Taylor, 2013). Although some follow-up studies have been performed from early to middle childhood in children with ASD (Chawarska, Klin, Paul, & Volkmar, 2007; Kleinman, et al., 2008; Lord, et al., 2006; Malhi & Singhi, 2011; Rondeau, et al., 2010; Starr, Szatmari, Bryson, & Zwaigenbaum, 2003; Turner & Stone, 2007), there is paucity in studies that evaluated the outcome in adolescence or adulthood of individuals with ASD and an IQ above 70. Knowledge regarding stability in this group would be valuable for parents, clinicians and policy makers who want to understand and cope with these children's long-term prospects.

Besides the diagnostic stability of ASD, several studies focused on other outcomes later in life of children with ASD, such as societal participation and societal burden. These studies indicate that individuals diagnosed with ASD continued to show impairments in daily functioning in adulthood (Billstedt, et al., 2005; Cederlund, Hagberg, Billstedt, Gillberg, & Gillberg, 2008; Gillberg & Steffenburg, 1987; Gotham, Pickles, & Lord, 2012; Howlin, Goode, Hutton, & Rutter, 2004; McGovern & Sigman, 2005; Woolfenden, Sarkozy, Ridley, & Williams, 2012). Especially individuals with AD and a cognitive impairment had a poor or very poor prognosis (Ballaban-Gil, Rapin, Tuchman, & Shinnar, 1996; Gillberg & Steffenburg, 1987; Rutter, Greenfeld, & Lockyer, 1967). These individuals were in need of special education, financial support

and they remained dependent upon others in adulthood (Howlin, et al., 2004; Mawhood, Howlin, & Rutter, 2000). In contrast, individuals with ASD (i.e. these studies mainly included individuals with AD) and an IQ above 70 had a better prognosis; they were less dependent upon others and more likely to have a job in adulthood (Billstedt, et al., 2005; Billstedt, Gillberg, & Gillberg, 2007; Cederlund, et al., 2008; Howlin, et al., 2004; McGovern & Sigman, 2005). However, less is known about the societal participation in adolescence of individuals with an ASD classification and without a cognitive impairment in childhood. This information is important for the planning of education or services to meet the specific needs of these adolescents with ASD to prepare them for better societal participation in adulthood.

Thus, the focus of previous research on the outcome in adolescence of children ASD has been on individuals with AD and a cognitive impairment. More follow-up studies are needed on individuals with ASD without a cognitive impairment. Besides including individuals with an ASD classification in childhood, it would also be beneficial to evaluate the outcome of individuals within the BAP. Including this specific sample would provide information on whether screen positives reflect normal variation in social development which may be without long-term consequences *or* whether some of these screen positives might meet criteria for ASD later in life, which might have consequences regarding daily functioning and desired care (i.e. societal participation and burden) of these individuals (Guthrie, Swineford, Nottke, & Wetherby, 2012). Thus, more research is needed to evaluate the outcome in adolescence of children with the BAP or an ASD classification. Therefore, these topics are addressed in Chapter 2 and 3.

Perception of and responses to social stimuli

The second line of investigation of this thesis focused on underlying mechanisms of the problems in social functioning of individuals with ASD and an IQ above 70. The majority of theories concerning ASD claim that atypical processing of social information is associated with the problems in social functioning (Frith, 1989; Hutt, Hutt, Lee, & Ounsted, 1964; Iarocci & McDonald, 2006; Kanner, 1943; Senju & Johnson, 2009; van Engeland, Roelofs, Verbaten, & Slangen, 1991). Previous studies therefore investigated how individuals with ASD *perceived* social information, how they processed this information and how they subsequently *responded* to social information. The *perception* of social stimuli can be assessed by investigating gaze behaviour: i.e. measuring visual fixation duration by means of eye-tracking. *Responses* to social stimuli can be retrieved by measuring for instance autonomic and subjective responses to social stimuli. Therefore, the next paragraphs provide information about visual fixation duration and autonomic responses to social stimuli in individuals with ASD.

Perception of social stimuli: visual fixation duration

In the past decade, there has been an increased interest in gaze behaviour towards social stimuli in individuals with ASD (for two reviews see: Boraston & Blakemore, 2007; Senju &

Johnson, 2009). Eye-tracking methods make it possible to study visual fixation duration very precisely by continuously tracking gaze behaviour, i.e. visual fixation durations. In experiments including individuals with ASD, eye-tracking techniques have been used to evaluate the visual fixation duration regarding various social stimuli, such as pictures of faces and people. Visual fixation duration of individuals with ASD is usually compared to the visual fixation duration of typically developing (TD) individuals. Differences between the two groups in visual fixation duration may reveal underlying differences in attention towards social information and may thus provide insight into the mechanisms underlying the problems in social functioning which individuals with ASD experience.

Most eye-tracking studies in individuals with ASD studied visual fixation duration to faces. Contrasting study results were reported. Some studies found that individuals with ASD looked shorted to the eye region of faces than TD individuals (Dalton, et al., 2005; Jones, Carr, & Klin, 2008; Klin, Jones, Schultz, Volkmar, & Cohen, 2002; Neumann, Spezio, Piven, & Adolphs, 2006), whereas others did not find differences between individuals with and without ASD (Freeth, Chapman, Ropar, & Mitchell, 2010; Sawyer, Williamson, & Young, 2012; van der Geest, Kemner, Verbaten, & van Engeland, 2002). These contrasting previous finding might be the result of the nature of the included social stimuli. Shorter fixation duration when looking towards the eye-region of faces was more often reported in studies that used dynamic stimuli (i.e. videos) than in studies that used static stimuli (de Wit, Falck-Ytter, & von Hofsten, 2008; Klin, Jones, Schultz, Volkmar, & Cohen, 2002; Speer, Cook, McMahon, & Clark, 2007; Spezio, Adolphs, Hurley, & Piven, 2007). The dynamic stimuli were more complex than static stimuli; they often included shifts in eye gaze direction, complex interactions between people, use of audio, etc. The dynamic/complex stimuli might therefore be experienced as more distressing than static stimuli. The complexity of the stimuli makes it hard to understand which aspect(s) of a stimulus triggers the shortened fixation duration towards the eye region. More studies are thus needed to disentangle which aspect(s) of the social stimulus triggers shortened fixation duration to the eye-region in individuals with ASD. Besides, more studies are needed to evaluate whether atypical fixation duration is specific for facial stimuli or that it could be general to other social or even non-social stimuli. Studies that compare gaze duration to social versus non-social stimuli are currently scarce (Klin, et al., 2002; Nakano, et al., 2010). Therefore, Chapter 4 and 5 regarded these topics.

There are several potential explanations why individuals with ASD show atypical gaze behaviour (i.e. visual fixation duration) towards social stimuli. For example, individuals with ASD might not be aware of the significance of social versus non-social information for social interaction (Baron-Cohen, Campbell, Karmiloff-Smith, Grant, & Walker, 1995; Klin, et al., 2002). Another explanation might be that several brain areas of importance for social information processing are underdeveloped in individuals with ASD (Dalton, et al., 2005). Finally, some researchers suggested that atypical fixation durations to social stimuli result from higher or lower levels of arousal (i.e. hyper or hypo arousal) that individuals with ASD experience when

they fixate on social stimuli. This last potential explanation will be investigated in the current thesis, and therefore more background information concerning autonomic responses to social stimuli will be provided in the next paragraph.

Responses to social stimuli: autonomic responses

Overreactivity to social stimuli (i.e. hyper arousal) suggests elevated autonomic responses to social stimuli in individuals with ASD compared to TD adolescents. In this 'heightened autonomic state' the autonomic nervous system (ANS) of individuals is on a maximum alert, and they experience high levels of arousal when attending to social stimuli (Bal, et al., 2010; Hirstein, Iversen, & Ramachandran, 2001). To illustrate this hypothesis; a girl with ASD described that looking at the eyes of another person was painful: "It was not quite like a broken bone or a burn but it can only be described as pain" (Nony, 1993). Thus, due to hyper arousal, individuals with ASD might avoid social stimuli. On the other hand, underreactivity to stimuli (i.e. hypo arousal) may indicate that individuals with ASD experience less arousal or reward when attending to stimuli in their environment (Mathersul, McDonald, & Rushby, 2012; Rimland, 1968). Therefore social stimuli might not be of particular interest in individuals with ASD, which is in contrast with the preferred attention towards social stimuli in TD individuals (Rimland, 1968). Differences between individuals with ASD and TD individuals in reaction to environmental stimuli might interfere with attending to, and learning from, the environment. Albeit these suggestions of hyper- or hypo arousal in ASD, research on indices of arousal in reaction to social or non-social stimuli is still scarce. In addition, few studies focused on arousal levels during rest (i.e. resting arousal) in individuals with ASD versus TD individuals.

Autonomic responses to social stimuli and resting autonomic activity can be measured using indices of activity of the autonomic nervous system (ANS). To facilitate the interpretation of ANS (re)activity and its indices, I will first provide some basic information on the ANS.

The ANS is a physiological system that is concerned with the regulation of the internal environment of the body. The goal of the ANS is to maintain a stable, constant condition of the internal organs (i.e. homeostasis). The ANS consists of two main branches: the sympathetic and the parasympathetic nervous system. The sympathetic nervous system is mostly involved in regulating activation, mobilization, and arousal, and is therefore often referred to as the 'fight-or-flight' system. The parasympathetic branch of the ANS is concerned with the maintenance of organ function during relaxation periods and the conservation of energy, and is therefore often referred to as the 'rest-and-digest' system. Both branches are constantly active, but the amount of activity varies dependent upon the internal and environmental circumstances. The ANS controls for instance activity in the sweat glands, and cardiovascular functioning. Thus, indices of ANS activity, such as skin conductance level (SCL) and heart rate (HR), provide insight in the activity in the sympathetic and parasympathetic branches of the ANS. These indices can be measured in response to environmental stimuli (i.e. as an index of

autonomic *re*activity), but also during resting periods without specific environmental stimuli (i.e. as an index of resting autonomic activity).

The SCL reflects sweat gland activity which is under control of the sympathetic branch of the ANS. The SCL can be used as an index for the level of physiological arousal experienced by an individual. A higher SCL indicates higher sympathetic activity. Skin conductance responses (SCR) can be defined as changes in sweat gland activity caused by environmental stimuli. In general, a high SCR is associated with high arousing stimuli.

HR reflects the balance between activity of the sympathetic and the parasympathetic branches of the ANS. The HR response to a stimulus consists of a triphasic pattern; an initial deceleration (i.e. orienting response), an acceleration and a second deceleration (Bradley & Lang, 2000). The initial deceleration is primarily mediated by parasympathetic processes whereas the acceleration phase is primarily related to sympathetic processes (Turpin, Schaefer, & Boucsein, 1999). Heart rate variability (HRV) is a measure of the fluctuations in HR during spontaneous breathing (Porges, 1995b). HRV is calculated by converting the beat-to-beat HR intervals into a signal in the frequency domain (i.e. power spectral analyses, Appelhans & Luecken, 2006). In the frequency domain, specific frequencies of the oscillating physiological rhythms per second (i.e. Hertz [Hz]) can be identified. HRV in the high frequency domain (between .15 and .40 Hz) is largely determined by the respiratory variations, which is a marker for parasympathetic input. Greater parasympathetic control of the heart suggests a soothed autonomic state and a more healthy cardiovascular control mechanism (Bar, et al., 2005).

Study findings concerning indices of ANS reactivity to social stimuli in individuals with ASD are divergent. In response to social stimuli, both higher and lower levels of SCR have been reported in individuals with ASD compared to TD individuals (Hirstein, et al., 2001; Hubert, Wicker, Monfardini, & Deruelle, 2009; Joseph, et al., 2008; Kaartinen, et al., 2012; Kylliäinen & Hietanen, 2006; Kylliäinen, et al., 2012; Mathersul, et al., 2012; Sasson, Dichter, & Bodfish, 2012). For HR responses, Mathersul et al. (2012) found larger deceleration responses to affective stimuli versus neutral stimuli in individuals with ASD, however these differences were not found in TD adolescents. Most studies concerning HR responses did not take into account the triphasic pattern of the HR response, but reported the mean HR. The mean HR reflects activity in the sympathetic as well as the parasympathetic branch of the ANS, which makes it difficult to disentangle hyper- versus hypo activity from these systems when using HR as the main ANS indice.

Findings regarding HRV in ASD are more equivocal, since HRV is considered a rather 'pure' marker of merely parasympathetic activity. Most HRV studies in individuals with ASD were based on assessment during a resting period. Resting HRV was reported to be lower in individuals with ASD compared to TD individuals (Bal, et al., 2010; Mathewson, et al., 2011; Ming, Julu, Brimacombe, Connor, & Daniels, 2005; Vaughan Van Hecke, et al., 2009). However, Duwallette et al. (2012) and Toichi & Kamio (2003) did not find significant differences in resting HF-HRV between individuals with ASD and TD individuals. Duwallette et al. (2012) were one

of the first to address and investigate the influence of medication on HRV in a sample of individuals with ASD. They argued that contrasting study findings in this field of research might be associated with psychotropic medicine use of individuals with ASD. Thus, it is important to take psychotropic medication into consideration when studying resting autonomic activity.

Importantly, contrasting findings concerning ANS reactivity to social stimuli might be also due to the amount of attention (i.e. visual fixation) that was paid to the stimuli (Coull, 1998). For example, when a picture of young people in a roller-coaster is presented, and a participant looks at this picture for the total duration of the presentation of the picture, he might have different levels of arousal than another participant that is paying attention to the picture for a shorter duration of time. However, until now, studies concerning autonomic arousal in individuals with ASD did not use an eye-tracking device to measure visual fixation duration. The combination of a measure of visual fixation duration and autonomic responses leads to a better understanding of how individuals with ASD *perceive* social information, and how they subsequently *respond* to social information.

Measuring visual fixation duration and autonomic responses simultaneously

Both the *perception of* and *reaction to* social information seems to be atypical in adolescents with ASD. However, although these two mechanisms both are part of the overall process of social information processing, they have not yet been investigated simultaneously. In previous experimental studies, the focus was on either one of these putative underlying mechanisms of ASD, i.e. on 'atypical visual fixation duration' or on 'atypical autonomic responses'. Although these previous studies contributed to the current field by uncovering the separate underlying mechanism involved in problems in social functioning in ASD, it remains difficult to integrate these previous findings into one picture, since the study designs and samples vary between studies. Therefore, to integrate and extend previous findings, it is an important next step to study visual fixation duration and autonomic responses simultaneously in one experimental design (Campatelli, Federico, Apicella, Sicca, & Muratori, 2013).

Research aims and questions

Based upon the state-of-the art of the ASD literature discussed above, the general aims of the current thesis are twofold.

The first aim of this thesis is to gain insight in the long-term (i.e. seven-year/adolescent) outcome of clinically referred children with symptoms of ASD. The main research questions concerning this first aim are:

1. Are ADOS ASD total scores and classifications stable from childhood to adolescence?
2. How do individuals with an ADOS ASD classification in childhood participate in society in adolescence? Does this level of societal participation differ from that of individuals with the BAP in childhood and from reference data from the general population?

The second aim of this thesis is to increase our understanding of putative underlying mechanisms of the problems in social functioning in adolescents with ASD. The research questions concerning this aim are:

3. Do eye gaze directions of facial images affect gaze behaviour and autonomic responses in adolescents with ASD as compared to TD adolescents?
4. Are there differences in autonomic responses to social versus non-social affective pictures in adolescents with ASD as compared to TD adolescents?
5. Do individuals with ASD differ from TD individuals with regard to resting ANS activity? And is resting ANS activity associated with HR responses to social stimuli or to social interaction abilities?

Sample

The selection of the individuals participating in respectively the clinical cohort studies (i.e. prospective design, part 1), and the lab studies (i.e. cross-sectional design, part 2) are described in the current section.

The outcome in adolescence (age 12-19) of clinically referred children (age 6-13) with ASD symptoms was studied in a clinical cohort study (Figure 2). This clinical cohort was retrieved from consecutive referrals for psychiatric evaluation to the outpatient clinic of the Department of Child and Adolescent Psychiatry/psychology of the Erasmus MC-Sophia in Rotterdam. Between July 2002 and September 2004, all parents of 6-13 year old children whom were referred to this outpatient clinic (n=503, de Bruin, de Nijs, Verheij, Hartman, & Ferdinand, 2007; de Bruin, Ferdinand, Meester, de Nijs, & Verheij, 2007) completed the CSBQ (Hartman, et al., 2006). From this group of referrals, the scores of 151 individuals were above the clinical cut-off of the CSBQ. Individuals with an IQ below 70 were excluded (n=41), which resulted in a study sample at the first assessment wave of 110 children (mean age = 9.5 years, SD = 1.7). The second assessment wave took place approximately seven years later between June 2009 and May 2011 (mean follow-up time of 6.9 years, SD = .7). During this assessment wave, 96 adolescents and their parents agreed to participate, resulting in a follow-up rate of 86%. The individuals were aged between 12 and 19 years when participating during this second assessment wave (mean age = 16.5 years, SD = 1.8). There was no selective attrition (please see Chapter 3 for details). Within this cohort, the ADOS was assessed both in childhood (wave 1) and in adolescence (wave 2) in 72 individuals.

The cases with ASD participating in the lab studies described in part 2 were drawn from the clinical cohort described in the previous paragraph. Additional inclusion criteria were (a) exceeding the cut-off for ASD on the Autism Diagnostic Interview – Revised (ADI-R, Bildt, et al., 2013; Rutter, et al., 2003; Sung, et al., 2005); and (b) being male. Thus, the adolescents participating in the lab studies exceeded the cut-off for ASD both on the ADOS and on the ADI-R. Thirty-nine male adolescents with ASD met the inclusion criteria and agreed to participate in the lab session. The adolescents with ASD were compared to a control **group of TD**

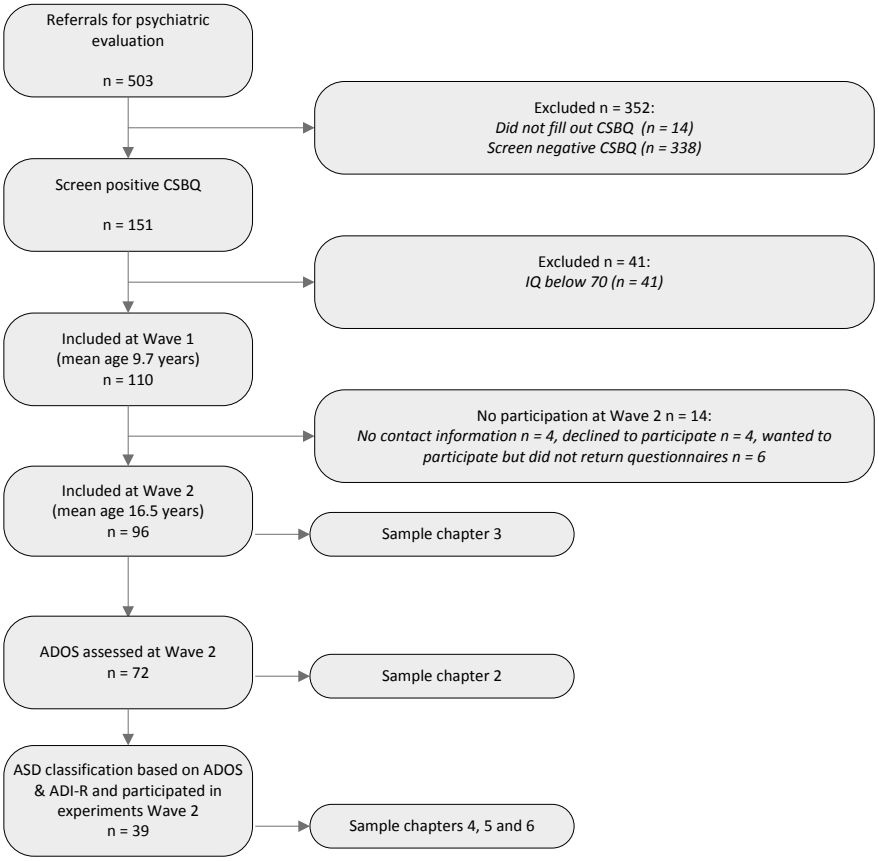


Figure 2. Inclusion of individuals with ASD symptoms in the studies described in the chapters of this thesis.

adolescents. These TD adolescents were selected from a general population sample (Tick, van der Ende, & Verhulst, 2008). Inclusion criteria for this control group of TD adolescents were: (a) no history of neurodevelopmental disorders; (b) no ASD symptoms exceeding the clinical cut-off of the CSBQ (Hartman, et al., 2006); (c) an IQ above 70; and (d) being male. Forty-two TD adolescents met these inclusion criteria and agreed to participate in the lab session. Thus, 39 boys with ASD and 42 TD boys participated in the lab session. There were no significant group differences in age or IQ between the boys with ASD and the TD boys (please see Chapter 4 for details).

Outline of the thesis

In the first part of this thesis (Chapter 2 and 3), the adolescent outcome of clinically referred individuals with ASD symptoms is investigated. In *Chapter 2* we investigate the stability of ADOS ASD total scores and classifications from childhood to adolescence. In *Chapter 3* we

evaluate societal participation in adolescence of individuals with ASD in childhood, and compare this to the societal participation of individuals with the BAP in childhood and reference data from the general population. In the second part of this thesis (Chapter 4-6), we examine possible underlying mechanisms of problems in social functioning in adolescents with ASD, by assessing gaze behaviour and autonomic activity simultaneously during a lab session. In this part of the thesis we compare adolescents with ASD with TD adolescents. We investigate whether eye gaze directions of facial images affect gaze behaviour and autonomic responses in adolescents with ASD in *Chapter 4*. In *Chapter 5* autonomic responses to social versus non-social stimuli are investigated. Besides the social content of the stimuli, we also account for the affective content (i.e. pleasantness) of the picture. The autonomic and gaze behaviour responses to the affective stimuli are compared between adolescents with ASD and TD adolescents. To evaluate the basal autonomic activity levels of adolescents with ASD, we describe these levels in adolescents with ASD and compare them to the levels in TD adolescents in *Chapter 6*. Also, we test whether these basal levels of autonomic activity are related to autonomic responses during a stimulus viewing task. Finally, in *Chapter 7* the main findings and conclusions of the studies presented in the foregoing chapters are presented and discussed. Research and clinical implications and recommendations for future studies are given.

PART 1

Outcome in Adolescence

CHAPTER 2

Brief report: a seven year follow-up of a clinical cohort using the Autism Diagnostic Observation Schedule

Anneke Louwerse, Mart Eussen, Pieter de Nijs, Arthur Van Gool, Fop Verheij, Jan van der Ende, Frank Verhulst, Kirstin Greaves-Lord

Submitted for publication

CHAPTER 3

Societal participation in adolescence: a follow-up study of children with autism spectrum disorder and the broader autism phenotype

Anneke Louwerse, Jan van der Ende, Mart Eussen, Arthur Van Gool, Linda Dekker, Fop Verheij, Frank Verhulst, Kirstin Greaves-Lord

Submitted for publication

PART **2**

Perception of and responses to social stimuli

CHAPTER 4

Effects of eye gaze directions of facial images on looking behavior and autonomic responses in adolescents with autism spectrum disorders

Anneke Louwerse, Jos van der Geest, Joke Tulen, Jan van der Ende, Arthur Van Gool, Frank Verhulst, Kirstin Greaves-Lord

Research in Autism Spectrum Disorders, 2013,7(9), 1043-1053.

ABSTRACT

It has been suggested that atypical eye contact of individuals with autism spectrum disorders (ASD) arises from an unusually high level of autonomic activity elicited by another person's gaze. The present study investigated visual fixation duration and autonomic reactivity (heart rate, skin conductance response) simultaneously, while adolescents looked towards photographs of neutral faces, with either direct eye gaze, averted eye gaze or closed eyes. Both cognitively able adolescents with ASD (n = 31, mean age = 16 years, mean IQ = 104) and typically developing (TD) adolescents (n = 34, mean age = 16 years, mean IQ = 108) looked significantly longer towards the eye region of faces with direct eye gaze compared with faces with averted eye gaze or closed eyes. The adolescents with ASD did not show higher levels of autonomic activity than TD adolescents while they were instructed to look at the eye region. This suggests that looking at the eye region of static faces does not particularly trigger high autonomic arousal in adolescents with ASD.

INTRODUCTION

Human eye gaze is an important cue for social interaction and communication. Gaze is used to provide and obtain information, to regulate conversations, and to express intimacy and control (Kleinke, 1986). Individuals prefer to fixate on the eye region when looking at faces of others (Maurer & Salapatek, 1976). This preference is present from early development: a newborn baby's attention is attracted by the eye gaze of another person (Hainline, 1978). Atypical attention towards eye gaze is a prominent feature of the qualitative impairment in social interaction in individuals with an autism spectrum disorder (ASD, APA, 2000).

Gaze behavior in ASD is studied in experimental settings using eye-tracking methods (for a review see: Senju & Johnson, 2009). Some studies reported that individuals with ASD looked shorter towards the eye region of faces than typically developing (TD) individuals (Dalton, et al., 2005; Jones, Carr, & Klin, 2008; Klin, Jones, Schultz, Volkmar, & Cohen, 2002; Neumann, Spezio, Piven, & Adolphs, 2006), whereas others did not find such an effect (Freeth, Chapman, Ropar, & Mitchell, 2010; Sawyer, Williamson, & Young, 2012; van der Geest, Kemner, Verbaten, & van Engeland, 2002). These contrasting findings might be related to the nature of the stimuli used: static images or dynamic movies (Riby & Hancock, 2009; Speer, Cook, McMahon, & Clark, 2007). With static images, fixation duration is usually not atypical in individuals with ASD (e.g. Sawyer, et al., 2012). Dynamic movies, on the other hand, do result in shorter fixation duration to the eye region in individuals with ASD compared to TD individuals (e.g. Klin, et al., 2002). However, such dynamic stimuli are usually more complex, with variation in eye gaze direction, social interactions between people, and the use of audio; all this makes it hard to determine which specific aspect of the stimuli triggers atypical gaze behavior in individuals with ASD.

The underlying mechanisms of atypical gaze behavior in individuals with ASD are also still unclear. Some researchers suggested that reduced gaze duration can be explained by a lower attentional priority to social stimuli in individuals with ASD (Riby & Hancock, 2009). Atypical fixation might be the result of reduced understanding of the significance of the eyes for social interaction (Baron-Cohen, Campbell, Karmiloff-Smith, Grant, & Walker, 1995; Klin, et al., 2002). Others suggest that individuals with ASD fixate less on the eye region to regulate their levels of arousal. They state that fixation to eyes triggers over-arousal in individuals with ASD, which can be reduced by looking less to the eye region of others (Dalton, et al., 2005; Hutt & Ounsted, 1966).

Arousal in response to social stimuli can be measured using several indices of the activity of the autonomic nervous system, such as heart rate (HR) or skin conductance level (SCL). Various recent studies investigated such measures in individuals with ASD in response to social stimuli (Joseph, Ehrman, McNally, & Keehn, 2008; Kylliäinen & Hietanen, 2006; Kylliäinen, et al., 2012; Riby, Whittle, & Doherty-Sneddon, 2012). However, results have been inconsistent. Some studies showed higher autonomic arousal levels (Joseph, et al., 2008),

while others showed lower levels of autonomic arousal in individuals with ASD compared to TD individuals (Hubert, Wicker, Monfardini, & Deruelle, 2009; Vaughan Van Hecke, et al., 2009). When autonomic responses to direct eye gaze were compared to averted eye gaze or closed eyes, some studies found higher autonomic arousal to direct eye gaze stimuli than to averted eye gaze stimuli in individuals with ASD, but not in TD adolescents (Kylliäinen & Hietanen, 2006; Kylliäinen, et al., 2012). Other studies did not find this higher level of arousal in reaction to direct eye gaze stimuli in individuals with ASD (Joseph, et al., 2008).

Until now, studies concerning autonomic arousal in individuals with ASD did not use an eye-tracking device to measure how long individuals looked at the eye region. It thus remains to be clarified whether autonomic responses in individuals with ASD are the result of looking towards the eye region. Visual fixation duration and autonomic responses should be measured simultaneously to unravel whether atypical gaze behavior is associated with autonomic activity in individuals with ASD (Riby, et al., 2012; Vaughan Van Hecke, et al., 2009). In TD individuals, fixation durations towards the eye region depend on the direction of the eye gaze in the stimulus. Direct eye gaze triggers longer fixation durations than averted eye gaze or closed eyes (Batki, Baron-Cohen, Wheelwright, Connellan, & Ahluwalia, 2000; Caron, Caron, Roberts, & Brooks, 1997; Farroni, Menon, & Johnson, 2006), which suggests that direct eye gaze is more socially relevant. To our knowledge, no eye-tracking studies compared fixation durations of direct eye gaze to averted eye gaze and closed eyes in ASD. Examining specific mechanisms behind atypical eye gaze in individuals with ASD is needed to determine which aspects of social situations triggers atypical gaze behaviour in individuals with ASD.

The current study aims to extend the current research by examining fixations to the eye region and the consequent arousal levels in individuals with ASD versus TD individuals. This study investigated the effect of gaze direction (direct eye gaze, averted eye gaze, and closed eyes) on visual fixation duration and autonomic arousal responses in ASD. Eye-tracking measures, measures of autonomic arousal (HR and SCL) and subjective ratings were combined in one experimental session. In two related tasks, fixation duration towards the eye region and the associated autonomic and subjective responses were assessed. In addition, the association between autonomic responses during direct eye gaze and the severity of social deficits was evaluated for the ASD group. We hypothesized that under spontaneous viewing conditions in the first task, the effect of gaze direction would have similar effects on fixation durations for individuals with ASD and TD individuals. When instructed to look at the eye region in the second task, however, we expected that direct eye gaze of the stimulus would elicit stronger autonomic and subjective responses than averted eye gaze or closed eyes in the ASD group. We also expected that the autonomic responses during direct eye gaze were correlated with social deficits in the ASD group (Kaartinen et al., 2012).

METHODS

Participants

ASD. Thirty-nine adolescents with ASD and 42 TD adolescents participated in this study, which was approved by the Medical Ethical Committee of the Erasmus MC. Informed consent was obtained from all adolescents and also from their parents if the adolescent was younger than 16 years of age. Only male adolescents with an IQ above 70 were included. To confirm an IQ above 70, the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999), was administered. There was no significant difference between the mean total IQ of the adolescents with ASD (mean IQ = 103.7 ± 13.6 SD) and the TD adolescents (108.1 ± 10.6; $t(79) = 1.6$, $p = .11$). The mean age of the adolescents with ASD (16.0 ± 1.9 SD, *Range* = 12-19 years) and the TD adolescents (16.2 ± 2.5, *Range* = 12-21 years), was also not significantly different ($t(79) = .4$, $p = .71$). All adolescents had normal, or corrected to normal, vision.

ASD adolescents were recruited from the outpatient's department of Child and Adolescent Psychiatry of a large university hospital. The ASD adolescents in the current sample were part of a larger follow-up study. Initially, all participants in this follow-up study were referred for psychiatric evaluation to an outpatient clinic of Child and Adolescent Psychiatry when they were 6 to 13 years old (de Bruin, Ferdinand, Meester, de Nijs, & Verheij, 2007). In the period of July 2002 to September 2004, 503 individuals were consecutively referred to this outpatient clinic. Of this group, 295 referrals scored above the cut-off of the Children's Social Behavior Questionnaire (CSBQ, Hartman, Luteijn, Serra, & Minderaa, 2006) or the Child and Adolescent Functional Assessment Scale (CAFAS, Bates, 2001), and thus met inclusion criteria for the larger follow-up study. Of these eligible participants, 244 individuals agreed to participate in the first assessment wave of the study. Seven years later, all participants were asked to participate in a second assessment wave. Of these adolescents, 124 were willing to participate in both diagnostic assessment and the current experimental task. Of these 124 participants, 39 adolescents met the inclusion criteria for the current study, namely (a) male gender, (b) IQ above 70 and (c) meeting the diagnostic cut-off of both the Autism Diagnostic Observation Schedule (Lord, Rutter, DiLavore, & Risi, 1999) and the Autism Diagnostic Interview – Revised (ADI-R; Rutter, Le Couteur, & Lord, 2003. On the ADOS, 21 adolescents met the Autistic Disorder (AD) cut-off and eighteen adolescents met the ASD cut-off (Lord et al., 1999). The mean total score on the communication and reciprocal social interaction domains was 10.3 (± 3.0). The total scores on the ADOS can vary from 0 to 22, with a cut-off for ASD of 7 (Lord et al., 1999). As for the ADI-R, seventeen adolescents met the AD cut-off and twenty-two adolescents met the ASD cut-off (Lainhart, et al., 2006). The mean score on the communication domain was 14.1 (± 4.3), the score on this domain can vary from 0 to 26, with a cut-off for AD of 8 and a cut-off of 6 for ASD (Lainhart, et al., 2006). The mean score on the reciprocal social interaction domain of the ADI-R was 17.5 (± 5.4), the score on this domain can vary from 0 to 30, with a cut-off for AD of 10 and a cut-off of 8 for ASD (Lainhart, et al., 2006). Parents were asked to fill out the CSBQ

and they were asked if their child had used medication in the week before testing. Fifteen adolescents used psychotropic medication, of which eight took methylphenidate, five took antipsychotics, one used an antidepressant and one used antiepileptic medication.

TD. The 42 TD participants were selected from a general population sample (Evans, et al., 2012, Tick, van der Ende, & Verhulst, 2008). This general population sample was randomly drawn between 2003 and 2005 from 35 representative municipalities in the Dutch province of South Holland. This sample included 2567 six to 18-year-old children. In total, 42 adolescents met the inclusion criteria of the current study and agreed to participate. The inclusion criteria were (a) male gender, (b) IQ above 70, (c) no history of neurodevelopmental disorders, (d) parental scores on the CSBQ (Hartman et al., 2006) below the clinical threshold, (e) approached for separate stress and EEG procedures (see Huizink et al., 2012).

Experimental design

The experimental design consisted of two related tasks. The same stimuli were presented during the first and the second task. However, a different instruction was given. In the first task ('spontaneous fixation'), the adolescent was instructed to look to the stimuli and to avoid extensive head movements. This task was designed to assess spontaneous fixation duration towards faces. In the second task ('sustained eye fixation'), the subject was explicitly instructed to look at the eyes. This task was designed to measure changes in autonomic responses related to increased fixation to the eye region of the faces. This design provided the opportunity to investigate autonomic reactivity of the participants when they increased their fixation duration to the eye region. Thus, for the autonomic measures, data was only included of participants with longer fixation duration at the eye region in the sustained eye fixation task compared to the spontaneous fixation task.

Stimuli

The stimuli consisted of eighteen photographs of faces with a neutral expression seen from the front (Figure 1). These photographs were taken from six young adult actors (three males and three females). The actors posed for three conditions, pertaining three different eye gaze conditions: direct eye gaze, averted eye gaze and closed eyes. For the averted eye gaze condition, three actors posed while looking to the left, and the other three actors posed while looking to the right. The eye region was defined as the region of interest. The eye region included a rectangle around the eye region (Figure 1). There were 18 distinct stimuli in total: six actors each showing three eye gaze conditions. All 18 pictures were presented once in each task in one out of two pseudo-random orders which were counterbalanced across adolescents.

Procedure

At the start of the procedure, the adolescent received general instructions on the procedure and was then seated in a fixed chair approximately 60 cm in front of the computer screen

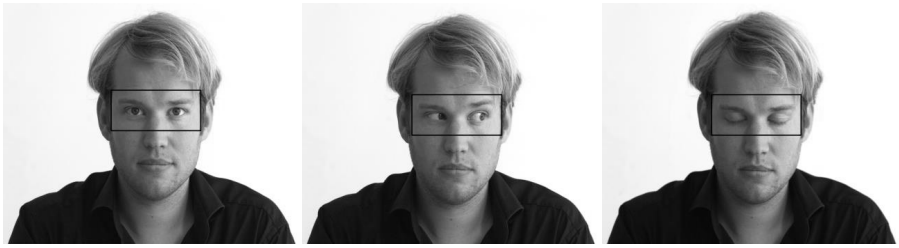


Figure 1. An example of an actor showing the three eye gaze conditions: direct eye gaze, averted eye gaze and closed eyes.

of the eye tracker. Electrodes for the recordings of autonomic reactivity (HR and SCL) were applied according to standard procedures (Greaves-Lord, et al., 2007; N. J. Lang, et al., 2007). The entire experimental procedure lasted about 1.5 h. The adolescent was presented with a series of eighteen photographs of faces twice, in two separate tasks. There was an intermediate other task of 30 min between the current two tasks. During the first task, spontaneous fixation duration towards the eye region was measured. During the second task sustained fixation to the eye region was evaluated. Instructions were given prior to each task. After instructions, a five-point calibration routine was used to ensure validity of the eye-tracking data. The examiner evaluated the calibration and the calibration routine was repeated in case of unsatisfactory data. Subsequently, two additional stimuli were shown as practice stimuli. After the presentation of each stimulus in the second task, the participant was asked to rate his subjective impression of valence and arousal (please see *Subjective ratings* for more information).

Stimulus presentation was designed and controlled by E-Prime software (version 2.0 including extensions for Tobii: PST-100777), which is used for computerized psychological tasks (Psychology Software Tools, Inc.). Each presentation of a stimulus lasted 6 seconds (s) and the interval between stimuli varied between 15 and 25 s. During this interval, a fixation cross was presented on the screen. The duration of the stimulus and inter stimulus interval was based on previous studies that included HR and/or SCL responses (Ben Shalom et al., 2006; Bölte et al., 2008; Kaartinen et al., 2012). SCL studies use relatively long intervals to make sure the skin conductance level has returned to baseline levels before a new trial starts (Figner & Murphy, 2011). During the two tasks, the examiner was seated behind a screen when instruction and calibration were completed.

Measures

Visual fixation duration. Visual fixation was recorded using a remote eye tracker (Tobii120; 60 Hz acquisition rate), which allowed for small head movements. Eye tracking data was processed using custom software written in Matlab (The Mathworks, Natick MA, United States). For each adolescent and each stimulus, the locations and durations of the fixation were determined. Subsequently, the total fixation duration towards the stimulus and the fixation

duration towards the eye region were calculated separately. A stimulus was discarded from further analysis if an adolescent fixated less than 3 s (i.e., half the presentation time) at the stimulus. If more than four out of six pictures in one eye gaze condition were discarded, the adolescent was excluded from further analysis. After exclusions, the mean total fixation duration towards the stimulus as a whole and the mean fixation duration towards the eye region were computed for each of the three conditions (direct eye gaze, averted eye gaze and closed eyes). These calculations were done for both tasks ('spontaneous fixation' and 'sustained eye fixation').

Autonomic measures. The HR and SCL data was sampled and stored on a flashcard by means of a portable digital recorder (Vitaport™ System; TEMEC Instruments B.V., Kerkrade, the Netherlands). Upon completion of the recording, all autonomic data was imported and processed on a laptop using the Vitascore™ software module (TEMEC Instruments B.V., Kerkrade, the Netherlands). Both HR and SCL data were visually inspected for detection and removal of artefacts.

HR was recorded continuously using a precordial lead, and was sampled at 512 Hz. The interbeat intervals were calculated, using R-top detection. This resulted in HR series of beats per minute. The classical HR response to emotional stimuli consists of an initial deceleration followed by an acceleration phase (Bradley & Lang, 2000; Hempel, Tulen, van Beveren, Mulder, & Hengeveld, 2007). The initial deceleration is defined as the orienting response (OR) and is considered to represent attention directed towards a stimulus. The following acceleration, defined as the acceleration response (AR), is indicative of the amount of autonomic arousal. The mean of the heart rates measured 1 s before stimulus onset was defined as the baseline value. This baseline value was subtracted from the minimum value of the HR measured between 1 and 3 s after stimulus onset, to retrieve the HR deceleration response (Hempel, et al., 2007; P. J. Lang, et al., 1993). The HR acceleration responses was defined as the maximum value between the third and sixth second, subtracted by the minimum value between the first and third second. Mean HR deceleration and mean HR acceleration responses were computed for each condition (direct eye gaze, averted eye gaze and closed eyes) and for each adolescent.

SCL was measured using two Ag/AgC1 electrodes attached to the volar surfaces of the medial phalanxes of the index and ring fingers of the non-dominant hand. The level of skin conductance was sampled at 8 Hz and stored in μSiemens. SCL reflects the fluctuations in sweat gland activity. This activation of the sweat glands occurs when external events and internal physiological states interact (Riby, Doherty-Sneddon, & Whittle, 2013). The SCL has been considered a good index of autonomic arousal experienced by a specific individual at a specific moment, since it is under direct control of the sympathetic branch of the autonomic nervous system. The skin conductance response (SCR) was defined as the largest change relative to baseline (the skin conductance level at stimulus onset) between 1 and 6 s after stimulus onset. Stimuli for which the SCR was below .01 μSiemens were marked as zero-responses.

For each adolescent, the mean values of SCR for direct eye gaze, averted eye gaze and closed eyes were computed.

Subjective ratings. During the second task, after presentation of each stimulus, the adolescent was asked to evaluate, firstly, how pleasant or unpleasant (valence) and, secondly, how calm or aroused (arousal) he felt. The subjective ratings were based on the Self-Assessment Manikin (SAM). The SAM is a visual 9-point rating scale with icons depicting values along the dimensions of valence and arousal in which the observer reports the number that indicates his or her level of valence or arousal (Lang, Bradley, & Cuthbert, 2001). An adaptation of the instruction about the SAM, used in studies with individuals with ASD, was also used in this study (Ben Shalom, et al., 2006; Bölte et al., 2008). To give the subjective rating, the adolescent responded with his dominant hand using a keyboard.

Social deficits. The CSBQ (Hartman, et al., 2006) was used to measure the amount of social deficits in the adolescents with ASD. This parental questionnaire consists of 49 items, which are scored on a 3-point scale (0: behaviour does not apply; 1: behaviour sometimes or somewhat applies; 2: behaviour clearly or often applies to the child). Higher scores on the CSBQ indicate more social deficits. Estimates for internal, test-retest, inter-rater reliability, convergent and divergent validity of the CSBQ were good.

Statistical analyses

Preliminary, to avoid confounding effects of possible differences in overall fixation durations between adolescents, the 'relative eye fixation duration' was defined as the percentage of the fixation duration for the eye region relative to the fixation duration for the whole stimulus. In addition, to account for possible effects of age, IQ and medication use on the main outcome variables (fixation duration, HR, SCR, and subjective rating scores), correlations were computed between those variables. If significant correlations were found, these parameters were taken into account in further analysis as covariates.

To determine differences in spontaneous fixation duration towards the eye region between the ASD and TD group, a repeated-measures analysis of variance (ANOVA) was performed with eye gaze condition (direct eye gaze, averted eye gaze and closed eyes) as the within-subjects factor and group (ASD versus TD) as the between-subjects factor. The Huynh-Feldt correction was used to adjust for sphericity violations when necessary.

To study changes in autonomic responses during sustained fixation to the eye region, we first determined which adolescents spent more time looking towards the eyes region (for all three eye gaze conditions) during the second task ('sustained eye fixation') compared to the first task ('spontaneous fixation'). Only adolescents with longer fixations to the eye region in the second task were included in the subsequent analyses regarding autonomic responses. Differences in autonomic measures (i.e. HR deceleration response, HR acceleration response and SCR) between the ASD and TD groups were investigated with respect to the eye gaze conditions. Box-Cox transformations were used to reduce the skewness of the distributions

of the HR deceleration response, HR acceleration response, and SCR (Osborne, 2010). After these transformations, the autonomic measures were analyzed by means of three separate repeated-measures ANOVAs, with condition (direct eye gaze, averted eye gaze and closed eyes) as the within-subjects factor and group (ASD versus TD) as the between-subjects factor. The subjective ratings of valence and arousal were log-transformed to reduce the skewness of the distributions. After these transformations, two separate repeated-measures ANOVA's were conducted with condition (direct eye gaze, averted eye gaze and closed eyes) as the within-subject factor and group (ASD versus TD) as the between-subject factor.

To determine whether autonomic responses during sustained fixation to direct eye gaze were associated with social deficits among individuals with ASD, we calculated the association between HR deceleration, HR acceleration, SCR and social deficits as measured with the total score on the CSBQ.

For all analyses SPSS software (Version 20.0) was used. All analyses were two-tailed with a significance level alpha of 0.05. Values are reported as mean ± standard deviation (SD).

RESULTS

Preliminary data inspection

Due to technical problems, SCL data of two adolescents with ASD and of four TD adolescents and HR data of four TD adolescents had to be excluded from the analyses. Fixation data were obtained successfully in all adolescents. On average, adolescents in both groups spent more than half of the stimulus duration looking at the stimulus for at least five out of six pictures in each of the three eye gaze conditions. However, more stimuli had to be discarded in the ASD group (mean number of stimuli discarded 1.0 ± 1.4 SD) compared to the TD group (.2 ± .7; $t(79) = -3.1$ $p < .01$). Furthermore, the fixation data of five adolescents with ASD and of three TD adolescents was discarded since these adolescents spend less than half of the stimulus presentation looking at the stimuli, for at least five out of six stimuli in an eye gaze condition. After exclusions, full data sets of 31 ASD and 34 TD adolescents were available.

Total fixation duration towards the stimuli as a whole was significantly shorter for adolescents with ASD (4.6 ± .5 s) than for TD adolescents (i.e. 5.1 ± .5 s, $t(63) = 3.8$, $p < .001$). In all subsequent analysis, the relative eye fixation duration (defined as the ratio between fixation duration to the eye region and fixation duration to the whole stimulus) was used.

Five adolescents with ASD and four TD adolescents did not look longer to the eye region in the second experiment ('sustained eye fixation') than in the first task ('spontaneous fixation'). As this task was used in order to achieve sustained fixation at the eye region, autonomic activity data of these adolescents was discarded, yielding full data sets of 26 ASD and 30 TD adolescents for the analyses of autonomic responses. The mean IQ and mean age of these adolescents (ASD; IQ = 105.4 ± 10.6, age = 16.2 ± 2.0, TD; IQ = 108.9 ± 10.8, age = 16.5 ± 2.4)

was comparable between the groups. It was also comparable to the mean IQ and mean age in the total included sample.

Age and IQ were not significantly correlated with the outcome measures (fixation durations, HR deceleration response, skin conductance level and SAM scores), except for a significant correlation between age and the HR acceleration response (r = -2.7, p = .05). Psychotropic medicine use in the ASD group was not significantly correlated with the outcome measures. Therefore, age, IQ and medication were not included as covariates in the main analyses, except for age in the analysis of the HR acceleration response.

Spontaneous fixation

Both adolescents with ASD and TD adolescents spent more time looking towards the eye region when the eyes of the presented actor in the stimulus were open (i.e. direct or averted) versus closed (Table 1 and Figure 2). The ANOVA on the relative fixation durations showed a significant main effect of eye gaze condition (F(2,126) = 122.9, p < .001, partial η^2 = .66). Contrasts revealed that relative fixation duration to the eye region was significantly longer in the direct eye gaze condition (64.3 ± 16.4%) than in the averted eye gaze condition (57.8 ± 18.3%, F(1,63) = 15.5, p <.001) or in the closed eyes condition (40.6 ± 14.0%, F(1,63) = 259.4, p <.001). Relative fixation duration was also significantly longer in the averted eye gaze condition than in the closed eyes condition (40.6 ± 14.0%, F(1,63) = 124.6, p <.001). The ANOVA did not indicate a main effect of group (F(1,63) = .2, p = .69) or an interaction effect of eye gaze condition by group (F(2,126) = 2.7, p = .07).

Table 1. Raw (in seconds) and relative (%) fixation durations to the eye region of faces for the two tasks ('spontaneous fixation' and 'sustained eye fixation') and the three conditions (direct eye gaze, averted eye gaze, and closed eyes) for the two groups (ASD and TD).

	Task	'Spontaneous fixation'		'Sustained eye fixation' (*)	
Condition		ASD (n = 31)	TD (n = 34)	ASD (n = 26)	TD (n = 30)
Direct eye gaze	Raw	3.24 s	3.39 s	4.30 s	4.71 s
	Relative	64%	65%	84%	87%
Averted eye gaze	Raw	2.87 s	3.03 s	4.05 s	4.61 s
	Relative	57%	58%	81%	87%
Closed eyes	Raw	2.14 s	1.96 s	3.25 s	3.31 s
	Relative	44%	38%	65%	63%

ASD = Autism Spectrum Disorder, TD = Typically Developing.

*Adolescents were excluded if they did not look longer to the eye region in the 'sustained eye fixation' task than in the 'spontaneous fixation' task.

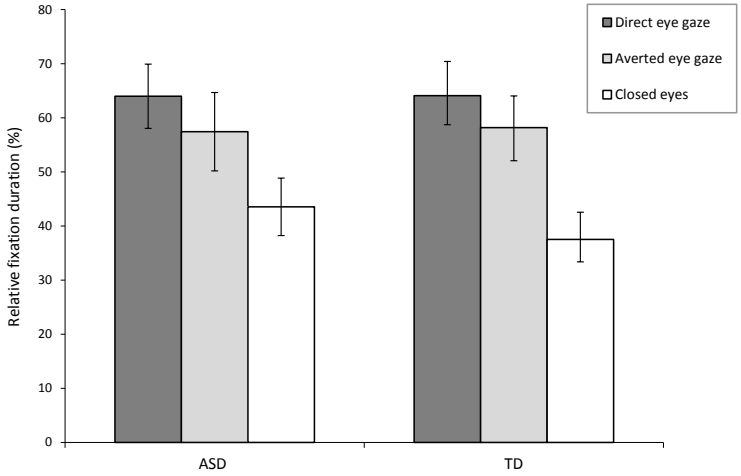


Figure 2. Relative fixation durations to the eye region (as a percentage of total fixation duration to the whole stimulus) in the 'spontaneous fixation' task for the three conditions (direct eye gaze, averted eye gaze and closed eyes) in adolescents with ASD and TD adolescents (mean and 95% confidence interval).

Autonomic responses

The relative fixation duration to the eye region was not significantly different between the remaining 26 adolescents with ASD and 30 TD adolescents in this task.

The autonomic responses for the two groups in the three conditions are presented in Table 2. The HR deceleration response, which represents the attention towards a stimulus,

Table 2. Average non-transformed heart rate (HR, in beats per minute) and skin conductance (SCR, in µSiemens) responses in the 'sustained eye fixation' task for the three conditions (direct eye gaze, averted eye gaze, and closed eyes) for the two groups (ASD and TD).

	ASD (n = 26) Mean (SD)	TD (n = 30) Mean (SD)
HR deceleration response (bpm)		
Direct eye gaze	-5.61 (4.81)	-6.52 (4.20)
Averted eye gaze	-5.07 (3.82)	-6.26 (6.03)
Closed eyes	-4.66 (4.08)	-6.34 (5.89)
HR acceleration response (bpm)		
Direct eye gaze	7.12 (3.26)	8.20 (4.44)
Averted eye gaze	7.52 (3.73)	8.22 (3.61)
Closed eyes	8.73 (4.31)	9.06 (5.25)
SCR (µSiemens)		
Direct eye gaze	0.08 (0.15)	0.05 (0.08)
Averted eye gaze	0.06 (0.10)	0.04 (0.06)
Closed eyes	0.04 (0.06)	0.04 (0.09)

ASD = Autism Spectrum Disorder, TD = Typically Developing, HR = Heart Rate, bpm = beats per minute , SCR = Skin Conductance Rate.

did not differ between conditions ($F(2,108) = .6$, $p = .55$), or groups ($F(1,54) = 1.0$, $p = .33$). Also the interaction of condition by group was not significant ($F(2,108) = .5$, $p = .64$). A significant main effect of condition was found for the HR acceleration response ($F(2,106) = 3.9$, $p = .02$, partial $\eta^2 = .07$), which is indicative of increased affective arousal. Contrasts indicated that the HR acceleration response was larger in the closed eyes condition than in the direct eye gaze condition ($F(1,53) = 7.3$, $p = .01$, $r = .12$). No interaction effect ($F(2,106) = .3$, $p = .75$) or group effect ($F(1,53) = .3$, $p = .45$) were found for the HR acceleration response.

Overall, the SCR was low (Table 2). On average, in 12 of the 18 presented stimuli, responses of smaller than .01 μSiemens were found. The number of stimuli with such a low response did not differ between the adolescents with ASD and TD adolescents. The SCR did not differ between conditions ($F(2,108) = .4$, $p = .64$), or groups ($F(1,54) = .8$, $p = .39$), or the interaction of condition by group ($F(2,108) = .8$, $p = .46$).

Subjective ratings

Figure 3 shows the mean rating scores of valence and arousal for the three eye gaze conditions per group. Eye gaze condition had a significant effect on both valence ratings ($F(2,108) = 7.4$, $p < .01$, partial $\eta^2 = .12$) and arousal ratings ($F(2,108) = 6.7$, $p < 0.01$, partial $\eta^2 = .11$). Contrasts indicated that the valence rating was higher in the direct eye gaze condition than

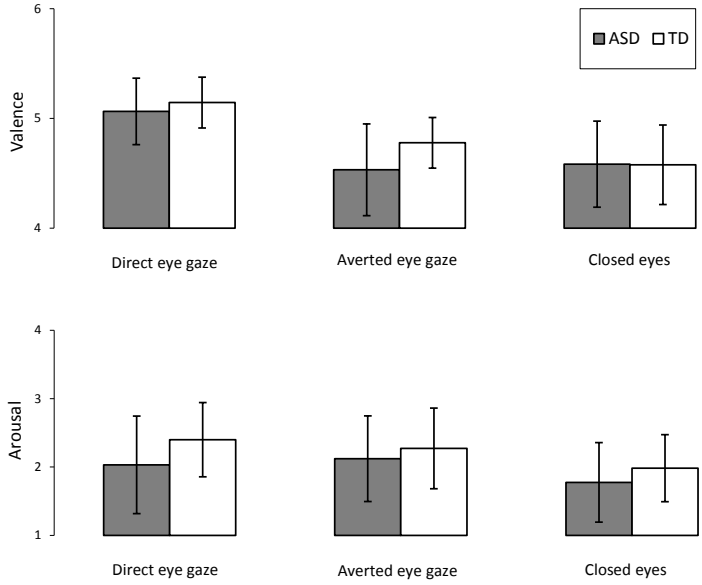


Figure 3. Non-transformed valence and arousal ratings (mean and 95% CI's) for the three conditions (direct eye gaze, averted eye gaze and closed eyes) in adolescents with ASD and TD adolescents.

in both the averted eye gaze condition ($F(1,54) = 10.0$, $p < .01$, $r = .16$) and the closed eyes condition ($F(1,54) = 7.6$, $p < .01$, $r = .12$). Contrasts also indicated that the averted eye gaze condition yielded a higher arousal rating than the closed eyes condition ($F(1,54) = 9.1$, $p < .01$, $r = .14$). For both ratings, no main effects of group (valence: $F(1,54) = 0.4$, $p = .51$; arousal: $F(1,54) = 0.7$, $p = .40$) or interaction effects were found (valence: $F(2,108) = 0.7$, $p = .50$; arousal: $F(2,108) = 2.0$, $p = .15$).

Autonomic responses and social deficits

No significant associations were found between the total CSBQ score and SCRs ($r = .11$, $p = .56$), HR deceleration responses ($r = .17$, $p = .35$) and HR acceleration responses ($r = .08$, $p = .67$).

DISCUSSION

The present study investigated the effect of eye gaze direction of facial images on gaze behavior and autonomic responses in cognitively able adolescents with autism spectrum disorders (ASD). Adolescents with ASD did not have significantly different fixation durations towards the eye region than typically developing (TD) adolescents. Both adolescents with ASD and TD adolescents showed longer spontaneous fixation durations towards the eye region of faces portraying direct eye gaze than faces with averted or closed eyes. During sustained fixation at the eye region, similar autonomic responses were found for adolescents with ASD and TD adolescents. Thus, results of this study suggest that gaze direction by itself does not account for the atypical spontaneous gaze behavior that is often observed in individuals with ASD (Dalton, et al., 2005; Jones, et al., 2008; Klin, et al., 2002; Neumann, et al., 2006). Also, sustained fixation to the eye region did not trigger higher levels of autonomic arousal for faces with direct eye gaze compared to faces with averted eye gaze or closed eyes.

In our study, cognitively able adolescents with ASD spontaneously looked at the eye region of faces as much as TD adolescents. This finding is in line with previous studies that used static stimuli (Freeth, et al., 2010; Sawyer, et al., 2012; van der Geest, et al., 2002). The present study showed that eye gaze direction significantly influenced the time spent looking towards the eye region in both the TD and the ASD group. Direct eye gaze evoked longer fixation durations than averted eye gaze, which in turn evoked longer fixation durations than closed eyes. This finding is in line with previous studies in TD individuals (Batki, et al., 2000; Farroni, et al., 2006) and indicates that cognitively able adolescents with ASD seem to be aware of the salience of eyes.

Both autonomic measures and subjective ratings were taken into account to evaluate arousal during sustained fixation on the eye region. However, no differences between groups were observed. For the HR acceleration response, an effect of eye gaze condition was found.

Contrary to our hypotheses, the closed eyes condition revealed larger HR acceleration response than direct eye gaze condition. Both adolescents with and without ASD seemed to be more aroused when they had to look to a photograph of a face with closed eyes than when they had to look to a photograph of a face with direct eye gaze. The closed eye condition also revealed shorter fixation times towards the eyes than the direct eye gaze or averted eye gaze condition. It could be, that participants - when instructed to look at the eyes - have to put more effort to sustain fixation on closed eyes than in the direct eye gaze condition. Such an increased effort, in turn, might lead to higher HR acceleration response, which is a measure of affective arousal (Wieser, Pauli, Alpers, & Muhlberger, 2009). However, this higher level of autonomic arousal for closed eyes was not found when analyzing the SCR data. The SCRs to all three conditions were low, which might explain why we did not find similar results for the SCR and the HR acceleration response data. In contrast to the HR acceleration response, the *subjective* ratings of the adolescents showed *higher* levels of arousal for faces with open eyes (i.e. direct eye gaze and averted eye gaze). When speculating on this discrepancy between the HR acceleration response and subjective rating of arousal, we presume that the subjective arousal level is probably a reflection of positive arousal. The adolescents gave higher valence and arousal ratings to faces with open eyes compared to closed eyes, which could indicate that they subjectively experienced these stimuli as positively arousing. The higher HR acceleration response for the closed eyes condition might be the result of the amount of effort to sustain looking at closed eyes, and could possibly reflect negative arousal. However, these speculations should be interpreted with caution, since HR acceleration response is indicative of arousal to both positive and negative stimuli (Bradley & Lang, 2000).

Thus, in the current study, sustained looking at the eye region of static pictures of neutral faces did not trigger higher levels of autonomic arousal for ASD adolescents compared to TD adolescents. Previous studies concerning autonomic responses to social stimuli did find higher (Joseph, et al., 2008) or lower (Hubert, et al., 2009; Vaughan Van Hecke, et al., 2009) autonomic arousal levels in individuals with ASD compared to TD. As described in the previous paragraph, we found contrasting results concerning the effect of eye gaze condition on arousal. Past studies that evaluated the effect of eye gaze direction on autonomic arousal levels also reported contrasting results. Some studies found higher levels of autonomic arousal for direct eye gaze compared to averted eye gaze or closed eyes for individuals with ASD but not for TD individuals (Kylliäinen & Hietanen, 2006; Kylliäinen, et al., 2012), while others studies did not (Kaartinen, et al., 2012). The level of autonomic arousal during sustained looking at direct eye gaze was not significantly associated with the amount of social deficits in adolescents with ASD as measured with the CSBQ. Kaartinen et al. (2012) did find a significant association between autonomic arousal during looking at the eye region and social impairment; however, they used different measures for autonomic arousal and social deficits. In general, all previous described studies did not measure visual fixations. Therefore, previous

results might be confounded by variations in fixation times towards the eye region. Fixation duration was taken into account in the current study.

Hajcak et al. (2011) indicated that fixation durations influence the amount of autonomic arousal. For example, when individuals focused on more arousing parts of a picture, their arousal levels (as indicated by electrocortical measures) were also higher. Thus, individuals in previous studies might have fixated longer on more arousing parts of the picture (allegedly the eye region), which in turn leads to higher autonomic arousal levels. Alternatively, they could have regulated their autonomic arousal levels by fixating on other parts of the pictures than the eye region. In the current study, the effect of reduced fixation duration towards the eye region was circumvented by specifically instructing participants to look at the eyes; participants were excluded if they did not look longer to the eye region in the sustained eye fixation task than in the spontaneous viewing task. Such differences between study designs might (partly) explain the differences in reported autonomic responses. Reactions to stimuli are expressed in three different output systems: behavior (e.g. fixation duration), autonomic activity (e.g. HR and SCR) and emotional content (e.g. subjective reports, Lang, et al., 1993). Therefore, we want to emphasize the importance of integrating measures of all three output systems in future studies.

There were some limitations to our study. Inclusion of cognitively able male participants limits the generalization of our results. It should also be noted that we used static facial images and not real life interactions, thus our observations do not imply that cognitively able adolescents with ASD have no difficulties in making eye contact during real life social situations. The clinical scores on the ADOS in fact revealed that 68% of the adolescents in the ASD group used poorly modulated eye contact to initiate, terminate or regulate social interaction (Lord, et al., 1999). Furthermore, we used static photographs of faces with neutral expressions. Other studies suggest that dynamic videos of faces and faces with emotional expressions do trigger higher levels of arousal in individuals with ASD compared to TD (Hietanen, Leppanen, Peltola, Linna-Aho, & Ruuhiala, 2008; Hubert, et al., 2009). However, in these studies it was hard to determine which specific aspect of the complex dynamic stimuli employed in the various study designs. In addition, these studies did not include a measure of fixation duration. Therefore, it remains unclear whether the higher level of autonomic arousal previous studies found was due to fixation at the eye region. We recommend further studies to elaborate on the design of the current study, by measuring fixation duration and autonomic arousal levels simultaneously, while presenting stimuli that vary in their complexity and naturalistic validity, to better understand the social information processing of individuals with ASD. This knowledge might be helpful in designing interventions at improving social skills in individuals with ASD. Finally, another limitation of the current study is that we did not have information on the recent medicine use of the TD individuals.

Aside from these limitations, our findings do have several implications. Our findings revealed that cognitively able adolescents with ASD showed typical fixation durations to the

eye region when viewing photographs of faces. Direct gaze plays an important role in face and social information processing (Farroni, Massaccesi, Menon, & Johnson, 2007). Typical looking behavior to faces with direct eye gaze suggests that cognitively able adolescents with ASD are aware of the social relevance of eye contact. Contrary to our hypothesis, sustained fixation to direct eye gaze did not elicit stronger autonomic responses than sustained fixation to averted or closed eyes in the ASD group compared to the TD group. Yet, the subjective reports of both groups revealed higher valence and arousal ratings for direct eye gaze compared to closed eyes. Thus, cognitively able adolescents with ASD, like TD adolescents, spent more time looking towards direct eye gaze which they subjectively reported as more pleasant and more arousing than closed eyes. However, on the level of autonomic responses, gaze direction by itself did not seem to trigger higher levels of arousal for direct eye gaze compared to averted or closed eyes.

CHAPTER **5**

Autonomic responses to social and non-social pictures in adolescents with autism spectrum disorder

Anneke Louwerse, Joke Tulen, Jos van der Geest, Jan van der Ende, Frank Verhulst, Kirstin Greaves-Lord

Autism Research, 2014, 7, 17-27.

ABSTRACT

It remains unclear why individuals with autism spectrum disorder (ASD) tend to respond in an atypical manner in social situations. Investigating autonomic and subjective responses to social versus non-social stimuli may help to reveal underlying mechanisms of these atypical responses. This study examined autonomic responses (skin conductance level and heart rate) and subjective responses to social versus non-social pictures in 37 adolescents with an ASD and 36 typically developing (TD) adolescents. Thirty-six pictures from the International Affective Picture System (IAPS) were presented, divided into six categories based on social content (social versus non-social) and pleasantness (pleasant, neutral, and unpleasant). Both in adolescent with ASD as well as TD adolescents, pictures with a social content resulted in higher skin conductance responses (SCRs) for pleasant and unpleasant pictures than for neutral pictures. No differences in SCRs were found for the three non-social picture categories. Unpleasant pictures, both with and without a social content, showed more heart rate deceleration than neutral pictures. Self-reported arousal ratings were influenced by the social and affective content of a picture. No differences were found between individuals with ASD and TD individuals in their autonomic and subjective responses to the picture categories. These results suggest that adolescents with ASD do not show atypical autonomic or subjective responses to pictures with and without a social content. These findings make it less likely that impairments in social information processing in individuals with ASD can be explained by atypical autonomic responses to social stimuli.

INTRODUCTION

Individuals with ASD show atypical behavioural responses in social situations (APA, 2000). For example, in response to social stimuli, individuals with ASD look less at faces (Chawarska & Shic, 2009; Osterling & Dawson, 1994; Riby & Hancock, 2009) and tend to avoid eye contact (Klin, Jones, Schultz, Volkmar, & Cohen, 2002; Rice, Moriuchi, Jones, & Klin, 2012). Previous studies have suggested that atypical behavioural responses to social stimuli of individuals with ASD could be explained by the level of arousal that individuals with ASD experience when attending to social stimuli (Hutt, Hutt, Lee, & Ounsted, 1964; Kylliäinen, et al., 2012; Levine, et al., 2012; Riby, Whittle, & Doherty-Sneddon, 2012; Rimland, 1968; Rogers & Ozonoff, 2005; Senju & Johnson, 2009; van Engeland, Roelofs, Verbaten, & Slangen, 1991). Two distinct models have been proposed based on this idea; the hyperarousal model and the hypoarousal model (Senju & Johnson, 2009). The hyperarousal model suggests that individuals with ASD are in a 'heightened' autonomic state, in which their autonomic system is constantly on a maximum alert (Bal, et al., 2010; Hirstein, Iversen, & Ramachandran, 2001). In this state, individuals with ASD might be more easily aroused by social stimuli than TD individuals and they may fail to habituate to social stimuli in the environment (Dalton, et al., 2005; Hutt, et al., 1964; Joseph, Ehrman, McNally, & Keehn, 2008; Rogers & Ozonoff, 2005). On the other hand, the hypoarousal model hypothesizes that individuals with ASD experience less arousal or reward when attending to social stimuli in their environment (Mathersul, McDonald, & Rushby, 2012; Rimland, 1968). This lack of autonomic arousal might interfere with attaching positive reward to social stimuli, which, in turn, allegedly hampers learning from the social environment (Senju & Johnson, 2009). Albeit these suggestions of hyper- or hypoarousal in ASD, research on indices of arousal in reaction to social stimuli is still scarce.

Arousal can be measured at various levels; at the level of brain functioning, autonomic arousal or subjective responses. First, at the level of brain functioning, several studies found atypical neural responses to social or affective stimuli in individuals with ASD (Adolphs, Sears, & Piven, 2001; Dalton, et al., 2005; Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2007; Kleinhans, et al., 2011; Kliemann, Dziobek, Hatri, Baudewig, & Heekeren, 2012). The structure and connectivity of the amygdala seems to influence these arousal levels (for overview see: Senju & Johnson, 2009).

Second, levels of arousal can be measured with indices of the autonomic nervous system, e.g. skin conductance level (SCL) or heart rate (HR). SCL reflects the level of, and fluctuations in, sweat gland activity. Sweat gland activity is under control of the sympathetic branch of the autonomic nervous system, and, therefore, SCL can be considered as a good index for autonomic arousal. In individuals with ASD, both higher and lower levels skin conductance responses (SCR) have been reported (Hirstein, et al., 2001; Hubert, Wicker, Monfardini, & Deruelle, 2009; Joseph, et al., 2008; Kaartinen, et al., 2012; Kylliäinen & Hietanen, 2006; Kylliäinen, et al., 2012; Mathersul, et al., 2012; Sasson, Dichter, & Bodfish, 2012). HR responses

are also used as an indicator of autonomic activity. HR is the resultant of the combined activity of the sympathetic and parasympathetic branches of the autonomic nervous system. Only few studies investigated HR responses to social stimuli in ASD (Bal, et al., 2010; Bölte, Feineis-Matthews, & Poustka, 2008; Mathersul, et al., 2012; Sigman, Dissanayake, Corona, & Espinosa, 2003). Almost all of these studies used the averaged heart rate response during the stimulus relative to the baseline, without taking into account the triphasic pattern of the cardiac response, which is a classical HR response to arousing pictures that consists of an initial deceleration (i.e. orienting response), an acceleration and a second deceleration (Bradley & Lang, 2000). The initial deceleration response (i.e. orientation response) is thought to reflect the intake of stimulus information and is part of an attention processing mechanism (Turpin, Schaefer, & Boucsein, 1999). The initial deceleration response is primarily mediated by parasympathetic processes. The acceleration phase is interpreted as a defensive response, primarily related to sympathetic arousal. Mathersul et al. (2012) did take this HR pattern into account and reported a higher HR deceleration response for pleasant and unpleasant stimuli compared to neutral stimuli in individuals with ASD. This pronounced deceleration response for affective stimuli was not found for the TD group. However, their pleasant and unpleasant stimuli were all social stimuli, whereas their neutral stimuli contained all non-social pictures. Therefore, Mathersul et al. (2012) were unable to clarify whether the higher HR deceleration response in individuals with ASD was due to the affective content or to the social content of the selected stimuli. Mathersul et al. (2012) therefore argued that future studies should consider studying responses to "pleasant, unpleasant, and neutral stimuli of both a social and non-social nature" (p. 20).

Third, the level of arousal can be measured at the level of subjective report of the participant. Only few studies examined self-reported arousal ratings for stimuli with a social content versus a non-social content. These studies did not find differences in self-reported arousal ratings between individuals with ASD and TD individuals (Mathersul, et al., 2012; Sasson, et al., 2012). Other studies focused on the self-reported arousal ratings of affective stimuli. In TD individuals, affective (pleasant or unpleasant) pictures triggered stronger subjective arousal ratings than neutral pictures (Bradley & Lang, 2000). Individuals with ASD reported lower levels of subjective arousal for affective pictures versus neutral pictures than TD individuals (Ben Shalom, et al., 2006; Bölte, et al., 2008). Interestingly, Bölte et al. (2008) found this lower reported arousal levels of individuals with ASD for sad pictures that were exclusively represented by social situations. Although their study did not focus on differences between social and non-social stimuli, the authors suggested that the atypical self-reported arousal ratings might have been influenced by the social content of the stimuli.

When combining study results at the level of brain functioning, autonomic responses and subjective responses, there is some evidence that individuals with ASD show atypical levels of arousal while attending to social stimuli. However, with claims of both hyper- and hypoarousal, previous results are divergent. It is important to unravel which aspects of the environment

trigger levels of hyper- or hypoarousal in individuals with ASD compared to TD individuals. First, the *social content* of the stimuli varied between studies; some studies included social and non-social stimuli, while others only included social stimuli (e.g. faces). Previous studies found lower levels of arousal for individuals with ASD in reaction to social stimuli versus non-social stimuli, while TD individuals did show higher levels of arousal to the social stimuli (Hirstein, et al., 2001; Hubert, et al., 2009). However, the results were contrasting for studies that only included social stimuli, but with a varying degree of social relevance (e.g. stimuli including direct eye gaze versus closed eyes). Some of these studies found higher levels of arousal for the more social relevant stimuli for individuals with ASD (Kylliäinen & Hietanen, 2006; Kylliäinen, et al., 2012), while others did not (Joseph, et al., 2008; Kaartinen, et al., 2012). Second, the *affective content* of the stimuli seemed to influence the level of autonomic and subjective arousal. Previous studies have indicated that looking to stimuli with an affective content (pleasant or unpleasant) was associated with higher levels of arousal than neutral stimuli for individuals with TD (Bradley & Lang, 2000). This typical higher response to affective compared to neutral pictures was not reported for individuals with ASD (Ben Shalom, et al., 2006; Bölte, et al., 2008; Hubert, et al., 2009; Mathersul, et al., 2012). However, some studies did not report differences in arousal to affective stimuli in individuals with ASD compared to TD individuals (Riby, et al., 2012; Sasson, et al., 2012). Thus, previous studies focused either on the social content or the affective content of stimuli, while these concepts often overlap. This overlap makes it hard to determine whether the social content, the affective content or both triggered autonomic and subjective responses. To our knowledge, there are no direct comparisons of stimuli with and without a social content, accounting for the affective content (i.e. pleasantness) of the stimuli. The integration of these two concepts into one study design would be beneficial, since this would make it possible to detect whether either the social content, the affective content or both trigger hyper-or hypoarousal in individuals with ASD.

When measuring levels of arousal, we must bear in mind the relationship of arousal with attention (Coull, 1998). The amount of attention paid to a stimulus might influence the level of autonomic arousal (Hajcak, Macnamara, Foti, Ferri, & Keil, 2011). Therefore, when studying reactions to specific stimuli, not only subjective and autonomic arousal measures, but also the attention towards the stimulus should be considered (P. J. Lang, Greenwald, Bradley, & Hamm, 1993). Attention towards the stimulus can be detected through eye-tracking. Yet, studies that combine measures of arousal and eye-tracking are presently scarce.

The aim of the current study is to determine autonomic activity, subjective experience and fixation durations in adolescents with ASD and TD individuals, while showing social versus non-social pictures, with a pleasant, neutral and unpleasant affective content. Since several previous studies indicated that both social stimuli and affective stimuli triggered higher levels of arousal in TD individuals, we expect that looking to pleasant and unpleasant social stimuli will be associated with higher levels of autonomic and subjective responses than non-social neutral stimuli for TD individuals. In line with some previous studies, we expect

that adolescents with ASD will show less arousal for affective versus neutral pictures than TD individuals (Ben Shalom, et al., 2006; Bölte, et al., 2008; Hubert, et al., 2009; Mathersul, et al., 2012). Also we expect less arousal to social stimuli versus non-social stimuli for individuals with ASD compared to TD individuals (Hirstein, et al., 2001; Hubert, et al., 2009). Therefore, for the combination of a social and affective content of stimuli, we expect less differential autonomic and subjective responses in individuals with ASD compared to TD individuals. This hypothesis would underline hypoarousal to social affective stimuli in individuals with ASD. To be able to evaluate whether attention (i.e. visual fixation time to the total picture) was related to the autonomic and subjective responses, we simultaneously recorded eye movements during stimulus presentation.

METHODS

Participants

Thirty-nine adolescents with ASD and 42 TD adolescents participated in this study, which was approved by the Medical Ethical Committee of the Erasmus MC. Informed consent was obtained from all adolescents and also from their parents if the adolescent was younger than 16 years of age. Only male adolescents with an IQ above 70 were included. To confirm an IQ above 70, the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) was administered. There was no significant difference regarding the mean total IQ of the adolescents with ASD (Mean = 103.7, SD = 13.6) and the TD adolescents (Mean = 108.1, SD = 10.6; $t(79)= 1.6$, $p = .11$). The ages of the adolescents with ASD (Mean = 16.0, SD = 1.9) and the TD adolescents (Mean = 16.2, SD = 2.5) were also not significantly different ($t(79= .4$, $p = .71$). All adolescents had normal, or corrected to normal, vision.

ASD adolescents were recruited from the outpatient's department of Child and Adolescent Psychiatry/Psychology of the Erasmus MC-Sophia Rotterdam, the Netherlands. All 39 adolescents with ASD met the diagnostic criteria (i.e. algorithm) of both the Autism Diagnostic Observation Schedule (ADOS; Bastiaansen, et al., 2011; Gotham, Risi, Pickles, & Lord, 2007; Lord, et al., 2000) and the Autism Diagnostic Interview – Revised (ADI-R; Bildt, et al., 2013; Lainhart, et al., 2006; Rutter, Le Couteur, & Lord, 2003; Sung, et al., 2005). The parents of ASD adolescents were asked if their child had used medication in the week before testing. Sixteen adolescents used psychotropic medication; eight took methylphenidate, six took antipsychotics, one used an antidepressant and one used antiepileptic medication.

The TD participants were selected from a general population sample (Tick, van der Ende, & Verhulst, 2008). These adolescents had no history of neurodevelopmental disorders. In addition, TD individuals were excluded if their parents reported ASD problems within the clinical range of the Children's Social Behavior Questionnaire (CSBQ, Hartman, Luteijn, Serra, & Minderaa, 2006).

Procedure

The experimental procedure lasted about 1.5 hours. The participant was presented with three tasks. The results described in the current paper are based on the second task. In this task, the adolescent was presented with a series of 36 pictures. The adolescent was seated in a fixed chair approximately 60 cm from the computer screen on which the stimuli were displayed. Electrodes for the autonomic recordings (HR and SCL) were applied according to standard procedures (Greaves-Lord, et al., 2007; N. J. Lang, et al., 2007). Eye movements were recorded during all tasks (please see *Measurement* for more information).

After instructions, a five-point calibration routine was used to ensure validity of the eye-tracking data. The examiner evaluated the calibration and the calibration routine was repeated in case of unsatisfactory data. Subsequently, two additional pictures were shown as practice stimuli. After instructions, the calibration procedure and the practice stimuli, the examiner sat behind a screen. The subject was instructed to look at 36 pictures, and to rate his subjective impression of valence and arousal (see *Subjective ratings*) after the presentation of each picture.

The stimulus presentation was designed and controlled by E-Prime software (version 2.0 including extensions for Tobii: PST-100777; Psychology Software Tools, Inc., Sharpsburg, PA, USA), which is used for computerized psychological tasks. Each presentation of a stimulus lasted six seconds and the interval between stimuli varied between 15 to 25 seconds. During this interval, a fixation cross was presented on the screen.

Stimuli

The stimuli consisted of 38 color pictures from The International Affective Picture System (IAPS; P. J. Lang, Ohman, & Vaitl, 1988); two of which were used as practice pictures and the other 36 were used as target stimuli. The pictures were selected based on social content (social versus non-social) and pleasantness (pleasant, neutral or unpleasant). Pictures with a social content depicted humans (e.g. individuals in a rollercoaster), while pictures with a non-social content did not depict humans (e.g. fireworks). Pleasantness was based on normative ratings provided with the IAPS (P. J. Lang, Bradley, & Cuthbert, 2001). There were six categories, containing six pictures each[1]:

1) Social – pleasant (valence rating > 6, arousal rating > 4)
2) Social – neutral (valence rating 4-6, arousal rating 0-4)
3) Social – unpleasant (valence rating 0-4, arousal rating > 4)
4) Non-social – pleasant (valence rating > 6, arousal rating > 4)
5) Non-social – neutral (valence rating 4-6, arousal rating 0-4)
6) Non-social – unpleasant (valence rating 0-4, arousal rating > 4)

The pictures were shown in a different random order for each participant.

The numbers of the IAPS pictures used were: social-pleasant (2070, 2216, 4599, 5830, 8490, 8540), social-neutral (2190, 2191, 2235, 2383, 2393, 2480), social-unpleasant (2683, 2800, 3500, 6250, 9050, 9220), non-social-pleasant (5480, 5600, 5982, 7330, 7470, 8510), non-social-neutral (5390, 5530, 7010, 7170, 7175, 7217), non-social-unpleasant (7380, 9000, 9470, 9600, 9630, 9911), practice stimuli (7150, 8021).

Measurement

Eye-tracking. Eye movements were recorded using a remote eye tracker (Tobii120, Tobii, Danderyd, Sweden) with a 17-inch display and data rate of 60 Hz. Adolescents were free to move their head throughout the tasks; the accuracy of recording was maintained as long as the adolescents kept their eyes with a virtual space measuring 44 x 22 x 30 cm. Eye movement data were processed using custom software written in Matlab (The Mathworks, Natick MA, United States). For each stimulus, the total fixation time for the whole picture was determined. For each of the six stimulus categories, fixation duration was defined as the average fixation time across the pictures in that category.

Subjective ratings. After the presentation of each stimulus, the adolescent was asked to evaluate how pleasant or unpleasant (i.e. valence) the picture made him feel, and how calm or aroused (i.e. arousal) the picture made him feel. The instruction for the participant was to "rate each picture in terms of how they made you feel while viewing it" (Bölte, et al., 2008, p.779). These subjective ratings were based on the Self-Assessment Manikin (SAM). The SAM is a visual 9-point rating scale with icons depicting values along the dimensions of valence and arousal (Lang, Bradley, & Cuthbert, 2001). The observer first reported the number on the 9-point scale that indicated his level of valence. Thereafter, he reported the number that indicated his degree of arousal. An adaptation of the instruction of the SAM, used specifically in studies with individuals with ASD, was also used in the current study (Ben Shalom, et al., 2006; Bölte, Feineis-Matthews, & Poustka, 2008). To give the subjective ratings, the adolescent responded with his dominant hand using a keyboard. For each of the six stimulus categories, SAM scores of valence and arousal were defined as the average valence and arousal scores across the pictures in that category.

Autonomic measures. Two indices of autonomic activity were measured: HR and SCL. All HR and SCL data was sampled and stored on a flashcard by means of a portable digital recorder (Vitaport™ System; TEMEC Instruments B.V., Kerkrade, the Netherlands). Upon completion of the recording, all autonomic data was imported and processed on a laptop using the Vitascore™ software module (TEMEC Instruments B.V., Kerkrade, the Netherlands). The autonomic measures were visually inspected for detection and removal of artefacts.

HR was recorded continuously using a precordial lead, and was sampled at 512 Hz. The interbeat intervals were calculated, using R-top detection. This resulted in HR series of beats per minute. The mean heart rate in the second before each picture onset was defined as the baseline value. This baseline value was subtracted from the minimum value of the HR

between the first and third second of the picture, to retrieve the HR deceleration response (Hempel, Tulen, van Beveren, Mulder, & Hengeveld, 2007; P. J. Lang, et al., 1993). The HR acceleration response was defined as the maximum value between the third and sixth second of the picture, subtracted by the minimum value between the first and third second. For each adolescent, mean HR deceleration responses and mean HR acceleration responses were computed for each stimulus category.

SCL was measured using two Ag/AgC1 electrodes attached to the volar surfaces of the medial phalanxes of the index and ring fingers of the non-dominant hand. The level of skin conductance was sampled at 8 Hz and stored in μSiemens. The skin conductance response (SCR) was defined as the largest amplitude relative to baseline (the skin conductance level at stimulus onset) from one to six seconds after picture onset. Changes of .01 or higher μSiemens were considered as responses, changes below .01 μSiemens were marked as zero-responses. For each adolescent, the mean value of SCR amplitude was computed for each stimulus category.

Statistical analyses

The power of the current study was calculated with G*Power 3 (Faul, Erdfelder, Lang, & Buchner, 2007). These analyses revealed that the power was between .96 and 1 to detect large between-subject effects and between .99 and 1 to detect medium to large within-group effects. These medium and large effect sizes were based on Cohen's population effect sizes (Cohen, 1988). The estimation of these effect sizes should be based on the results from previous studies. However, the heterogeneity within the results of current literature hampered the estimation of a population effect size.

Since the autonomic responses (HR deceleration response, HR acceleration response, SCR) and the total fixation durations were not normally distributed, we used the Box-Cox transformation to reduce the skewness of the distributions (Osborne, 2010). For the SCR a lambda (λ) of -33.4 reduced skewness of the data most. For the HR deceleration response, the lambda of 2.2 fitted best and for the amplitude of the HR acceleration response a lambda of .2 was used to reduce the skewness of the distribution.

To check for possible effects of age, IQ on the main outcome variables (fixation duration, HR deceleration response, HR acceleration response, SCR, and subjective rating scores), correlations between the putative confounding variables and the outcome variables were computed. If significant correlations were found, the parameters were taken into account in further analysis as covariates. In addition, to be able to evaluate whether attention (i.e. visual fixation time to the total picture) was related to the autonomic and subjective responses, we also computed correlations between fixation duration and the outcome variables.

The outcome variables (fixation duration, HR deceleration response, HR acceleration response, SCR, self-reported valence and self-reported arousal) were analysed using separate repeated measures ANOVAs with social content (social versus non-social) and pleasantness

(pleasant versus unpleasant versus neutral) as the within-subjects factors and group (ASD versus TD) as the between subjects factor. If an interaction effect or a main effect of condition was found, simple contrasts were reported to clarify the nature of the effect. All factors were entered in a single model. We did not conduct further model selection steps. A univariate approach, with a compound-symmetry structure of the variance-covariance matrix, was used if the assumption of sphericity was not violated. The Huynh-Feldt correction was used to adjust for sphericity violations if necessary. For each ANOVA, the results of the Box's Tests of Equality of Covariance Matrices was reported.

For all analyses we used SPSS software (Version 20.0; IBM SPSS Statistics, Chicago, IL, USA). All analyses were two-tailed, and the alpha was set at 0.05.

RESULTS

Preliminary data inspection

Due to technical problems, SCL data of two adolescents with ASD and four TD adolescents and HR data of three TD adolescents could not be analysed. After exclusion of these participants, full data sets of 37 ASD and 36 TD adolescents were available.

IQ and fixation duration were not significantly related with the outcome measures. Age was significantly correlated with SCR ($r = -.3$, $p < .01$), HR deceleration response ($r = .3$, $p < .01$), and HR acceleration response ($r = -.3$, $p = .03$). Therefore, age was included as a covariate in the subsequent analyses regarding SCR and HR deceleration responses. To reduce multicollinearity between age and group, age was centred by subtracting the mean age of the total group from the age of the participants.

Fixation duration

Fixation duration was not significantly related with the outcome variables (HR deceleration response, HR acceleration response, SCR, and subjective rating scores). The repeated measures ANOVA revealed that adolescents with ASD fixated significantly shorter to the total set

Table 1. Fixation duration in seconds (Mean, SD) towards the total stimuli for social versus non-social pictures with a pleasant, neutral and unpleasant affective content, for adolescents with ASD and TD adolescents.

	Category					
	Social			Non-social		
	Pleasant	Neutral	Unpleasant	Pleasant	Neutral	Unpleasant
ASD	4.2 (.9)	4.0 (.7)	4.3 (.8)	4.0 (.8)	3.8 (1.0)	4.1 (.9)
TD	4.7 (.6)	4.4 (.9)	4.8 (.8)	4.5 (.8)	4.5 (.9)	4.6 (.8)

ASD n = 37, TD n = 36.

ASD = Autism Spectrum Disorder, TD = Typically Developing.

of stimuli than TD adolescents ($F(1, 71) = 10.3$, $p = .002$, $\eta_p^2 = .13$; Table 1). Also, a significant interaction effect between social content and pleasantness on fixation duration was found ($F(2, 142) = 3.8$, $p = .03$, $\eta_p^2 = .05$). The within-subject contrasts indicated longer fixation durations to pleasant versus neutral pictures with a social content, but not for pictures with a non-social content ($F(1,71) = 6.1$, $p = .02$, $\eta_p^2 = .08$) and longer fixation durations to unpleasant versus neutral pictures for the social pictures, but not for the non-social pictures ($F(1,71) = 4.1$, $p = .05$, $\eta_p^2 = .05$). The Box's Tests of Equality of Covariance Matrices was not significant.

Subjective ratings

Concerning valence ratings, a significant interaction effect between social content and pleasantness of the pictures was found ($F(2,142) = 21.7$, $p < .001$, $\eta_p^2 = .23$; Figure 1). The within-subjects contrasts revealed that the decrease in valence rating for a neutral picture, compared to a pleasant picture, was larger for non-social pictures than for social pictures ($F(1,71) = 29.7$, $p < .001$, $\eta_p^2 = .30$), and that the decrease in valence rating for unpleasant pictures compared to neutral pictures was larger for social than for non-social pictures ($F(1,71) = 45.8$, $p < .001$, $\eta_p^2 = .39$). There was no significant main effect of group ($F(1,71) = .3$, $p = .60$, $\eta_p^2 < .01$).

For the arousal ratings, a significant interaction effect between social content and pleasantness was found ($F(2,142) = 28.5$, $p < .001$, $\eta_p^2 = .29$). The within-subjects contrast revealed that the decrease in arousal rating for a neutral picture, compared to a pleasant picture, was larger for non-social pictures than for social pictures ($F(1,71) = 42.7$, $p < .001$, $\eta_p^2 = .38$). The decrease in arousal rating for a neutral picture, compared to an unpleasant picture, was also

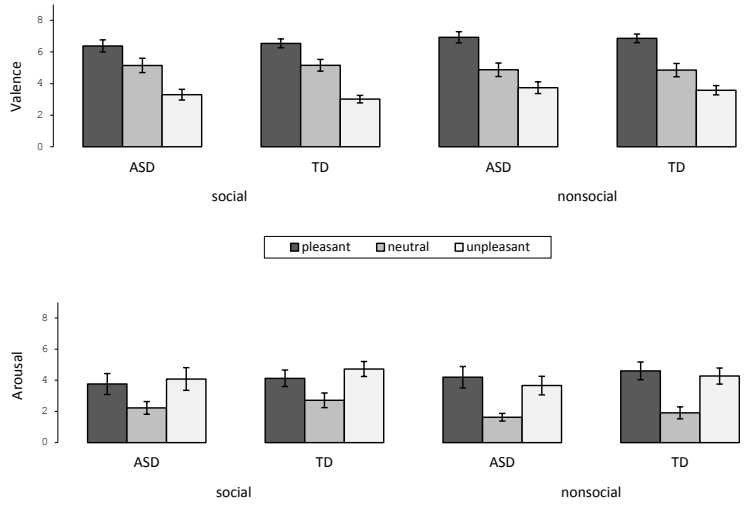


Figure 1. Valence and arousal SAM ratings (Mean and 95% CI's) for social versus non-social pictures with a pleasant, neutral and unpleasant affective content, for adolescents with ASD and TD adolescents.

somewhat larger for the non-social pictures than for the social pictures (trend significant effect: ($F(1,71) = 3.1$, $p = .08$, $\eta_p^2 = .04$)). There was no significant effect of group for arousal ratings ($F(1,71) = 2.2$, $p = .14$, $\eta_p^2 = .03$). For both the ANOVAs concerning valence and arousal ratings, the Box's Tests of Equality of Covariance Matrices was not significant.

Autonomic responses

SCR. There was a significant interaction effect between social content and pleasantness of the picture ($F(2,140) = 3.2$, $p = .05$, $\eta_p^2 = .04$). Affective pictures with a social content resulted in higher SCR than neutral social pictures (Figure 2). Within-subjects contrasts indicated higher SCR for unpleasant versus neutral pictures for the social pictures, versus more similar SCR for unpleasant and neutral pictures for the non-social pictures ($F(1,70) = 5.6$, $p = .02$, $\eta_p^2 = .07$) and a trend towards higher SCR for pleasant versus neutral pictures for social pictures, compared to more similar SCR responses to pleasant and neutral non-social pictures ($F(1,70) = 3.7$, $p = .06$, $\eta_p^2 = .05$). There was no significant effect of group ($F(1,70) = 1.0$, $p = .33$, $\eta_p^2 =$

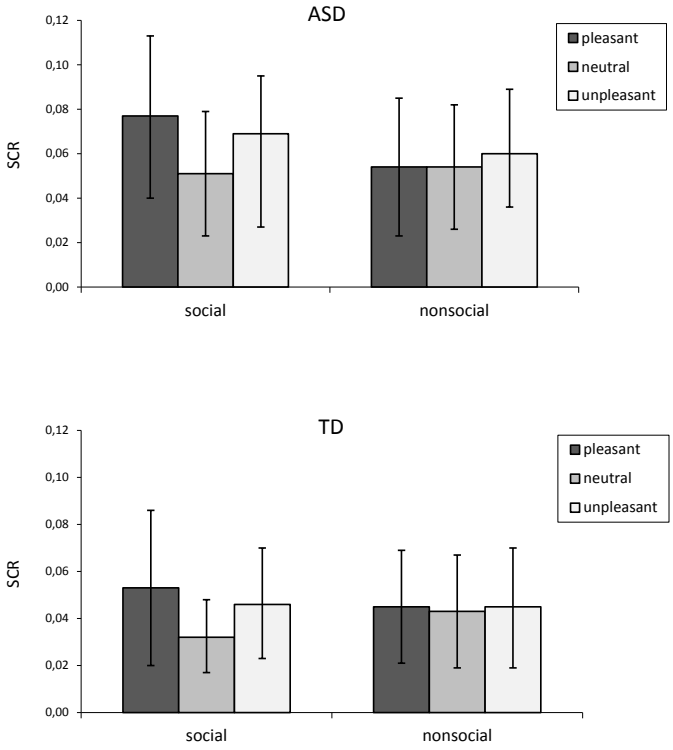


Figure 2. Non-transformed SCRs (mean and 95% CI's) for social versus non-social pictures with a pleasant, neutral and unpleasant affective content, for adolescents with ASD and TD adolescents.

Table 2. HR deceleration response and HR acceleration response in beats per minute (Mean, SD) for social versus non-social pictures with a pleasant, neutral and unpleasant affective content, for adolescents with ASD and TD adolescents.

		Category					
		Social			Non-social		
		Pleasant	Neutral	Unpleasant	Pleasant	Neutral	Unpleasant
HR DR	ASD	-4.1 (3.3)	-4.8 (3.5)	-7.9 (4.7)	-4.4 (3.6)	-4.5 (4.3)	-6.0 (4.9)
	TD	-4.4 (2.5)	-4.8 (4.1)	-6.8 (4.0)	-5.1 (3.6)	-4.0 (3.4)	-5.8 (4.3)
HR AR	ASD	8.7 (3.5)	8.3 (3.8)	9.2 (3.8)	9.7 (5.1)	8.7 (3.5)	9.1 (3.9)
	TD	9.2 (3.7)	9.9 (5.3)	8.7 (3.6)	9.5 (4.1)	9.5 (4.3)	9.2 (4.3)

ASD n = 37, TD n = 36.
HR DR = Heart Rate Deceleration Response, HR AR = Heart Rate Acceleration Response, ASD = Autism Spectrum Disorder, TD = Typically Developing.

.01), nor any interaction involving group on SCR. The Box's Tests of Equality of Covariance Matrices was not significant.

HR responses. For the HR deceleration response, a significant main effect of pleasantness was found ($F(2,140) = 18.1$, $p < .001$, $\eta_p^2 = .21$; Table 2). Unpleasant pictures triggered a larger HR deceleration response than neutral pictures ($F(1,70) = 29.9$, $p < .001$, $\eta_p^2 = .30$). No significant effects were found for social content ($F(1,70) = 3.8$, $p = .06$, $\eta_p^2 = .05$), or group ($F(1,70) = .1$, $p = .82$, $\eta_p^2 < .01$). The HR acceleration response was not significantly associated with social content ($F(1,70) = .6$, $p = .43$, $\eta_p^2 = .01$), pleasantness ($F(2,140) = .1$, $p = .93$, $\eta_p^2 < .01$), or group ($F(1,70) = .6$, $p = .44$, $\eta_p^2 = .01$). For the HR deceleration response, the Box's Tests of Equality of Covariance Matrices was not significant. However, for the HR acceleration response, the Box's Test of Equality of Covariance was significant ($p = .04$). To evaluate the violation of this assumption, we also looked at the Levene's tests for the HR acceleration response in the separate categories. These Levene's Tests were not significant, suggesting no significant differences between the group variances. Therefore we did not take further steps to equalize the variance of the HR acceleration response.

DISCUSSION

The current study investigated whether high functioning adolescents with ASD (i.e. an IQ above 70) showed atypical autonomic and subjective responses when looking at social versus non-social pictures, with a pleasant, neutral and unpleasant affective content. For both adolescents with ASD and TD adolescents, subjective responses, autonomic responses and fixation durations were influenced by *both social content and affective content* (i.e. pleasantness) of the pictures. However, no differences in autonomic and subjective arousal levels were found between the ASD and TD groups, suggesting typical subjective and autonomic

responses to static social stimuli in high functioning adolescents with ASD. Thus, both the social and affective content of the picture influenced subjective and autonomic arousal levels. However, for high-functioning adolescents with ASD these pictures did not trigger hypo- or hyperarousal compared to TD adolescents.

In line with the norm data of the IAPS, the subjective ratings of the pictures used in this study were significantly affected by the social and affective content of the pictures (P. J. Lang, et al., 2001), which underlines the validity of the six selected categories (social pleasant, social neutral, social unpleasant, non-social pleasant, non-social neutral, and non-social unpleasant). Mathersul et al. (2012) reported higher levels of subjective arousal for social pictures versus the non-social pictures. Their finding, however, might have been due to the more extreme affective pictures in the social condition (erotica, mutilations) compared to the less arousing pictures in the non-social category. The present study replicated the finding of higher subjective arousal ratings for social versus non-social pictures in a design that accounted for the level of pleasantness. Based on previous studies (Ben Shalom, et al., 2006; Bölte, et al., 2008), we expected that individuals with ASD would report similar levels of arousal for the pleasant, neutral and unpleasant pictures. However, the responses of the adolescents with ASD, like TD adolescents, did differentiate in their arousal ratings for these categories of pleasantness. This suggests that cognitively able adolescents with ASD show a typical subjective report of arousal to social and non-social stimuli.

The autonomic responses of both adolescents with ASD and TD adolescents were influenced by social content and pleasantness of the pictures. Both adolescents with ASD and TD adolescents displayed the typical pattern of higher SCRs to pleasant and unpleasant social pictures (Bradley, Codispoti, Cuthbert, & Lang, 2001; Mathersul, et al., 2012; Phillips, Drevets, Rauch, & Lane, 2003). These results elaborate on earlier findings by indicating that social content significantly influences the level of SCRs. Pleasant and unpleasant social pictures resulted in higher SCRs than neutral social pictures. For non-social pictures, no significant differences in SCR were found between pleasant, neutral and unpleasant pictures. This typical pattern of higher SCRs for social affective pictures was not found for the HR acceleration response. Yet, the HR deceleration responses were larger for unpleasant than for neutral pictures, which was line with previous studies (Alpers, Adolph, & Pauli, 2011; Bradley, et al., 2001; Mathersul, et al., 2012). This larger HR deceleration response indicates that unpleasant pictures triggered more attention and a larger initial orienting response compared to neutral pictures. This finding is in line with the eye-tracking data, since unpleasant pictures also triggered significantly longer fixation durations than neutral pictures.

No significant differences in SCR and HR responses between individuals with ASD and TD individuals were found. Previous studies indicated that individuals with ASD had lowered SCRs, to social stimuli but not to non-social stimuli than TD adolescents (Hirstein, et al., 2001; Mathersul, et al., 2012). Our findings did not seem to support our hypotheses that looking to social stimuli was associated with hypoarousal in adolescents with ASD compared to TD

adolescents. The models of hyper- and hyperarousal in ASD try to explain why individuals with ASD show impairment during social interactions. Both models of hyper- or hypoarousal in ASD emphasize the role of attached reward value to stimuli; i.e. negative reward in the hyperarousal model or neutral reward in the hypoarousal model. Both negative and neutral rewards hampers reinforcement learning about the environment (Senju & Johnson, 2009). Taken together, the current study did not find significant differences in autonomic responses between adolescents with ASD and TD adolescents. However, the null hypothesis cannot be completely ruled out, because we found several differences of small effect size between the groups (Cohen, 1988). Thus, it is hard to make theoretical claims of hyper- or hypoarousal based on the current data. Divergent study results concerning autonomic responses in individuals with ASD versus TD individuals might be due to study characteristics or to the heterogeneity of ASD.

We should keep in mind that the pictures selected in the current sample were probably not as extreme in the arousal they triggered as the stimuli used in the previous mentioned studies (i.e. extreme pictures with violent or sexual content in Mathersul, et al., 2012). This might have resulted in rather similar levels of SCR and HR in the current study between the adolescents with ASD and TD adolescents, in contrast to the results of previous studies. Since the current study however included a clinical and adolescent sample, it was not considered ethical to use more extreme pictures from the International Affective Pictures System (IAPS, Lang et al. 1998). In addition, we depicted arousal levels while individuals watched pictures of social versus non-social content. These levels of arousal do not represent arousal levels during real life interaction. Thus, these findings can not be generalized to real life interactions and, therefore, further research is necessary to extend these findings to more ecological valid stimuli.

A strength of this study was that we included an eye-tracker for the registration of the total fixation duration towards the pictures. Therefore, we were able to evaluate whether arousal levels were affected by the amount of attention that individuals paid to the pictures. Total fixation durations were not significantly correlated with the autonomic and subjective arousal measures. However, individuals with ASD spend significantly less time looking towards the pictures than TD individuals. This diminished gaze duration was apparent in both social and non-social pictures with a pleasant, neutral and unpleasant content. Still, one might question whether the overall shorter amount of fixation duration towards the stimuli in individuals with ASD implies a failure to optimally respond to stimuli in their environment. The interpretation of the current study results is limited to high functioning adolescents with ASD, since all participants in the current study had an IQ above 70. The autonomic responses of the adolescents in the current study might have been influenced by the instruction to rate their subjective valence and arousal levels after the stimulus presentation. A previous study found that spontaneous viewing was associated with differences in responses between individuals with ASD versus TD, whereas this difference was not apparent when a task was given to both

groups (Oberman, Winkielman, & Ramachandran, 2009). Another limitation of the current study was that we did not account for medication effects due to the small sample and the variety of psychotropic medicine use in the sample. However, the use of medicines might have influenced our results, since previous studies revealed that differences in autonomic responses in individuals with ASD versus TD individuals might be related to the effects of medication (Daluwatte, et al., 2012; Mathewson, et al., 2011). The influence of SAM-ratings and medication on autonomic arousal measures in individuals with ASD requires further investigation.

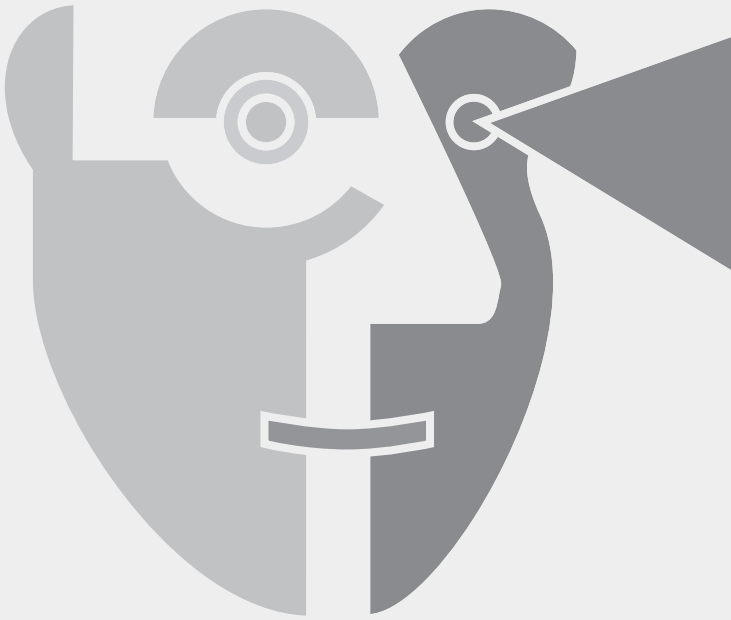
The present results nevertheless elucidate our understanding of autonomic responses and subjective arousal ratings to social and affective stimuli. This study underlines that autonomic and subjective responses are dependent upon both the social and affective content of a stimulus. Adolescents with ASD, like TD adolescents, do respond to both affective and social information. We did not find significant differences in autonomic and subjective responses between adolescents with ASD and an IQ above 70 and TD adolescents. Based on the current findings, we cannot make firm statements about the hyperarousal theory that suggests that individuals with ASD show higher levels of arousal than TD individuals while attending to social stimuli (Hutt, et al., 1964) or the hypoarousal theory that suggests that individuals with ASD show lower levels of arousal than TD (Rimland, 1968). Divergent study results concerning autonomic and subjective responses might be the result of study characteristics or could be related to the heterogeneity of ASD. We encourage researchers investigating hyper- and hypoarousal to social affective stimuli to pay close attention to their study designs to disentangle the different constructs.

CHAPTER 6

Heart rate variability in adolescents with autism spectrum disorder

Anneke Louwerse, Joke Tulen, Hugo van Steenis, Arthur Van Gool, Frank Verhulst, Julian Thayer, Jos van der Geest, Kirstin Greaves-Lord.

Submitted for publication



CHAPTER 7

General discussion

INTRODUCTION

The present thesis aimed to contribute to the autism spectrum disorder (ASD) literature by examining behavioural outcome and underlying mechanisms of ASD. The aim of the first line of investigation of this thesis was to evaluate outcome in adolescence of individuals with ASD symptoms in childhood. The aim of the second line of investigation was to get a better understanding of two specific mechanisms that may be associated with the problems in social functioning in individuals with ASD. Namely the *visual perception of* social stimuli and the *autonomic responses to* social stimuli. As part of this second line of investigation, we also investigated autonomic nervous system (ANS) activity during a resting period.

In the current chapter, the main results (Table 7.1) will be discussed in the light of the existing literature. Then, methodological considerations concerning the studies in the current thesis are discussed, and clinical implications and recommendations for future research are given.

PART 1: OUTCOME IN ADOLESCENCE OF CHILDREN WITH SYMPTOMS OF ASD

The first aim of the current thesis was to investigate the outcome in adolescence (age 12-19) of clinically referred individuals with ASD symptoms in childhood (age 6-13). Two aspects of outcome in adolescence were evaluated in the current thesis, namely stability of the ADOS ASD total scores and classifications (Chapter 2) and societal participation (Chapter 3).

Main findings

The findings of Chapter 2 showed that ADOS ASD scores and classifications (ASD, see Figure 1 in the Introduction) were considerably stable over a seven-year period from childhood to adolescence in a group of cognitively able (i.e. an Intelligence Quotient [IQ] above 70) individuals. We found a correlation between ADOS total scores in childhood and adolescence of .58 in individuals with ASD symptoms or an ASD classification in childhood. In our study, 81% of the individuals with an Autism Diagnostic Observation Schedule (ADOS) classification of ASD in childhood still met criteria for an ADOS ASD classification seven years later in adolescence. We also evaluated the stability of the ADOS classification in a BAP group (i.e. individuals who were referred for psychiatric evaluation and who were screen positive for ASD on a screenings measure, but who did not meet the criteria for an ADOS ASD classification during childhood). In adolescence, 37% of this group of individuals did meet the ADOS criteria of ASD in adolescence.

Individuals with an ADOS ASD classification in childhood showed lower levels of societal participation during adolescence compared to reference data from the general population (Chapter 3). Only one fourth of the ASD group in this sample attended regular schools in

Table 1. Overview of research questions and findings of the current thesis

	Research Questions	Main findings
Part I: outcome in adolescence of children with symptoms of ASD in childhood		
1	Are ADOS ASD total scores and classifications stable from childhood to adolescence?	The stability of ADOS ASD total scores and classifications until adolescence was fairly high (i.e. a correlation of .58) in individuals with ASD symptoms or an ASD classification in childhood, although approximately thirty percent of the sample had a discontinuous classification (i.e. from ASD to no ASD or vice versa).
2	How do individuals with an ADOS ASD classification in childhood participate in society in adolescence? Does this level of societal participation differ from that of individuals with the BAP in childhood and from reference data from the general population?	Individuals with ASD showed significantly lower levels of societal participation in adolescence when compared to reference data from the general population. Individuals with ASD and individuals with the BAP in childhood showed similar levels of societal participation in adolescence, except for the amount of friendships and romantic relationships, which was significantly lower in the ASD group.
Part II: perception of and responses to social stimuli in adolescents with ASD		
3.	Do eye gaze directions of facial images affect gaze behaviour and autonomic responses in adolescents with ASD as compared to TD adolescents?	Both adolescents with ASD and TD adolescents looked significantly longer to the eye region of faces with direct eye gaze, compared to faces with averted eye gaze or closed eyes. Similar autonomic responses were found for adolescents with ASD and TD adolescents.
4.	Are there differences in autonomic responses to social versus non-social affective pictures in adolescents with ASD as compared to TD adolescents?	Both adolescents with ASD and TD adolescents showed a somewhat higher skin conductance response to social pictures compared to non-social pictures. Unpleasant pictures triggered a larger heart rate deceleration than neutral pictures in both groups. No differences were found between the ASD and TD groups in their autonomic and subjective responses.
5.	a. Do individuals with ASD differ from TD individuals with regard to resting ANS activity?	a. No, resting HR and HF-HRV were similar in adolescents with ASD and TD adolescents. However, medication influenced the level of resting HR; medicated adolescents with ASD had a significantly higher mean HR than non-medicated adolescents with ASD.
	b. Is resting ANS activity associated with HR responses to social stimuli and/or social interaction abilities?	b. Yes, resting HF-HRV was significantly associated with HR reactivity to stimuli. No, resting HR and HF-HRV were not significantly associated with social interaction abilities. These findings were similar in the ASD and the TD group.

ASD = Autism Spectrum Disorder, ADOS = Autism Diagnostic Observation Schedule, BAP = Broader Autism Phenotype (i.e. elevated ASD symptoms in childhood but who did not meet criteria for an ADOS ASD classification), TD = Typically Developing, ANS = Autonomic Nervous System, HR = Heart Rate, HF-HRV = High Frequency – Heart Rate Variability.

adolescence. Eighty-five percent of the parents reported that these adolescents had no reciprocal friendships. More than half of the group of individuals with an ASD classification received professional mental health care. Interestingly, when the outcome in adolescence of individuals with an ASD classification in childhood was compared to that of individuals with the BAP in childhood, similar levels of societal participation and societal burden were found. However, when regarding friendships and romantic relationship, adolescents with an ASD classification in childhood had limited social relationships in adolescence when compared to adolescents with the BAP or general population data.

Definition and classification issues

In the introduction I described the changes that occurred in the conceptualisation and diagnostic criteria of autism over the past decades. Whereas previous follow-up studies from childhood to adolescence/adulthood included their samples before 1990 (Billstedt, et al., 2005, 2007; McGovern & Sigman, 2005), the first assessment of the current studies took place between 2002 and 2004. Before 1990, the operationalization of autism was defined through rather strict criteria (APA, 1980); individuals were diagnosed with 'infantile autism' or 'autistic disorder'. Also, the majority of these individuals had a concurrent cognitive impairment. After 1990, the operationalization of autism was more lenient, resulting in more individuals with an ASD classification (compared to an AD classification) and an IQ above 70. Our studies included individuals with an ADOS classification of ASD and an IQ above 70 (CDC, 2012; Fombonne, 2009). The differences between previous versus the current study sample might explain why we found a somewhat lower level of stability and a somewhat higher level of societal participation compared to previous studies. However, the reader should keep in mind that even in this sample of individuals with an ASD classification without a cognitive impairment, the ASD classification was persistent in the majority of the individuals, and more than half of the individuals received support in adolescence (i.e. special education, mental health care, and/or medication).

Comparison between studies on the outcome of individuals with ASD is also hampered by the differences in diagnostic assessment procedures used to classify ASD. Almost all previous studies based their ASD classification (i.e. outcome measure) on clinical judgement with regard to a DSM-III or DSM-IV diagnosis (Cederlund, et al., 2008; Chawarska, et al., 2007; Lord, et al., 2006). It remains unclear how exactly this clinical judgement (i.e. Best Estimate Clinical diagnoses: BEC) was retrieved from the available standardized and non-standardized assessment tools. Although BEC diagnosis might add information to predict later outcome in single-site studies, recently researchers have expressed scepticism about the utility of the BEC diagnosis in evaluating groups across different sites and across studies. Lord et al. (2012) conducted a multisite study in which BEC diagnoses versus classifications based on standardized assessment tools were compared between individuals across twelve different sites. They found that BEC diagnoses varied largely between regional sites.

In contrast, when evaluating standardized assessment tools between sites, almost no significant differences were found. Since scores on standardize assessment tools are more consistent between regions, among clinicians, and over time (i.e. changes in DSM criteria) than BEC judgement, I chose to report ASD classifications in childhood and adolescence based on the ADOS in the current study. The use of the outcome on this uniform assessment tool makes it easier to replicate the current findings and translate them to findings of other studies. However, one might argue that an ASD classification on the ADOS is not as relevant for clinical practice as an overall clinical BEC ASD diagnosis, since it only includes the observation of the child and does not include information from multiple sources (i.e. provided by the parent and school), while a BEC diagnosis does. Agreeably, integrating information from different information sources leads to a more complete diagnostic picture of the child. In other words, the overall clinical diagnosis of a child should indeed represent more than the classification on one assessment tool. However, currently, no transparent rules have been agreed upon as to how to combine the information from multiple assessment tools into one final overall BEC diagnosis. If clearer BEC decision rules were agreed upon in the field of ASD clinical practice as well as research, the BEC diagnosis would have additional value above the separate test results on standardized assessment tools.

The broader autism phenotype: individuals with ASD symptoms but no classification

In the studies concerning outcome in adolescence (Chapter 2 and 3), we also included individuals with ASD symptoms but who did not meet the criteria for an ADOS ASD classification in childhood. Previous research concerning outcome in adolescence/adulthood was restricted to individuals with an ASD classification in childhood. The adolescent outcome of clinically referred individuals with ASD symptoms but without an ASD classification in childhood was surprising. About one third of these individuals did meet criteria for an ASD classification (i.e. based on the ADOS) in adolescence. The ADOS total score in this group on average increased from three to nine, suggesting a clinically relevant elevation in symptoms rather than a merely artificial passing of the classification threshold. Thus, clinically referred individuals with ASD symptoms who do not meet criteria for ASD in childhood, may meet criteria for ASD classification later in life. This finding might feel counter intuitive, since ASD is generally regarded as a lifelong neurodevelopmental disorder that is present from early childhood onwards. Indeed, the majority of individuals are diagnosed with ASD in childhood. However, for some individuals, ASD may only clearly manifest itself later in life, when social demands exceed limited capacities (APA, 2013). Findings from a school-based population study in the United Kingdom revealed that for every three identified cases with ASD in childhood, there were two cases without a diagnosis who might need assessment, support and interventions for ASD symptoms at later in their lives (Baron-Cohen, et al., 2009). Thus, children with the BAP warrant further research and clinical attention.

Splitters and lumpers

In the studies in this thesis I focused on the outcome in adolescence of the classifications and symptoms of one psychiatric disorder: ASD. However, the majority of the individuals in the clinical cohort received (additional) classifications for other psychiatric diagnoses (i.e. ADHD, anxiety disorder), in childhood and/or in adolescence (de Bruin, Ferdinand, et al., 2007). Thus, we should keep in mind that besides the spectrum of autistic traits (Figure 1), other psychiatric symptoms or disorders might have influenced outcome in adolescence. Whether co-occurring symptoms or disorders should be regarded as separate from ASD or as related phenomena, has been a topic of debate (e.g. in Volkmar, 2013). In the follow-up study described in Chapter 2, we found that some individuals shifted from a primary ASD classification in childhood to another primary psychiatric classification in adolescence, and vice versa. These shifts in the primary psychiatric classification might thus not best be labelled as 'co-morbidity'. Rather, these findings might suggest that these formally distinct psychiatric classifications are closely related and mainly reflect subtle differences in expression of the underlying disorder. Indeed, high correlations have been found between autistic and other psychiatric traits (Bölte, Holtmann, & Poustka, 2008). The question has been raised whether it is useful to distinguish these distinct psychiatric classifications (e.g. in Volkmar, 2013), given the considerable amount of overlap, not only at one given time point, but also over time? This separation versus overlap between psychiatric categories is an ongoing debate in taxonomic research between 'splitters' versus 'lumpers' (Kendell & Jablensky, 2003). Splitters focus on the differences between groups, resulting in a complex classification system with many categories. Lumpers emphasize the similarities between phenomena and classify them in large inclusive groups (Mandy, Skuse, Charman, & Frazier, 2012). In the current thesis, by splitting ASD from other psychiatric disorders, my focus was first and foremost on differences between the classifications, as a result of this, the conclusions might suggest that ASD is a completely discrete entity that is divided from other psychiatric disorders, an assumption that is probably not true (DSM-IV, p. xxii). Thus, when studying ASD symptoms and classifications, one should be aware of the tendencies of 'splitting' versus 'lumping' in the field of psychiatry. In a first and modest attempt to be able to take a perspective from both viewpoints, we not only assessed ASD symptoms and classifications in Chapter 2, but also symptoms and classifications from other psychiatric disorders.

Societal participation

The current results indicated that not only individuals with an ASD classification in childhood, but also individuals with ASD symptoms but without an ASD classification in childhood (i.e. the BAP group) generally showed limited societal participation in adolescence. This finding suggests that the general limited levels of societal participation (i.e. education, leisure activities) and the use of mental health care are probably not specifically the result of ASD symptoms, but are also associated with symptoms of other psychiatric disorders (Biederman,

et al., 2008). However, participation in social relationships (i.e. friendships and romantic relationships) was more limited in adolescence in the ASD group than in the BAP group. This underlines that problems in social interaction and communication are more specific to ASD. The finding that these problems were more prominent in the ASD group than in the BAP group could be regarded as an indirect validation of the initial diagnostic classification and clearly illustrate that these features should be regarded as the core diagnostic criteria for ASD, especially in adolescence (APA, 2013).

PART 2: PERCEPTION OF AND RESPONSES TO SOCIAL STIMULI

The aim of this part of the thesis was to study the *perception of* and the *responses to* social stimuli simultaneously in adolescents with ASD versus Typically Developing (TD) adolescents. Therefore, in an experimental setup, visual perception and autonomic responses were measured simultaneously in male adolescents with ASD and in TD adolescents. All individuals had an IQ of 70 or higher. Visual fixation duration and autonomic responses to facial stimuli (Chapter 4) and social versus non-social stimuli (Chapter 5) were investigated. Since autonomic responses might be influenced by the resting levels of ANS activity, we also investigated the association between ANS activity during a resting period and autonomic responses during a task (Chapter 6).

Main findings

The current studies indicated that our sample of cognitively able boys with ASD, like TD boys, fixated significantly longer on socially relevant parts of the presented stimuli. In the facial picture viewing task (Chapter 4), both groups looked significantly longer to direct eye gaze stimuli than to stimuli with averted eye gaze or closed eyes. In the social affective picture viewing task (Chapter 5), both groups looked significantly longer to social pictures than to non-social pictures. Looking to social stimuli (i.e. facial pictures and social affective pictures) did not trigger higher levels of autonomic activity in individuals with ASD than in TD individuals. Also, the adolescents with ASD gave similar subjective ratings of valence and arousal to the presented pictures as TD adolescents.

The resting autonomic activity level (i.e. High Frequency – Heart Rate Variability [HF-HRV]) was significantly associated with the autonomic responses (i.e. Heart Rate [HR]) to a social picture viewing task (Chapter 6). This association was significant, regardless of type of stimulus (i.e. social versus non-social) or group (i.e. adolescents with ASD versus TD adolescents).

Hyper- or hypo arousal in ASD

As described in the introduction of this thesis, previous studies have suggested that atypical gaze behaviour in social situations of individuals with ASD might be explained by the

level of arousal that individuals with ASD experience when attending to social stimuli (Hutt, et al., 1964; Rimland, 1968). Some researchers stated that individuals with ASD experience relatively high levels of arousal when attending to social stimuli (i.e. hyperarousal, Bal, et al., 2010; Hirstein, et al., 2001), and therefore they avoid looking at these stimuli. In contrast, other researchers stated that individuals with ASD experience less arousal when attending to social stimuli (i.e. hypo arousal) than TD individuals (Mathersul, McDonald, & Rushby, 2012; Rimland, 1968), and therefore social stimuli are of no particular interest to them.

In the current studies, we did not find differences between adolescents with ASD and TD adolescents in either fixation duration or in autonomic responses to social stimuli. Thus, we did not find evidence for either hypo- or hyper arousal in ASD. Previous findings on gaze behavior and arousal were also unequivocal. Some studies did find differences in fixation durations to social stimuli in individuals with ASD compared to TD individuals (Dalton, et al., 2005; Jones, Carr, & Klin, 2008; Klin, Jones, Schultz, Volkmar, & Cohen, 2002; Neumann, Spezio, Piven, & Adolphs, 2006) while other studies did not find differences in fixation durations between individuals with ASD and TD adolescents (Freeth, Chapman, Ropar, & Mitchell, 2010; Sawyer, Williamson, & Young, 2012; van der Geest, Kemner, Verbaten, & van Engeland, 2002). Also, some studies reported significant differences between individuals with ASD and TD adolescence in autonomic response to social stimuli (Hirstein, et al., 2001; Hubert, Wicker, Monfardini, & Deruelle, 2009; Joseph, Ehrman, McNally, & Keehn, 2008; Vaughan Van Hecke, et al., 2009) while other studies did not report differences in autonomic responess to social stimuli (Joseph, et al., 2008; Kaartinen, et al., 2012). When these contrasting study findings concerning visual fixation duration and autonomic responses to social stimuli are interpreted, several factors are relevant to consider

First, the *age* of the included samples could have influenced differences in study results concerning fixation durations and autonomic arousal levels. For example, two reviews reported different findings in fixation duration to social stimuli for adolescents and adults versus children (Falck-Ytter & von Hofsten, 2011; Senju & Johnson, 2009). In children, no differences were found in fixation durations to faces, whereas significant differences (i.e. less fixation durations the eye region, combined with longer fixation durations to the mouth region) were reported for adults with ASD compared to TD adults. In the current studies with adolescent samples, we did not find differences in fixation duration to social stimuli in adolescents with ASD and TD adolescents. Thus, differences in age of the sample might underly differences in the findings.

Second, the presented social stimuli varied largely between studies. In general, it seemed that the *complexity of the stimuli* influenced visual fixation durations and autonomic arousal levels. For studies that included complex social stimuli (e.g. movies including sound, emotional stimuli including a task-condition, or real-life stimuli) visual fixation durations were shorter (de Wit, et al., 2008; Klin, et al., 2002; Speer, et al., 2007; Spezio, et al., 2007) and autonomic responses were higher in individuals with ASD compared to TD individuals (Hietanen,

Leppanen, Peltola, Linna-Aho, & Ruuhiala, 2008; Hubert, et al., 2009). However, the drawback of studying evaluated autonomic responses to complex dynamic stimuli is that it remains unknown what element of the stimuli triggered the higher level of autonomic responses in individuals with ASD compared to TD individuals. Althoug findings support overarousal to these stimuli, it remains unclrear if the eyes, the emotional expressions, the task-condition, or the sounds, or the combination of all these aspects the trigger of shorter gaze durations or higher autonomic responses? Research is needed in which the role of these seperate aspects are studied, not only insolated but also in a combined experimental setup with multiple conditions.

Finally, previous studies did not *simultaniously assess* measures of fixation duration and autonomic responses. Previous studies focused either on visual fixation duration *or* on autonomic responses, they did not measure both constructs at the same time. Hajcak et al. (2011) have indicated that the fixation duration on specific parts of a stimuli influenced the amount of autonomic arousal. Fixation to more arousing parts of the stimuli triggered higher levels of autonomic arousal in TD individuals (Hajcak, et al., 2011). Previous studies concerning autonomic responses to social stimuli did not take into account a measure of fixation duration. Therefore, it remained unclear how long the individuals fixated to the socially salient – and perhaps more arousing - parts of the stimuli, and how this fixation duration was associated to autonomic arousal levels. In the current thesis, by combining eye-tracking and autonomic activity measurements, the experiments were designed in such a way that we could evaluate arousal levels to specific aspects of stimuli (i.e. different conditions; direct gaze versus averted/closed or social versus non-social). The findings of the current study indicated that looking to direct eye gaze (i.e. 'into the eyes') in itself did not trigger shorter gaze duration or higher levels of arousal in adolescents with ASD versus TD adolescents. Also direct eye gaze did not trigger more arousal than looking at averted or closed eyes for both groups. More studies are needed to build unto these findings, by evaluating which aspects of social stimuli (or the integration of aspects) trigger possible differences in fixation durations and autonomic responses in individuals with ASD versus TD individuals.

Taken together, in our current studies we did not find differences in fixation duration between adolescents with ASD and TD adolescents, which contradicts the hypothesis of diminished attention to social stimuli in individuals with ASD. At the same time we did not find differences in autonomic responses to social stimuli between adolescents with ASD and TD adolescents, which does not indicate relative hypo- or hyper arousal, at least not in reaction to static social stimuli in adolescents with ASD without a cognitive impairment.

Integrating underlying mechanisms

As mentioned in the introduction and the previous paragraph, the focus of experimental studies is usually on one underlying mechanism of ASD. This underlying mechanism is studied isolated from other putative underlying mechanisms. Isolated evaluation of specific un-

derlying mechanisms does contribute to uncovering the different neurobiological roots of ASD. The next step, the integration of these pieces of information into a coherent picture of the combined underlying mechanisms, remains complicated. One step forward in achieving a more integrated picture is to measure more than one underlying mechanism in a single experimental design (Campatelli, et al., 2013). The current studies combined measures of two putative underlying mechanisms; perception of social stimuli (fixation duration) and responses to social stimuli (autonomic responses), in one experimental design. However, one should keep in mind that even the combination of two putative underlying mechanisms is just a first step in trying to capture a more complete and integrated picture of the overall underlying neurobiological roots of social problems in individuals with ASD. Including other putative underlying mechanisms that have shown to be related to ASD, for example the perception of sound (Magnee, de Gelder, van Engeland, & Kemner, 2007) or the activation patterns of certain brain areas during the attendance to social stimuli (Dalton, Nacewicz, Alexander, & Davidson, 2007), would lead to more detailed understanding of the underlying biology of social problems in individuals with ASD. Although I now know from experience that integrating multiple measures in one experimental set-up comes with several methodological challenges, I encourage researchers to so nonetheless.

Resting ANS activity

Previous studies concerning ANS activity in individuals with ASD mainly focused on autonomic responses (i.e. *re*activity) to social stimuli. Less attention has been paid to resting levels of autonomic activity (i.e. baseline/basal activity), although this resting autonomic activity level is hypothesized to mediate social and affective behavioural responses (Bazhenova, et al., 2001; Porges, 2003; Thayer & Lane, 2000).

A few studies reported that resting ANS activity (i.e. measures of heart rate variability; HRV) is lower in individuals with ASD (Bal, et al., 2010; Mathewson, et al., 2011; Ming, et al., 2005; Vaughan Van Hecke, et al., 2009), whereas other studies, including our own study (i.e. Chapter 6), did not find differences in HRV in the high frequency band (HF-HRV) between individuals with ASD and TD adolescents (Daluwatte, et al., 2012; Toichi & Kamio, 2003). These contradicting findings might be the result of differences in *age* of the selected study samples. In general, children participated in the studies that reported significant differences in HF-HRV between individuals with ASD and TD individuals (Bal, et al., 2010; Ming et al., 2005), while adolescents or adults participated in the studies that did not find significant differences between ASD and TD (Daluwatte et al., 2012; Toichi & Kamio, 2003). It has been indicated there are differences in HF-HRV between children versus adolescents in TD populations (Evans, et al., 2013). Possibly, HF-HRV is lower in individuals with ASD compared to TD individuals in childhood, but becomes more similar between individuals with ASD and TD individuals in adolescence. This influence of age on HF-HRV needs to be further explored in, preferably longitudinal, research.

Our finding that higher HF-HRV was associated with higher levels of autonomic reactivity to stimuli during a social picture viewing task (Chapter 6) is in line with the findings of previous studies (Althaus, et al., 1999; Patriquin, et al., 2011). Althaus et al. (1999) reported that this association was significantly weaker in individuals with ASD than in TD individuals. Whereas the current study (Chapter 6) and Patriquin et al. (2013) found similar associations between resting HF-HRV and autonomic reactivity to stimuli in individuals with ASD and TD individuals. However, all study findings suggest that autonomic responses to stimuli are associated with resting vagal activity. Thus, I suggest that future research concerning autonomic responses to specific stimuli should consider whether measures of resting ANS activity should be included as a covariate.

Methodological considerations

The specific methodological considerations and limitations in relation to each study have been discussed in the previous chapters. In this section, some more general overarching methodological considerations are discussed.

Firstly, we included a specific group of individuals with ASD symptoms in the current studies, i.e. individuals that were referred to only one university outpatient department of child and adolescent psychiatry/psychology. A university outpatients' department is usually not the first mental health service that individuals with psychiatric problems are referred to, thus cases usually had complex problems that needed specialist diagnostics or treatment. In addition, the current clinical samples consistent of individuals with an IQ above 70 and with at least a score above the clinical threshold on the CSBQ. Therefore, the current findings and conclusions should interpreted while bearing in mind the particular characteristics of our samples.

Secondly, in part one of this thesis, the outcome in adolescence of individuals with ASD symptoms in childhood was described. We only performed two assessment waves, one in childhood and one in adolescence. Therefore, the developmental course of the ASD symptoms between childhood and adolescence could not be investigated using more sophisticated techniques, as we did not have data of later or intermediate assessment waves. At least three assessment waves are needed to estimate the rate of change of developmental trajectories (Szatmari, et al., 2009). Tracking changes in ASD symptoms over more than two assessment waves may lead to more detailed prognostic estimates, as well as opportunities to study the course of this disorder more precisely (Gotham, et al., 2012).

Finally, the highly controlled experimental settings used in the studies of part two complicate relating the results concerning visual fixation duration and autonomic reactivity to behaviour of individuals with ASD in more naturalistic settings (i.e. challenge the ecological validity). Thus, one should be cautious when translating findings from the current experiments to the behaviour of individuals with ASD in more naturalistic settings and daily life.

Recommendations for future research

Based on the main findings and methodological considerations that I discussed in the previous paragraphs, several suggestions for future research are described in the current section.

First, I encourage future studies to include participants from several centers or even countries, thus including individuals with a wide range of ASD symptom severity, IQ, socio-economic backgrounds etc., which would lead to a more representative sample, and thus more generalizable conclusions.

Second, follow-up studies should try to include more than two assessment waves to be able to draw more extensive conclusions concerning the developmental course of ASD symptoms. Assessing ASD symptoms over three or more assessment waves may lead to more detailed prognostic estimates and the opportunity to study the developmental course of ASD more precisely (Gotham, et al., 2012; Szatmari, et al., 2009). It would be useful to continue to measure outcome into adulthood and beyond. Given the growing number of individuals with ASD that are aging into adulthood, the Interagency Autism Coordination Committee (IACC) encouraged researchers to conduct longitudinal studies, as results are relevant for the planning of adult services that can meet the specific needs of adults or even the elderly with ASD (AICC, 2012). I also encourage future studies not only to assess autistic traits but also to assess traits of other psychiatric disorders.

Third, for research regarding underlying mechanisms of ASD, I would like to stress the importance of integrating measures. In the current studies, we integrated measures of fixation duration and autonomic arousal to learn more about underlying mechanisms of problems in social functioning in individuals with ASD. Combining these two measures in response to static pictures is just a first step in trying to capture the complete picture of the underlying neurobiological roots of social information processing in individuals with ASD. I thus encourage future research to build onto our model by 1. integrating multiple underlying mechanisms into a single experimental design; 2. taking into account resting levels of autonomic activity, and 3. introducing more ecologically valid stimuli.

Clinical implications

The clinical implications of the two parts of the current thesis will be discussed below.

The results of part one suggested that for individuals with ASD symptoms, re-evaluation of ASD symptoms as well as other psychiatric symptoms later in life might be beneficial. I think that it is beneficial for clinicians to know that, although ASD is a fairly stable construct, shifts in primary classifications and symptoms can and do occur over time. Societal participation in adolescence is lower in clinically referred individuals with ASD symptoms (i.e. BAP and ASD) in childhood compared to individuals from the general population. Also, more than half of the clinically referred individuals with ASD symptoms in childhood received mental health care in adolescence. Mental health care professionals should use these longitudinal findings to provide adequate counselling regarding adolescent prospects for children with ASD

symptoms. Firstly, it is important for professionals to recognise the heterogeneity in outcome of individuals with ASD in order to provide adequate support. Secondly, professionals should be aware that the majority of individuals with ASD symptoms in childhood need support later in life. Therefore, follow-up contacts seem beneficial to evaluate whether interventions or extra support might be needed.

Since the experiments in part two of this thesis were highly controlled and thus far from naturalistic, we should be conservative concerning the clinical implications of these findings. Static social pictures did not seem to trigger atypical fixation durations or autonomic arousal levels in adolescents with ASD. Thus, the perception of and the reaction to static social pictures in adolescents with ASD does not seem to be impaired. Possibly, problems in social interaction of adolescents with ASD in more naturalistic settings are due to difficulties in processing and combining complex social stimuli and constantly changing situations. More studies are needed to unravel which particular processes or aspects within complex social situations trigger atypical behavior in individuals in ASD. If I do take the liberty to further speculate on the current findings, the data seems to suggest that adolescents with ASD and an IQ above 70 did show motivation for attending to social static stimuli. Like TD adolescents, they spent longer time looking at social pictures versus non-social pictures. Also, they spent more time looking at socially relevant information (i.e. direct eye gaze) compared to less relevant social information (i.e. closed eyes). A developmental explanation for this finding might be that in adolescence looking at social stimuli is not aberrant for adolescents with ASD and that the basic underlying dysfunctions in social contact only manifest in more complex real world situations. Whereas a complex social situation might overwhelm individuals with ASD, our findings suggest that basic social pictures do not trigger altered visual attention or physiological arousal. This finding may have some clinical relevance, when I freely translate it to previous studies that showed that a simplification of complex social situations might be a beneficial way to teach social skills to individuals with ASD (Golan, et al., 2010; Robins, Dautenhahn, & Dickerson, 2009). For example, Robins et al. (2009), studied a therapeutic intervention in which children with ASD worked with a small minimally expressive humanoid robot, which can be regarded as a simplified person. Findings showed that children with ASD were able to learn to interact with this robot (Robins, 2009). In turn, the interaction with the robot facilitated interaction with other people. This example shows that a simplification of complex social stimuli or situations can be used to learn individuals with ASD new skills that can subsequently be used to deal with more complex situations. To translate this back to the current findings; since we found that a) basic social stimuli were not perceived differently in high functioning adolescents with ASD, compared to TD adolescents. b), these stimuli did not trigger higher levels of arousal. c) longer gaze durations to basic stimuli seemed to reveal motivation for static social stimuli in adolescents with ASD, basic social stimuli can possibly be used in therapeutic interventions in adolescents with ASD. These simple stimuli might serve as stepping stones to acquire social skills and to subsequently deal with more complex

social situations. Thus, the use of static social stimuli in therapeutic interventions might be a worthwhile avenue.

CHAPTER **8**

References
Summery
Samenvatting
CV
Publications
PhD-portfolio
Dankwoord

REFERENCES

Adolphs, R., Sears, L., & Piven, J. (2001). Abnormal processing of social information from faces in autism. *Journal of Cognitive Neuroscience, 13*(2), 232-240.

Alpers, G. W., Adolph, D., & Pauli, P. (2011). Emotional scenes and facial expressions elicit different psychophysiological responses. *International Journal of Psychophysiology, 80*(3), 173-181.

Althaus, M., Mulder, L. J., Mulder, G., Aarnoudse, C. C., & Minderaa, R. B. (1999). Cardiac adaptivity to attention-demanding tasks in children with a pervasive developmental disorder not otherwise specified (PDD-NOS). *Biological Psychiatry, 46*(6), 799-809.

Althaus M., Van Roon A. M., Mulder L. J., Mulder G., Aarnoudse C.C., Minderaa R.B. (2004). Autonomic response patterns observed during the performance of an attention-demanding task in two groups of children with autistic-type difficulties in social adjustment. *Psychophysiology*, 41(6):893-904.

Amone-P'Olak, K., Ormel, J., Oldehinkel, A. J., Reijneveld, S. A., Verhulst, F. C., & Burger, H. (2010). Socioeconomic Position Predicts Specialty Mental Health Service Use Independent of Clinical Severity: The TRAILS Study. *Journal of the American Academy of Child and Adolescent Psychiatry, 49*(7), 647-655.

APA. (1952). *Diagnostic and Statistical Manual of Mental Disorders, 1th ed. (DSM-I)*. Washington, DC: American Psychiatric Association.

APA. (1968). *Diagnostic and Statistical Manual of Mental Disorders, 2th ed. (DSM-II)*. Washington, DC: American Psychiatric Association.

APA. (1980). *Diagnostic and Statistical Manual of Mental Disorders, 3th ed. (DSM-III)*. Washington, DC: American Psychiatric Association.

APA. (1994). *Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV)*. Washington, DC: American Psychiatric Association.

APA. (2000). *Diagnostic and Statistical Manual of Mental Disorders, 4th ed. text rev (DSM-IV-TR)*. Washington, DC: American Psychiatric Association.

APA. (2000). *Diagnostic and Statistical Manual of Mental Disorders, 4th ed. text rev (DSM-IV-TR)*. Washington, DC: American Psychiatric Association.

APA. (2013). *Diagnostic and Statistical Manual of Mental Disorders, 5th ed. (DSM-5)*. Washington, DC: American Psychiatric Association.

Appelhans, B. M., & Luecken, L. J. (2006). Heart rate variability as an index of regulated emotional responding. *Review of General Psychology, 10*(3), 229-240.

Asperger, H. (1994). Die "autistischen psychopathen" in kindersalter. *Communication, 13*, 45-52.

Bal, E., Harden, E., Lamb, D., Van Hecke, A. V., Denver, J. W., & Porges, S. W. (2010). Emotion recognition in children with autism spectrum disorders: relations to eye gaze and autonomic state. *Journal of Autism and Developmental Disorders, 40*(3), 358-370.

Ballaban-Gil, K., Rapin, I., Tuchman, R., & Shinnar, S. (1996). Longitudinal examination of the behavioral, language, and social changes in a population of adolescents and young adults with autistic disorder. *Pediatric Neurology, 15*(3), 217-223.

Bar, K. J., Letzsch, A., Jochum, T., Wagner, G., Greiner, W., & Sauer, H. (2005). Loss of efferent vagal activity in acute schizophrenia. *Journal of Psychiatric Research, 39*(5), 519-527.

Baron-Cohen S., Hoekstra R.A., Knickmeyer R., Wheelwright S. (2006). The Autism-Spectrum Quotient (AQ)-adolescent version. *Journal of Autism and Developmental Disorders*, *36*(3), 343-50.

Baron-Cohen, S., Campbell, R., Karmiloff-Smith, A., Grant, J., & Walker, J. (1995). Are children with autism blind to the mentalistic significance of the eyes? *British Jorunal of Developmental Psychology, 13*, 379-398.

Baron-Cohen, S., Scott, F. J., Allison, C., Williams, J., Bolton, P., Matthews, F. E., et al. (2009). Prevalence of autism-spectrum conditions: UK school-based population study. *British Journal of Psychiatry, 194*(6), 500-509.

Bastiaansen, J. A., Meffert, H., Hein, S., Huizinga, P., Ketelaars, C., Pijnenborg, M., et al. (2011). Diagnosing autism spectrum disorders in adults: the use of Autism Diagnostic Observation Schedule (ADOS) module 4. *Journal of Autism and Developmental Disorders, 41*(9), 1256-1266.

Bates, M. P. (2001). The Child and Adolescent Functional Assessment Scale (CAFAS): review and current status. *Clinical Child and Family Psychology Review, 4,* 63-84.

Batki, A., Baron-Cohen, S., Wheelwright, S., Connellan, J., & Ahluwalia, J. (2000). Is there an innate gaze module? Evidence from human neonates. *Infant Behavior and Development, 23*, 223-229.

Bazhenova, O. V., Plonskaia, O., & Porges, S. W. (2001). Vagal reactivity and affective adjustment in infants during interaction challenges. *Child Development, 72*(5), 1314-1326.

Ben Shalom, D., Mostofsky, S. H., Hazlett, R. L., Goldberg, M. C., Landa, R. J., Faran, Y., et al. (2006). Normal physiological emotions but differences in expression of conscious feelings in children with high-functioning autism. *Journal of Autism and Developmental Disorders, 36*(3), 395-400.

Biederman, J., Petty, C. R., Fried, R., Kaiser, R., Dolan, C. R., Schoenfeld, S., et al. (2008). Educational and occupational underattainment in adults with attention-deficit/hyperactivity disorder: a controlled study. *Journal of Clinical Psychiatry, 69*(8), 1217-1222.

Bildt, A., Oosterling, I., Lang, N. J., Kuijper, S., Dekker, V., Sytema, S., et al. (2013). How to Use the ADI-R for Classifying Autism Spectrum Disorders? Psychometric Properties of Criteria from the Literature in 1,204 Dutch Children. *Journal of Autism and Developmental Disorders*, Advance online publication. doi: 10.1007/s10803-013-1783-1.

Billstedt, E., Gillberg, I. C., & Gillberg, C. (2005). Autism after adolescence: population-based 13- to 22-year follow-up study of 120 individuals with autism diagnosed in childhood. *Journal of Autism and Developmental Disorders, 35*(3), 351-360.

Billstedt, E., Gillberg, I. C., & Gillberg, C. (2007). Autism in adults: symptom patterns and early childhood predictors. Use of the DISCO in a community sample followed from childhood. *Journal of Child Psychology and Psychiatry, 48*(11), 1102-1110.

Billstedt, E., Gillberg, I. C., & Gillberg, C. (2011). Aspects of quality of life in adults diagnosed with autism in childhood: a population-based study. *Autism, 15*(1), 7-20.

Bölte, S., Feineis-Matthews, S., & Poustka, F. (2008). Brief report: Emotional processing in high-functioning autism - physiological reactivity and affective report. *Journal of Autism and Developmental Disorders, 38*(4), 776-781.

Bölte, S., Holtmann, M., & Poustka, F. (2008). The Social Communication Questionnaire (SCQ) as a screener for autism spectrum disorders: additional evidence and cross-cultural validity. *Journal of the American Academy of Child & Adolescent Psychiatry, 47*(6), 719-720; author reply 720-711.

Boraston, Z., & Blakemore, S. J. (2007). The application of eye-tracking technology in the study of autism. *Journal of Physiology Online, 581*(3), 893-898.

Bradley, M. M., & Lang, P. J. (2000). Measuring emotion: behavior, feeling, and physiology. In R. D. Lane & L. Nadel (Eds.), *Cognitive neuroscience of emotion* (pp. 242-276): Oxford University Press, Inc.

Bradley, M. M., Codispoti, M., Cuthbert, B. N., & Lang, P. J. (2001). Emotion and motivation I: defensive and appetitive reactions in picture processing. *Emotion, 1*(3), 276-298.

Bagwell, C. L., Schmidt, M. E., Newcomb, A. F., Bukowski, W. M. (2001). Friendship and peer rejection as predictors of adult adjustment. *New Directions for Child and Adolescent Development, 91,* 25-49.

Cadman, T., Eklund, H., Howley, D., Hayward, H., Clarke, H., Findon, J., et al. (2012). Caregiver Burden as People With Autism Spectrum Disorder and Attention-Deficit/Hyperactivity Disorder Transition into

Adolescence and Adulthood in the United Kingdom. *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(9), 879-888.

Campatelli, G., Federico, R. R., Apicella, F., Sicca, F., & Muratori, F. (2013). Face processing in children with ASD: Literature review. *Research in Autism Spectrum Disorders, 7*, 444-454.

Caron, A. J., Caron, R., Roberts, J., & Brooks, R. (1997). Infant sensitivity to deviations in dynamic facial-vocal displays: The role of eye regard. *Developmental Psychology, 33*, 802-813.

CDC. (2012). Prevalence of autism spectrum disorders - Autism and Developmental Disabilities Monitoring Network, United States, 2008. *Morbidity and mortality weekly report. Surveillance Summaries,* pp. 1-19.

Cederlund, M., Hagberg, B., Billstedt, E., Gillberg, I. C., & Gillberg, C. (2008). Asperger syndrome and autism: a comparative longitudinal follow-up study more than 5 years after original diagnosis. *Journal of Autism and Developmental Disorders, 38*(1), 72-85.

Central Bureau of Statistics. (2010). Statline Databank, Central Bureau of Statistics. Retrieved 21 August 2013, from Central Bureau of Statistics.

Chawarska, K., & Shic, F. (2009). Looking But Not Seeing: Atypical Visual Scanning and Recognition of Faces in 2 and 4-Year-Old Children with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders, 39*(12), 1663-1672.

Chawarska, K., Klin, A., Paul, R., & Volkmar, F. (2007). Autism spectrum disorder in the second year: stability and change in syndrome expression. *Journal of Child Psychology and Psychiatry, 48*(2), 128-138.

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. Hillsdale, New Jersey: Lawrence Erlbaum Associates.

Constantino J. N. (2011). The quantitative nature of autistic social impairment. *Pediatric Research, 69*(5), 55-62.

Constantino J. N., Yang D., Gray T. L., Gross M. M., Abbacchi A. M., Smith S. C. et al. (2007). Clarifying the associations between language and social development in autism: a study of non-native phoneme recognition. *Journal of Autism and Developmental Disorders*, *37*(7), 1256-63.

Constantino, J. N., & Todd, R. D. (2003). Autistic traits in the general population: a twin study. *Archives of General Psychiatry, 60*(5), 524-530.

Coull, J. T. (1998). Neural correlates of attention and arousal: insights from electrophysiology, functional neuroimaging and psychopharmacology. *Progress in Neurobiology, 55*(4), 343-361.

Dalton, K. M., Nacewicz, B. M., Alexander, A. L., & Davidson, R. J. (2007). Gaze-fixation, brain activation, and amygdala volume in unaffected siblings of individuals with autism. *Biological Psychiatry, 61*(4), 512-520.

Dalton, K. M., Nacewicz, B. M., Johnstone, T., Schaefer, H. S., Gernsbacher, M. A., Goldsmith, H. H. et al. (2005). Gaze fixation and the neural circuitry of face processing in autism. *Nature Neuroscience, 8*, 519-526.

Daluwatte, C., Miles, J., Christ, S., Beversdorf, D., Takahashi, T. N., & Yao, G. (2012). Atypical Pupillary Light Reflex and Heart Rate Variability in Children with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders, 43*(8), 1910-1925.

de Bruin, E. I., de Nijs, P. F., Verheij, F., Hartman, C. A., & Ferdinand, R. F. (2007). Multiple complex developmental disorder delineated from PDD-NOS. *Journal of Autism and Developmental Disorders, 37*(6), 1181-1191.

de Bruin, E. I., Ferdinand, R. F., Meester, S., de Nijs, P. F., & Verheij, F. (2007). High rates of psychiatric comorbidity in PDD-NOS. *Journal of Autism and Developmental Disorders, 37*(5), 877-886.

de Wit, T. C. J., Falck-Ytter, T., & von Hofsten, C. (2008). Young children with Autism Spectrum Disorder look differently at positive versus negative emotional faces. [doi: 10.1016/j.rasd.2008.01.004]. *Research in Autism Spectrum Disorders, 2*(4), 651-659.

DiCicco-Bloom, E., Lord, C., Zwaigenbaum, L., Courchesne, E., Dager, S. R., Schmitz, C., et al. (2006). The developmental neurobiology of autism spectrum disorder. *Journal of Neuroscience, 26*(26), 6897-6906.

Esbensen A. J., Greenberg J. S., Seltzer M. M., Aman M. G. (2009). A longitudinal investigation of psychotropic and non-psychotropic medication use among adolescents and adults with autism spectrum disorders. *Journal of Autism and Developmental Disorders*, *39*(9), 1339-49.

European Agency for Development in Special Needs Education (2010). *Special Needs Education Country Data 2010*, Odense, Denmark: European Agency for Development in Special Needs Education.

Evans, B. E., Greaves-Lord, K., Euser, A. S., Tulen, J. H. M., Franken, I. H. A., Huizink, A. C. (2012). Alcohol and tobacco use and heart rate reactivity to a psychosocial stressor in an adolescent population. *Drug and Alcohol Dependence, 126,* 296-303.

Evans, B. E., Greaves-Lord, K., Euser, A. S., Tulen, J. H. M., Franken, I. H. A., Huizink, A. C. (2013). Determinants of physiological and perceived physiological stress reactivity in children and adolescents. *PlosOne, 8*(4), e61724.

Falck-Ytter, T., & von Hofsten, C. (2011). How special is social looking in ASD: a review. *Progress in Brain Research, 189*, 209-222.

Farley, M. A., McMahon, W. M., Fombonne, E., Jenson, W. R., Miller, J., Gardner, M., et al. (2009). Twenty-year outcome for individuals with autism and average or near-average cognitive abilities. *Autism Research, 2*(2), 109-118.

Farroni, T., Massaccesi, S., Menon, E., & Johnson, M. H. (2007). Direct gaze modulates face recognition in young infants. *Cognition, 102*, 396-404.

Farroni, T., Menon, E., & Johnson, M. H. (2006). Factors influencing newborns' preference for faces with eye contact. *Journal of Experimental Child Psychology, 95*, 298-308.

Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods, 39*(2), 175-191.

Figner, B., & Murphy, R. O. (2011). Using skin conductance in judgment and decision making research. In M. Schulte-Mecklenbeck, A. Kuehberger, & R. Ranyard (Eds.), *A handbook of process tracing methods for decision research* (pp. 163-184). New York, NY: Psychology Press.

Fombonne, E. (2009). Epidemiology of pervasive developmental disorders. *Pediatric Research, 65*(6), 591-598.

Fombonne, E. (2010). Estimated prevalence of autism spectrum conditions in Cambridgeshire is over 1%. *Evidence-Based Mental Health, 13*(1), 32.

Freeth, M., Chapman, P., Ropar, D., & Mitchell, P. (2010). Do Gaze Cues in Complex Scenes Capture and Direct the Attention of High Functioning Adolescents with ASD? Evidence from Eye-tracking. *Journal of Autism and Developmental Disorders, 40*, 534-547.

Frith, U. (1989). *Autism: Explaining the enigma*. Oxford: Basil Blackwell.

Gezondheidsraad. (2009). *Autismespectrumstoornissen: een leven lang anders* (publicatienr: 2009/09 ed.). Den Haag: Gezondheidsraad.

Gillberg, C., & Steffenburg, S. (1987). Outcome and prognostic factors in infantile autism and similar conditions: a populationbased study of 46 cases followed through puberty. *Journal of Autism and Developmental Disorders, 17*(2), 273-287.

Golan, O., Ashwin, E., Granader, Y., McClintock, S., Day, K., Leggett, V., et al. (2010). Enhancing Emotion Recognition in Children with Autism Spectrum Conditions: An Intervention Using Animated Vehicles with Real Emotional Faces. *Journal of Autism and Developmental Disorders, 40*(3), 269-279.

Gotham, K., Pickles, A., & Lord, C. (2012). Trajectories of Autism Severity in Children Using Standardized ADOS Scores. *Pediatrics, 130*(5), 1278-1284.

Gotham, K., Risi, S., Pickles, A., & Lord, C. (2007). The Autism Diagnostic Observation Schedule: revised algorithms for improved diagnostic validity. *Journal of Autism and Developmental Disorders, 37*(4), 613-627.

Greaves-Lord K., Eussen M. J. M., Verhulst F. C., Minderaa R., Mandy W., Hudziak J., et al. (2012). Empirically Based Phenotypic Profiles of Children with Pervasive Developmental Disorders: Interpretation in the Light of the DSM-5. *Journal of Autism and Developmental Disorders, 43*(8), 1784-97.

Greaves-Lord, K., Ferdinand, R. F., Sondeijker, F. E. P. L., Dietrich, A., Oldehinkel, A. J., Rosmalen, J. G. M. et al. (2007). Testing the tripartite model in young adolescents: Is hyperarousal specific for anxiety and not depression? *Journal of Affective Disorders, 102*, 55-63.

Gresham F. M., Elliot S. N. (1990). *Social skills rating system manual.* Circle Pines, MN: American Guidance Service.

Guthrie, W., Swineford, L. B., Nottke, C., & Wetherby, A. M. (2012). Early diagnosis of autism spectrum disorder: stability and change in clinical diagnosis and symptom presentation. *Journal of Child Psychology and Psychiatry*, *54*(5), 582-590.

Hadjikhani, N., Joseph, R. M., Snyder, J., & Tager-Flusberg, H. (2007). Abnormal activation of the social brain during face perception in autism. *Human Brain Mapping, 28*(5), 441-449.

Hainline, L. (1978). Developmental changes in visual scanning of face and nonface patterns by infants. *Journal of Experimental Child Psychology, 25*, 90-115.

Hajcak, G., Macnamara, A., Foti, D., Ferri, J., & Keil, A. (2011). The dynamic allocation of attention to emotion: Simultaneous and independent evidence from the late positive potential and steady state visual evoked potentials. *Biological Psychology*, *92*(3), 447-455.

Hartman, C. A., Luteijn, E., Moorlag, A., de Bildt, A., & Minderaa, R. (2007). *Manual for the CSBQ [Handleiding voor de VISK]*. Amsterdam: Harcourt.

Hartman, C. A., Luteijn, E., Serra, M., & Minderaa, R. (2006). Refinement of the Children's Social Behavior Questionnaire (CSBQ): an instrument that describes the diverse problems seen in milder forms of PDD. *Journal of Autism and Developmental Disorders, 36*(3), 325-342.

Hempel, R. J., Tulen, J. H., van Beveren, N. J., Mulder, P. G., & Hengeveld, M. W. (2007). Subjective and physiological responses to emotion-eliciting pictures in male schizophrenic patients. *International Journal of Psychophysiology, 64*(2), 174-183.

Henault, I., & Attwood, T. (2002). *The Sexual Profile of Adults with Asperger's Syndrome: The Need for Understanding, Support and Sex Education*. Paper presented at the Inaugural Word Autism Congress.

Henninger, N. A., & Taylor, J. L. (2013). Outcomes in adults with autism spectrum disorders: a historical perspective. *Autism, 17*(1), 103-116.

Hietanen, J. K., Leppanen, J. M., Peltola, M. J., Linna-Aho, K., & Ruuhiala, H. J. (2008). Seeing direct and averted gaze activates the approach-avoidance motivational brain systems. *Neuropsychologia, 46*, 2423-2430.

Higashida, N. (2013). The reason I jump. One boy's voice from the silence of autism. London: Sceptre.

Hirstein, W., Iversen, P., & Ramachandran, V. S. (2001). Autonomic responses of autistic children to people and objects. *Proceedings of the Royal Society, 268*(1479), 1883-1888.

Hoekstra R. A., Bartels M., Cath D. C., Boomsma D.I. (2008). Factor structure, reliability and criterion validity of the Autism-Spectrum Quotient (AQ): a study in Dutch population and patient groups. *Journal of Autism and Developmental Disorders, 38*(8), 1555-1566.

Howlin, P., Goode, S., Hutton, J., & Rutter, M. (2004). Adult outcome for children with autism. *Journal of Child Psychology and Psychiatry, 45*(2), 212-229.

Howlin, P., Moss, P., Savage, S., & Rutter, M. (2013). Social outcomes in mid- to later adulthood among individuals diagnosed with autism and average nonverbal IQ as children. *Journal of the American Academy of Child and Adolescent Psychiatry, 52*(6), 572-581.

Hubert, B. E., Wicker, B., Monfardini, E., & Deruelle, C. (2009). Electrodermal reactivity to emotion processing in adults with autistic spectrum disorders. *Autism, 13*, 9-19.

Huizink, A. C., Greaves-Lord, K., Evans, B. E., Euser, A. S., van der Ende, J., Verhulst, F. C., et al. (2012). Youth in the Netherlands Study (JOiN): Study design. *BMC Public Health, 12,* 350.

Hutt, C., & Ounsted, C. (1966). The biological significance of gaze aversion with particular reference to the syndrome of infantile autism. *Behavioral Science, 11*, 346-356.

Hutt, C., Hutt, S. J., Lee, D., & Ounsted, C. (1964). Arousal and childhood autism. *Nature, 204*, 900-919.

Hyndman B. W., Mohn R. K, editors (1973*). A pulse modulator model of pacemaker activity.* Proceedings of the Digest of the 10th International Conference on Medical and Biological Engineering, Dresden, Germany.

Iarocci, G., & McDonald, J. (2006). Sensory integration and the perceptual experience of persons with autism. *Journal of Autism and Developmental Disorders, 36*(1), 77-90.

Jones, W., Carr, K., & Klin, A. (2008). Absence of preferential looking to the eyes of approaching adults predicts level of social disability in 2-year-old toddlers with autism spectrum disorder. *Archives of General Psychiatry, 65*, 946-954.

Joseph, R. M., Ehrman, K., McNally, R., & Keehn, B. (2008). Affective response to eye contact and face recognition ability in children with ASD. *Journal of the International Neuropsychological Society, 14*, 947-955.

Kaartinen, M., Puura, K., Makela, T., Rannisto, M., Lemponen, R., Helminen, M., et al. (2012). Autonomic Arousal to Direct Gaze Correlates with Social Impairments Among Children with ASD. *Journal of Autism and Developmental Disorders, 42,* 1917–1927.

Kamp-Becker, I., Smidt, J., Ghahreman, M., Heinzel-Gutenbrunner, M., Becker, K., & Remschmidt, H. (2010). Categorical and dimensional structure of autism spectrum disorders: the nosologic validity of Asperger Syndrome. *Journal of Autism and Developmental Disorders,, 40*(8), 921-929.

Kanner, L. (1943). Autistic disturbances of affective contact. *Nervous Child, 2*(217-250).

Kendell, R., & Jablensky, A. (2003). Distinguishing between the validity and utility of psychiatric diagnoses. *American Journal of Psychiatry, 160*(1), 4-12.

Kleinhans, N. M., Richards, T., Johnson, L. C., Weaver, K. E., Greenson, J., Dawson, G., et al. (2011). fMRI evidence of neural abnormalities in the subcortical face processing system in ASD. *Neuroimage, 54*(1), 697-704.

Kleinke, C. L. (1986). Gaze and eye contact: a research review. *Psychological Bulletin, 100*, 78-100.

Kliemann, D., Dziobek, I., Hatri, A., Baudewig, J., & Heekeren, H. R. (2012). The role of the amygdala in atypical gaze on emotional faces in autism spectrum disorders. *Journal of Neuroscience, 32*(28), 9469-9476.

Klin, A., Jones, W., Schultz, R., Volkmar, F., & Cohen, D. (2002). Visual fixation patterns during viewing of naturalistic social situations as predictors of social competence in individuals with autism. *Archives of General Psychiatry, 59*(9), 809-816.

Knapp, M., Romeo, R., & Beecham, J. (2009). Economic cost of autism in the UK. *Autism, 13*(3), 317-336.

Kuo, M. H., Orsmond, G. I., Cohn, E. S., & Coster, W. J. (2013). Friendship characteristics and activity patterns of adolescents with an autism spectrum disorder. *Autism, 17*(4), 481-500.

Kylliäinen, A., & Hietanen, J. K. (2006). Skin conductance responses to another person's gaze in children with autism. *Journal of Autism and Developmental Disorders, 36*, 517-525.

Kylliäinen, A., Wallace, S., Coutanche, M. N., Leppanen, J. M., Cusack, J., Bailey, A. J., et al. (2012). Affective-motivational brain responses to direct gaze in children with autism spectrum disorder. *Journal of Child Psychology and Psychiatry*, *53*(7), 790-797.

La Greca, A. M., Moore Harrison, H. (2005) Adolescent peer relations, friendships, and romantic relationships: do they predict social anxiety and depression? *Journal of Clinical Child & Adolescent Psychology, 34*(1), 49-61.

Lainhart, J. E., Bigler, E. D., Bocian, M., Coon, H., Dinh, E., Dawson, G., et al. (2006). Head circumference and height in autism: A study by the collaborative program of excellence in autism. *American Journal of Medical Genetics Part A, 140A*(21), 2257-2274.

Lang, N. J., Tulen, J. M., Kallen, V., Rosbergen, B., Dieleman, G., & Ferdinand, R. (2007). Autonomic reactivity in clinically referred children attention-deficit/hyperactivity disorder versus anxiety disorder. *European Child & Adolescent Psychiatry, 16*(2), 71-78.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1998). Emotion and motivation: measuring affective perception. *Journal of Clinical Neurophysiology, 15*(5), 397-408.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2001). *International Affective Pictures System (IAPS): Instruction Manual and Affective Ratings* (Technical Report A-5 ed.). Gainsville, FL: The Center for Research in Psychophysiology.

Lang, P. J., Greenwald, M. K., Bradley, M. M., & Hamm, A. O. (1993). Looking at pictures: affective, facial, visceral, and behavioral reactions. *Psychophysiology, 30*(3), 261-273.

Lang, P. J., Ohman, A., & Vaitl, D. (1988). *The international affective picture system [photographic slides]*. Gainsville FL: Center for Research in Psychophysiology.

Leventhal, B. L. (2012). Lumpers and splitters: who knows? Who cares? *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(1), 6-7.

Levine, T. P., Sheinkopf, S. J., Pescosolido, M., Rodino, A., Elia, G., & Lester, B. (2012). Physiologic Arousal to Social Stress in Children with Autism Spectrum Disorders: A Pilot Study. *Research in Autism Spectrum Disorders, 6*(1), 177-183.

Levy, A., & Perry, A. (2011). Outcomes in adolescents and adults with autism: A review of the literature. *Research in Autism Spectrum Disorders, 5*(4), 1271-1282.

Lord, C., Petkova, E., Hus, V., Gan, W., Lu, F., Martin, D. M., et al. (2012). A multisite study of the clinical diagnosis of different autism spectrum disorders. *Archives of General Psychiatry, 69*(3), 306-313.

Lord, C., Risi, S., DiLavore, P. S., Shulman, C., Thurm, A., & Pickles, A. (2006). Autism from 2 to 9 years of age. *Archives of General Psychiatry, 63*(6), 694-701.

Lord, C., Risi, S., Lambrecht, L., Cook, E. H., Jr., Leventhal, B. L., DiLavore, P. C., et al. (2000). The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders, 30*(3), 205-223.

Lord, C., Rutter, M., DiLavore, P. C., & Risi, S. (1999). *Autism Diagnostic Observation Schedule: Manual*. Los Angeles: Western Psychological Services.

Lord C., Rutter M., DiLavore P. C., Risi S., Gotham K., Bishop S. (2012). *Autism Diagnostic Observation Schedule - Second Edition: Manual.* Los Angeles: Western Psychological Services.

Louwerse A., Tulen J. H. M., van der Geest J. N., van der Ende J., Verhulst F.C., Greaves-Lord K. (2014). Autonomic Responses to Social and Nonsocial Pictures in Adolescents With Autism Spectrum Disorder. *Autism Research*, 7 (1), 17-27.

Louwerse A., van der Geest J. N., Tulen J. H. M., van der Ende J., Van Gool A. R., Verhulst F. C., et al. (2013). Effects of eye gaze directions of facial images on looking behaviour and autonomic responses in adolescents with autism spectrum disorders. *Research in Autism Spectrum Disorders, 7*(9), 1043-53.

Maenner, M. J., Rice, C. E., Arneson, C. L., & et al. (2014). Potential impact of dsm-5 criteria on autism spectrum disorder prevalence estimates. [doi: 10.1001/jamapsychiatry.2013.3893]. *JAMA Psychiatry*.

Magnee, M. J., de Gelder, B., van Engeland, H., & Kemner, C. (2007). Facial electromyographic responses to emotional information from faces and voices in individuals with pervasive developmental disorder. *Journal of Child Psychology and Psychiatry, 48*(11), 1122-1130.

Mandy, W. P., Skuse, D. H., Charman, T., & Frazier, T. W. (2012). In defense of lumping (and splitting). *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(4), 441-442; author reply 442-443.

Mathersul D., McDonald S., Rushby J. A. (2013). Autonomic arousal explains social cognitive abilities in high-functioning adults with autism spectrum disorder. *International Journal of Psychophysiology, 89(3),* 475-482.

Mathersul, D., McDonald, S., & Rushby, J. A. (2012). Automatic facial responses to affective stimuli in high-functioning adults with autism spectrum disorder. *Physiology & Behavior, 109C*, 14-22.

Mathewson, K. J., Drmic, I. E., Jetha, M. K., Bryson, S. E., Goldberg, J. O., Hall, G. B., et al. (2011). Behavioral and cardiac responses to emotional stroop in adults with autism spectrum disorders: influence of medication. *Autism Research, 4*(2), 98-108.

Maurer, D., & Salapatek, P. (1976). Developmental Changes in the Scanning of Faces by Young Infants. *Child Development, 47*, 523-527.

Mawhood, L., Howlin, P., & Rutter, M. (2000). Autism and developmental receptive language disorder-a comparative follow-up in early adult life. I: Cognitive and language outcomes. *Journal of Child Psychology and Psychiatry, 41*(5), 547-559.

McGovern, C. W., & Sigman, M. (2005). Continuity and change from early childhood to adolescence in autism. *Journal of Child Psychology and Psychiatry, 46*(4), 401-408.

Ming, X., Julu, P. O. O., Brimacombe, M., Connor, S., & Daniels, M. L. (2005). Reduced cardiac parasympathetic activity in children with autism. *Brain and Development, 27*(7), 509-516.

Murphy, N., & Young, P. C. (2005). Sexuality in children and adolescents with disabilities. *Developmental Medicine & Child Neurology, 47*(9), 640-644.

Nakano, T., Tanaka, K., Endo, Y., Yamane, Y., Yamamoto, T., Nakano, Y., et al. (2010). Atypical gaze patterns in children and adults with autism spectrum disorders dissociated from developmental changes in gaze behaviour. *Proceedings of the Royal Society, 277*(1696), 2935-2943.

Neumann, D., Spezio, M. L., Piven, J., & Adolphs, R. (2006). Looking you in the mouth: Abnormal gaze in autism resulting from impaired top-down modulation of visual attention. *Social Cognitive & Affective Neuroscience, 1*, 194-202.

Nony (1993). Speculation on light sensitivity. *Our Voice, 3*(1).

Oberman, L. M., Winkielman, P., & Ramachandran, V. S. (2009). Slow echo: facial EMG evidence for the delay of spontaneous, but not voluntary, emotional mimicry in children with autism spectrum disorders. *Developmental Science, 12*(4), 510-520.

Orsmond, G. I., Krauss, M. W., & Seltzer, M. M. (2004). Peer relationships and social and recreational activities among adolescents and adults with autism. *Journal of Autism and Developmental Disorders, 34*(3), 245-256.

Orsmond, G. I., Shattuck, P. T., Cooper, B. P., Sterzing, P. R., & Anderson, K. A. (2013). Social Participation Among Young Adults with an Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders, 43*(11), 2710-9.

Osborne, J. (2010). Improving your data transformations: Applying the Box-Cox transformation. *Practical Assessment, Research & Evaluation, 15*. Retrieved from http://pareonline.net/getvn.asp?v=15&n=12.

Osterling, J., & Dawson, G. (1994). Early recognition of children with autism: a study of first birthday home videotapes. *Journal of Autism and Developmental Disorders, 24*(3), 247-257.

Patriquin, M. A., Scarpa, A., Friedman, B. H., & Porges, S. W. (2013). Respiratory sinus arrhythmia: A marker for positive social functioning and receptive language skills in children with autism spectrum disorders. *Developmental Psychobiology*, 55(2), 101-112.

Phillips, M. L., Drevets, W. C., Rauch, S. L., & Lane, R. (2003). Neurobiology of emotion perception II: Implications for major psychiatric disorders. *Biological Psychiatry, 54*(5), 515-528.

Porges S. W. (1995). Cardiac vagal tone: a physiological index of stress. *Neuroscience Biobehavioral Review, 19*(2), 225-33.

Porges S. W. (1995). Orienting in a defensive world: mammalian modifications of our evolutionary heritage. A Polyvagal Theory. *Psychophysiology,* 32(4), 301-18.

Porges S. W. (2003). Social engagement and attachment: a phylogenetic perspective. *Annals of the New York Academy of Science, 1008,* 31-47.

Porges S. W. (2009). The polyvagal theory: new insights into adaptive reactions of the autonomic nervous system. *Cleveland Clinic Journal of Medicine*, 76(2), 86-90.

Riby, D. M., & Hancock, P. J. (2009). Do faces capture the attention of individuals with Williams syndrome or autism? Evidence from tracking eye movements. *Journal of Autism and Developmental Disorders, 39*(3), 421-431.

Riby, D. M., Doherty-Sneddon, G., & Whittle, L. (2012). Face-to-face interference in typical and atypical development. *Developmental Science, 15*, 281-291.

Riby, D. M., Whittle, L., & Doherty-Sneddon, G. (2012). Physiological reactivity to faces via live and video-mediated communication in typical and atypical development. *Journal of Clinical and Experimental Neuropsychology, 34*(4), 385-395.

Rice, K., Moriuchi, J. M., Jones, W., & Klin, A. (2012). Parsing Heterogeneity in Autism Spectrum Disorders: Visual Scanning of Dynamic Social Scenes in School-Aged Children. [doi: 10.1016/j.jaac.2011.12.017]. *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(3), 238-248.

Rimland, B. (1968). On the objective diagnosis of infantile autism. *Acta Paedopsychiatrica, 35*(4), 146-161.

Robins, B., Dautenhahn, K., & Dickerson, P. (2009, 1-7 Feb. 2009). *From Isolation to Communication: A Case Study Evaluation of Robot Assisted Play for Children with Autism with a Minimally Expressive Humanoid Robot.* Paper presented at the Advances in Computer-Human Interactions, 2009 ACHI '09 Second International Conferences.

Rogers, S. J., & Ozonoff, S. (2005). Annotation: what do we know about sensory dysfunction in autism? A critical review of the empirical evidence. *Journal of Child Psychology and Psychiatry, 46*(12), 1255-1268.

Rosenberg R. E., Mandell D. S., Farmer J. E., Law J. K., Marvin A. R., Law P. A. (2010). Psychotropic medication use among children with autism spectrum disorders enrolled in a national registry, 2007-2008. *Journal of Autism and Developmental Disorders, 40*(3), 342-351.

Rutter, M. (2000). Genetic studies of autism: from the 1970s into the millennium. *Journal of Abnormal Child Psychology, 28*(1), 3-14.

Rutter, M., Greenfeld, D., & Lockyer, L. (1967). A five to fifteen year follow-up study of infantile psychosis: II. Social and behavioral outcome. *British Journal of Psychiatry, 113*, 1183-1189.

Rutter, M., Le Couteur, A., & Lord, C. (2003). *The Autism Diagnostic Interview-Revised: Manual*. Los Angeles: CA: Western Psychological Services.

Sasson, N. J., Dichter, G. S., & Bodfish, J. W. (2012). Affective responses by adults with autism are reduced to social images but elevated to images related to circumscribed interests. *PLoS One, 7*(8), e42457.

Saul J. P. (1990). Beat-To-Beat Variations of Heart Rate Reflect Modulation of Cardiac Autonomic Outflow. *Physiology, 5*(1), 32-37.

Sawyer, A. C., Williamson, P., & Young, R. L. (2012). Can gaze avoidance explain why individuals with Asperger's syndrome can't recognise emotions from facial expressions? *Journal of Autism and Developmental Disorders, 42*, 606-618.

Schaffer, D., Fisher, P., & Lucas, C. (1998). *NIHM DISC-IV. Diagnostic Interview Schedule for Children, Parent-informant*. New York: Columbia University.

Senju, A., & Johnson, M. H. (2009). Atypical eye contact in autism: Models, mechanisms and development. *Neuroscience & Biobehavioral Reviews, 33*, 1204-1214.

Shaffer, D., Fisher, P., & Lucas, C. (1998). *NIMH DISC-IV. Diagnostic Interview Schedule for Children. Parent-informant*. New York, NY: Columbia University.

Sigman, M., & McGovern, C. W. (2005). Improvement in cognitive and language skills from preschool to adolescence in autism. *Journal of Autism and Developmental Disorders, 35*(1), 15-23.

Sigman, M., Dissanayake, C., Corona, R., & Espinosa, M. (2003). Social and cardiac responses of young children with autism. *Autism, 7*(2), 205-216.

Silverman, J. M., Smith, C. J., Schmeidler, J., Hollander, E., Lawlor, B. A., Fitzgerald, M., et al. (2002). Symptom domains in autism and related conditions: evidence for familiarity. *American Journal of Medical Genetic, 114*(1), 64-73.

Simonoff, E., Pickles, A., Charman, T., Chandler, S., Loucas, T., & Baird, G. (2008). Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. *Journal of the American Academy of Child and Adolescent Psychiatry, 47*(8), 921-929.

Skuse, D. H., Mandy, W., Steer, C., Miller, L. L., Goodman, R., Lawrence, K., et al. (2009). Social communication competence and functional adaptation in a general population of children: preliminary evidence for sex-by-verbal IQ differential risk. *Journal of the American Academy of Child and Adolescent Psychiatry, 48*(2), 128-137.

Speer, L. L., Cook, A. E., McMahon, W. M., & Clark, E. (2007). Face processing in children with autism: effects of stimulus contents and type. *Autism, 11*(3), 265-277.

Spezio, M., Adolphs, R., Hurley, R., & Piven, J. (2007). Abnormal Use of Facial Information in High-Functioning Autism. *Journal of Autism and Developmental Disorders, 37*(5), 929-939.

Stigler, K. A., & McDougle, C. J. (2013). Chapter 3.1 - Structural and Functional MRI Studies of Autism Spectrum Disorders *The Neuroscience of Autism Spectrum Disorders* (pp. 251-266). San Diego: Academic Press.

Sung, Y. J., Dawson, G., Munson, J., Estes, A., Schellenberg, G. D., & Wijsman, E. M. (2005). Genetic investigation of quantitative traits related to autism: use of multivariate polygenic models with ascertainment adjustment. *American Journal of Human Genetics, 76*(1), 68-81.

Szatmari, P., Bryson, S., Duku, E., Vaccarella, L., Zwaigenbaum, L., Bennett, T., et al. (2009). Similar developmental trajectories in autism and Asperger syndrome: from early childhood to adolescence. *Journal of Child Psychology and Psychiatry, 50*(12), 1459-1467.

Tantam, D. (2003). The challenge of adolescents and adults with Asperger syndrome. *Child & Adolescent Psychiatric Clinics of North America, 12*(1), 143-163, vii-viii.

Thayer J. F., Rossy L. A., Ruiz-Padial E., Johnsen B. H. (2003). Gender Differences in the Relationship Between Emotional Regulation and Depressive Symptoms. *Cognitive Therapy and Research, 27*(3), 349-64.

Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders, 61*(3), 201-216.

Tick, N. T., van der Ende, J., & Verhulst, F. C. (2007). Twenty-year trends in emotional and behavioral problems in Dutch children in a changing society. *Acta Psychiatrica Scandinavica, 116*(6), 473-482.

Tick, N. T., van der Ende, J., & Verhulst, F. C. (2008). Ten-year increase in service use in the Dutch population. *European Child & Adolescent Psychiatry, 17*(6), 373-380.

Toichi, M., & Kamio, Y. (2003). Paradoxical autonomic response to mental tasks in autism. *Journal of Autism and Developmental Disorders, 33*(4), 417-426.

Turpin, G., Schaefer, F., & Boucsein, W. (1999). Effects of stimulus intensity, risetime, and duration on autonomic and behavioral responding: implications for the differentiation of orienting, startle, and defense responses. *Psychophysiology, 36*(4), 453-463.

Urbano, M. R., Hartmann, K., Deutsch, S. I., Polychronopoulos, G. M. B., & Dorbin, V. (2013). *Relationships, Sexuality, and Intimacy in Autism Spectrum Disorders - Volume I*, Prof. Michael Fitzgerald (Ed.), ISBN: 978-953-51-1021-7, InTech, DOI: 10.5772/53954. Available from: http://www.intechopen.com/books/recent-advances-in-autism-spectrum-disorders-volume-i/relationships-sexuality-and-intimacy-in-autism-spectrum-disorders.

van der Geest, J. N., Kemner, C., Verbaten, M. N., & van Engeland, H. (2002). Gaze behavior of children with pervasive developmental disorder toward human faces: a fixation time study. *Journal of Child Psychology and Psychiatry, 43*, 669-678.

van Engeland, H., Roelofs, J. W., Verbaten, M. N., & Slangen, J. L. (1991). Abnormal electrodermal reactivity to novel visual stimuli in autistic children. *Psychiatry Research, 38*(1), 27-38.

van Steenis H. G., Tulen J. H., Mulder L. J. (1994). Heart rate variability spectra based on non-equidistant sampling: the spectrum of counts and the instantaneous heart rate spectrum. *Medical Engeneering and Physics, 16*(5), 355-62.

van Steensel, F. J., Bogels, S. M., & de Bruin, E. I. (2013). Psychiatric Comorbidity in Children with Autism Spectrum Disorders: A Comparison with Children with ADHD. *Journal of Child and Family studies, 22*(3), 368-376.

Vander Stoep, A., Weiss, N. S., McKnight, B., Beresford, S. A. A. (2002). Which measure of adolescent psychiatric disorder – diagnosis, number of symptoms, or adaptive functioning – best predicts adverse young adult outcomes? *Journal of Epidemiological & Community Health, 56*, 56-65.

Vaughan Van Hecke, A., Lebow, J., Bal, E., Lamb, D., Harden, E., Kramer, A., et al. (2009). Electroencephalogram and heart rate regulation to familiar and unfamiliar people in children with autism spectrum disorders. *Child Development, 80*, 1118-1133.

Wechsler, D. (1999). *Wechsler Abbreviated Scale of Intelligence, manual*. San Antonio, TX Harcourt Assessment.

Wieser, M. J., Pauli, P., Alpers, G. W., & Muhlberger, A. (2009). Is eye to eye contact really threatening and avoided in social anxiety? - An eye-tracking and psychophysiology study. *Journal of Anxiety Disorders, 23*, 93-103.

Wing, L. (1988). The continuum of autistic characteristics. In E. Schopler & G. Mesibov (Eds.), *Diagnosis and Assessment in Autism* (pp. 91-110). New York: Plenum.

Woolfenden, S., Sarkozy, V., Ridley, G., & Williams, K. (2012). A systematic review of the diagnostic stability of Autism Spectrum Disorder. *Research in Autism Spectrum Disorders, 6*, 345-354.

Zito, J., Safer, D., Berg, L., Janhsen, K., Fegert, J., Gardner, J., et al. (2008). A three-country comparison of psychotropic medication prevalence in youth. *Child and Adolescent Psychiatry and Mental Health, 2*(1), 26.

SUMMARY

The aim of the current thesis was twofold. The first aim was to provide insight in the outcome in adolescence (age 12-19) of clinically referred individuals with an autism spectrum disorder (ASD) classification or ASD symptoms in childhood (age 6-13). The second aim of the current thesis was to gain a better understanding of the mechanisms - namely *perception of* and *responses to* social stimuli - that might underlie problems in social functioning in individuals with ASD.

In *Chapter 1*, general background information concerning adolescent outcome of ASD and mechanisms that may underlie problems in social functioning in individuals with ASD was provided. Also the main research questions were presented. The research questions concerning the first part of the thesis were as follows: 1. Are ADOS ASD total scores and classifications stable from childhood to adolescence? 2. How do individuals with an ADOS ASD classification in childhood participate in society in adolescence ? Does this level of societal participation differ from that of individuals with the broader autism phenotype (BAP) in childhood and from reference data from the general population? The research questions concerning the second part of the thesis were: 1. Do eye gaze directions of facial images affect gaze behaviour and autonomic responses in adolescents with ASD as compared to Typically Developing (TD) adolescents? 2. Are there differences in autonomic responses to social versus non-social affective pictures in adolescents with ASD as compared to TD adolescents? 3. Do individuals with ASD differ from TD individuals with regard to resting ANS activity? And is resting autonomic nervous system (ANS) activity associated with HR responses to social stimuli or to social interaction abilities? To answer the research questions, data was used from a prospective follow-up study of 96 individuals that were referred for psychiatric evaluation to the outpatient Department of Child and Adolescent Psychiatry/psychology of the Erasmus MC-Sophia in Rotterdam.

In *Chapter 2*, we examined the 7-year stability of Autism Diagnostic Observation Schedule (ADOS) ASD total scores and classifications from childhood (age 6-13) to adolescence (age 12-19) in a sample of individuals with an ASD screen positive on the Child Social Behavior Questionnaire (CSBQ) and an IQ above 70. We found a correlation between ADOS total scores in childhood and adolescence of .58. Eighty-one percent of the individuals with an ADOS ASD classification in childhood still met criteria for an ASD classification seven years later in adolescence. The remaining 19 percent did no longer meet criteria for an ADOS ASD classification, but ADOS total scores were just below the classification threshold and the majority did meet criteria for internalizing or externalizing disorder. Also, we found that 37 percent of the individuals with above-threshold ASD symptoms on the CSBQ but without an ADOS ASD classification in childhood did receive an ADOS ASD classification in adolescence. These individuals seemed to show an increase in the ADOS total score together with decrease in concurrent externalizing disorder. The reported seven-year stability in the current study was

lower than reported in previous long-term follow-up studies that mainly included individuals with an Autistic Disorder (AD) and a cognitive impairment. Although the findings should be interpreted with caution given the sample size of this study, the findings suggest a relatively lower stability in individuals with ASD symptom levels above the screening threshold of the CSBQ and without a cognitive impairment compared to individuals with an AD. For these individuals it seems to be important to perform follow-up assessments on autistic and other psychiatric traits later in life.

In *Chapter 3*, societal participation in adolescence of individuals classified with ASD in childhood was investigated. The level of societal participation was compared with reference data from the general population and with individuals with the BAP (i.e. Broader Autism Phenotype, screen positives on the CSBQ) in childhood. In general, the individuals with an ASD classification showed a lower level of societal participation compared to the reference data from the general population. Only one fourth of the ASD sample attended regular schools in adolescence. The majority of the parents reported that their child had no reciprocal relationships. Half of the individuals within the ASD sample received professional mental health care. Not only the adolescents within the ASD sample, but also the adolescents with the BAP showed limited societal participation in adolescence. They showed similar levels of special education needs and mental health care. Differences were found between the ASD and the BAP groups with regard to reciprocal relationships; individuals with an ASD classification were significantly less likely than individuals in the BAP group to have a reciprocal friendship or a romantic relationship, and they spend significantly less time with friends. Thus, individuals with an ASD classification in childhood seemed to especially experience impairments in the field of social relationships during adolescence.

In *Chapter 4*, fixation durations towards the eye region of photographs of faces were examined in cognitively able adolescents with ASD and TD adolescents. The stimuli in this lab session were divided into three categories: direct eye gaze (which might be experienced as 'eye contact'), averted eye gaze or closed eyes. Adolescents with ASD as well as TD adolescents looked longer towards faces with direct eye gaze than towards faces with averted eye gaze or closed eyes. This finding seems to suggest that cognitively able adolescent with ASD are aware of the social relevance of direct eye gaze. Sustained fixation to direct eye gaze did not elicit stronger autonomic responses than sustained fixation to averted eye gaze or closed eyes in the ASD group compared to the TD group. The subjective reports revealed that both groups experienced direct eye gaze as more pleasant and more arousing than closed eyes. Thus, both cognitively able adolescents with ASD and adolescents with TD spent more time looking towards direct eye gaze compared to averted eye gaze or closed eyes. Looking towards direct eye gaze was subjectively reported as more pleasant and more arousing than closed eyes, while on the level of autonomic responses, direct eye gaze did not trigger higher levels of arousal compared to averted or closed eyes in both groups.

In *Chapter 5*, we investigated autonomic and subjective responses to social and affective stimuli in cognitively able adolescents with ASD and TD adolescents. We found that the autonomic and subjective responses of both adolescents with ASD and TD adolescents were dependent upon the social and the affective content of a stimulus. Both groups showed a stronger autonomic response (SCR) and higher subjective arousal scores for social pictures versus non-social pictures. These findings suggest that cognitively able individuals with ASD are neither hypo aroused nor hyper aroused when attending to social affective static stimuli. However, firm statements about the hypo- or hyper arousal theory in individuals with ASD cannot be drawn from the current study, since we the selected stimuli did not represent real-life interactions. Additional research is necessary to disentangle autonomic responses of individuals with ASD to social affective stimuli.

In *Chapter 6*, we extended the existing literature concerning resting levels of ANS activity in ASD, by reporting significant associations between resting levels of ANS activity and ANS responses to social and non-social stimuli in cognitively able adolescents with ASD versus TD adolescents. The significant associations between resting high frequency – heart rate variability (HF-HRV) and heart rate (HR) responses to stimuli were found in both groups. Therefore, we suggest future research regarding ANS responses to stimuli to pay attention to the putative influence of basal vagal activity. This chapter also revealed that resting HR was affected by medication. Adolescents with ASD who used medication had significantly higher levels of resting HR than adolescents with ASD who did not use medication or TD adolescents. This higher level of resting HR in adolescents with ASD who used medication suggests an increased sympathetic tone, decreased parasympathetic control or both that warrants further research and clinical attention.

In the last chapter of this thesis, *Chapter 7*, the main results and conclusions of this thesis were presented. We have gained further insight into the outcome in adolescence of cognitively able individuals with ASD symptoms and classifications in childhood. The main findings of the first part of this thesis were that children with an ASD classification in childhood, showed limited societal participation in adolescence, and that changes in classifications and symptoms occurred over time. The findings of the two seven-year follow-up studies hopefully raise research and clinical awareness of the usefulness to perform follow-up assessments later in life, not only to evaluate the level of ASD symptoms, but also to assess other psychiatric symptoms and the level of societal participation. In the second part of the thesis, insight was gained into the *perception of* and the *responses to* social stimuli of cognitively able male adolescents with ASD and TD adolescents. The simultaneous investigation of two putative underlying mechanisms (i.e. perception of and responses to social stimuli) within one experimental design provided the unique opportunity to integrate findings and insights from two lines of investigation that have been related to one-and-other on theoretical grounds, but thus far were not yet examined in a combined study design. This combined investigation of two putative underlying mechanisms of problems in social behaviour is just a first step in

trying to accomplish a more coherent and complete picture of the social problems which individuals with ASD experience.

SAMENVATTING

Het doel van dit proefschrift was tweeledig. Het eerste doel van het proefschrift was om inzicht te krijgen in het functioneren in de adolescentie van individuen met een autismespectrumstoornis (ASS) of symptomen van een ASS in de kindertijd. Het tweede doel van het proefschrift was om meer zicht te krijgen op mechanismen die mogelijk onderliggend zijn aan problemen in het sociaal functioneren van individuen met ASS, namelijk *waarneming van* en *reacties op* sociale stimuli.

In *hoofdstuk 1* werd de achtergrond van het huidige onderzoek beschreven. Tevens werden de onderzoeksvragen gepresenteerd. De onderzoeksvragen betreffende het eerste deel van het proefschrift waren als volgt: 1. Zijn ADOS ASS scores en classificaties stabiel van de kindertijd tot in de adolescentie? 2. Hoe participeren individuen, die in de kindertijd werden geclassificeerd met een ADOS classificatie ASS, in de samenleving als zij adolescent zijn? Verschilt de mate van participatie van individuen die een ADOS ASS classificatie kregen in de kindertijd van de mate van participatie van individuen die in de kindertijd symptomen van ASS hadden, maar niet voldeden aan een ADOS ASS classificatie (bredere autisme fenotypering) en van de mate van participatie zoals bekend bij jongeren uit de algemene populatie? De onderzoeksvragen behorend bij het tweede deel van het proefschrift waren: 1. Is de kijkrichting van ogen in afbeeldingen van gezichten van invloed op het kijkgedrag en de autonome reacties van adolescenten met ASS in vergelijking met typisch ontwikkelende adolescenten? 2. Verschillen adolescenten met ASS van typisch ontwikkelende adolescenten in hun autonome reacties op sociale versus niet-sociale affectieve afbeeldingen? 3. Verschilt de activiteit van het autonome zenuwstelsel tijdens rust tussen adolescenten met ASS en typisch ontwikkelende adolescenten? Hangt de activiteit van het autonome zenuwstelsel tijdens rust samen met de hartslag reactie tijdens het kijken naar afbeeldingen van sociale situaties *of* met sociale karakterkenmerken? Om de bovenstaande onderzoeksvragen te beantwoorden, werd een prospectieve vervolgstudie uitgevoerd waarbinnen tijdens het tweede meetmoment (in de adolescentie) een lab sessie werd verricht. De onderzoeksgroep bestond uit individuen die in de kindertijd (tussen de 6 en 13 jaar oud), werden verwezen voor diagnostisch onderzoek naar de polikliniek Kinder- en Jeugdpsychiatrie/psychologie van het Erasmus MC-Sophia. De kinderen met symptomen van een ASS (met een verhoogde score op de screenings Vragenlijst voor Inventarisatie Sociaal gedrag van Kinderen; VISK) en waarbij een Autisme Diagnostisch Observatie Schema (ADOS) werd afgenomen. In de adolescentie werden deze individuen (inmiddels tussen de 12 en 19 jaar oud) uitgenodigd om mee te doen aan vervolgonderzoek, waarin opnieuw een uitgebreid diagnostisch onderzoek plaatsvond. In de adolescentie werden eveneens een lab sessie uitgevoerd. Deze lab sessie werd ook uitgevoerd bij een controlegroep, welke bestond uit typisch ontwikkelende jongeren.

In *hoofdstuk 2* onderzochten we de stabiliteit van de ADOS totaalscore en classificatie van de kindertijd tot in de adolescentie. Voor deze studie includeerden we zowel 32 individuen

die in de kindertijd voldeden aan een ADOS ASS classificatie, als 40 individuen die in de kindertijd kenmerken van een ASS hadden (een verhoogde score op de VISK) maar die niet voldeden aan een ADOS ASS classificatie. Alle deelnemers in deze studie hadden een Intelligentie Quotiënt (IQ) boven de 70. De correlatie tussen de ADOS totaalscore in kinderleeftijd en de ADOS totaalscore in de adolescentie was 0,58. Eén-en-tachtig procent van de individuen met een ADOS ASS classificatie in de kindertijd voldeed eveneens aan de criteria voor een ADOS ASS classificatie in de adolescentie. De overige negentien procent voldeed niet langer aan de criteria voor een ADOS ASS classificatie. Echter, de gemiddelde totaalscore op de ADOS van deze individuen lag net onder de grenswaarde voor een ASS classificatie en de meerderheid van deze individuen voldeed aan de criteria voor een internaliserende of een externaliserende stoornis. Daarnaast vonden we dat 37 procent van de individuen met ASS symptomen (een verhoogde score op de VISK) maar zonder een ADOS ASS classificatie in de kindertijd, voldeed aan de criteria voor een ADOS ASS classificatie in de adolescentie. Deze groep was klein, maar liet een stijging zien in de totale score op de ADOS en een daling in het aantal individuen dat voldeed aan een classificatie van een externaliserende stoornis. De in dit proefschrift gerapporteerde stabiliteit van de ADOS classificaties over een periode van zeven jaar was lager dan de stabiliteit gerapporteerd in eerdere onderzoeken naar de stabiliteit tot in de adolescentie waarin met name individuen met een autistische stoornis (AS) en een cognitieve beperking werden geïncludeerd. Hoewel de resultaten van het huidige onderzoek gezien de kleine onderzoeksgroep met voorzichtigheid moeten worden geïnterpreteerd, kan op basis van de huidige onderzoeksresultaten worden gesuggereerd dat de stabiliteit van ADOS classificaties lager is in een groep individuen met kenmerken van ASS (een verhoogde score op de VISK) of een ASS classificatie, zonder een cognitieve beperking dan bij individuen met een AS. Het lijkt belangrijk om bij deze groep cliënten later in de ontwikkeling opnieuw diagnostisch onderzoek uit te voeren waarin zowel de kenmerken van ASS, als kenmerken van andere psychiatrische problemen in kaart worden gebracht.

In *hoofdstuk 3* beschreven we hoe individuen die in de kindertijd aan een ADOS ASS classificatie voldeden, meedraaiden in de samenleving in de adolescentie. Om deze cijfers in perspectief te plaatsen, werd de maatschappelijke participatie in de adolescentie van individuen met een ADOS ASS classificatie in de kindertijd vergeleken met de maatschappelijke participatie van individuen met ASS kenmerken (verhoogde score op de VISK), maar zonder ASS classificatie in de kindertijd en met cijfers die reeds bekend waren over adolescenten uit de algemene populatie. Individuen die in de kindertijd aan een ADOS ASS classificatie voldeden, participeerden in beperktere mate in de maatschappij in de adolescentie. Slechts een kwart van deze groep adolescenten volgde regulier onderwijs, 85% van de ouders rapporteerden dat hun kind geen wederkerige vriendschap had en meer dan 50% van de adolescenten maakte gebruik van geestelijke gezondheidszorg. Deze waarden verschilden significant van de cijfers bekend over individuen uit de algemene populatie. Echter, de maatschappelijke participatie van de groep met een ADOS ASS classificatie verschilde voor het merendeel niet

significant van maatschappelijke participatie van de groep individuen met ASS kenmerken maar zonder een ASS classificatie in de kindertijd, met uitzondering van vriendschappen en intieme relaties. Individuen met een ASS classificatie hadden significant minder vaak een wederkerige vriendschap of een romantische relatie dan individuen met ASS kenmerken maar zonder ASS classificatie in de kindertijd.

In *hoofdstuk 4* werd onderzocht hoe lang adolescenten met een ASS en typisch ontwikkelende adolescenten naar de oogregio van gezichten keken en hoe zij hier fysiologisch op reageerden (autonome activiteit). Beide groepen bestonden uit jongens met een IQ boven de 70. Voor dit onderzoek werden de adolescenten tijdens een lab sessie gevraagd om te kijken naar de oogregio van foto's van gezichten, die op een computerscherm werden gepresenteerd. De kijkrichting van de ogen op de foto's was verdeeld in drie condities: ogen die recht vooruit 'de camera in' keken (ervaren als 'oogcontact'), ogen die opzij keken (wel blootstelling aan ogen, maar geen oogcontact) en ogen dicht (geen blootstelling aan ogen en geen oogcontact). Zowel adolescenten met ASS als typisch ontwikkelende adolescenten met een IQ boven de 70 keken langer naar de oogregio van gezichten waarbij de ogen op de adolescent waren gericht (oogcontact), dan naar gezichten waarbij de ogen opzij keken of gezichten waarbij de ogen dicht waren. Op basis van deze bevinding zou gesuggereerd kunnen worden dat adolescenten met ASS en zonder een cognitieve beperking zich bewust lijken te zijn van de sociale relevantie van ogen die in jouw richting kijken (oogcontact). In een tweede taak werden de jongeren gevraagd gedurende de gehele presentatie hun aandacht en blik op de ogen in de afbeelding te richten. Het (langdurig) blijven kijken naar de ogen die op de adolescent waren gericht, was niet geassocieerd met sterkere autonome reacties (hartslag en huidgeleiding) bij adolescenten met ASS versus typisch ontwikkelende adolescenten. Tevens was er voor beide groepen geen sprake van sterkere autonome reacties tijdens het kijken naar oogcontact versus het kijken naar de ogen in de andere twee condities (opzij en ogen dicht). Hoewel er geen verschil werd gevonden met betrekking tot de fysiologische maten, werden er wel verschillen gevonden in de subjectieve beoordelingen van de adolescenten met ASS en de typisch ontwikkelende adolescenten. Het kijken naar de oogregio van gezichten die de adolescenten aankeken, werd door beide groepen subjectief als meer plezierig en meer opwindend ervaren dan het kijken naar de oogregio van gezichten met ogen dicht. De discrepanties in bevindingen en methoden tussen het huidige en eerder onderzoek werden in tot slot dit hoofdstuk bediscussieerd.

In *hoofdstuk 5* werden autonome reacties en subjectieve reacties op sociaal-affectieve plaatjes onderzocht van jongens met een ASS versus typisch ontwikkelende jongens met een IQ boven de 70. De autonome reacties en de subjectieve reacties hingen samen met de sociale en de affectieve inhoud van de stimuli, zowel bij adolescenten met ASS als voor typisch ontwikkelende adolescenten. Beide groepen lieten sterkere autonome activiteit (huidgeleiding) en hogere subjectieve scores van mate van overprikkeling zien bij sociale plaatjes ten opzichte van niet-sociale plaatjes. Deze resultaten suggereren dat adolescenten

met ASS zonder cognitieve beperking niet hyper of hypo geprikkeld zijn wanneer zij kijken naar sociaal affectieve statische plaatjes. Wij willen benadrukken dat een stellige aanname ten opzichte van over- of onderprikkeling bij ASS niet kan worden gedaan op basis van deze onderzoeksresultaten, aangezien in het huidige onderzoek statische stimuli (plaatjes) werden gebruikt, en geen onderzoek werd gedaan naar de reactie op sociale/affectieve situaties in het dagelijks leven. Aanvullend onderzoek is daarom nodig om verder inzicht te krijgen in hoeverre autonome reacties op sociale/affectieve situaties verschillen tussen individuen met ASS en typisch ontwikkelende individuen.

In *hoofdstuk 6* werd getracht om de huidige beperkte literatuur op het gebied van de activiteit van het autonome zenuwstelsel tijdens rust bij adolescenten met ASS uit te breiden. In dit hoofdstuk werd een significante samenhang gevonden tussen de activiteit van het autonome zenuwstelsel tijdens rust en autonome reacties tijdens het kijken naar sociaal affectieve stimuli bij een groep adolescenten met ASS en bij een groep typisch ontwikkelende adolescenten. De groepen bestonden uit jongens met een IQ boven de 70. Er werd een significante associatie gevonden tussen de parasympatische reactiviteit (de reactiviteit van het 'remmende, rusten en verteren' systeem, gemeten middels de hartslag variabiliteit in de hoogfrequente band) tijdens rust en de hartslag tijdens het kijken naar sociaal affectieve stimuli. Deze samenhang werd in de beide groepen gevonden. Voor toekomstig onderzoek is het belangrijk om te weten dat autonome activiteit tijdens rust samenhangt met de autonome reacties tijdens taaksituaties. Daarnaast werd in dit hoofdstuk een associatie gevonden tussen de hartslag tijdens rust en het gebruik van medicatie. In de groep adolescenten met een ASS was het gebruik van medicatie geassocieerd met een hogere hartslag tijdens rust. Deze samenhang suggereert verhoogde sympathische activiteit, verlaagde parasympathische controle, of beide in adolescenten met ASS die medicatie gebruiken. Deze bevinding behoeft aandacht in toekomstig onderzoek en in de klinische praktijk.

In het laatste hoofdstuk, *hoofdstuk 7*, werden de belangrijkste resultaten en conclusies van het huidige proefschrift weergegeven. Het huidige promotieonderzoek heeft inzicht gegeven in de uitkomsten in de adolescentie van individuen met ASS symptomen en een ASS classificatie in de kindertijd, zonder een cognitieve beperking. De belangrijkste bevinding van deel 1 van het proefschrift is dat kinderen met een ASS classificatie of symptomen van ASS in de adolescentie een beperktere mate van participatie in de samenleving laten zien, en dat er veranderingen kunnen optreden in de aard van de classificaties en het aantal symptomen. Daarom lijkt het in de klinische praktijk belangrijk om bij de groep kinderen die een verhoogde mate van ASS symptomen vertoont later in de ontwikkeling opnieuw diagnostisch onderzoek te verrichten, omdat de ASS symptomen, symptomen van andere psychiatrische stoornissen en het functioneren in de samenleving kunnen veranderen en daarmee de indicatie voor de benodigde zorg/begeleiding. In het tweede deel van het proefschrift was de belangrijkste bevinding dat het *waarnemen van* en de autonome *reacties op* sociale stimuli van jongens met ASS en typisch ontwikkelende jongens met een IQ boven de 70 niet

significant van elkaar verschilden. Het integreren van metingen betreffende waarneming van én autonome reacties op sociale stimuli in één design creëerde een unieke mogelijkheid om twee lijnen van eerder van elkaar gescheiden onderzoek naar mogelijke onderliggende mechanismen van ASS aan elkaar te verbinden. Deze integratie heeft informatie opgeleverd over de manier waarop adolescenten met een ASS naar sociale statische plaatjes kijken én hoe zij daarop reageren. Echter, gezien het feit dat in deze studies gebruik werd gemaakt van gecontroleerde lab sessies waarin gebruik werd gemaakt van statische plaatjes, kunnen de uitkomsten van deze studies niet direct worden vertaald naar het gedrag van adolescenten met ASS tijdens sociale situaties in het dagelijks leven. Meer onderzoek is nodig om de beperkingen in de sociale interacties in het dagelijks leven van individuen met ASS beter te leren begrijpen.

CURRICULUM VITAE

Suzanne Christine (roepnaam: Anneke) Louwerse werd op 29 mei 1985 geboren te Middelburg. In 2003 behaalde zij haar VWO diploma aan de Christelijke Scholengemeenschap Walcheren te Middelburg. In hetzelfde jaar startte zij met de studie Pedagogiek aan de Universiteit Utrecht. In 2005 volgde zij enkele vakken Psychologie aan de universiteit van Århus (Denemarken). In 2006 startte zij de master Orthopedagogiek en voerde ze zowel haar diagnostiekstage als haar afstudeeronderzoek uit binnen de polikliniek Kinder- en Jeugdpsychiatrie/psychologie van het Erasmus MC-Sophia. In 2007 behaalde zij haar master en bleef zij werkzaam als orthopedagoog bij de dezelfde polikliniek.

In 2008 werd zij aangesteld als promovendus binnen de afdeling Kinder- en Jeugdpsychiatrie/psychologie van het Erasmus MC-Sophia (hoofd: Prof. Dr. Frank Verhulst). In deze periode werd in samenwerking tussen deze afdeling en Yulius de onderzoekslijn Autisme vormgegeven (hoofdonderzoeker: Dr. Kirstin Greaves-Lord). Anneke gaf binnen deze onderzoekslijn haar promotieonderzoek vorm door met succes een subsidie bij de Sophia Stichting te verkrijgen. Dit promotieonderzoek genaamd GAME (towards Genotypes in Autism, Measuring Endophenotypes) betrof een vervolgstudie naar kinderen met (kenmerken van) een autismespectrumstoornis. In deze vervolgstudie werd uitgebreide diagnostiek uitgevoerd bij adolescenten die zeven jaar eerder naar de polikliniek Kinder- en Jeugdpsychiatrie/psychologie van het Erasmus MC-Sophia werden verwezen. In het kader van deze diagnostiek behaalde Anneke de certificaten trainer van het Autisme Diagnostisch Observatie Schema (ADOS) en trainer van het Autisme Diagnostisch Interview (ADI-R). Naast de diagnostiek werd bij deze jongeren een lab sessie uitgevoerd, welke in samenwerking met de afdelingen Neurowetenschappen en Volwassenpsychiatrie van het Erasmus MC werden vormgegeven. De resultaten van dit promotieonderzoek staan in dit proefschrift beschreven. Vanaf 2012 was Anneke naast haar promotie werkzaamheden ook werkzaam bij de polikliniek Autisme van Yulius. Sinds september 2013 werkt zij als orthopedagoog in opleiding tot GZ-psycholoog bij Yulius.

PUBLICATIONS

Eussen, M., de Bruin, E., Van Gool, A., **Louwerse, A.**, van der Ende, J., Verheij, F., Verhulst, F., Greaves-Lord, K. (2014). Formal thought disorder in autism spectrum disorder predicts future symptom severity, but not psychosis prodrome. *European Child & Adolescent Psychiatry, May 2013 [Epub ahead of print]*

Louwerse, A., Tulen, J., van der Geest, J., van der Ende J., Verhulst, F., Greaves-Lord, K. (2014). Autonomic Responses to Social and Nonsocial Pictures in Adolescents With Autism Spectrum Disorder. *Autism Research, 7(1),* 17-27.

Louwerse, A., van der Geest, J., Tulen, J., van der Ende, J., Van Gool, A., Verhulst, F., Greaves-Lord, K. (2013). Effects of eye gaze directions of facial images on looking behaviour and autonomic responses in adolescents with autism spectrum disorders. *Research in Autism Spectrum Disorders, 7(9)*, 1043-53.

Louwerse, A., Greaves-Lord, K. (2013) Congres Review International Meeting for Autism Research (IMFAR) 2012. *Wetenschappelijk Tijdschrift Autisme, 1*, 15-16.

Louwerse, A., Eussen, M. en Greaves-Lord, K. (2012) Het beloop van autismespectrumstoornissen: een sneak preview van 4 casussen uit een vervolgonderzoek. *Wetenschappelijk Tijdschrift Autisme, 4*, 119-126.

Louwerse, A., Greaves-Lord, K. (2012) Congres Review: Nationaal Autisme Congres 2012. *Wetenschappelijk Tijdschrift Autisme, 3*, 99-101.

Greaves-Lord, K., Eussen, M., Duvekot, J., **Louwerse, A.**, ten Hoopen, L., de Nijs, P. (2012) Dimensionele en categoriale diagnostiek als solide basis voor zorg en onderzoek. *Wetenschappelijk Tijdschrift Autisme, 2*, 68-71.

Berkien, M., **Louwerse, A**., van der Ende, J., Verhulst, F. (2012). Children's perceptions of dissimilarity in parenting styles are associated with internalizing and externalizing behavior. *European Child & Adolescent Psychiatry, 21(2),* 79-85.

PHD PORTFOLIO

Summary of PhD training and teaching

Name of PhD student:	Anneke Louwerse
PhD period:	2008-2013
Erasmus MC Department:	Child and Adolescent Psychiatry/psychology
Research school:	NIHES
Promotor:	Prof. Dr. F.C. Verhulst
Supervisor:	Dr. K. Greaves-Lord

1. PhD-training	Year	Workload (ECTS)
General Academic Skills		
Research Integrity in Medical Research	2009	2
Basiscursus regelgeving en organisatie voor klinische onderzoekers	2010	1
Biomedical English Writing and Communication	2012	4
Training presentatievaardigheden	2012	0.5
Research Skills		
Introduction into data-analyses	2009	0.9
Regression analyses for Clinicians	2010	1.9
Clinical Epidemiology	2011	5.7
In-depth courses		
Structure and organization of the nervous system	2008	1
Sensory systems	2008	1
Neurocognition	2009	1
Didactic skills		
Training ADOS module 3 en 4	2008	1.5
Training CAARMS	2008	1
Training E-prime	2009	1
Training ADI-R	2009	1.5
Train de trainer ADI-R	2010	1.5
Train de trainer ADOS module 3 en 4	2010	1.5
Training ADOS module 1 en 2	2012	1.5
Train de trainer ADOS module 1 en 2	2013	1.5
Conferences and symposia		
Wetenschapsmarkt de Grote Rivieren, Hendrik Ido Ambacht, oral presentation	2008	1
Symposium Generation-R, Rotterdam	2008	0.3
Zestig jaar Sectie Kinder- en Jeugdpsychiatrie, Rotterdam	2008	0.3
Nationaal Autisme Congres, Rotterdam	2009	0.3
Voorjaarscongres NVvP, Groningen, oral presentation	2009	1
Behandeling Centraal, Erasmus MC, afdeling KJP, Rotterdam, oral presentation	2009	1
Symposium over de toekomst van psychiatrische diagnostiek	2010	0.2
Nationaal Autisme Congres, Rotterdam	2010	0.3
Voorjaarscongres NVvP, Maastricht, oral presentation	2010	1
Autisme Congres, Utrecht	2010	0.3

Nationaal Autisme Congres, Rotterdam	2011	0.3
Wetenschapsmarkt Yulius, Hendrik Ido Ambacht, oral presentation	2011	1
Voorjaarscongres NVvP, Amsterdam, oral presentation	2011	1
Symposium 'Vroege interventie bij psychosen: een regionale aanpak', Ridderkerk	2011	0.2
Kracht van Autisme, Barendrecht, 2 oral presentations	2011	1
Nationaal Autisme Congres, Rotterdam, poster presentation	2012	1
Wetenschapsmarkt Yulius, Hendrik Ido Ambacht, oral presentation	2012	1
International Meeting of Autism Research, Toronto, oral and poster presentation	2012	2
Society for Psychophysiological Research, New Orleans, poster presentation	2012	1
Nationaal Autisme Congres, Rotterdam	2013	0.3
Wetenschapsmarkt Yulius, Hendrik Ido Ambacht, oral presentation	2013	1
Voorjaarscongres NVvP, Maastricht, oral presentation	2013	1
Other		
Presentation Sophia Foundation, Grand assigned	2008	2
2. Teaching	**Year**	**Workload (ECTS)**
Lecturing		
Vaardigheidsonderwijs medische studenten, Erasmus MC, Rotterdam	2008-2012	3
ADOS en ADI-R trainingen, Assen, Rotterdam en Willemstad	2010-2013	6
Supervising Master's theses		
Marjan Blok, Erasmus MC, Rotterdam, Review Eyetracking in ASD	2008	1
Annelot Blankenberg, Institute of Psychology, EUR, Pilot-study eyetracking	2010	3
Linda Dekker, Leiden University, Change Blindness Paradigm	2010	3
Rhea Cruden, Erasmus MC, Rotterdam, Pilot-study physiology	2010	3
Dennison Silva, Leiden University, Physiology in IAPS task	2011	3
Carolien Verheij, Erasmus MC, Rotterdam, Follow-up of co-morbidity in ASD	2011	3

DANKWOORD

"*What you see, is what you get*"

Het proefschrift dat u voor u ziet, is met hulp van velen tot stand gekomen. Zonder deze mensen had u dit proefschrift niet kunnen vasthouden. Daarom wil ik graag iedereen bedanken die mij heeft geholpen.

Als eerste richt ik mijn blik op de jongeren en ouders die hebben deelgenomen aan dit onderzoek. Deze deelnemers zijn jaren geleden, tussen 2002 en 2004, in zorg gekomen bij het Erasmus MC – Sophia, waar zij destijds meededen aan een onderzoek naar contact- en denkproblemen. Toen wij hen zeven jaar later benaderden om deel te nemen aan een vervolgonderzoek, stapten we ineens weer binnen in het leven van deze jongeren en hun ouders. De bereidheid die zij hebben getoond om ons te laten zien hoe op dat moment met hen ging, was groot. Graag wil ik jullie bedanken voor de tijd die jullie hebben geïnvesteerd, maar vooral voor de openhartigheid waarmee jullie je verhaal met ons hebben gedeeld. Daarnaast ben ik de deelnemers uit de controlegroep dankbaar voor hun enthousiasme en inzet. Uiteraard wil ik ook het dataverzamelingsteam bedanken voor het bellen van deelnemers, opsturen van vragenlijsten, afnemen van (telefonische) interviews, uitvoeren van de experimenten en het invoeren van data. In het bijzonder wil ik Roshenie Bhola bedanken. Het geduld waarmee jij per dag meerdere interviews afnam, die per stuk meer dan twee uur duurden, getuigd van doorzettingsvermogen. Dat je je naast deze taak ook verantwoordelijk voelde voor vele andere taken, maakte jou onmisbaar tijdens de dataverzamelingsperiode. Ook wil ik graag de volgende studenten bedanken: Marjan Blok, Annelot Blankenberg, Linda Dekker, Rhea Cruden, Carolien Verheij en Danny Silva. Niet meer dan eens liepen er dingen anders dan gepland, maar al met al hebben we er samen voor gezorgd dat alle data werd verzameld en dat deze netjes werd ingevoerd. Ik wil graag Dr. Esther de Bruin bedanken voor de zorgvuldige dataverzameling tussen 2002 en 2004, waarmee zij de basis heeft gelegd voor de huidige beloopstudies.

Mijn kijk op wetenschappelijk onderzoek veranderde ruim zes jaar geleden door Prof. dr. Frank Verhulst. Voordat Frank mijn kamer op de polikliniek binnenstapte dacht ik dat het doen van wetenschappelijk onderzoek iets was voor in de verre toekomst. Ik dacht destijds dat ik pas geschikt zou zijn voor het uitvoeren van wetenschappelijk onderzoek nadat ik veel kennis zou hebben opgebouwd over een fascinerende doelgroep. Frank, jij liet mij op een andere manier naar wetenschappelijk onderzoek kijken, je vertelde mij dat het doen van onderzoek in plaats van 'later' ook 'nu' zou kunnen èn dat je al een fascinerende doelgroep in gedachten had. Ik wil je bedanken voor dit heldere inzicht, maar vooral voor het vertrouwen dat je daarmee in mij uitsprak. Je gaf mij veel ruimte om dit onderzoek op te zetten, maar je

was altijd beschikbaar voor een kritisch overleg of voor het lezen van de artikelen. Hartelijk bedankt.

Toen ik het inzicht had dat een promotieproject in de nabije toekomst haalbaar was, maakte ik kennis met Prof. dr. Anja Huizink, die mij in de eerste periode begeleide. Anja, bedankt voor je vertrouwen.

Voor de dagelijkse begeleiding kwam ik onder de hoede van Dr. Kirstin Greaves-Lord. Kirstin, toen ik jou voor het eerst zag, dacht ik 'jij bent heerlijk enthousiast'. Dit enthousiasme was er vanaf het begin en dit is niet meer weggegaan. Je schroomde niet om contacten te leggen met andere afdelingen of om 'out-of-the-box' te denken. Dit leidde tot ambitieuze plannen, die we samen met veel enthousiasme uitvoerden. Ik waardeer het enorm dat je dit 'samen-gevoel' even belangrijk vond als ikzelf. We hebben samen wat afgelachen, maar ook wanneer ik een tegenslag te verwerken kreeg, stond je voor me klaar met relativerende opmerkingen als "*Promoveren is leren incasseren*" (vrije interpretatie Wildred, december 2012). Bedankt dat ik altijd bij je terecht kon.

Mijn blik werd op de juiste zaken gericht door ervaren onderzoekers. Prof. dr. Fop Verheij, bedankt voor het delen van jouw expertise op het gebied van (poli) klinisch onderzoek. Dr. Joke Tulen en Dr. Jos van der Geest, jullie waren beiden vanaf het begin van het ontwikkelen van de experimentele taken betrokken. Joke, jij introduceerde mij in de wereld van de fysiologie; Jos, jij deelde je expertise op het gebied van kijkgedrag. Naast jullie inhoudelijke betrokkenheid ben ik jullie ook dankbaar voor jullie persoonlijke betrokkenheid. Dr. Hugo van Steenis, bedankt dat je de software schreef om de data goed op te kunnen slaan. Toen de data verzameld was, keek Jan van der Ende over mijn schouder mee, om mij op weg te helpen met de analyses. Dr. Arthur van Gool, jij verbreedde mijn blik, door mij verder te laten kijken dan het autismespectrum. Ik wil jullie allen bedanken voor jullie goede adviezen, die jullie (gelukkig gedoseerd) met mij hebben gedeeld.

Tijdens een promotietraject kan een kritische blik niet ontbreken. Ik wil graag de leden van de kleine commissie, Prof. dr. Frank Verhulst, Prof. dr. Ingmar Franken, Dr. Joke Tulen en Dr. Catharina Hartman, van harte bedanken. Prof. dr. Ingmar Franken, bedankt dat u ook de secretaris van de leescommissie wilde zijn. Ook de overige leden van de commissie wil ik van harte bedanken. Prof. dr. Ina van Berckelaer-Onnes, u heeft u blik al lange tijd op autismespectrumstoornissen gericht en ik vind het daarom een eer om hierover met u van gedachten te mogen wisselen. Prof. dr. Henning Tiemeier, bedankt dat u tijdens mijn promotie wil opponeren. Jos en Arthur, wat fijn dat jullie ook tijdens de promotie een belangrijke rol willen vervullen.

Twee zien meer dan één. Wat was het dan ook fijn om tegelijkertijd met iemand aan een promotietraject te beginnen. Mart, samen vormden we de 'eerste promovendi' binnen de onderzoekslijn autismespectrumstoornissen. Jij had al een staat van verdienste in de Kinder-

en Jeugdpsychiatrie, ik kwam net kijken in dit werkveld. Toch gaf jij mij het gevoel dat we in hetzelfde schuitje zaten. Ik heb veel geleerd van de rust die je uitstraalt en tegelijkertijd van de gedrevenheid die je hebt om klinisch werk en onderzoek met elkaar te integreren.

Vele collega's hebben mijn beeld de afgelopen jaren mooi gekleurd. Bedankt daarvoor. Tijdens mijn stageperiode werd ik hartelijk ontvangen door Mariëlle en Myra. Natuurlijk als allereerst door Q, die niet alleen mij, maar ook alle deelnemers van het onderzoek hartelijk heeft ontvangen. Na mijn stage begon ik tegelijkertijd met Frida in het ontwikkelingsstoornissenteam, samen gingen we op (ADOS) onderzoek uit, bedankt voor het delen van de leuke onderzoeken en autoritjes. Janneke, Vief, Marijke en Rianne wil ik ook graag bedanken. Fijn dat we nog steeds onze ervaringen delen, ook al werken we nu bijna allemaal op andere poli's. Daarnaast wil ik alle andere collega's van de polikliniek bedanken.

Aan het begin van mijn promotietraject werd ik door mijn research-collega's hartelijk onthaald op de Westzeedijk, onder andere door Sylvana, Hanneke en Inge. Tijdens het analyseren van mijn data werd ik bijgestaan door enthousiaste collega's op 'de opbouw'; Karolijn, Jens, Willem, Petra, Jorieke, Linda, Gerbrich, Hanan, Marcus, Brittany, Mark Patrick, Maartje, Ryan, Pauline, Nathalie, Floor B., Ilse, Johanna, Lisette, Nikita, Nita, Geerte, Suzanne, Janneke, Vandhana, Floor van O., Tonya, Laureen, Helene, Mireille en Andriene. Ik wil jullie allemaal bedanken voor jullie interesse, de gezellige lunches, borrels, research work meetings, maar vooral bedankt voor de leuke grappen die bij onderzoekers kunnen ontstaan ten tijde van het doen van pittige analyses of het schrijven van een moeilijke discussie. Gelukkig werd ik bij het schrijven van deze discussie, maar ook van de overige delen van de papers bijgestaan door intelligente co-auteurs, waarvan ik Prof. Julian Thayer en Dr. Pieter de Nijs nog niet heb genoemd. Bedankt!

Alle ogen waren gericht op de onderzoekslijn autismespectrumstoornissen, die in samenwerking tussen het Erasmus MC en Yulius werd opgericht. Graag wil ik Dr. Jan-Luc Klompenhouwer, Dr. Athanasios Maras, Ad van der Sijde en Ellen Hoogervorst van Yulius bedanken voor het vertrouwen dat ze hadden in deze onderzoekslijn en in mij. Gaandeweg het project leerde ik meer Yulius-collega's kennen, bij de Academie, op de polikliniek autismespectrumstoornissen in Dordrecht en bij het Yulius Autisme Expertisecentrum. In het bijzonder wil ik Kirsten, Frieda, Linda B., Julliette, Esther, Laura en Annette bedanken voor hun getoonde interesse.

Vanuit Yulius kwam ook de kans om mijn blik te verbreden, doordat ik mocht starten met de GZ-opleiding. Tijdens mijn GZ-opleidingsjaar voelde ik me welkom op de polikliniek Langdurige Zorg. Ik heb veel geleerd van mijn nieuwe collega's die me op weg hielpen in mijn nieuwe functie, in het bijzonder wil ik Eveline, Gabriëlla, Hans, Ankie, Inge, Gerda, Mijke, Rachna, Wilma, Agnes, Diederik, Ron, Lenny, Marjan en Charlotte bedanken. Ook wil ik mijn GZ-opleidingsgroep bedanken voor hun medeleven tijdens de laatste loodjes van het schrijven van mijn proefschrift.

Samen onze blik op oneindig, zo voelt het als ik met mijn Zeeland-vriendinnen ben. Xandra, Marieke, Roos, Lieneke en Leonie, jullie kennen mij door én door. Wat is het heerlijk om al een leven lang in het gezelschap te zijn van zulke lieve vriendinnen, en dat daar in de loop van de tijd zulke leuke vrienden bijkwamen: Danny, Marijn, Eric, Roderick en Peter. Bedankt voor alle leuke momenten samen! Marieke, zo zit je samen in de box, zo sta je samen op een promotie. Jij was degene die het eerst kon staan. Wat ben ik blij dat je vandaag letterlijk en figuurlijk achter mij staat.

Tijdens mijn studententijd was het tijd om mijn blik te verbreden. Samen met Willemijn, Inge en Liesbeth bekeken we niet alleen de bibliotheek van de UU, maar richten we ons ook op de gezelligheid die Utrecht te bieden had. Vele leuke eet-dates volgden en samen vierden we het leven (en de behaalde tentamens). Bedankt voor jullie gezelligheid.

Toen daarna Rotterdam in zicht kwam, kwamen ook Stefan en Anne steeds duidelijker in beeld. Naast veel plezier maken, hadden we samen vaak goede gesprekken. Jullie staan mooi in het leven, samen met Morris en Maas. Bedankt voor het plezier, maar ook voor de steun die jullie mij gaven. Het plezier maken deden we vaak samen met Sietse, Christa, Lennart, Elwin, Gillis, Franka en Marèn, bedankt daarvoor.

Mijn familie kijkt al lange tijd met mij mee. Bedankt voor jullie steun en interesse in de afgelopen jaren. Ina, John, Jolanda, Annemieke en Jos wil ik graag bedanken voor de Zeeuwse gezelligheid en de leuke vakanties.

Lieve grote broer Tom en kleine zus Linda, bedankt dat jullie vanaf jongs af aan samen met mij de wereld inkijken en ontdekken. We doen dit op onze eigen manier, maar samen vormen we een sterk 3-TAL. Bedankt voor jullie steun en liefde en voor de leuke etentjes met Hadewych en Igie erbij. Tom, bedankt dat je me ook vandaag wil steunen, dit maal in de rol van paranimf.

De positieve kijk op het leven heb ik aan twee bijzondere mensen te danken. Lieve mama en papa, jullie hebben mij geleerd om te kijken naar al het moois wat het leven te bieden heeft. Ik wil jullie ontzettend bedanken voor de fijne basis die jullie mij hebben gegeven en voor de liefde die jullie met mij delen. De quote aan het begin van het boekje is voor jullie.

Aan één blik genoeg hebben. Lieve JW, wat ben ik blij dat jij er altijd voor mij bent. Ik ben je dankbaar voor je onvoorwaardelijke steun, maar ook voor je perfecte timing voor ontspanning en vakanties. We slaagden erin om voor en tijdens dit promotietraject prachtige reizen te maken. Ik kijk uit naar onze toekomstige avonturen!

"So how do people with autism see the world, exactly? We, and only we, can ever know the answer to that one! Sometimes I actually pity you for not being able to see the beauty of the world in the same way we do. Really, our vision of the world can be incredible, just incredible..."

Naoki Higashida, 2013, page 91