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# Autonomic Arousal to a Direct Gaze, Cooperative Behavior and Reactive Aggression among Children with Autism Spectrum Disorders

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UNIVERSITY OF TAMPERE

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*To those I love to the moon and back*

# ABSTRACT

The present dissertation explored autonomic arousal and response habituation to a direct gaze (Studies I and II), cooperative behavior (Study III) and the regulation of reactive aggression (Study IV), and their associations to social impairments (Studies I-III) among children with autism spectrum disorder (ASD).

Autonomic arousal responses and response habituation were examined in Studies I and II among 23 children with ASD and 21 neurotypical children by measuring skin conductance responses (SCR) while the participants viewed different gaze directions on the live face of another person. In Studies III and IV, 27 boys and eight girls with ASD and 35 neurotypical children matched for gender, age and total score intelligence were studied with a modified version of the Prisoner's dilemma task and with the Pulkkinen Aggression Machine (PAM) to examine cooperative behavior and the regulation of reactive aggression, respectively. In Study III, only the results of the boy participants were presented. In Studies I-III, impairments in social skills were measured by interviewing the parents of the participants with the Developmental, Dimensional and Diagnostic Interview (3Di).

Children with and without ASD did not differ significantly from each other in autonomic arousal responses or response habituation to a direct gaze. However, the level of autonomic response habituation was observed to be inversely associated with the level of social impairments among the children with ASD. The more the response habituation to a direct gaze was attenuated, the more the children with ASD had impairments in social skills in the Use of Language and Other Social Communication Skills, in the Use of Gesture and Non-verbal Play, and in Social Expressiveness.

The boys with ASD showed cooperative behavior as frequently as the neurotypical boys in the PD task. However, when cooperative choices were investigated within gender-, age- and total IQ-matched pairs, the cooperative

choices of pair members had a tendency to be opposite, especially when cooperating with an unknown cooperator.

The boys with ASD reacted with a greater intensity of aggression than their matched pairs when they were faced with attacks of minor intensity or an assailant of the opposite gender. The girls with ASD, on the other hand, were able to regulate their aggressive responses similarly to the girls without ASD. ASD-related social impairments were not observed to be associated with cooperative behavior or the level of reactive aggression among the boys with ASD.

The results of the present study imply that attenuated autonomic response habituation during eye contact might be one mechanism that relates to ASD severity. Future studies are needed to investigate whether attenuated autonomic response habituation to a direct gaze has a role in ASD pathogenesis.

According to the findings, boys with ASD are able to follow the rule of equity in cooperation when inequity represents harm to the self or another. However, more information is needed about strategies that are utilized in cooperative decisions among boys with and without ASD. Future studies are also needed to investigate mechanisms that may yield less regulated responses to minor attacks and to attacks from assailants of the opposite gender among the boys with ASD in comparison to neurotypical boys.

Keywords: autism spectrum disorders; ASD; eye contact; gaze; skin conductance; autonomic arousal; habituation; cooperation; aggression; gender; social skills

# TIIVISTELMÄ

Tässä tutkimuksessa selvitettiin autismikirjon lasten autonomisia virittymisreaktioita suoraan katseeseen (osatyöt I ja II), yhteistyökäyttäytymistä (osatyö III) ja reaktiivisen aggressiivisuuden säätelyä (osatyö IV). Lisäksi tutkittiin edellä mainittujen tekijöiden yhteyksiä sosiaalisten taitojen vaikeuksiin, jotka ovat autismikirjon häiriöille ominaisia (osatyöt I-III).

Osatöissä I ja II autonomisia virittymisreaktioita ja niiden vaimenemista (habituaatiota) tutkittiin 23 autismikirjon lapselta sekä 21 verrokilta mittaamalla muutoksia ihonsähköjohtavuudessa lapsen nähdessä elävän ihmisen kasvoilla esitettynä katseärsykkeitä (suora katse, sivulle käännetty katse, ja suljetut silmät). Osatöissä III ja IV 27 autismikirjon poikaa ja kahdeksan autismikirjon tyttöä sekä 35 sukupuolen, iän ja kokonaisälykkyysosamäärän suhteen kaltaistettua verrokkia tekivät yhteistyökäyttäytymistä tutkivan Vangin dilemmasta (engl. the Prisoner's dilemma) muokatun tehtävän ja reaktiivisen aggressiivisuuden säätelyä kartoittavan Pulkkinen Aggression Machine (PAM) –tehtävän. Osatyön III tulokset raportoitiin ainoastaan autismikirjon poikien ja heidän verrokkiensa osalta. Sosiaalisten taitojen vaikeuksia selvitettiin haastattelemalla lasten vanhempia 3Di-haastattelulla (engl. the Developmental, Dimensional and Diagnostic Interview) osatöissä I-III.

Autismikirjon lapset eivät eronneet verrokeista tilastollisesti merkitsevästi suoran katseen aikaansaamien autonomisten virittymisreaktioiden tai näiden virittymisreaktioiden vaimenemisen suhteen. Tutkimuksessa kuitenkin havaittiin, että mitä vähemmän autonomiset virittymisreaktiot vaimenivat toistettaessa suora katse -ärsykettä, sitä enemmän autismikirjon lapsilla oli vaikeuksia sosiaalisissa taidoissa kielen ja muun sosiaalisen kommunikaation käytön, eleiden käytön ja ei-kielellisen leikin sekä sosiaalisen ilmaisukyvyn alueilla.

Autismikirjon pojat sitoutuivat yhteistyöhön yhtä usein kuin verrokkipojat. Tilanteessa, missä päätös yhteistyöhön sitoutumisesta piti tehdä tietämättä parin

valintaa, autismikirjon pojan ja verrokkipojan valinnat yhteistyöhön sitoutumisen suhteen erosivat toisistaan yli puolessa kaltaistetuista pareista.

Autismikirjon pojat reagoivat voimakkaammilla aggressiivisilla puolustusreaktioilla kuin tavanomaisesti kehittyneet pojat tilanteissa, joissa heitä vastaan kohdistui voimakkuudeltaan heikko hyökkäys, tai joissa hyökkääjä oli vastakkaista sukupuolta. Autismikirjon tytöt eivät eronneet tilastollisesti merkitsevästi kaltaistetuista pareistaan aggressiivisten reaktioiden suhteen. Sosiaalisten taitojen vaikeuksien ei havaittu olevan yhteydessä yhteistyökäyttäytymiseen tai reaktiivisen aggressiivisuuden tasoon autismikirjon pojilla.

Tutkimustulokset viittaavat siihen, että poikkeava autonomisten virittymisreaktioiden vaimeneminen katsekontaktin aikana voi olla yksi niistä mekanismeista, jotka liittyvät epätyypilliseen sosiaaliseen käyttäytymiseen autismikirjon häiriöissä. Lisää tutkimusta tarvitaan siitä, liittyykö poikkeava autonomisten virittymisreaktioiden vaimeneminen katsekontaktin aikana autismikirjon häiriöiden patogeneesiin.

Tutkimuksen perusteella autismikirjon pojat kykenevät huomioimaan tasapuolisuutta yhteistyökäyttäytymisessä sellaisissa tilanteissa, joissa epäoikeudenmukaisuus aiheuttaa vahinkoa itselle tai toiselle. On kuitenkin epäselvää, millaisia strategioita autismikirjon pojat hyödyntävät yhteistyövalintoja tehdessään, ja missä määrin nämä strategiat mahdollisesti eroavat tavanomaisesti kehittyneiden poikien käyttämistä strategioista. Lisätietoa tarvitaan myös mekanismeista, jotka voivat aiheuttaa aggressiivisten puolustusreaktioiden voimakkuuden lisääntymistä autismikirjon pojilla.

Avainsanat: autismikirjon häiriöt, katsekontakti, katse, ihonsähköjohtavuus, autonominen virittyminen, habituaatio, yhteistyö, aggressiivisuus, sukupuoli, sosiaaliset taidot

# CONTENTS

# LIST OF ORIGINAL COMMUNICATIONS

This dissertation is based on original communications referred to in the text by their Roman numerals (I-IV).

I. Kaartinen M, Puura K, Mäkelä T, Rannisto M, Lemponen R, Helminen M, Salmelin R, Himanen S-L, Hietanen JK. (2012). Autonomic arousal to direct gaze correlates with social impairments among children with ASD. Journal of Autism and Developmental Disorders, 42(9), 1917-1927.

II. Kaartinen M, Puura K, Himanen SL, Nevalainen J, Hietanen JK. (2016). Autonomic arousal response habituation to social stimuli among children with ASD. Journal of Autism and Developmental Disorders, 46(12), 3688-3699.

III. Kaartinen M, Puura K, Pispa P, Helminen M, Salmelin R, Pelkonen E, Juujärvi P, Kessler EB, Skuse DH. (Submitted). Associations between cooperation, reactive aggression, and social impairments among boys with autism spectrum disorder.

IV. Kaartinen M, Puura K, Helminen M, Salmelin R, Pelkonen R, Juujärvi P. (2014). Reactive aggression among children with and without Autism Spectrum Disorder. Journal of Autism and Developmental Disorders, 44(10), 2383-2391.

# ABBREVIATIONS

| | |
|---|---|
| ADHD | Attention deficit and hyperactive disorder |
| ASD | Autism spectrum disorder |
| BLUP | Best linear unbiased prediction |
| DSM-5 | The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition |
| ECG | Electrocardiography |
| EDA | Electrodermal activity |
| EEG | Electroencephalography |
| ICD-10 | International Classification of Diseases, tenth revision |
| ISI | Inter-stimulus interval |
| IQ | Intelligence |
| M | Mean |
| Md | Median |
| $P_A$ | The probability of SCR to an averted gaze being greater than SCR to a direct gaze and closed eyes |
| $P_C$ | The probability of SCR to closed eyes being greater than SCR to a direct gaze and an averted gaze |
| $P_D$ | The probability of SCR to a direct gaze being greater than SCR to an averted gaze and closed eyes |
| PAM | The Pulkkinen Aggression Machine task |
| PD | Prisoner's dilemma task |
| PDD | Pervasive Developmental Disorder |
| $Q_1$ | Lower quartile |
| $Q_3$ | Upper quartile |
| SCR | Skin conductance responses |
| SD | Standard deviation |
| ToM | Theory of Mind |
| WISC-III | Wechsler Intelligence Scale for Children, Third Edition |
| Yrs | Years |
| 3Di | Developmental, Dimensional and Diagnostic Interview |

# 1 INTRODUCTION

Humans interact socially with each other in more complex and extensive ways than other species, including non-human primates (Bickham, 2008). The origins of humans' superior social interaction skills may lie in ancient cooperative behavior (Hayes and Sanford, 2014; Moll and Tomasello, 2007; Nowak, 2006; Pennisi, 2009; Vogel, 2004; Warneken and Tomasello, 2006). Among our ancestors, cooperation increased the survival of infants and personal fitness to such a degree that it may have guided natural selection to favor socially cooperative individuals over individuals with a high self-interest (Hayes and Sanford, 2014; Moll and Tomasello, 2007; Nowak, 2006; Pennisi, 2009; Vogel, 2004; Warneken and Tomasello, 2006). For example, shared childcare may have enabled the increased rate of survival of offspring born immature and dependent on caregivers for a long period (Dunsworth, 2016). A need to secure cooperation might have further boosted the development of features and skills that increase effective cooperative communication, attention preference for the social world, a desire to experience pleasure in social interactions, and an urge to maintain social relationships (Chevallier et al., 2014).

Humans' gaze expertize may be one of the social domains that evolved to meet the demands of effective cooperative communication. Today, humans are able to use eye contact flexibly to communicate in social situations according to cultural norms, but it is likely that among our human ancestors, the direct gaze of another served mainly as a threatening and arousal-eliciting signal (Akechi et al., 2013; Emery, 2000). Engagement in eye contact probably served such major advantages for effective communication that natural selection favored morphological and functional changes in the neural system, enabling the regulation of autonomic arousal induced by the direct gaze of another (Bickham, 2008; Emery, 2000; Skuse, 2003; Tomasello et al., 2007). It is also possible that increased cooperation decreased competition and increased protection, which led to reducing the costs of revealing the target of one's own interest to others by gaze direction (Bickham, 2008; Boucher et al., 2012). As a result, the human eye evolved toward a structure revealing gaze direction, and today the human

eye has a unique morphology with a clearly visible eye outline and iris position (Bickham, 2008; Boucher et al., 2012; Kobayashi and Kohshima, 1997).

Social communication and interaction skills are extensively impaired in autism spectrum disorder (ASD), which also include restricted, repetitive patterns of behavior, interests, or activities (American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders, 5th edition). It is estimated that ASD occurs in 0.84 in a hundred children in Finland, and in 0.48–2.12 in a hundred children in the United Kingdom and the United States of America (Baird et al., 2006; Baron-Cohen et al., 2009; Christensen et al., 2016; Mattila et al., 2007; Mattila et al., 2011). ASD is diagnosed more often among males than among females (gender ratio 3-4:1), which may partly result from difficulties in recognizing ASD in females due to the gender differences in the clinical presentation of ASD (Kirkovski et al., 2013; Loomes et al., in press; Schaafsma and Pfaff, 2014). ASD-related social impairments include deficits in, e.g. expressed reciprocity in conversations and social interaction, nonverbal communicative behaviors and functioning in social relationships (American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders, 5th edition). The earliest symptoms of ASD appear during the first years of life and include – besides impairments in social communication – atypical sensory and repetitive behaviors (Bryson et al., 2007; Jones et al., 2014; Landa and Garrett-Mayer, 2006). The onset and developmental course of full-blown ASD varies between individuals, and it is estimated that ASD can be reliably diagnosed at the earliest at 24 months (Bryson et al., 2007; Daniels et al., 2014). However, ASD is frequently diagnosed markedly later (Daniels et al., 2014).

ASD is a neurodevelopmental disorder, with a liability related strongly to inherited and de novo polygenetic effects, and epigenetic regulatory mechanisms (de la Torre-Ubieta et al., 2016; Schanen, 2006; Vijayakumar and Judy, 2016). ASD-related impairments in social interaction, i.e. autistic traits, are also observed to a minor degree in the population, especially among the first-degree relatives of individuals with ASD, and in certain other psychiatric disorders, e.g. in schizophrenia, which implies a shared genetic liability of autistic traits between the population and autistic individuals (Bailey et al., 1998; Blain et al., 2016; Cassel et al., 2007; Colvert et al., 2015; Ronald and Hoekstra, 2011; Wahlsten, 2012; Westwood et al., 2016). It is likely that ASD results from complex gene-environment interaction, where altered epigenetic

regulation may have an important role in mediating the effects of the environment to gene expression (Loke et al., 2015; Mandy and Lai, 2016; Matelski and Van de Water, 2016). Environmental risk factors related to increased ASD liability include, e.g. high paternal and maternal age, exposure to elevated testosterone levels in utero, immunological processes, and obstetric complications (Mandy and Lai, 2016; Matelski and Van de Water, 2016; Young et al., 2016). It is likely that vulnerability to ASD is programmed already in utero, where the prenatal environment has the potential to alter epigenetic mechanisms that in turn regulate gene expression essential to the development of neural circuits (Babenko et al., 2015). Alteration in gene expression might result in, e.g. disrupted neurogenesis and neuronal proliferation, and synaptic dysfunction (Packer, 2016; Reiner et al., 2016). Altered structural and functional connectivity of the brain circuits responsible for social and communication skills, e.g. the prefrontal-thalamic circuitry, has been suggested to play an important role in ASD pathogenesis (Fakhoury, in press).

Several theories have tried to describe the mechanisms that cause atypical social development in ASD. Quite commonly, these theories share a similar assumption that in ASD, a certain core deficit limits the infant's engagement in social interaction, which causes social and communicational impairments by restricting opportunities to learn socio-cognitive skills in interaction (Chevallier, 2014; Dawson et al., 2005; Jones et al., 2014; Skuse, 2003; Tanaka and Sung, 2015; Zalla and Sperduti, 2013). The theories have suggested that the core deficit could be, e.g. an inability to represent the mind of another (Theory of Mind (ToM), Baron-Cohen et al., 1985); an inability to orient socially, to experience pleasure in social relationships and to long for maintained social relationships (the Social motivation theory, Chevallier et al., 2014); an inability to perceive information as a whole rather than as fragmented (the theory of Weak Central Coherence, Happe and Frith, 2006); or an inability to regulate autonomic arousal to a direct gaze (Hutt and Ounsted, 1966; Skuse, 2003). However, none of the above-mentioned theories so far has been sufficient alone in explaining ASD-related abnormalities in social interaction, and it is more likely that the explanations provided above are not mutually exclusive in the pathogenesis of ASD-related social impairments.

Even though ASD symptoms are persistent throughout life, behavioral and educational therapeutic interventions can be used in order to build new skills

and decrease the severity of ASD and comorbid symptoms, and their impact on daily life (Volkmar et al., 2014). Pharmaceutical interventions used in ASD are targeted mainly at comorbid symptomatology, e.g. irritability, impulsivity, aggression, and sleep disturbances, and they are used, if needed, alongside behavioral and educational interventions (Volkmar et al., 2014). Despite today's therapeutic possibilities, ASD-related social disturbances have a significant negative impact on adulthood outcomes among individuals with ASD (e.g. employment, social relationships, and quality of life) (Howlin and Moss, 2012; Lai et al., 2014). Increased knowledge of the mechanisms that relate to atypical social behavior in ASD is needed to decrease the negative impact social impairments have in the prognosis of ASD. The present study aimed to address this question by (i) investigating autonomic arousal and response habituation to direct gaze, and their relations to social impairments among children with ASD, and by (ii) investigating cooperative behavior and the regulation of reactive aggression, and their associations with social impairments among children with ASD.

# 2 REVIEW OF THE LITERATURE

## 2.1 Development of social impairments in autism spectrum disorders (ASD)

### 2.1.1 Early signs of autism spectrum disorders – difficulties in face and gaze perception

Already a few hours after birth, typically developing newborn babies show a capability to orient to faces, and especially open and straight-directed eyes attract and hold their attention effectively (Batki et al., 2000; Keehn et al., 2013; Morton and Johnson, 1991). The capability of newborns to orient to facial stimuli seems to be based on an innate attentional preference for stimuli with vertical asymmetry and a curved contour (Cassia, 2004; Simion et al., 2001; Simion et al., 2002; Turati, 2002). According to recent evidence, infants later to be diagnosed with ASD seem to show quite typical social orientation and eye contact engagement during the first months of life, implying that they do not have deficits in innate facial preference mechanisms (Bryson et al., 2007; Falck-Ytter et al., 2013; Jones et al., 2014; Jones and Klin, 2013; Ozonoff et al., 2010). However, whereas typically developing infants increase their looking times toward faces during the first year of life, a decline in eye contact engagement typically seems to appear in those later diagnosed with ASD by six months of age, and it continues through toddlerhood (Bryson et al., 2007; Chawarska et al., 2013; Chawarska and Shic, 2009; Courage et al., 2006; Frank et al., 2009; Jones and Klin, 2013; Osterling and Dawson, 1994; Sigman et al., 1992; Trillingsgaard et al., 2005; Wass et al., 2015; Zwaigenbaum et al., 2005). Decline in eye contact engagement seems to be accompanied with the appearance of marked irritability, intolerance of intrusions, and proneness to distress/negative affects (Bryson et al., 2007; Landa and Garrett-Mayer, 2006). A subgroup of infants later to be diagnosed with ASD seems to show earlier emerging and more prominent signs of autism, including a greater decline in gaze fixation, and the subgroup seems to be distinguishable from those infants

who develop autistic symptoms later on the basis of a marked decrease in IQ and poorer outcomes (Bryson et al., 2007; Jones and Klin, 2013; Wass et al., 2015).

### 2.1.2 Face and gaze fixation patterns later in life in ASD

Atypical facial fixation patterns later in life vary between individuals with ASD and may include diminished fixation frequency and time, enhanced latencies in fixation initiation, and atypical fixation patterns on different parts of a face, e.g. enhanced focusing on detailed information, diminished fixation to the eye region, and increased attention to the lower part of a face (Adolphs et al., 2001; Amestoy et al., 2015; Behrmann et al., 2006; Chawarska and Shic, 2009; Corden et al., 2008; Dalton et al., 2005; Deruelle et al., 2004; Freeth et al., 2010; Jones et al., 2008; Joseph and Tanaka 2003; Gillespie-Smith et al., 2014; Klin et al., 2002; Riby and Hancock, 2009; Speer et al., 2007; Spezio et al., 2007; Sterling et al., 2008; Yi et al., 2013). Even though some children with ASD seem to show intact proportional fixation to the eye region of faces, they have also been observed to show prolonged fixations below and outside of the eye region instead of attending to the pupil area compared to typically developing children (Yi et al., 2013). The capability to orient to a direct gaze may vary from one situation to another among children with ASD, who seem to be more motivated to engage in eye contact when they desire to gain something for themselves than in situations where eye contact is used to share interest (Stone et al., 1997).

### 2.1.3 Importance of engaging in eye contact for social development

Newborns' innate attentional preference to open and straight directed eyes has a vital function in guiding newborns' progress in social development and the development of the human social brain (Farroni et al., 2004; Farroni et al., 2006; Itier and Batty, 2009). Already a few days after birth, typically developing infants show a nascent ability to follow the gaze of another, but only if the gaze shift is preceded by a short moment of mutual gaze (Farroni et al., 2000; Farroni et al., 2003; Farroni et al., 2004; Frischen et al., 2007). An ability to follow a gaze gives rise to joint attention skills, which in turn are essential for subsequent social development, e.g. for development of language and theory of mind skills

(Frischen et al., 2007; Mundy et al., 2007; Strid et al., 2006; Vaughan Van Hecke et al., 2007).

Children with ASD fail to develop joint attention skills in a typical manner, which seems to have a negative impact on the development of language and other social and communication skills (Bruinsma et al., 2004; Bryson et al., 2007; Charman, 2003; Courage et al., 2006; Frank et al., 2009; Itier and Batty, 2009; Jones and Klin, 2013; Osterling and Dawson, 1994; Sigman et al., 1992; Trillingsgaard et al., 2005; Zwaigenbaum et al., 2005). Their joint attention skills are compromised, e.g. by difficulties in synchronizing gaze, gestures, and emotional expressions for communicative purposes; developmental delays in head and gaze following; and an inability to prefer social cues (eyes) over nonsocial cues (arrows) in attention orienting (Bruinsma et al., 2004; Leekam et al., 1998; Senju et al., 2004; Stone et al., 1997).

Engagement in social interaction is so essential for further social development that even infants with typical development are at risk of developing social impairments that resemble autistic features if they are faced with severe social deprivation (Rutter et al., 1999). The importance of eye contact engagement for social development is highlighted by observations showing that also among neurotypical children, gaze avoidance at the age of two months seems to predict impairments in social interaction establishment in toddlerhood, and the association between diminished engagement in eye contact and lessened social skills can still be detected in adulthood (Cherulnik et al., 1978; Itier and Batty, 2009; Keller and Zach, 1993; Kleinke, 1986).

Reduced eye fixation in infancy seems to relate to greater impairments in social communication skills in toddlerhood also among infants with a familiar risk for ASD (Wass et al., 2015). Among toddlers with ASD, diminished fixation to the eye region is observed as being most likely among those toddlers who show greater social disability; furthermore, reduced fixation to faces and the eye region later in childhood seems to be associated with higher autism severity, decreased social responsiveness, and a decreased level of social functioning in ASD (Gillespie-Smith et al., 2014; Jones et al., 2008; Riby and Hancock, 2009; Speer et al., 2007). In adulthood, reduced fixation to the eyes seems not to relate to greater severity in autism symptomatology, but seems to be associated with

greater impairments in fear recognition and enhanced social anxiety (Corden et al., 2008).

### 2.1.4 Autonomic arousal and response habituation to a direct gaze in ASD

Typically in adulthood, a direct gaze seems to increase autonomic arousal compared to seeing an averted gaze (McBride et al., 1965; Nicholas and Champness, 1971; Hietanen et al., 2008; Pönkänen and Hietanen, 2012; Pönkänen et al., 2011). Among typically developing children, picturized direct and averted gaze facial stimuli have been observed to elicit a similar level of autonomic arousal (Joseph et al., 2008; Kylliäinen and Hietanen, 2006). However, recent studies by Hietanen et al. (2008), Pönkänen and Hietanen (2012), and Pönkänen et al. (2011) have observed enhanced autonomic arousal responses to a direct gaze only when stimuli are presented on a live face. Thus, it is possible that enhanced autonomic arousal to a direct gaze in comparison to an averted gaze could also be observed among typically developing children if facial stimuli were presented as live.

It has been suggested that gaze avoidance behavior in ASD could result from an inability to regulate the autonomic arousal elicited in eye contact (Hutt and Ounsted, 1966; Skuse, 2003). According to Skuse (2003), this might result from enhanced activation in the amygdala. Indeed, Dalton et al. (2005) observed that the length of maintained eye contact seems to correlate positively with the level of amygdala activation among children and adults with ASD (Dalton et al., 2005). In a study by Nacewicz et al. (2006), decreased amygdala volumes among children and adults with ASD were positively associated with diminished eye fixation, slowed facial emotion discrimination, and greater impairments in social reciprocity and nonverbal communication in childhood (Nacewicz et al., 2006). Nacewicz et al. (2006) speculated that reduced amygdala volumes, i.e. amygdala atrophy, might have resulted from chronic amygdala hyperactivity in ASD. In addition, earlier studies have observed that some, but not all, children with ASD may show enhanced skin conductance responses, which are thought to reflect amygdala activity, to a direct gaze in comparison to an averted gaze (Critchley, 2002; Joseph et al., 2008; Kylliäinen and Hietanen, 2006; Kylliäinen et al., 2012; LaBar et al., 2008; Sequeira et al., 2009). A study by Joseph et al. (2008) also showed that enhanced autonomic arousal to a direct gaze may hamper facial information processing, especially

facial identification, among children with ASD. The evidence from the above mentioned studies implies that sustained eye contact might cause autonomic hyperarousal in ASD, which in turn might be related to greater social impairments.

One mechanism that could yield autonomic hyperarousal to a direct gaze is an attenuated or nonexistent response habituation. Habituation is a highly preserved evolutionary mechanism wherein repeated or sustained exposure to a non-threatening stimulus reduces the neural response over time (Schmid et al., 2015; Thompson and Spencer, 1966; Typlt et al., 2013). An exposure to repeated affective or facial stimuli leads to suppression in neural responses, with the valence and behavioral relevance of the stimuli modifying the level of neural suppression (Breitel et al., 1996; Ishai et al., 2004; Klorman and Ryan, 1980; Klorman et al., 1975; Klorman et al., 1977). Attenuated or nonexistent neural habituation to sensory input has the potential to cause hypo- or hypersensitivity to stimuli, which in turn may be regulated by maladaptive sensory-seeking or sensory-avoiding behavior, respectively (McIntosh et al., 1999).

Although maladaptive sensory-seeking and sensory-avoiding behavior are commonly observed among individuals with ASD and are noted to be strongly related to maladjustment in autism, there is no conclusive evidence on whether abnormal habituation to sensory input has a role in ASD pathogenesis (Iarocci and McDonald, 2006; Lane et al., 2010; O'Neill and Jones, 1997; Rogers and Ozonoff, 2005). The most recent evidence has shown that infants with a high familiar risk for ASD seem to show reduced neural habituation to auditory stimuli (Guiraud et al., 2011). Adaptation to familiarized social stimuli also seems to be slower among toddlers with ASD than among typically developing children (Webb et al., 2010). Even though not all individuals with ASD show abnormal habituation to sensory input, there is preliminary evidence that attenuated habituation to visual stimuli seems to be positively associated with autistic traits (Bruno et al., 2014; Ewbank et al., 2015; Guiraud et al., 2011; Rogers and Ozonoff, 2005; Webb et al., 2010). In addition, amygdala response habituation to a direct gaze seems to be reduced, and it correlates negatively with autism-related social impairments among children and adults with ASD (Kleinhans et al., 2009; Swartz et al., 2013). Together, these findings imply that autonomic response habituation to a direct gaze has been understudied so far in ASD.

## 2.2 Cooperation

### 2.2.1 Cooperation in typical development

In mutual cooperative activities, participants share a joint goal to which they are both committed, take reciprocal or complementary roles in order to achieve this joint goal, and are generally motivated and willing to help one another accomplish their respective roles if needed. Cooperation has an essential role in human social interaction (Moll and Tomasello, 2007). Humans have a tendency to help others to achieve goals, share valuable goods and inform others of meaningful things even though these behaviors do not provide immediate benefits to a performing individual, i.e. humans are altruistic (Stallen and Sanfey, 2013; Warneken and Tomasello, 2009). Whereas instrumental helping behavior can be observed to some degree also among non-human primates, other forms of altruistic behavior seem to be unique to humans (Warneken and Tomasello, 2006, 2009). Helping behavior may present one of the simplest domains of altruistic behavior and depend less than other forms of altruistic behavior on an ability to understand the mind of another that in general seems to explain humans' superior performance in cooperative behavior (Paal and Pereczkey, 2007; Stallen and Sanfey, 2013; Takagishi et al., 2010). While altruistic cooperative behavior may be rewarding per se, engagement in altruistic sharing behavior also requires an ability to foresee that a costly action may provide delayed gains in the future by providing later reciprocity, fitness and the secured transfer of genetic material through generations (Stallen and Sanfey, 2013; Vogel, 2004). For example, cooperators gain a reputation for trustworthiness as an opponent and are seen as more attractive, which increases the odds for continued cooperation and reproduction (Stallen and Sanfey, 2013; Vogel, 2004). On the other hand, besides rewarding cooperators, humans also tend to foster cooperation by punishing non-cooperators, and thus any disturbances in expected cooperativeness may have a negative impact on long-term social relationships (Stallen and Sanfey, 2013).

Cooperative abilities seem to emerge typically in early childhood (Fantasia et al., 2014; Moll and Tomasello, 2007; Tomasello and Carpenter, 2007; Southgate et al., 2007). Among neurotypical children, simple altruistic helping behavior toward adults can be observed at 18 months of age, and approximately six months later an ability to solve cooperative problem tasks with peers emerges

within the ability to represent the self and another as separate objects (Brownell and Carriger, 1990; Warneken and Tomasello, 2006). Within time, toddlers' coordination of actions and problem-solving skills become more successful, and an ability to guide peers to solve cooperative tasks emerges (Ashley and Tomasello, 1998; Brownell and Carriger, 1990). Neurotypical toddlers show a preference to share resources equally, and in the case of unequal resources, they allocate more resources to familiar than unfamiliar individuals, similarly to adults (Olson and Spelke, 2008). In addition, they are able to consider previous acts of an opponent when they are making choices of resource allocation and choices of cooperation, i.e. they reward those who have earlier benefited the self, or showed acts of generosity toward other individuals (Kenward and Dahl, 2011; Olson and Spelke, 2008). Typically developing children aged six to 12 years are also able to consider the morality of previous acts by an opponent in their cooperative choices (Li et al., 2014). By adulthood, neurotypical individuals seem to gain an ability to change their cooperative strategies flexibly, i.e. whether or not to cooperate, according to situational demands (Hill and Sally, 2003).

### 2.2.2 Cooperative abilities among children with ASD

As autism is characterized by extensive deficits in social communication and social interaction (DSM-5), one may assume that children with ASD also have impairments in cooperative behavior. Quite surprisingly, toddlers with ASD are able to show helping behavior at least in situations with simple goals, i.e. they are able to figure out another's goal and are motivated to help them to achieve it (Liebal et al., 2008). Some of them are able to perform even more complicated cooperative behavior that demands, e.g. an ability to share goals and intentions with another, performing respective roles, and coordinating actions in order to achieve a mutual goal in simple non-verbal tasks (Liebal et al., 2008). However, toddlers with ASD seem to succeed less often in cooperative tasks and orient less to a withdrawn opponent in order to maintain cooperative behavior when compared to toddlers with other developmental delays (Liebal et al., 2008). At school age, children with ASD are able to show an equal number of cooperative acts with typically developing children, but while they are able to make moral judgements in terms of nice and naughty acts, they seem to be impaired in exploiting the morality of the previous acts of an antagonist in cooperative choices, i.e. to cooperate more with nice rather than naughty peers (Dows and

Smith, 2004; Li et al., 2014; Schmitz et al., 2015). In addition, albeit in their pursuit of equality, children with ASD seem to be prone to choose unequal choices more often than typically developing children when their inequality is not related to harm to the self or another (Schmitz et al., 2015).

Earlier observations have shown that cooperative behavior among children with ASD seems to be determined by their joint attention and theory of mind skills (Colombi et al., 2009; Downs and Smith, 2004; Hill and Sally, 2003). These skills are reliably observed only among humans and provide remarkable benefits for effective cooperative communication among typically developed individuals (Hayes and Sanford, 2014; Moll and Tomasello, 2007; Nowak, 2006; Pennisi, 2009; Vogel, 2004; Warneken and Tomasello, 2006). Although human social interaction serves cooperative purposes to a remarkable degree, and cooperative behavior in turn seems to have an essential role in maintaining long-term relationships, cooperative behavior in ASD seems to be understudied (Paal and Pereczkey, 2007; Stallen and Sanfey, 2013; Takagishi et al., 2010; Vogel, 2004). Studies investigating associations between cooperation and ASD-related social impairments might have the potential to provide new insights into the difficulties that individuals with ASD may face in social relationships.

## 2.3 Aggressive behavior

### 2.3.1 Reinforcements behind aggressive behavior

According to evolutionary psychology, enhancement in cooperative behavior toward in-group members provided clear benefits, but it could not provide enough security from attacking out-group members in the ancestral environment. It is possible that readiness to act violently against out-group members co-evolved with cooperation, or even boosted the development of cooperative behavior in order to deal effectively with the threat caused by intergroup conflicts (Rusch, 2014; Van Vugt et al., 2007). Ancient social cooperative roles for males and females differed remarkably, and whereas males cooperated with in-group members in order to enhance defense toward out-group attacks, females formed close cooperative relationships with in-group female members in order to gain social and emotional support, e.g. for offspring rearing (Geary et al., 2003). Thus aggressive acts among males and females had

mainly different reinforcements –conflict with out-group members and resource allocation from in-group members, respectively (Geary et al., 2003). As severe violent behavior toward in-group members brought the risk of high costs for aggressive individuals, e.g. a risk of being excluded from a group, female aggressive behavior may have evolved to be less intense and have less severe consequences than male aggressive behavior (Geary et al., 2003).

Today, aggressive behavior may be adaptive, e.g. serve assertiveness and self-protection, or maladaptive, e.g. be destructive or hurtful (Bay-Hinitz et al., 1994; Dodge and Coie, 1987; Rosenzweig, 1977). Maladaptive aggression may be angry reactions to frustration, anger or threats (reactive aggression, also called impulsive, affective or hostile aggression) or planned and goal-directed actions in order to gain social or other valuable benefits (proactive aggression, also called predatory, instrumental, premeditated) (Bay-Hinitz et al., 1994; Dodge and Coie, 1987; Rosenzweig, 1977). Interestingly, even today boys with typical development seem to show more physical aggressive behavior than typically developing girls after toddlerhood, and to be more prone to engage in reactive aggression than girls, who instead more likely use aggressive behavior in order to gain social status or resources (Card et al., 2008; Loeber and Hay 1997; for negative results, see Pepler and Craig, 1995).

### 2.3.2 Typical development and maladaptive aggression

Every human is born with certain aggressive traits. In typical development, aggressive behavior peaks in prevalence during the first three years of life (Connor, 2002). Almost three out of four children with typical development show a modest or high amount of physical aggression between ages of 1.5 and 3.5 years (Tremblay et al., 2005). With age, children start to gain knowledge about the consequences (rewards and punishments) of aggressive behavior by observing and enacting with other people (Malamuth and Addison, 2002). In favorable cases, as a result of sensitive parenting and maturation of the central nervous system, the child's ability to regulate his/her aggressive behavior and emotions improves, leading to enhanced abilities to cope in social situations with less aggressive behavior (Calkins and Fox, 2002).

Some neurotypical children fail to achieve these abilities and remain overly aggressive. Several child-related factors predict a continuity of aggressive

behavior from infancy to later childhood, e.g. difficult temperament, low intelligence, reading problems, attention problems, the child's positive attitudes toward aggression, poor emotional regulation and recognition, impaired theory of mind skills, and callous-unemotional traits (e.g. lack of guilt, absence of empathy) (Best et al., 2002; Calkins and Fox, 2002; Crockenberg et al., 2008; Frick and White, 2008; Frith and Frith, 2005; Hage et al., 2009; Hoaken, 2007; Loeber and Hay, 1997; Pitzer et al., 2009; Tremblay et al., 2005; Walker, 2005). There is also a subgroup of neurotypical children who show low levels of aggressive behavior in infancy but start to show maladaptive aggressive behavior later in life (Loeber and Hay, 1997). The nature of aggressive behavior seems to change with growing age: older children seem to show more proactive aggression and less reactive aggression than younger children (Connor et al., 2004).

### 2.3.3 ASD and comorbid maladaptive aggression

In ASD, enhanced self-injurious behavior and maladaptive aggression can be observed at a similar level as among clinically referred non-ASD children with oppositional compulsive disorder or conduct disorder, at least when ASD is combined with intellectual disability (Dickerson Mayes et al., 2012; Farmer et al., 2015; Farmer and Aman, 2011; Fitzpatrick et al., 2016; Giacomo et al., 2016; Green et al., 2000; McClintock, 2003; Tsakanikos et al., 2007, for negative results, see Mahan and Matson, 2011). The prevalence of maladaptive aggressive behavior seems to be approximately 20-30% among children and adolescents with ASD, even though a prevalence up to almost 60% has also been observed (Dominick et al., 2007; Green et al., 2000; Hartley et al., 2008; Hill et al., 2014; Kanne and Mazurek, 2011; Ming et al., 2008, Presmanes Hill et al., 2014). In comparison, the prevalence for reactive aggression among typically developing school-aged children in Finland is estimated to be 5%, while the prevalence for proactive aggression is 9% (Mäki et al., 2010; Salmivalli and Nieminen, 2002).

Among children with ASD, comorbid maladaptive aggressive behavior seems to emerge during toddlerhood, but in some cases, later onset has also been reported (Dominick et al., 2007). Children with ASD seem to be prone to use mainly reactive aggression, e.g. react in frustrating situations with aggressive behavior (Farmer et al., 2015). When compared to non-ASD children with other

developmental and intellectual disabilities, children with ASD seem to use especially milder forms of aggressive behavior more often, i.e. pinching, biting and scratching, and head-butting behavior (Farmer and Aman, 2011). Even though the intensity of comorbid aggressive behavior may be predominantly mild among children with ASD, it may have a negative impact on their social functioning and relationships, as in ASD violent behavior seems to have a persistent and stable nature over development, seems to happen in multiple places, and seems to be targeted toward more than one person (Ambler et al., 2015; Dominick et al., 2007; Matson et al., 2010).

While the prevalence of aggression seems to hold a gender difference among typically developing children, the frequency of aggressive behavior in childhood or adolescence does not seem to differ between boys and girls with ASD (Farmer et al., 2015; Hartley et al., 2008; Kanne and Mazurek, 2011). However, the phenotype of aggressive behavior may be different among boys and girls with ASD, as suggested by Kopp and Gillberg (1992). Reese et al. (2005) observed that reinforcements behind aggressive behavior might differ between girls and boys with ASD. Whereas boys with ASD targeted disruptive behavior to secure repetitive behavior engagement or to avoid unpleasant sensory stimulation, the reinforcements behind disruptive behavior were similar among girls with ASD and typically developing girls and boys, including a need to gain attention and items, and a pursuit to escape demands in general (Reese et al., 2005).

### 2.3.4 Maladaptive aggression and cooperative behavior

Even though evolutionary psychology suggests that cooperative and aggressive behavior may have co-evolved, there are few studies that have studied the associations between cooperative and aggressive behavior in typical childhood. An association between maladaptive aggressive behavior and diminished engagement in cooperative activities can be observed already among three- to five-year-old children (Bay-Hinitz et al., 1994; Walker, 2005). However, at this age, co-occurrence of cooperative and aggressive behavior has also been observed among socially dominant children, and thus it seems that an association between cooperative and aggressive behavior is complex and may vary according to the reinforcements that behavior serves (Pellegrini et al., 2007). A study by Gallup et al. (2010) implies that an association between

maladaptive aggression and cooperation might hold a gender difference, at least in late adolescence. Whereas typically developing males previously involved in aggressive behavior toward peers seem to show diminished reciprocity and trustworthiness in cooperation, reduced trustworthiness in cooperation seems to be associated with earlier experiences of severe peer victimization among females with typical development (Gallup et al., 2010).

No study so far has investigated whether cooperative behavior and maladaptive aggressive behavior are associated among children with ASD. However, several other social functioning domains, e.g. adaptive behavior and cognitive and language performance, seem to positively relate to a decreased risk for aggressive behavior among children with ASD (Ambler et al., 2015; Boonen et al., 2014; Cervantes et al., 2013; Connor et al., 2004; Dominick et al., 2007; Hartley et al., 2008; Matson and Adams, 2014; Ming et al., 2008; Visser et al., 2014). Previous evidence of associations between ASD-related social impairments and aggressive behavior is ambiguous, showing no association in preschool age, and a positive association between maladaptive aggressive behavior and autism-related impairments in social behavior, communication, and repetitive behavior later in childhood (Dominick et al., 2007; Hartley et al., 2008; Kanne and Mazurek, 2011; Mazurek et al., 2013; de Giacomo et al., 2016; see Hill et al., 2014 for contradictory results). Especially social anxiety, anger rumination, fear of humiliation and social rejection seem to increase the risk for aggressive behavior among children with ASD (Ambler et al., 2015; Kanne and Mazurek, 2011; Matson and Rivet 2008; Pugliese et al., 2013, 2015).

## 2.4 Summary of literature review

In human evolution, cooperative skills, a readiness to act violently against assailants, and socio-cognitive skills, e.g. gaze behavior, likely co-evolved (Bickham, 2008; Moll and Tomasello, 2007; Nowak, 2006; Pennisi, 2009; Vogel, 2004; Warneken and Tomasello, 2006). It is possible that a need to enhance effective cooperative communication guided the development of the human social brain, and also the mechanisms that regulate autonomic arousal to a direct gaze evolved to enable eye contact engagement within this development process (Bickham, 2008; Emery, 2000; Skuse, 2003). As a result of this

evolutionary process, humans learned to cooperate and communicate in unique ways (Nowak, 2006; Pennisi, 2009; Vogel, 2004).

In ASD, impairments in social skills are persistent and even extensive, and may have a long-term negative impact on quality of life among individuals with ASD (DSM-5; Howlin and Moss, 2012; Lai et al., 2014). Social impairments in ASD concern also gaze behavior, and at least to some degree also cooperative abilities and the regulation of aggressive behavior (e.g. Chawarska et al., 2013; Courage et al., 2006; Dickerson Mayes et al., 2012; Farmer et al., 2015; Fitzpatrick et al., 2016; Giacomo et al., 2016; Li et al., 2014; Liebal et al., 2008; Pennisi, 2009; Schmitz et al., 2015). Previous evidence implies that in ASD, autonomic arousal to a direct gaze might be unregulated, which in turn might hamper eye contact engagement and social development (e.g. Dalton et al., 2005; Joseph et al., 2008; Kleinhans et al., 2009; Kylliäinen and Hietanen, 2006; Kylliäinen et al., 2012; Swartz et al., 2013). However, evidence of enhanced autonomic arousal (measured as skin conductance responses (SCR)) to a direct gaze among children with ASD is mixed, showing both equal and enhanced SCR to a direct gaze in comparison to SCR to other gaze stimuli (Joseph et al., 2008; Kylliäinen and Hietanen, 2006; Kylliäinen et al., 2012). Studies investigating possible associations between autonomic arousal to a direct gaze and ASD-related social impairments among children with ASD are scarce, but they have yielded preliminary evidence of inverse associations between enhanced autonomic arousal to a direct gaze (measured as SCR) and facial recognition skills, and between decreased arousal habituation to neutral and sad faces with a direct gaze (measured as amygdala activity) and autism severity (Joseph at al., 2008; Swartz et al., 2013). The present work aimed to extend the previous findings by studying both – autonomic arousal and response habituation to a direct gaze (measured as SCR) – and their associations to ASD-related social impairments among children with ASD.

Even though social impairments in ASD are extensive, studies investigating cooperative behavior among individuals are limited (Colombi et al., 2009; Downs and Smith, 2004; Hill and Sally, 2003; Li et al., 2014; Schmitz et al., 2015). Interestingly, the earlier findings have shown that basic cooperative abilities might not be hampered in ASD, at least among high-functioning individuals (Colombi et al., 2009; Downs and Smith, 2004; Hill and Sally, 2003; Li et al., 2014; Schmitz et al., 2015). The present study aimed to extend the

earlier findings by investigating the cooperative choices of children with ASD in situations where knowledge of a partner's cooperativeness varies. The present study also aimed to provide novel information of possible associations between cooperative behavior and reactive aggression among children with ASD. Previous evidence has shown an inverse association between these behaviors among neurotypical children, which supports the suggestion that the regulation of aggressive behavior might have co-evolved with cooperation (Bay-Hinitz et al., 1994; Gallup et al., 2010; Rusch, 2014; Van Vugt et al., 2007 Walker, 2005).

According to earlier findings, approximately 20-30% of children with ASD show comorbid reactive aggressive behavior (e.g. Hill et al., 2014; Kanne and Mazurek, 2011; Presmanes Hill et al., 2014). In order to provide effective interventions to reduce aggressive behavior in ASD, it is essential to recognize mechanisms that might yield enhanced reactive aggression. Previous studies have observed that heightened adaptive behavior and cognitive and language performance might decrease the risk for aggressive behavior among children with ASD (Ambler et al., 2015; Boonen et al., 2014; Cervantes et al., 2013; Connor et al., 2004; Dominick et al., 2007; Hartley et al., 2008; Matson and Adams, 2014; Ming et al., 2008; Visser et al., 2014). The present study aimed to yield novel information about the utilization of situational cues in the regulation of aggressive responses among children with ASD.

# 3 AIMS

The present dissertation aimed to explore autonomic arousal and response habituation to the direct gaze of a live person, cooperative behavior and regulation of reactive aggression, and their associations with social impairments among children with ASD. The specific aims were:

1. To study a) whether autonomic arousal – as measured by skin conductance responses (SCR) – to the direct gaze of a live person is greater among children with than without ASD, and b) whether the level of autonomic arousal to a direct gaze is associated with the level of social impairments among children with ASD (Study I).
2. To investigate a) whether the habituation of autonomic arousal responses to a repeated direct gaze stimulus is attenuated among children with ASD in comparison to children without ASD, and b) whether the level of autonomic response habituation to a direct gaze is associated with the level of social impairments among children with ASD (Study II).
3. To investigate a) whether boys and girls with ASD show a similar amount of cooperative behavior as boys and girls without ASD, and b) whether cooperative behavior is associated with the level of social impairments among boys and girls with ASD (Study III).
4. To explore a) whether boys and girls with ASD show enhanced responses of reactive aggression compared to boys and girls without ASD, and b) whether the level of reactive aggressive responses is related to the level of social impairments and/or cooperative behavior among boys and girls with ASD (Studies III and IV).

# 4 MATERIAL AND METHODS

## 4.1 Participants

### 4.1.1 Recruitment procedures

The children in the ASD groups were recruited from children with ASD who currently received or had previously received care at the Department of Child Psychiatry, Tampere University Hospital. The children in the control groups were recruited from local schools. The families of children with ASD and neurotypical children were contacted first with information letters providing basic information on the study. There were separate information letters for parents and children; the latter also included visual information on the study procedures. The families of neurotypical children were asked to express their interest in participating using letters that were returned via schoolteachers to the examiners. About one week after the information letters were sent an examiner contacted parents of children with ASD by phone to invite them to participate in the study. If a child with ASD was recruited, a neurotypical child of the same gender and most similar age was invited to participate in the study by phone call.

In all studies, children were considered to have ASD if they had been previously diagnosed with autism spectrum disorder (ASD) by experienced physicians using clinical procedures and according to the ICD-10 classification at the Department of Child Psychiatry, Tampere University Hospital. Exclusion criteria for children with ASD in all studies included the presence of a depression or anxiety diagnosis or a total IQ lower than 70.

The children in the control groups had no history of mental or neurological disorders or learning disabilities, i.e. they were typically developing, according to their parents. Exclusion criteria for children in the comparison groups included a total IQ lower than 70.

### 4.1.2 Studies I and II

Data collection took place between January 2007 and June 2007. The participants were 23 children with ASD and 21 typically developing children. The children with ASD were aged from eight to 16 years. Six of them had a diagnosis of childhood autism (F84.0), four had a diagnosis of atypical autism (F84.1), and 13 had a diagnosis of Asperger's syndrome (F84.5) (Table 1).

Skin conductance response (SCR) data from five children with ASD (three diagnosed with F84.0 and two with 84.5) and three typically developing children were excluded from the main analyses of the study; one boy with ASD and two typically developing boys were excluded due to technical problems in SCR data acquisition, two boys with ASD were excluded due to sleepiness, one boy with ASD due to a migraine attack, one girl with ASD due to restlessness during the SCR recordings, and one typically developing boy was excluded due to non-compliance during the direct gaze stimuli. After the exclusions, SCR data were available for 14 boys and 4 girls in both groups. Interview data used to measure impairments in social skills were missing for three boys with ASD (one diagnosed with F84.1 and two with F84.5) and two boys with typical development, as their parents did not participate in the Developmental, Dimensional and Diagnostic Interview (3di) (Skuse et al., 2004). After all exclusions, 15 children with ASD (11 boys and four girls) and 16 children without ASD (12 boys and four girls) had both the SCR and the 3di data (Figure 1).

### 4.1.3 Studies III and IV

Data collection took place between December 2008 and January 2009. The participants were 27 boys and eight girls with ASD and 35 typically developing children matched for gender, age and total score intelligence. The children with ASD were aged seven to 17 years old: seven children had a diagnosis of childhood autism (F84.0), six had a diagnosis of atypical autism (F84.1), and 22 had a diagnosis of Asperger's syndrome (F84.5). The matching criterion for age was +/- six months. The matching criterion for total IQ included that both children in the matched pair had to have a total IQ within the range of 80 to 120, or if one child in the matched pair scored a total IQ of 70-79 or over 120, the total IQ of the other child in the matched pair had to be within 30 units (but the

total IQ was always over 70). However, there was one boy pair where the age difference was 25 months. As the results in Studies III and IV were not changed after the exclusion of the pair from the analysis, the pair was not excluded from the sample (Table 1).

**Table 1.** Participant characteristics.

| | Studies I and II | | | Studies III and IV | | |
|---|---|---|---|---|---|---|
| | ASD | Controls | p | ASD | Controls | p |
| | (n = 23) | (n = 21) | | (n = 35) | (n = 35) | |
| Gender (n) | | | | | | |
| Boys | 18 | 17 | | 27 | 27 | |
| Girls | 5 | 4 | | 8 | 8 | |
| Age (mean (SD); min-max) | 12.0 (2.2); 8.1-15.6 | 12.1 (2.1); 8.5-15.9 | ns | 12.8 (2.4); 8.1-16.9 | 12.8 (2.4); 7.7-16.6 | ns |
| IQ (mean (SD); min-max) | | | | | | |
| Verbal | 101 (19); 69-145 | 105 (18); 71-137 | ns | 105 (13); 83-140 | 107 (18); 71-150 | ns |
| Performance | 94 (16); 56-119 | 99 (14); 66-122 | ns | 100 (20); 58-141 | 102 (13); 66-125 | ns |
| Total | 97 (13); 72-118 | 102 (15); 75-127 | ns | 102 (14); 77-138 | 104 (13); 75-127 | ns |
| Social impairments (*median; $Q_1$-$Q_3$*) | (n = 15) | (n = 16) | | (n = 34) | (n = 33) | |
| Reciprocal social interaction | *10.8; 8.5-17.6* | *3.7; 3.2-4.3* | < 0.001 | *13.9; 8.4-16.2* | *3.6; 3.0-4.5* | < 0.001 |
| Use of language and other social communication skills | *10.4; 8.0-14.1* | *1.4; 1.1-3.0* | < 0.001 | *12.4; 8.2-16.2* | *1.5; 1.0-2.7* | < 0.001 |
| Use of gesture and non-verbal play | *6.1; 4.7-8.8* | *1.1; 0.6-2.2* | < 0.001 | *7.5; 4.8-9.7* | *1.0; 0.5-2.1* | < 0.001 |
| Repetitive and stereotyped behavior | *4.0; 1.0-5.7* | *0.0; 0.0-0.2* | < 0.001 | *2.5; 0.9-5.4* | *0.0; 0.0-0.4* | < 0.001 |
| Social expressiveness | *1.8; 1.5-2.8* | *1.1; 0.9-1.6* | 0.025 | *1.9; 1.1-2.6* | *1.1; 0.9-1.4* | < 0.001 |

$Q_1$ = lower quartile
$Q_3$ = upper quartile

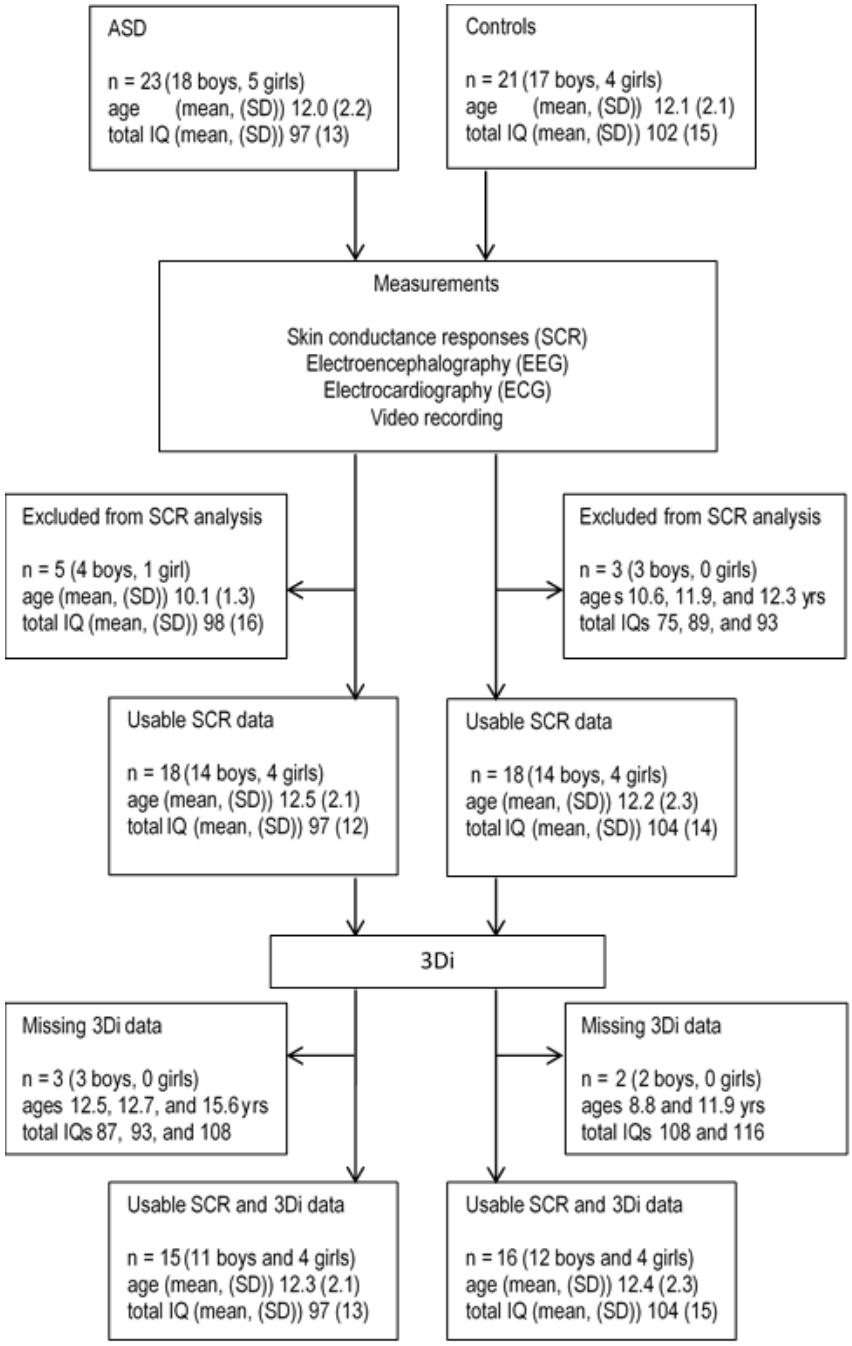


**Figure 1.** Subject flow chart of Studies I and II.

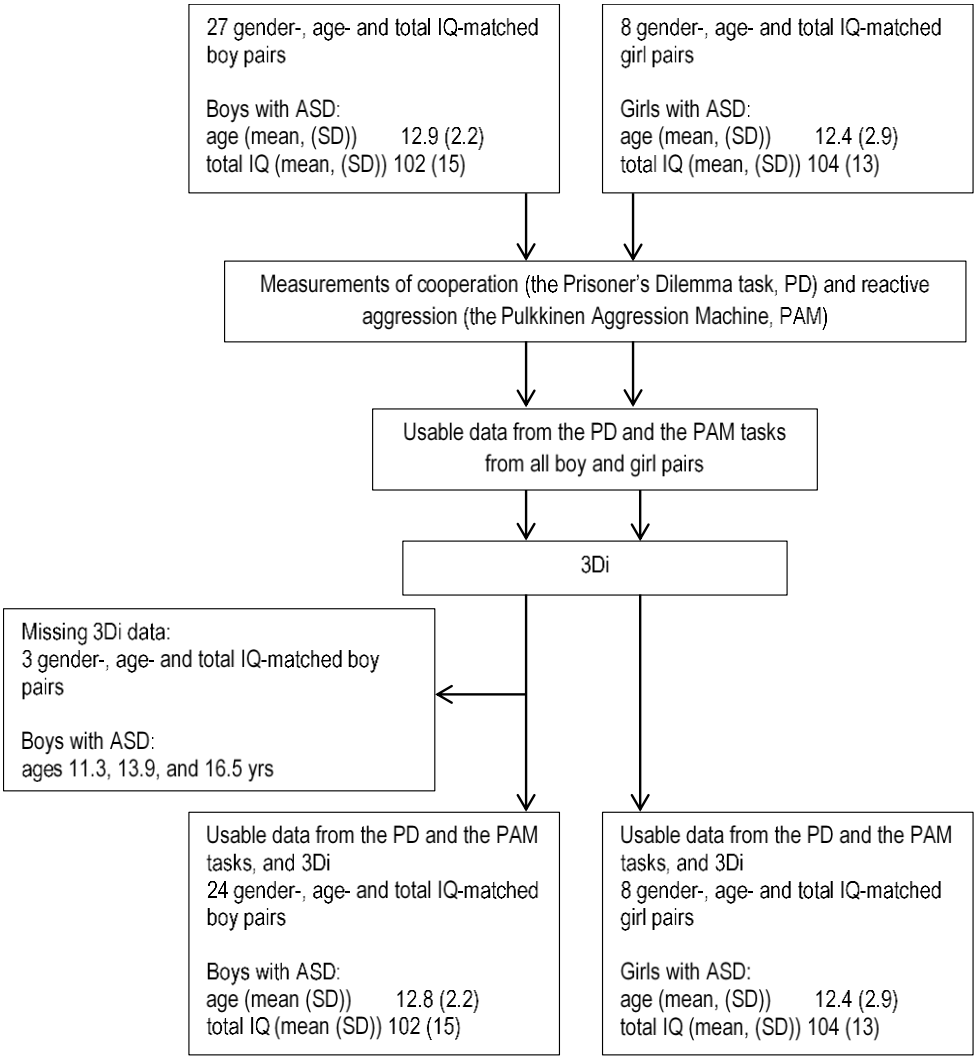


**Figure 2.** Subject flow chart of Studies III and IV.

The interview data (Developmental, Dimensional and Diagnostic Interview, 3di; Skuse et al., 2004) were missing for one boy with ASD and two boys without ASD as their parents did not participate in the interview, meaning that three boy pairs were excluded from further analysis (two of the boys with ASD had a diagnosis of F84.0 and one of F84.5). Thus, 24 boy pairs and eight girl pairs had the data for all measurements concerning cooperative behavior, reactive

aggression and social skills. Study IV included both the boy and girl participants, whereas Study III included only the boy participants (Figure 2).

## 4.2 Methods and procedures

### 4.2.1 Social impairments in ASD

The parents of the children were interviewed according to the Developmental, Dimensional and Diagnostic Interview (3di) (Skuse et al., 2004). The 3di is a computerized, standardized interview that was primarily developed to assess autistic symptoms, but it also covers other ICD-10 child psychiatric diagnostic categories to assess a full range of comorbidity. The interview includes 183 questions concerning demography, family background, developmental history, and motor skills; 266 questions concerning direct or indirect disorders of the autistic spectrum; and 291 questions that relate to current mental states and are relevant to other child psychiatric diagnoses. Besides recording answers to the structured questions, the interviewer can add unstructured information as written text if necessary. Immediately after the assessment, a computer generates a structured report including also all the possible unstructured answers. The concurrent validity of 3di (agreement with independent clinician formulation) seems to be very good, the criterion validity (a comparison with the Autism Diagnostic Interview) is excellent, and it has a sensitivity of 1.0 and a specificity of >0.97 (Skuse, 2004). The Finnish version of the interview was created (Kaija Puura) together with the original developer of the instrument (David Skuse). For the present work, five subscales from the interview's PDD (Pervasive Developmental Disorder) scale were used to measure impairments in social skills. These subscales (scale range in parentheses) are Reciprocal Social Interaction (0-30), Use of Language and Other Social Communication Skills (0-26), Use of Gesture and Non-verbal Play (0-14), Repetitive and Stereotyped Behaviour (0-12), and Social Expressiveness (0-4).

### 4.2.2 Autonomic arousal and response habituation to a direct gaze

Autonomic arousal responses were studied by measuring skin conductance responses (SCR) while participants saw the face of a female model or a control

stimulus (a vase) through a voltage-sensitive liquid crystal shutter window (Figure 3). The facial stimuli and the control stimulus were presented in two separate blocks. Half of the children in the ASD and comparison groups saw the block of the facial stimuli first and then the block of the control stimulus, while the other half saw the stimuli in reverse order.

Two young adult females of the same ethnicity as the participants modeled for the facial stimuli. The females modeling for the live face stimuli were not aware of the clinical status of the child. The model for the facial stimuli was the same throughout the whole experiment. The model's gaze direction was either direct or averted (left and right), or the model's eyes were closed. In the averted gaze condition, the gaze was 30° to the left or right. In each condition, the face was oriented directly toward the participant and bore a neutral expression. The model tried to avoid blinking. Stimuli were presented through a voltage-sensitive liquid crystal shutter (LC-TEC Displays AB) (30 cm width x 40 cm height) attached to a white panel between the participant and the model. The shutter could be changed between an opaque and a transparent state within a millisecond. The participant sat in a chair 70 cm away from the shutter and the model sat in a chair 40 cm away from the shutter, yielding a distance of 110 cm between the participant and the model. The model was positioned on the same level as the participant before the experiment started. The control stimulus was a 30cm high curved vase with two round handles on both sides, and it was located at the same height as the facial stimulus.

In the experiment, each of the three facial conditions (a direct gaze, an averted gaze and closed eyes) was presented six times in random order and the control stimulus was presented six times in a separate block. Thus, in total, each participant underwent 24 trials in the experiment. Each trial lasted for 5 seconds and the inter-stimulus interval (ISI) varied from 20 to 35 seconds. During the ISI, the voltage-sensitive liquid crystal shutter was opaque. The presentation of the stimuli was controlled with NeuroScan Stim software. The experimenter initiated every trial by pressing a button on a remote control, and was able to stop the experiment at any time without causing the experiment to fail. Before each trial, in order to confirm that the participant directed his/her attention toward the shutter and to prepare the model for the opening of the shutter, the experimenter said "Let's look at the next one!" [In Finnish "Katsotaan seuraava!"] After each facial stimulus presentation, the experimenter asked the

participant whether the model gazed directly ahead, sideways or whether the model's eyes were shut to be sure that the participant had looked at the facial stimulus and was able to distinguish the different gaze conditions. During the control stimulus block, the participants just viewed the stimulus.

**Figure 3.** Examples of the facial stimuli and the control stimulus used in the skin conductance measurements.

Before the experiment, picture cards were used to introduce the laboratory to the participant, and together with his/her parent, the participant signed a written consent form. The participants were told that during the experiment physiological signals would be measured while the participant performed a task in which they would view the face of a model through a window. The participant was asked to avoid large-scale movements during the experiment and to look at the stimuli when presented. Before each block in the experiment, verbal instructions were given to the participant with the assistance of picture cards and an example of each stimulus was shown by opening the shutter. The data collection included electroencephalography (EEG) and electrocardiography (ECG) measurements. The experiment started with a 4-minute period of baseline EEG activity recording, during which the participant was asked to keep his/her eyes closed and open in 30-second turns. After a short break, the participant saw the stimuli in two blocks as described above.

During the experiment, the experimenter sat behind the participant to be able to make observations of the participant's possible movements and also the possible movements and blinks of the model. In addition, in order to investigate whether the time the children viewed the stimuli differed between the children with ASD and the control children, the participants' eye movements were recorded during the experiment using a video camcorder. To eliminate any possible effects that video recording might have on autonomic arousal, the children were not

informed of the recording beforehand. The video camcorder was placed behind a partition next to the shutter. The recordings were made through a small hole in the partition, and due to this setup, opportunities to adjust the position of the video camcorder according to the height or sitting position of the participant were extremely limited. There were also technical difficulties in the recordings. As a result, the eye movements were recorded for only ten children with ASD and 17 control children. Furthermore, data from the recording had to be excluded if the participant changed his/her body position during the recordings so that his/her eyes were not sufficiently visible on the videotape. After exclusions, satisfactory video data were available for five boys with ASD (three with a diagnosis of 84.1 and two with F84.5; age (mean (SD)) 12.4 (2.5); total IQ (mean (SD)) 88 (13)) and for 13 boys without ASD (age (mean (SD)) 11.9 (2.1); total IQ (mean (SD)) 106 (14)). The gazing behavior during the stimulus presentation was coded with the Queen's video coder program (Baron et al., 2001). Each stimulus trial was analyzed frame by frame (0 = not looking at the stimulus, 1 = looking toward the stimulus). Thirty percent of the videos were rated by two independent researchers in order to calculate interrater reliability. Inter-rater reliability between the two independent researchers in the video coding of the looking time, analyzed by bivariate correlations, was excellent ($r = 0.96$, $p < 0.05$).

Skin conductance responses (SCR) were recorded from the left hand with two Ag-AgCl electrodes (diameter 8 mm) that were filled with paste and attached to the palmar surface of the medial phalanxes of the index and middle fingers (Lykken and Venables, 1971). The signal was acquired with a GSR amplifier supplying constant-voltage AC excitation (22 mV) (AD Instruments). Skin conductance was measured by Power Lab 400 equipment and collected by the Power Lab Chart v3.6 computer program running on a Power Macintosh 7100/80 computer. The system was calibrated prior to each session to detect activity in the range of 0–40 microSiemens (µS). The data sampling rate was 100 Hz.

In Study I, the skin conductance response (SCR) was defined as the maximum amplitude change from baseline (the level of electrodermal activity, EDA, at stimulus onset) between 1 s and 3.5 s from the stimulus onset. Changes of 0.05 µS or more were considered responses, whereas smaller changes were not considered responses and were marked as a zero response (Andreassi, 2006). In

Study II, all measured values of EDA between 0s and 5s were used to investigate habituation. SCR data contaminated by the child's body movements or technical problems with the measurements were excluded from the subsequent analysis. Due to this, 9% of trials in the group of children with ASD and 5% of trials in the control group were discarded. There was no significant difference between the groups in the percentage of eliminated trials (Mann–Whitney, $p = 0.440$).

### 4.2.3 Cooperation

Cooperation was measured with a variant of the Prisoner's dilemma (PD) task adapted from Herrmann et al. (2010), which combines a simultaneously played PD task with a strategy method. The first part of the experiment consisted of two conditional decisions (the strategy method) where the child had to decide whether he/she wants to cooperate or defect knowing that the other child has either 1) cooperated or 2) defected. The second part of the experiment consisted of an unconditional decision where the child had to make his/her decision without knowing the actual decision of his/her (gender-, age- and total IQ-matched) pair. The compensation for the PD task depended only on the unconditional decision, and it was calculated by combining the unconditional decision of the child with that of the matched pair. The following outcomes were possible: 1) if both participants cooperated with one another, both of them ended up with two tokens; 2) if one participant defected but the other cooperated, the non-cooperator ended up with three tokens and the co-operator ended up with none; 3) in the case of mutual defection, both participants ended up with one token (Study III, Figure 1). Afterwards, the tokens were exchanged for small prizes, e.g. comic books, that were worth approximately five euros.

Before starting the actual game, the experimenter – a registered nurse (EP) – introduced the game with verbal and visual instructions to the child, and informed that another child of the same age and gender would conduct the same PD task at a different time. The participants were informed that the task is played with imaginary tokens and that every participant will be given one token at the beginning of each of the three conditions of the PD task. They were instructed that in the game they would need to decide whether they would like to give their token to an unknown child or to keep it themselves. The participants were informed that the actual game played with another child would

only concern the unconditional condition and that the compensation for the game depended only on his/her and on the other child's decision in that condition, and that the tokens would be exchanged for small prizes afterwards. After this instruction, the nurse verbally and with picture cards presented all four possible outcomes of the game (keep-keep, give-keep, keep-give, and give-give) in random order and questioned the child about the compensation for each of the players in each case to confirm that the child clearly understood the rules and the outcomes of the game before the actual game was played. In addition, in each condition the nurse again went through the possible choices and their outcomes before the child gave an answer (Study III, Figure 1).

All participants were tested individually, without the opponent being present. This was done to avoid possible immediate or delayed punishment behavior toward the defecting matched pair. Playing against an imaginary opponent has been found to correlate strongly with a playing in a situation where an opponent is present (Knight and Kagan, 1977). The experiments were conducted either at Tampere University Hospital or at the home of the participant, according to the choice of the participants' parents. During the tasks, the children were seated in front of a laptop computer in a silent room, with only an experimenter present. She seated herself so that she could not see the responses chosen by the child. This was done in order to minimize any possible influence of her presence on the child's choices in the tasks.

### 4.2.4 Reactive aggression

Reactive aggression was measured by the Pulkkinen Aggression Machine (PAM), which is a computerized task designed to study the inhibition of reactive aggression in the absence and presence of situational cues (Juujärvi et al., 2001).The PAM task includes three conditions that are presented in the following fixed order: (I) the arbitrary condition, (II) the impulsive aggression condition, and (III) the controlled aggression condition. In every condition, two parallel columns of eight stimulus and nine response icons are shown respectively on the left and the right sides of the computer screen. In the arbitrary condition, the icons contain neutral black dots and there is no aggressive content in the stimulus or in the response icons. In the impulsive and controlled aggression condition, the stimulus icons represent violent aggressive acts of increasing degrees of intensity, while the response icons represent

retaliatory acts of aggression, also of increasing intensity. The levels of stimulus intensity are scaled as follows: row 0 = a harmless interaction, row 1 = you are slightly pushed, row 2 = you are pinched, row 3 = you are slapped, row 4 = you are knocked to the ground, row 5 = your hair is being pulled, row 6 = you are hit with a stick, and row 7 = you are punched in the face. The response icons are parallel to the stimulus icons with one exception: one icon is added on to the extreme end of the response scale to deal with possible ceiling effects (i.e. row 8 = the assailant is kicked while lying on the ground). The delivery of an attack is marked as a rectangle appearing around one of the stimulus icons and requires the participant to determine his/her response in retaliation. Stimulus presentation is self-paced, so that the generation of a response triggers the next stimulus at a constant interval of three seconds after the last response. Responses are given by clicking with a mouse one of the response icons on the right side of the computer screen.

The arbitrary condition serves as a training condition. Each stimulus icon is delivered once in the following predetermined order: 3-5-4-2-7-0-1-6.

In the impulsive aggression condition, participants are instructed to defend themselves against offensive attacks, as follows: “You are having a quarrel with somebody. A black rectangle around one of the icons on the left shows what the other person does to you. You may do to him or to her what you wish by touching one of the icons on the right and you do not need to worry about the consequences of your choice.” Participants are free to imagine the assailant. Each stimulus icon is delivered twice in a predetermined order: 3-5-4-2-7-0-1-6-2-5-7-3-1-4-6-0.

The controlled aggression condition is similar to the impulsive aggression condition except that the assailant is now identified (Figure 4). The identity of the assailant is presented as a picture in the center of the screen, and participants are told to imagine how they would behave in a real confrontation with the assailant. There are eight categories of assailants— a boy of the same size, a girl of the same size, a smaller boy, a smaller girl, a bigger boy, a bigger girl, a father and a mother. The assailants are presented in random order. Besides a picture on the screen, the participants are instructed verbally by the experimenter about the size and the gender of assailants. For each assailant, all

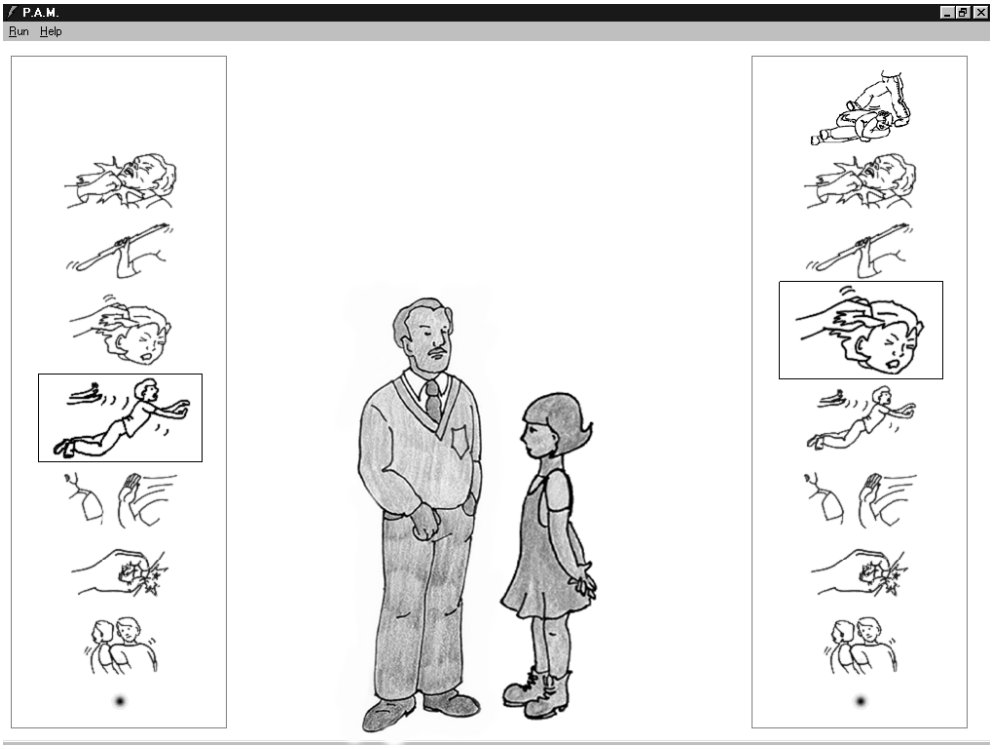


**Figure 4.** A screenshot of the controlled aggression condition of the Pulkkinen Aggression Machine (PAM).

eight stimulus icons are presented twice in the same fixed order as in the impulsive aggression condition.

### 4.2.5 Intelligence

In all the studies, the cognitive and verbal intelligence of the children with ASD was tested with WISC-III (Wechsler Intelligence Scale for Children, Third Edition) as a normal part of a clinical evaluation. The typically developing children had their IQ estimated by WISC-III during the same session in which the other data collection took place or at another session, depending on the schedules of their families.

## 4.3 Statistical methods

In all the studies, descriptive statistics were presented as means (M) and standard deviations (SD) for normally distributed continuous variables, as medians (Md) and quartiles ($Q_1$, $Q_3$) for other continuous variables, or as percentage frequencies for categorical variables. For comparing groups, statistical tests were used, as appropriate, according to the normality of the probability distribution of the measured variables and the relatedness of the samples (e.g. age-, gender- and IQ-matched groups in Studies III and IV). Student's *t*-test was used to analyze differences between the groups in age and total, verbal and performance IQs in Studies I and II. In Studies III and IV, Wilcoxon matched pairs test was used to analyze differences in age, IQ and social skills between the children with ASD and their gender-, age- and total IQ-matched pairs. In all studies, the limit for statistical significance was set at 0.05, but values up to 0.10 were reported and considered as indicative.

In Study I, bivariate Pearson correlations were used to analyze the inter-rater reliability in the video coding. The non-parametric Friedman test, which analyzes differences between dependent continuous variables, was used to investigate the possible effects of gaze direction on the stimulus looking times. Possible differences between the groups in the stimulus looking times were analyzed with the Mann–Whitney test separately for each stimulus condition. To analyze autonomic arousal to gaze stimuli, the mean values of SCR for a direct gaze, an averted gaze and closed eyes were computed separately for each individual. As there was a markedly positive skewing of the SCR distributions and in many cases the participants' responses within a condition showed wide intra-individual variation, the analysis was also based on the probability that, for each participant, SCR to a given gaze condition would be greater than SCR to the other two conditions. To study how often a direct gaze (D) elicited a greater SCR than averted (A) and closed eyes (C), a new measure, $P_D$, was created. For each participant, consider all possible triples of measurements D, A and C. (Most often, the number of triples is 6*6*6 = 216.) Then $P_D$ = the proportion of triples for which D>max(A,C). Similarly $P_A$ = the proportion of triples, for which A>max(D, C), and $P_C$ = the proportion of triples, for which C>max(D, A). Note that the new variables are assigned values between 0 and 1, and depend on original measurements only through their ranks. The Kruskall–Wallis test was used in Study I to investigate the possible effects of gaze direction on

SCR, but the Friedman test was used to re-analyze the data in order to take into account dependence between variables. Possible differences between the children with and without ASD in the probability for SCR to a direct gaze being greater than SCR to an averted gaze and closed eyes ($P_D$) was studied with the Mann–Whitney test. The correlation analyses (Kendall's tau-b coefficient) were separately run between the individual mean SCR values for a direct gaze, an averted gaze, and closed eyes and the variables of social impairments, and between $P_D$, $P_A$, $P_C$ and the variables of social impairments.

In Study II, to address habituation, a random coefficients linear mixed model was fitted to the logarithmically transformed SCR data assuming child-specific random intercepts and slopes, as it enables a flexible and realistic modeling of the mean and covariance structure across repeated measurements. Overall habituation to the facial stimuli was investigated in a time-ordered sequence of 18 measurements, with time and stimulus as fixed effects. Habituation for each gaze direction stimulus separately was investigated in a sequence of six measurements with time as a fixed effect. A negative slope represented habituation, whereas a positive slope represented enhancement of the SCR over stimulus repetitions. In order to determine differences between the children with ASD and control children, appropriate Wald tests accompanied with the Kenward-Roger adjustment were used to test equalities in group-wise slopes. Participant-specific slopes (best linear unbiased prediction; BLUP) were extracted from the models as quantities measuring the tendency for habituation, and they were then correlated with the social impairment measures of the child (Spearman's correlation coefficient).

Possible associations between mean SCR values for a direct gaze, $P_D$ and habituation slopes to a direct gaze stimulus were studied with the correlation analyses (Kendall's tau-b coefficient).

In Study III, to assess cooperative behavior in the Prisoner's dilemma task, the response of the child was coded (1) "cooperate" if the child decided to give the token or (2) "defect" if the child decided to keep the token. Variables representing all three choices of cooperation were generated; i.e. one variable for the conditioned "other keeps" decision, a second for the conditioned "other gives" decision and a third for the unconditioned decision. McNemar's test was used when age-, gender- and IQ-matched pairs were assessed in terms of one

dichotomous variable (cooperative choice). The modified Wald method (Agresti and Coull, 1998) was used to study confidence intervals for the probability of contrasting cooperative choices in the matched pairs.

In Study IV, variables of reactive aggression representing stimulus intensities were generated by first calculating a difference score by subtracting the stimulus intensity score from the respective response intensity score for each stimulus-response pair. Then the arithmetic mean of the differences of the two presentations of each pair (henceforth called response intensity) was used as the representative of the respective stimulus-response pair in further calculations. To study the participant's level of reactive aggression in the impulsive and controlled reactive aggression condition, one variable for the overall response intensity of impulsive aggression and another for overall controlled reactive aggression were generated. The variable value for impulsive aggression was calculated as an arithmetic mean of response intensities across all eight stimulus levels (i.e. from 0 to 7), and the variable value for controlled aggression was calculated similarly across both all eight stimuli and eight assailants. In order to study low and high levels of attack intensity separately, the above-mentioned variables were recalculated for minor attacks (i.e. stimulus levels 0 to 3: 0 = no provocation, 1 = a slight push, 2 = pinching, and 3 = slapping) and for major attacks (i.e. levels 4 to 7; 4 = knock to the ground, 5 = pulling hair, 6 = hitting with a stick, and 7 = punching in the face).

In Study IV, as the variable values for the reactive aggression had a tendency to be skewed to the right and could not be normalized even by a square root or logarithmic transformation, the nonparametric Wilcoxon test was used to investigate possible differences in the intensity of reactive aggression between the gender-, age- and total IQ-matched pairs.

In Studies III and IV, to investigate social impairments' associations with cooperation and reactive aggression, an individual social impairment score was generated for every matched pair by subtracting the social impairment scores of the boy with ASD from the social impairment scores from an age-, gender- and IQ-matched control. The Mann–Whitney test was used to investigate possible differences between the groups of cooperators and non-cooperators in the social impairment scores and in the intensity of reactive aggression. Possible correlations between impulsive and controlled reactive aggression and the social

impairment score were investigated with a non-parametric correlation analysis (Kendall's tau-b coefficient).

All statistical analyses were carried out using R (Software environment for statistical computing and graphics, version 2.5.1, The R Foundation for Statistical Computing, Vienna, Austria) and SPSS (versions 17.0-23.0, SPSS Inc., Chicago, Illinois, USA).

## 4.4 Ethics

All studies were approved by the Ethics Committee of the Pirkanmaa Hospital District. In all studies, participants and their parents received information concerning the methods and aims of the study and gave their written informed consent. The participants were informed beforehand that they have a right to refuse to participate in the study, and to withdraw from the participation at any time without need to declare any reason for withdrawal, and further that possible withdrawal would not be followed by any sanctions. They were also informed that their care was unrelated to participation in the study. In Studies I and II, participants afterwards received two cinema tickets and in Studies III and IV one cinema ticket as compensation for their participation.

# 5 SUMMARY OF RESULTS

## 5.1 Participant characteristics

In Studies I and II, the children with ASD and without ASD showed no statistically significant differences in chronological age, total IQ, verbal IQ, and performance IQ. The children with ASD showed significantly greater impairments in social skills than the children without ASD, as expected (Table 1).

In Studies III and IV, the boys with ASD and without ASD and the girls with ASD and without ASD did not differ from each other in chronological age, total IQ, or performance IQ. However, the difference between the boys with ASD and without ASD in verbal intelligence was indicative ($p$ = 0.082). Both the boys and the girls with ASD showed significantly more social impairments than their gender-, age- and total IQ-matched peers, as expected (Table 1).

## 5.2 Autonomic arousal and arousal response habituation to a direct gaze, and their associations with impairments in social skills (Studies I and II)

There were no statistically significant differences in either group regarding the time the children looked at each of three gaze direction stimuli. The median looking times for direct gaze, averted gaze, and closed eyes stimuli were 4.32 s, 4.38 s, and 4.62 s, respectively, among the children with ASD (n = 5), and 4.86 s, 4.78 s, and 4.74 s among the children without ASD (n = 13). The groups did not differ significantly from each other in the stimulus looking times in any stimulus condition, either. However, as the sample size was small, especially in the group of children with ASD, the analysis might be underpowered to show statistically significant differences between the groups.

Both the children with and without ASD were able to perform the identification of gaze direction almost faultlessly. In the entire data set, only one wrong answer to the question regarding the model's gaze direction was given, and this was given by a child with ASD.

### 5.2.1 Autonomic arousal to facial stimuli

The children with ASD (n = 15) seemed to show a lower level of overall autonomic arousal (SCR averaged over all facial stimuli) during the presentation of facial stimuli than the children without ASD (n = 16), but this difference was only indicative (Mann–Whitney, $p = 0.072$).

All three stimulus types – a direct gaze, an averted gaze and closed eyes – seemed to have a similar effect on the mean SCR when analyzed across all participants ($p = 0.111$), and among the children with ASD ($p = 0.288$) and without ASD ($p = 0.167$) with the Kruskall–Wallis test. However, when the data was re-analyzed with the Friedman test, the analysis showed that a direct gaze elicited a greater level of autonomic arousal (mean SCR) than an averted gaze or closed eyes among all participants ($p = 0.008$), and this difference also was indicative among the children with ($p = 0.065$) and without ASD ($p = 0.086$) (I, Figure 1).

No statistically significant differences were observed between the children with (n = 15) and without ASD (n = 16) in the probability of SCR to a direct gaze being greater than SCR to an averted gaze and closed eyes ($P_D$) (I, Figure 2).

### 5.2.2 Autonomic response habituation to a direct gaze

Among the children with ASD (n = 18), an overall SCR habituation to repeated presentations of facial stimuli, i.e. habituation analyzed across all 18 experimental trials of faces with three different gaze directions, was not observed. A further analysis conducted for each gaze direction separately showed that the SCR did not habituate in response to repetitions of direct or averted gaze stimuli, but the response habituation to closed eyes stimuli was indicative ($p = 0.080$) (II, Figure 1).

In the control group (n = 18), there was an overall habituation of the SCR to repeated presentations of facial stimuli ($p$ = 0.048). Further analysis revealed that even though the SCR habituation was observed to direct gaze stimuli ($p$ = 0.025), and indicatively to closed eyes stimuli ($p$ = 0.061), there was no habituation to averted gaze stimuli. However, the difference between the SCR habituation to direct gaze, averted gaze, and closed eyes stimuli was only indicative ($p$ = 0.097) (II, Figure 1).

Despite the seemingly different SCR habituation patterns to facial stimuli among the children with and without ASD shown above, a direct comparison of the group-wise habituation slopes did not yield evidence of significantly different overall SCR habituation between the groups. In addition, between-group comparisons for habituation to each gaze direction separately showed no statistically significant differences in SCR habituation to direct gaze, averted gaze or closed eyes stimuli.

### 5.2.3 Associations between autonomic arousal and response habituation to a direct gaze

The autonomic arousal variables mean SCR to a direct gaze and the probability of SCR to a direct gaze being greater than SCR to an averted gaze and closed eyes ($P_D$) showed significant positive correlations (Kendall's tau-b) in the ASD (n = 15) and control (n = 16) groups ($r$ = 0.542; $p$ = 0.006 and $r$ = 0.635; $p$ = 0.001, respectively). In addition, the probability of SCR to a direct gaze being greater than SCR to an averted gaze and closed eyes ($P_D$) was observed to have a marginally significant positive correlation with the habituation slopes of the SCR to repetitions of a direct gaze, but only among children with ASD (n = 15) ($r$ = 0.356; $p$ = 0.066).

### 5.2.4 Associations between impairments in social skills and autonomic arousal and response habituation to a direct gaze

Correlation analyses (Kendall's tau-b) showed no evidence that the mean SCR to a direct gaze, an averted gaze or closed eyes would be associated with the level of social impairments in either of the groups (Table 2). However, when the correlation analyses were based on the probability that SCR to a given gaze

condition would be greater than SCR to the other two conditions, significant positive correlations were observed between $P_D$ and two social skill impairment variables, Use of Language and Other Social Communication Skills and Use of Gesture and Non-verbal Play (identical correlations with both variables, $r$ = 0.55, $p$ = 0.005) among children with ASD (n = 15). The correlations between $P_D$ and the other variables of social impairments were indicative among the children with ASD ($r$ = 0.35-0.36, $p$ = 0.066-0.074) (Table 2). There were no observations of significant correlations between $P_A$ or $P_C$ and the social skills impairment variables. Among the children without ASD (n = 16) there were no correlations between $P_D$, $P_A$ or $P_C$ and the social skill impairment variables.

In order to investigate whether social impairments were associated with SCR habituation, the slopes of the SCR to repetitions of facial stimuli were correlated (Spearman's correlation) with social impairment scores. It should be noted that a negative slope represents habituation, whereas a positive slope represents enhancement of the SCRs over stimulus repetitions. When the analyses were conducted for each gaze direction separately, among the children with ASD (n = 15), the SCR habituation to a direct gaze was inversely associated with impairments in the Use of Language and Other Social Communication Skills ($r$ = 0.64; $p$ = 0.010), Use of Gesture and Non-verbal Play ($r$ = 0.55; $p$ = 0.032), and Social Expressiveness ($r$ = 0.61; $p$ = 0.015), i.e. attenuated SCR habituation to a direct gaze was related to greater social impairments (Table 2). SCR habituation to an averted gaze or closed eyes was not observed to be associated with impairments on any subscale of social skills among the children with ASD.

Among the children without ASD (n = 16), SCR habituation to a direct gaze or closed eyes did not correlate with any social skill impairment. However, SCR habituation to an averted gaze correlated positively with impairments in Use of Language and Other Social Communication Skills ($r$ = -0.59; $p$ = 0.016), Use of Gesture and Non-verbal Play ($r$ = -0.58; $p$ = 0.019), in Social Expressiveness ($r$ = -0.61; $p$ = 0.012), and indicatively with impairments in Reciprocal Social Interaction ($r$ = -0.46; $p$ = 0.076), i.e. a greater SCR habituation to an averted gaze was associated with more social impairments among the typically developing children (II, Table 3).

**Table 2.** Correlations (Kendall's tau-b) between mean skin conductance responses (SCR) to a direct gaze, the probability that a direct gaze evokes stronger SCR than an averted gaze and closed eyes ($P_D$), SCR habituation slopes of a direct gaze stimulus, and impairments in social skills among children with ASD (n = 15) and children without ASD (n = 16) (statistically significant correlations bolded).

| | Mean SCR to a direct gaze | | Probability that a direct gaze evokes stronger SCR than other gaze stimuli | | SCR habituation slopes of a direct gaze stimulus | |
|---|---|---|---|---|---|---|
| | ASD | Controls | ASD | Controls | ASD | Controls |
| Reciprocal Social Interaction | 0.29 | 0.07 | 0.36 | 0.17 | 0.41 | 0.25 |
| Use of Language and Other Social Communication Skills | 0.29 | 0.24 | **0.55** | 0.14 | **0.64** | -0.34 |
| Use of Gesture and Non-verbal Play | 0.27 | 0.22 | **0.55** | 0.10 | **0.55** | -0.38 |
| Repetitive and Stereotyped Behaviour | 0.24 | 0.11 | 0.35 | -0.01 | 0.07 | -0.01 |
| Social Expressiveness | 0.29 | -0.01 | 0.36 | -0.03 | **0.61** | 0.06 |

## 5.3 Cooperation and its relations to impairments in social skills (Study III)

### 5.3.1 Cooperative behavior

There was no evidence that the boys with ASD (n = 27) would cooperate less frequently in any of the three decisions in the Prisoner's dilemma task than their gender-, age- and total IQ-matched pairs (n = 27). The majority of boys with ASD (n = 22) and boys without ASD (n = 23) chose to cooperate with a known co-operator, i.e. preferred a maximal mutual payoff over maximal individual gain. In addition, all boys with ASD and the majority of boys without ASD (n = 23) choose not to cooperate with a known non-cooperator, which implies that they have the ability to make a strategic choice instead of self-sacrificing. When a choice of cooperation had to be made without knowing the choice of the opponent (the unconditional decision), the majority of boys with ASD (n = 18)

and a minority of boys without ASD (n = 11) did not cooperate. For further details of the cooperativeness of the matched pairs in the three conditions, see Table 3.

Even though the boys with ASD did not cooperate less frequently than the boys without ASD, there was a tendency for the choices of pair members to be opposite. In the choice of whether or not to cooperate with a known cooperator (the conditional decision of the PD task), in 26% (7/27) of boy pairs the choices of the boy with ASD and the boy without ASD were opposite (the 99% confidence interval for the proportion of matched pairs showing contrasting cooperative choices in the decisions was 10-51%). When the choice of cooperation had to be made without knowledge of the matched pair's choice (the unconditional decision), in 56% (15/27) of the boy pairs the choices of the matched pairs were opposite of each other's (the 99% confidence interval for the proportion of matched pairs showing contrasting cooperative choices in the decisions was 32-76%, respectively). (Table 3)

The results concerning cooperative choices among the girls with ASD are presented here as preliminary, due to the small number of girl participants (n = 8). For the same reason, differences between the girls with ASD and their gender-, age- and total IQ-matched peers in cooperative choices could not be studied.

In the choice of whether or not to cooperate with a known cooperator, seven of the girls in the both groups cooperated, i.e. showed an ability to prefer mutual maximum gain instead of pursuing maximized individual payoff. None of the girls with ASD or without ASD showed self-sacrifice behavior, i.e. would cooperate when they knew that an opponent had decided not to cooperate. When the actual game was played, i.e. the choice of cooperation needed to be made without knowing the choice of the matched opponent (the unconditional decision), two girls with ASD and three without ASD cooperated (Table 3).

**Table 3.** Distribution (number of cases) of cooperative choices of boys (n = 27) and girls (n = 8) with ASD and their gender-, IQ- and age-matched pairs.

| | Boys | | | | Girls | | | |
|---|---|---|---|---|---|---|---|---|
| | ASD cooperator | | ASD non-cooperator | | ASD cooperator | | ASD non-cooperator | |
| | matched pair | | matched pair | | matched pair | | matched pair | |
| | cooperator | non-cooperator | cooperator | non-cooperator | cooperator | non-cooperator | cooperator | non-cooperator |
| | n | n | n | n | n | n | n | n |
| Conditional, with a known cooperator | 19 | 3 | 4 | 1 | 7 | 0 | 0 | 1 |
| Conditional, with a known non-cooperator | 0 | 0 | 4 | 23 | 0 | 0 | 0 | 8 |
| Unconditional | 5 | 4 | 11 | 7 | 0 | 2 | 3 | 3 |

When cooperating with a known cooperator (the conditional decision), the cooperative and non-cooperative boys with ASD did not statistically differ from each other in age. Younger boys with ASD were less likely than older boys with ASD to cooperate with an unknown cooperator (the unconditional decision).The median ages of the non-cooperative and the cooperative boys with ASD were 11.8 years and 14.5 years, respectively ($p < 0.001$) (Figure 5).

The levels of total, verbal and performance intelligence were not observed to relate to cooperativeness when cooperating with a known cooperator (the conditional decision) or when cooperating with an unknown cooperator (the unconditional decision) among the boys with ASD.

Due to the small sample size, a similar statistical analysis concerning associations between cooperation, age and intelligence was not appropriate among the girls with ASD.

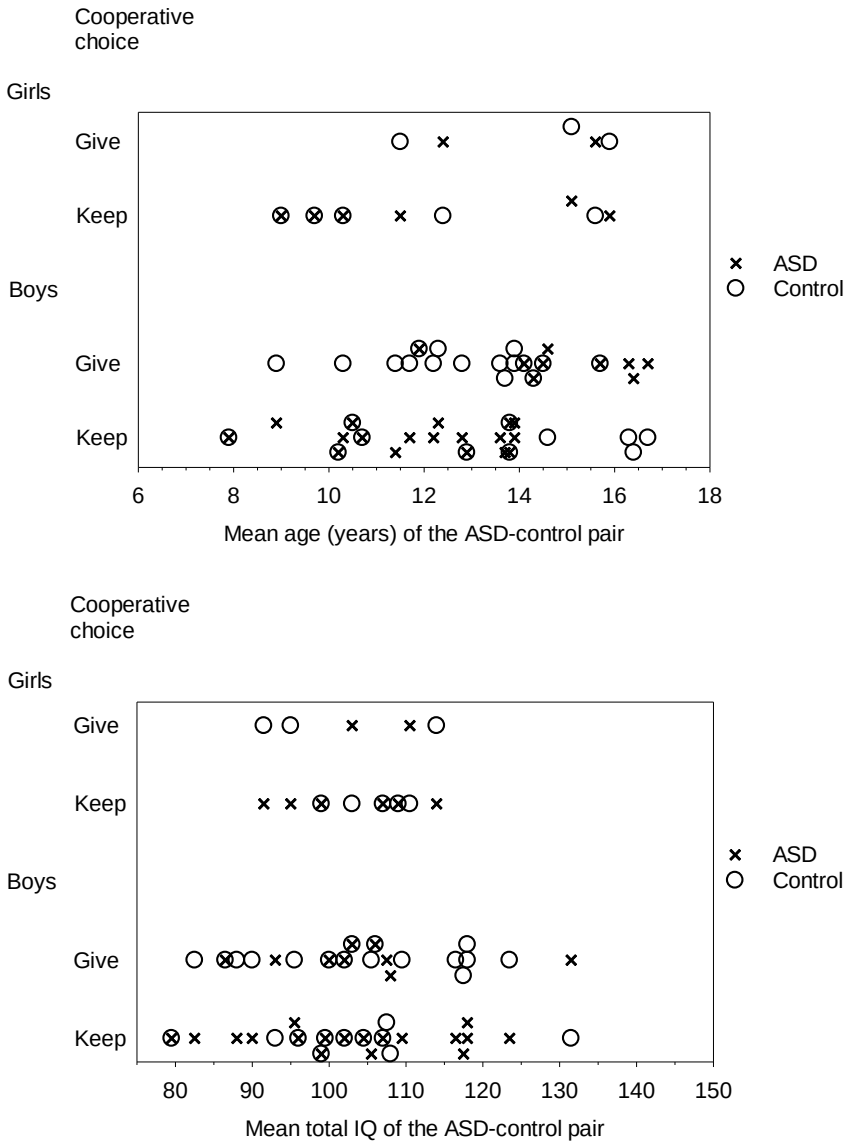


**Figure 5.** Distribution of matched pairs' cooperative choices in relation to the mean age and mean total intelligence of the ASD-control pair.

### 5.3.2 Associations between cooperation and impairments in social skills

In all three decisions of cooperation, the cooperative and the non-cooperative boys with ASD showed similar levels of social impairments in all subscales of 3Di. Due to small sample size, a similar statistical analysis was not conducted among the girls with ASD.

## 5.4 Reactive aggression and its associations with social impairments and cooperation (Studies III and IV)

### 5.4.1 Reactive aggression in the impulsive aggression condition

In the impulsive aggression condition where an assailant was not specified, the boys with and without ASD responded with equal intensity to the full range of attacks and to major provocations. However, the boys with ASD (n = 27) seemed to respond to the minor attacks with slightly more intense aggression than the boys without ASD (n = 27), but the difference between the groups was statistically only indicative ($p = 0.060$) (Figure 6). Further analysis revealed that while harmless interactions and slight pushes provoked equal reactions among all the boys, the boys with ASD reacted significantly more intensively to slaps ($p = 0.013$), and indicatively to pinches ($p = 0.084$) than the boys without ASD (Figure 7).

The girls with ASD (n = 8) and without ASD (n = 8) showed similar response intensities to the full range of attacks – minor and major – in the impulsive aggression condition (Figure 6), although that the girls with ASD responded with a lower intensity of aggression to slight pushes than their age-, gender- and IQ-matched peers ($p = 0.039$) (Figure 7).

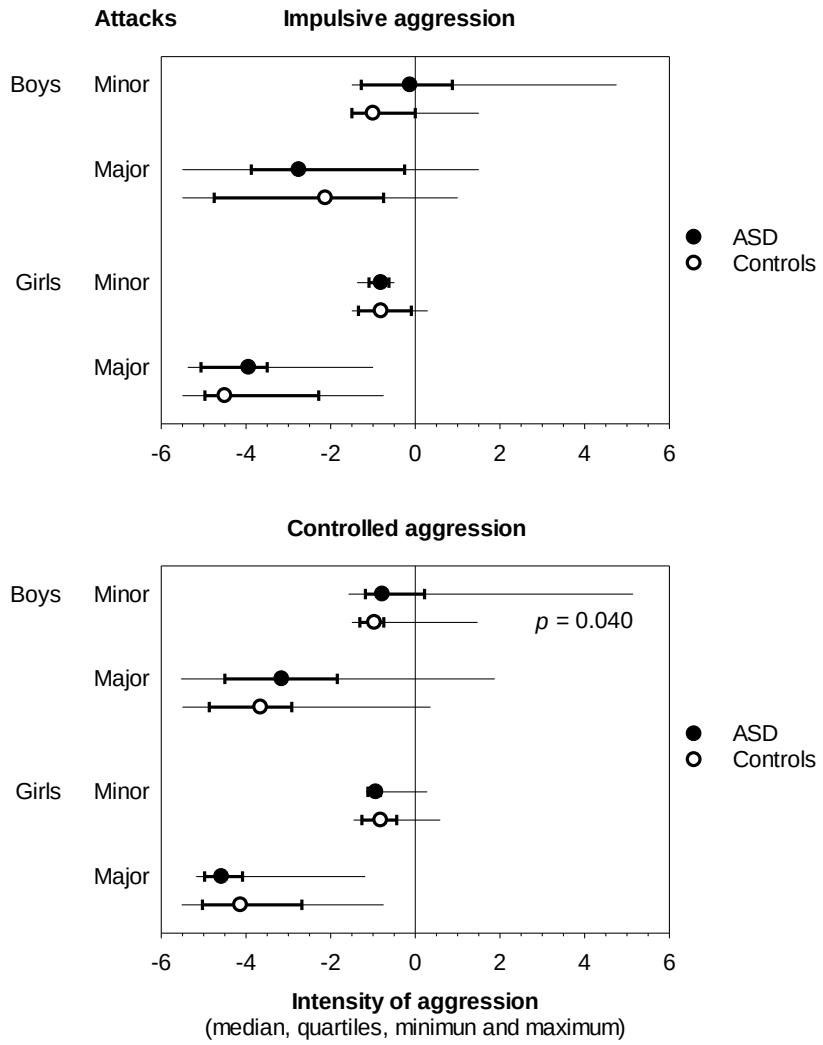


**Figure 6.** Intensity of reactive aggression in the Pulkkinen Aggression Machine (PAM) among the participants.

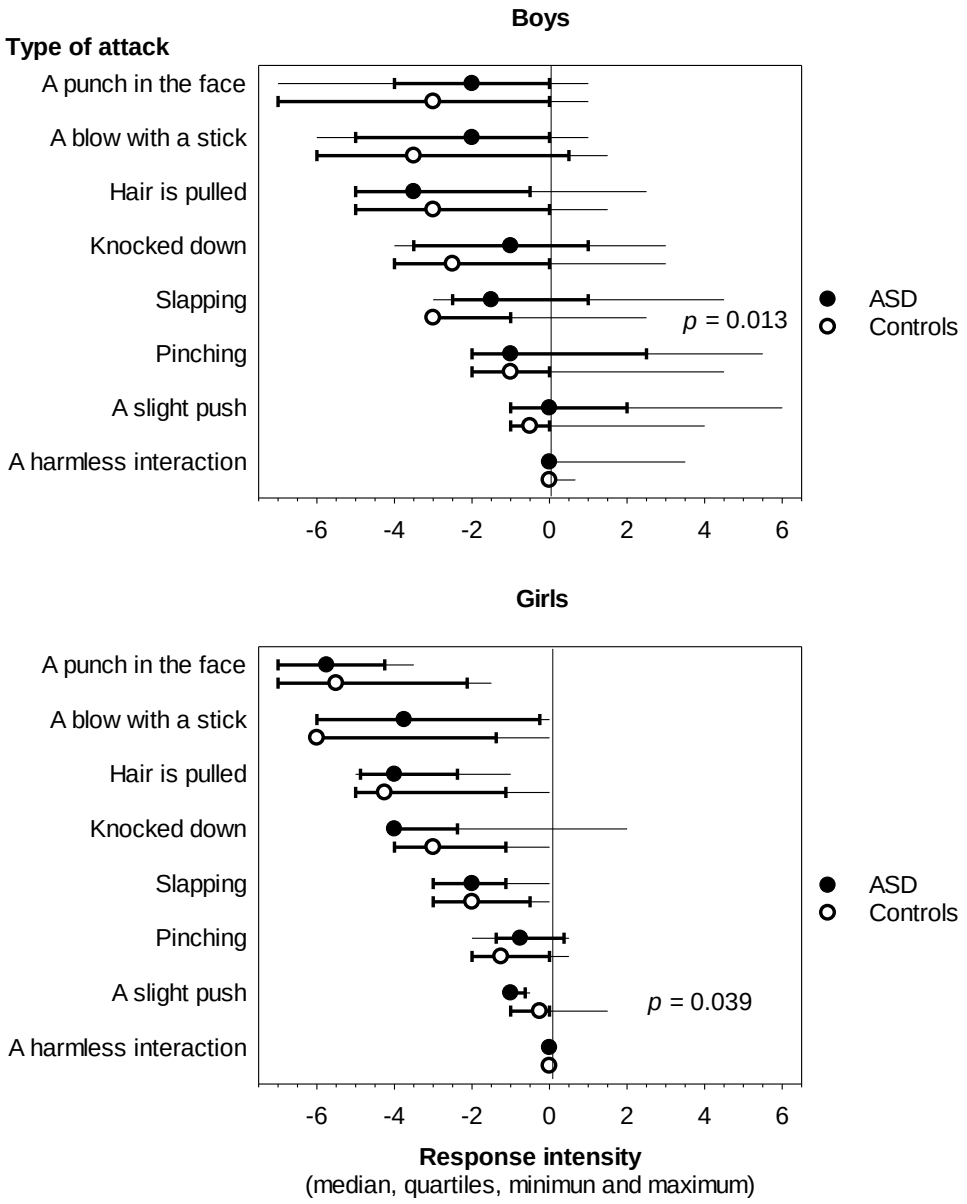


**Figure 7.** Intensity of reactive aggression responses to attack stimuli in the impulsive aggression condition.

### 5.4.2 Reactive aggression in the controlled aggression condition

In the controlled aggression condition where assailants were specified, the findings resembled the impulsive aggression condition, as the boys with ASD (n = 27) responded with more intense reactive aggression than the boys without ASD (n = 27) to the minor attacks ($p = 0.040$), and with equal intensity levels as the boys without ASD to the full range of attacks and major attacks (Figure 6).

A further analysis revealed that the boys with ASD reacted statistically significantly with more intense aggression than the boys without ASD to attacks from a same-sized, a smaller and a bigger child of the opposite gender ($p = 0.004$, $p = 0.007$ and $p = 0.012$, respectively) (Figure 8). Similar observations of more intensive responses among the boys with ASD compared to the matched peers were also made in the case of minor attacks from a same-sized, a smaller and a bigger child of the opposite gender ($p = 0.009$, $p = 0.001$ and $p = 0.002$, respectively) and in the case of major attacks from a same-sized and a smaller child of the opposite gender ($p = 0.008$ and $p = 0.019$ respectively) (Figures 9 and 10). The boys with ASD seemed to respond with a higher intensity of aggressive responses against major provocations by a bigger child of the opposite gender, but the difference between the boys with and without ASD was only indicative ($p = 0.058$) (Figure 10).

The girls with ASD (n = 8), on the other hand, did not differ significantly from their gender-, age- and total IQ-matched controls (n = 8) in response intensity in the full range of attacks and minor or major attacks in the controlled aggression condition (Figure 6). The comparisons between the girls with and without ASD showed that the physical strength and gender of the assailant affected the response intensity similarly in both groups of girls (Figures 8-10). The only difference between the girls with and without ASD was observed in major attacks from a same-sized child of the opposite gender, where the girls with ASD responded with a lower intensity than the girls without ASD ($p = 0.043$) (Figure 10).

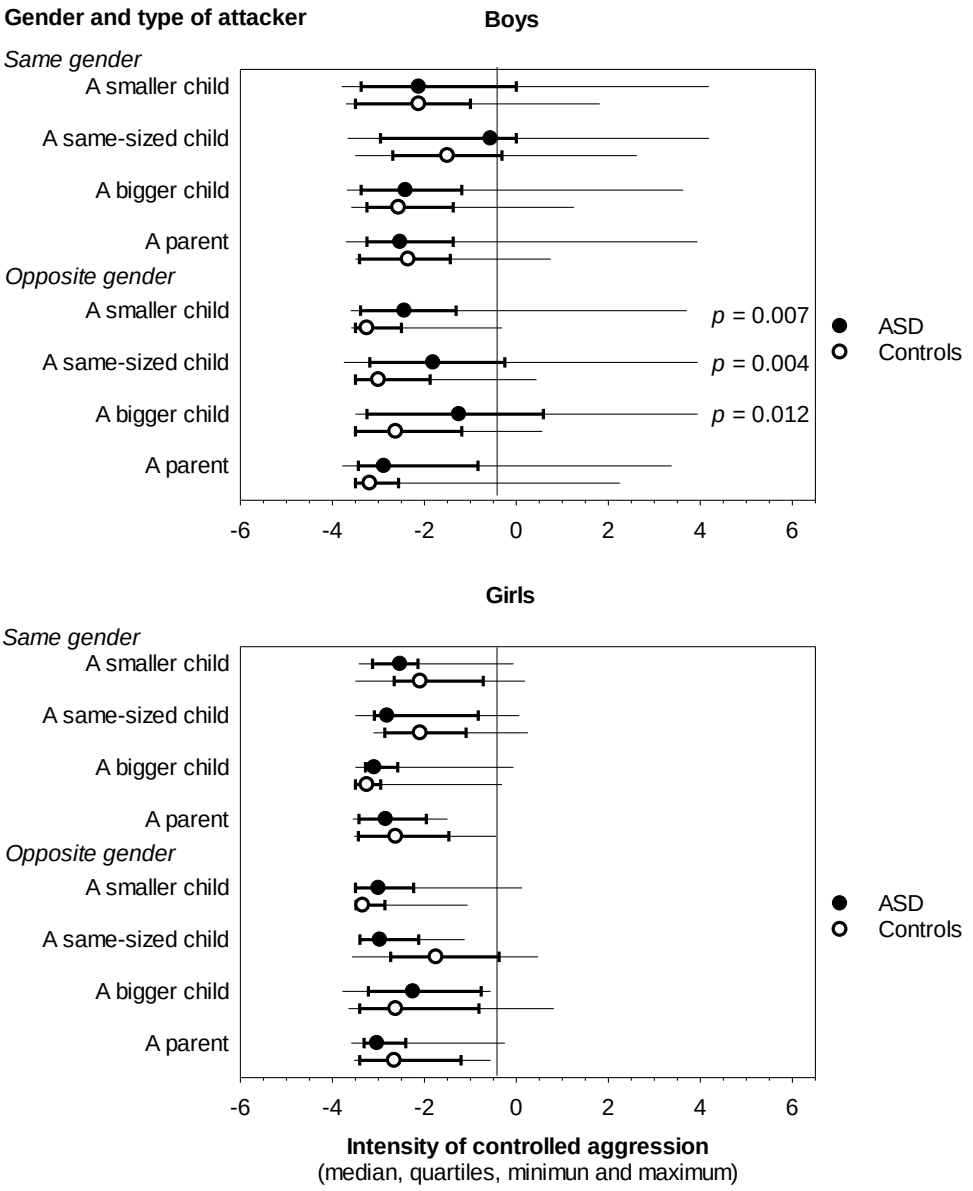


**Figure 8.** Intensity of reactive aggression responses to the attacks in the controlled aggression condition.

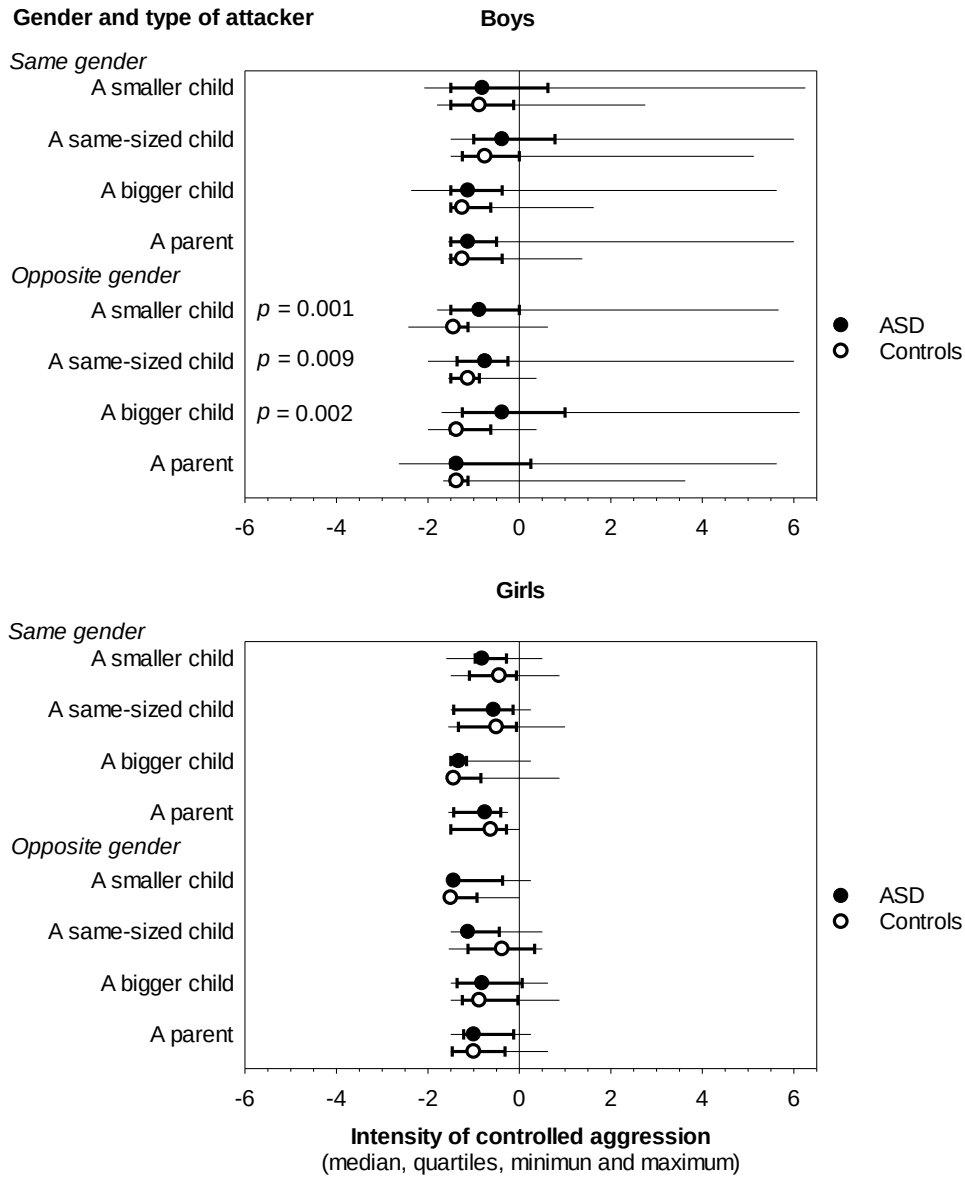


**Figure 9.** Intensity of reactive aggression responses to the minor attacks in the controlled aggression condition.

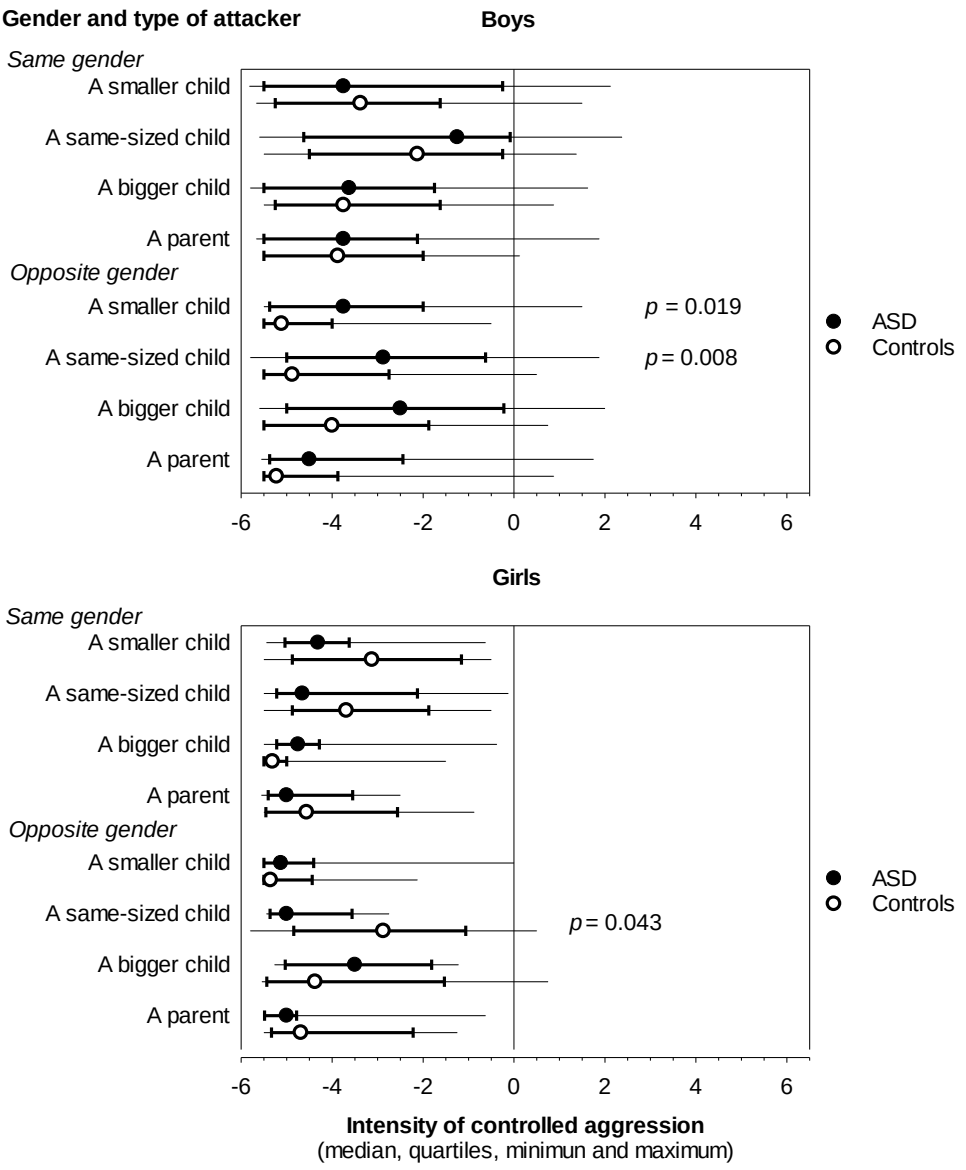


**Figure 10.** Intensity of reactive aggression responses to the major attacks in the controlled aggression condition.

### 5.4.3 Associations between reactive aggression and impairments in social skills

Impairments in social skills were not associated with the level of reactive aggression in either of the impulsive or the controlled aggression situation among the boys with ASD (n = 24) or the girls with ASD (n = 8).

### 5.4.4 Associations between reactive aggression and cooperation

None of the boys with ASD cooperated with a known non-cooperator (i.e. in a conditional decision of the PD task). Consequently, it was not possible to investigate the associations between cooperative and aggressive choices in that conditional decision.

When cooperativeness was based on a decision whether or not to cooperate with a known cooperator (a conditional decision of the PD task), the cooperative (n = 22) and the non-cooperative boys (n = 5) with ASD did not statistically differ in the level of reactive aggression in the controlled aggression condition of PAM. However, a further analysis revealed that the non-cooperative boys with ASD did show a higher level of reactive aggression than the cooperative boys with ASD in the impulsive aggressive situation in the PAM task ($p$ = 0.039) (III, Figure 3). The non-cooperative boys with ASD responded with greater aggression in PAM to attacks of minor intensity ($p$ = 0.015), and indicatively also to higher intensity attacks ($p$ = 0.096) when compared to the cooperative boys with ASD (Figure 11).

When the choice of cooperation was made without knowing what the opponent would do (the unconditional decision of the PD task), the non-cooperative boys with ASD (n = 9) showed a greater level of reactive aggression than the cooperative boys with ASD (n = 18) in the impulsive condition in the PAM task ($p$ = 0.002), and the difference between the cooperators and the non-cooperators was indicative even in the controlled condition in the PAM task ($p$ = 0.085) (III, Figure 3). The non-cooperative boys with ASD responded with a greater level of reactive aggression than the cooperative boys with ASD to minor attacks in both impulsive and controlled conditions ($p$ = 0.006 and $p$ = 0.020, respectively), and to major attacks in the impulsive condition ($p$ = 0.002) (Figure 11).

In addition, a combined analysis of all independently significant predictors of cooperative behavior, i.e. aggression and age, was carried out. Binary logistic regression analyses, with aggression as the only explanatory variable and non-cooperativeness as the reference category, also showed that a greater level of aggression in the impulsive aggression condition significantly predicted non-cooperativeness with an unknown cooperator (OR = 2.1, 95% CI =1.1-4.1, $p$ = 0.032). The odds ratio for the level of reactive aggression in the controlled condition in the PAM task was quite similar compared to the impulsive aggression condition, although not statistically significant (OR = 1.8, CI = 0.8-3.7, $p$ = 0.128). When age, which was observed to relate to cooperativeness among the boys with ASD, was added as an explanatory variable, it was found to be a significant predictor of non-cooperativeness in both models (III, Table 3). The greater level of reactive aggression in the impulsive condition did not remain a significant predictor of non-cooperativeness after adding age as an explanatory variable in the model.

Due to the small sample size, a similar statistical analysis was not appropriate for the girls with ASD.

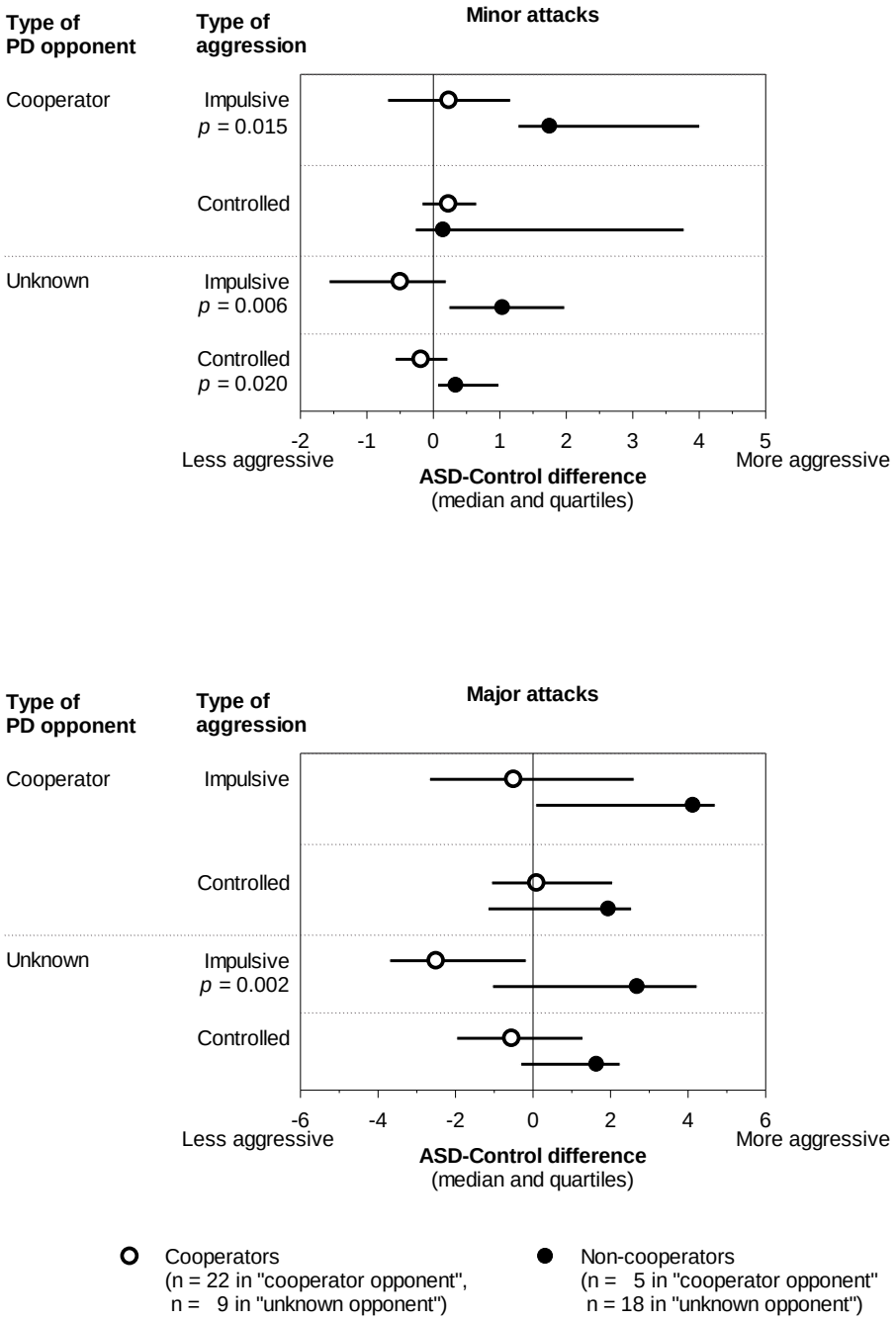


**Figure 11.** Associations between cooperative choices in the Prisoner's dilemma task and aggressive responses to minor and major attacks in the Pulkkinen Aggression Machine among the boys with autism spectrum disorder (ASD).

## 5.5 Summary of findings

Studies I and II did not find evidence that children with ASD in general would show enhanced autonomic arousal or attenuated autonomic response habituation to a direct gaze, as they did not differ from neurotypical children in the level of autonomic arousal or in the level of autonomic response habituation to a direct gaze. When autonomic arousal was determined as arousal responses averaged across trials, autonomic arousal to a direct gaze was not observed to be associated with any social skill impairment. However, when autonomic arousal to a direct gaze was studied as a probability that a direct gaze would evoke stronger skin conductance responses (SCR) than an averted gaze and closed eyes, statistically significant positive correlations were observed between this probability and impairments in Use of Language and Other Social Communication Skills, and Use of Gesture and Non-verbal Play, but only among the children with ASD (Study I). Further analysis also revealed an inverse association between the level of autonomic arousal response habituation to a direct gaze and the level of impairments in social skills among the children with ASD (Study II). A similar association was not observed between social impairments and autonomic response habituation to any other gaze conditions among the children with ASD (Figure 12).

The Study III did not find evidence that the boys with ASD would be impaired in reciprocating in a simple cooperative dilemma. The majority of boys with ASD followed the rule of equity in their cooperative choices when cooperating with a known cooperator when their choice of non-cooperation would represent harm to another. In addition, all the boys with ASD were also able to protect themselves from inequity by withdrawing from cooperation instead of self-sacrificing by cooperating with a known non-cooperator. When the choice of cooperation was made without knowing the choice of the opponent, the boys with ASD did not cooperate less frequently than the boys without ASD. However, when choices of cooperation were investigated within the gender-, age- and total IQ-matched pairs, there was a tendency for the cooperative choices of the boy with ASD and his matched pair to be opposite, especially when they were cooperating with an unknown cooperator. Cooperativeness seemed to increase with age among the boys with ASD, but the level of social impairments was not related to the choices of cooperation. Due to the small

sample size, possible differences in cooperativeness between the girls with and without ASD could not be examined.

In Study IV, the boys with ASD were able to regulate their aggressive responses according to the varying intensity of major provocations and the varying size of the assailant similarly to the boys without ASD. However, they seemed to respond with a higher level of aggression than the boys without ASD when they were faced with minor aggressive attacks or an assailant of the opposite gender. The girls with ASD instead showed similar – or even attenuated – responses of reactive aggression to all attacks and regulated their responses according to situational cues (gender and size of an assailant) similar to the girls without ASD.

Autism severity, determined as the level of impairments in social skills, did not relate to the cooperative choices in the PD task or the level of reactive aggression responses in the PAM task among the boys with ASD (Study III). However, non-cooperativeness in the PD task seemed to be related to a higher level of reactive aggression in the PAM task, especially when the choice of cooperation was made without knowing the opponent's choice (Study III) (Figure 12).

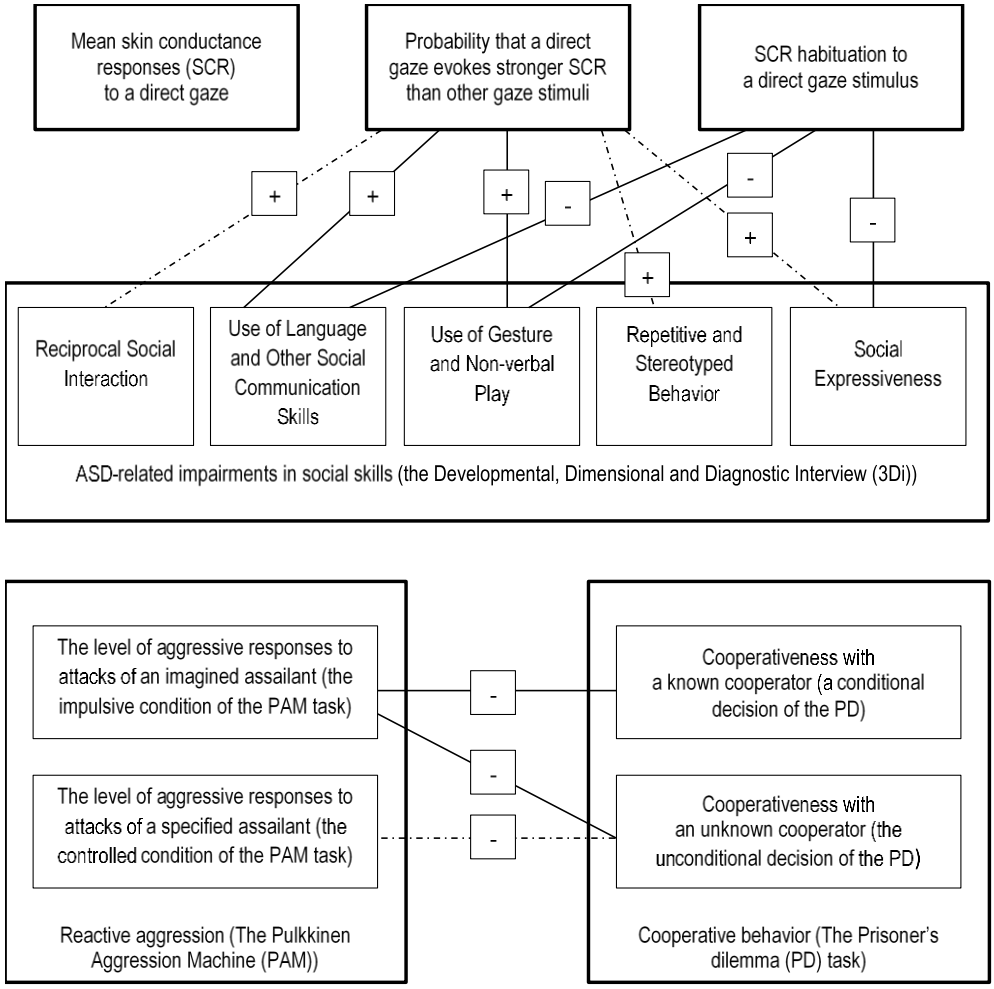


**Figure 12.** Summary of relations between autonomic arousal to a direct gaze, cooperative behavior, reactive aggression and impairments in social skills among children with ASD.

# 6 DISCUSSION

## 6.1 Autonomic arousal and response habituation to a direct gaze

It has been suggested (Hutt and Ounsted, 1966; Skuse, 2003) that regulation of amygdala-induced autonomic arousal to a direct gaze enables eye contact engagement among humans. Attenuated engagement in eye contact is one of the earliest symptoms of ASD, and one mechanism that could have the potential to cause this early arising impairment in ASD is enhanced autonomic arousal to a direct gaze (Bryson et al., 2007; Hutt and Ounsted, 1966; Osterling and Dawson, 1994; Sigman et al., 1992; Skuse, 2003; Trillingsgaard et al., 2005; Zwaigenbaum et al., 2005). Study I did not find evidence that autonomic responses to a direct gaze are enhanced among children with ASD when compared to neurotypical children. Furthermore, while it seemed that autonomic response habituation to facial stimuli could be observed only among children without ASD, the children with and without ASD did not differ statistically significantly from each other in response habituation to facial stimuli overall or to any gaze stimuli (direct gaze, averted gaze, closed eyes) (Study II).

In the present work, when the children with and without ASD were combined, a greater level of autonomic arousal (mean SCR) to a direct gaze was observed in comparison to an averted gaze or closed eyes presented on a live face. This finding was only marginally significant when groups were analyzed separately. Joseph et al. (2008) did not find evidence of enhanced autonomic responses to direct gaze in relation to averted gaze either among children with or without ASD when they used computerized gaze stimuli. It is possible that the discrepancy between the findings could be explained by observations implying that greater autonomic arousal to a direct gaze in comparison to autonomic arousal to other gaze directions can be observed among neurotypical adults only when live gaze stimuli are used (Hietanen et al., 2008; Pönkänen and Hietanen, 2012; Pönkänen et al., 2011). Contrasting this suggestion, studies by Kylliäinen and Hietanen (2006) and Kylliäinen et al. (2012) showed with computerized stimuli that autonomic responses to a direct gaze were enhanced in comparison

to an averted gaze and closed eyes among children with ASD, but not among neurotypical children. It is possible that the stimuli used in studies by Kylliäinen and Hietanen (2006) and Kylliäinen et al. (2012) were especially arousing, as they were looming pictures of faces that may have given the impression of an approaching person. It is also possible that the participants in the present study and in the studies by Joseph et al. (2008), Kylliäinen and Hietanen (2006), and Kylliäinen et al. (2012) were unevenly selected from different subgroups of ASD that are suggested to show different developmental courses (Bryson et al., 2007; Jones and Klin, 2013; Nacewicz, 2006; Wass et al., 2015). Particularly when sample sizes are small, as in the above-mentioned studies, differences in participant selection have the potential to affect results and cause contradictory findings between studies.

According to the present results, enhanced autonomic arousal and attenuated response habituation to a direct gaze do not seem to be core deficits in ASD, i.e. they are not observed among all children with ASD. However, it should be noted that in Studies I and II, the participants' fixation time to eye region was not monitored. An earlier study by Dalton et al. (2005) showed that the level of amygdala activity was positively associated with eye fixation time in ASD. Thus, it is possible that some children with ASD and some without ASD only gazed at the facial stimuli or shortened their fixation time on the eye region of the live model in order to regulate the level of autonomic arousal. Future studies combining measurements with eye tracking are needed to investigate autonomic arousal and response habituation to a direct gaze in relation to gaze behavior in ASD.

In Study II, the level of response habituation to a direct gaze was observed to be associated with the level of social impairments only among the children with ASD, so that the more response habituation to a direct gaze was attenuated, the more the children had impairments in social skills in the fields of Use of Language and Other Social Communication Skills, Use of Gesture and Non-verbal Play, and Social Expressiveness. The result implies that the attenuated habituation of autonomic responses during eye contact might be one mechanism that relates to atypical social behavior in ASD. Considering that amygdala is thought to play an essential role in mediating the physiological arousal elicited by eye contact (Senju and Johnson, 2009), the above-mentioned suggestion is supported by evidence from previous studies by Kleinhans et al.

(2009) and Swartz et al. (2013) showing that attenuated amygdala response habituation to facial stimuli with a direct gaze is related to greater autism severity among children and adults with ASD.

In addition, enhancement in autonomic arousal to a direct gaze in relation to the two other gaze conditions showed a moderate positive correlation with attenuated autonomic response habituation to a direct gaze. As a similar association was not observed among the children without ASD, it is possible that autonomic response habituation plays a greater role in determination of the level of autonomic arousal to a direct gaze in ASD than in neurotypical development. Future studies are needed to clarify this issue.

The present work does not provide answers to how autonomic response habituation to a direct gaze and autism severity are causally related. For example, a greater level of social impairments in childhood might cause enhanced social anxiety, which might in turn disturb autonomic response habituation to a direct gaze in ASD (Corden et al., 2008). In addition, in infancy attenuated autonomic response habituation to a direct gaze could result in active avoidance of social interaction due to uncomfortable feelings of arousal at eye contact, which in turn might lead to social impairments by diminishing an infant's opportunities to learn essential social skills in interaction (Hutt and Ounsted, 1966; Skuse, 2003).

One mechanism that could have the potential to yield atypical autonomic response habituation to a direct gaze is the abnormal functioning of the oxytocin system. The oxytocin system plays an important role in facilitating social motivation, approach behavior and social engagement from early on in life, and besides increasing the social reward, it seems to diminish amygdala hyperactivity to stimuli signaling social threat (Clark et al., 2013; Feldman et al., 2010; Kirsch et al., 2005; Lim and Young, 2006; Shamay-Tsoory and Abu-Akel, 2016; Petrovic et al., 2008; Preti et al., 2014; Weisman et al., 2012). Interestingly, the administration of nasal oxytocin seems to enhance eye contact behavior among individuals with and without ASD (Andari et al., 2010; Auyeung et al., 2015; Kirsch et al., 2005). Future studies are needed to investigate whether atypical functioning of the oxytocin system is related to attenuated autonomic response habituation to a direct gaze among children with ASD.

Taking into account the heterogeneity in ASD symptoms, it is likely that multiple factors are working side by side to cause impairments in gaze behavior in ASD (Dalton et al., 2005; Joseph and Tanaka, 2003; Klin et al., 2002; Osterling and Dawson, 1994; Rice et al., 2012; Spezio et al., 2007; Zwaigenbaum et al., 2005). For example, infants later diagnosed with ASD might involuntarily maintain eye contact for longer than typically developing infants, e.g. due to impairments in flexible visual orienting and face processing, which in combination with attenuated response habituation to a direct gaze could exaggerate enhancement of autonomic arousal in eye contact (Bryson et al., 2007; Elison et al., 2013; Elsabbagh et al., 2013; Jones et al., 2014; Keehn et al., 2013; Webb et al., 2010). Furthermore, in ASD the motivation to engage in eye contact might be decreased due to impairments in experiencing a social reward in social interaction (Bar-Haim et al., 2006; Berger, 2006; Chevallier et al., 2014; Hutt and Ounsted, 1966; Kamio et al., 2006; Kylliäinen et al., 2012; Mundy, 1995; Skuse, 2003). As a result, an active avoidance of eye contact would serve as a natural solution for an infant to avoid unpleasant experiences in social interaction. However, it is also possible that eye contact is not unpleasant to all individuals with ASD, and also other mechanisms, e.g. diminished social saliency of a direct gaze, might hamper engagement in eye contact (Helminen et al., in press). Mechanisms that yield atypical eye contact behavior in ASD may relate to altered functioning in multiple neurological systems responsible for, e.g. regulation of autonomic arousal to a direct gaze, attention orientation to a direct gaze, facial information processing, social saliency evaluation of stimuli and experienced social reward (e.g. Bar-Haim et al., 2006; Berger, 2006; Bryson et al., 2007; Chevallier et al., 2014; Helminen et al., 2017; Joseph et al., 2008; Keehn et al., 2013; Kylliäinen and Hietanen, 2006; Webb et al., 2010).

Earlier studies investigating gaze direction-related autonomic arousal among typically developing children have reported that a direct and an averted gaze elicit a similar level of autonomic arousal when presented as pictures on a computer screen (Joseph et al., 2008; Kylliäinen and Hietanen, 2006). The present dissertation extends earlier findings by showing that similarly to adults, enhanced autonomic arousal to a direct gaze in comparison to an averted gaze or closed eyes is also observed in childhood when live gaze stimuli are used (Hietanen et al., 2008; Pönkänen and Hietanen, 2012; Pönkänen et al., 2011). It is possible, as suggested by Myllyneva and Hietanen (2015) that only live gaze

stimuli are able to activate a participant's mental attributions of being the target of another's attention, which results in different neural processing of socially relevant information from live and picture stimuli.

## 6.2 Cooperation

In Study III, the boys with ASD did not cooperate less frequently than their gender-, age- and total IQ-matched peers in the PD task. This finding is in line with the earlier findings of Dows and Smith (2004), Li et al. (2014) and Schmitz et al. (2015), showing that children with ASD are able to make an equal number of cooperative choices in cooperative tasks when compared to children without ASD. In a game-theoretic dilemma, the boys with ASD were observed to follow the basic rules of cooperation, e.g. preferring maximal mutual gain for being a member of a cooperating pair instead of pursuing the highest individual payoff, and declining to cooperate when it would mean self-sacrificing. When the boys with ASD had to choose whether or not to cooperate with an unknown cooperator, approximately two-thirds of them did not choose to cooperate, which should be considered the most strategic choice in the one-round PD task in order to minimize the risk of personal costs (Engel and Zhurakhovska, 2016; Hill and Sally, 2003).

Cooperation in the present dissertation and the previous studies by Dows and Smith (2004), Li et al. (2014), and Schmitz et al. (2015) has been measured by structured tasks with predictable outcomes and limited social interaction, which probably make them easier to master than social cooperative situations in real life. Indeed, when cooperative abilities have been assessed with more naturalistic study settings, toddler-aged children with ASD have shown deficits in succeeding in and maintaining mutual cooperative behavior (Liebal et al., 2008). It is also possible that, in spite of their basic cooperative abilities, children with ASD continue having difficulties in following more complicated rules in cooperation, e.g. taking into account another's fairness and previous acts when making cooperative choices (Li et al., 2014). This might make them vulnerable to engaging in unfair cooperation with exploitative individuals. They might also continue to show impairments in understanding the importance of the pursuit of equality for social relationship maintenance, even in situations when unequal choices would not directly harm the self or another (Schmitz et al.,

2015). “Advantageous inequity aversion” – negativity toward getting more than one’s opponents – seems to be unique to humans and apes, and may represent an important behavioral pattern that ensures continuity in long-term social cooperation (Brosnan and de Waal, 2015). The pursuit of equity seems to have a lessened role in the maintenance of family relationships, and it thus may be most effectively learned only by acting with peers (Brosnan and de Waal, 2015). Children with ASD may demure from self-advantageous inequity less often as they might have limited opportunities to estimate the reactions of an opponent and the long-term social consequences of their actions due to their socio-cognitive disabilities and lessened experiences from long-term peer relationships (Brosnan and de Waal, 2015). If this suggestion is supported by findings in future studies, children with ASD might benefit from interventions aimed at increasing their acknowledgement of the rules of cooperation, e.g. the pursuit of equity in peer relationships in order to maintain long-term friendships.

When cooperative choices were compared within gender-, age- and total IQ- matched boy pairs, the cooperative choices of boy pair members had a tendency to be opposite, especially when cooperating with an unknown cooperator. It is possible that the decisions on cooperation were based on different strategies among the boys with ASD than among the neurotypical boys. Cooperation was measured in the Study III with a modified version of the Prisoner’s dilemma task (PD), which combines a simultaneously played PD task with a strategy method (Herrmann et al., 2010). Even though cooperation in the decisions of the PD task could signal reciprocity/trustworthiness (cooperation with a known cooperator), self-sacrifice (cooperation with a known non-cooperator) and trust behavior (cooperation with an unknown cooperator), as suggested by Gallup et al. (2010), choices to cooperate could also be based on, e.g. empathy-induced altruism, mental reasoning, rational decision-making or even random selection (Batson and Ahmad, 2001; Pantelis and Kennedy, 2017; Tayama et al., 2012). For example, in Study III, the participants’ motivation to win a prize in the PD task was not studied. It has been noticed that the increased value of the potential gains is related to enhanced betrayal in the PD task (Engel and Zhurakhovska, 2016). As the prizes for the PD task were small, e.g. comic books, it is possible that the motivation to gain the prize differed between the younger and the older participants, and between boys with and without ASD. It is possible that the older participants considered the costs of an opponent’s betrayal to be lower to themselves, and thus they were more

willing to take a risk to achieve the maximum mutual gain, i.e. to cooperate in an unconditional decision. It has also been noticed that whereas neurotypical adolescents tend to pursue the highest score in cooperative game dilemmas, adolescents with ASD might instead be prone to make their cooperative choices according to, e.g. self-generated rules and the external characteristics of the choices (Tayama et al., 2012).

The evidence from Study III implies that cooperative behavior among boys with ASD is not determined by ASD severity, even though according to previous studies it might relate to their imitation, joint attention and theory of mind skills (Colombi et al., 2009; Downs and Smith, 2004; Hill and Sally, 2003). Thus, it seems that if cooperative abilities are at risk of being compromised in ASD, it is due to impairments in certain socio-cognitive skills that are essential for effective cooperation, not to ASD-related social impairments in general. This suggestion could also explain why oxytocin, which is thought to mediate unique variance in human cooperativeness, seems to intensify engagement in eye contact, emotion recognition, and the processing of socially relevant cues in cooperative situations, but not to induce remarkable improvements in more extensive social impairments related to ASD (Andari et al., 2010; Auyeung et al., 2015; Dadds et al., 2014; De Dreu and Kret, 2016; Guastella et al., 2010; Haas et al., 2013; Kosfeld et al., 2005; Watanabe et al., 2015; Yamasue, 2016; Yatawara et al., 2016).

Study III failed to investigate cooperation among girls with ASD due to the very limited number of girl participants. Possible gender differences in cooperation among children with ASD should be studied in future, as observations made among typically developed individuals have provided evidence that precursors for cooperative choices might differ between males and females (Geary et al., 2003).

## 6.3 Reactive aggression

Since maladaptive aggression is a common comorbidity in ASD (Dominick et al., 2007; Green et al., 2000; Ming et al., 2008), at least the boys with ASD were expected to show a general deficit in reactive aggression regulation. Against this expectation, in Study IV, both the boys and the girls with ASD showed similar levels of reactive aggression to their gender-, age- and total IQ-matched peers

when the intensity of provocations and the inhibitory cues of the assailant were not considered in the PAM task.

However, when the responses to the attacks of varying intensities were investigated, the boys with ASD were observed to be prone to respond with enhanced reactive aggression, especially to attacks of milder intensity, even though they regulated their responses to the higher intensity attacks similarly to the boys without ASD in the PAM task. The girls with ASD instead did not differ significantly from the girls without ASD in the level of reactive aggression to the attacks of varying intensity, except by showing a lower intensity of reactive aggression to slight pushes. Taking these results together with an earlier observation of more frequent mild aggressive behavior, i.e. pinching, biting and scratching, and head-butting among children with ASD, it seems that children with ASD – especially boys – might have specific difficulties in responding appropriately to provocations of minor intensity and refraining from minor aggressive acts (Farmer and Aman, 2011).

This dissertation contains the first study to measure reactive aggression regulation in ASD with the PAM task. As the PAM task is not validated in ASD, and as the present study did not include parallel measures of aggressive behavior, it is impossible to estimate whether the findings resemble behavior in naturalistic situations of conflict. Neurotypical children have been observed to modify their response intensity according to the intensity of provocation in the PAM task so that the stronger the attack is, the stronger the response is (Juujärvi et al., 2001). Low self-control of emotions in real life seems to relate to more intense responses, especially to attacks of minor intensity in the PAM task among neurotypical children (Juujärvi et al., 2001). Future studies are needed to investigate whether a similar association between the tendency to respond with enhanced reactive aggression in the PAM task and low self-control of emotions also holds for boys with ASD (Juujärvi et al., 2006). There is also no knowledge as to whether boys with ASD are able to discriminate between the intensity levels of mild aggressive responses; they may rather see them as equally intense responses to assaults of minor intensity. For example, the ability to estimate the consequences of different mild aggressive acts on a target, e.g. pain and emotional discomfort, might rely more on an ability to understand the mind of the other, i.e. theory of mind skills, than an estimation of consequences from serious aggressive acts that include more obvious physical injuries, and thus be

particularly difficult for children with ASD (Baron-Cohen et al., 1985). In addition, compared to conflicts with serious aggressive acts, using mild aggressive acts, e.g. pinching, might more often provide benefits and desired outcomes, e.g. getting a toy from a peer, and be less often accompanied with the threat of being confronted and sanctioned by caregivers. If the results of the present study are replicated in the future and are observed to relate to aggressive behavior in real life, comprehension of those mechanisms that count toward enhanced responses to minor aggressive acts among boys with ASD might aid in diminishing maladaptive aggressive behavior in ASD.

According to the results of Study IV, boys and girls with ASD are able to regulate their aggressive responses according to the physical strength of an assailant similarly to their gender-, age- and total IQ-matched peers in the PAM task. However, boys with ASD might have difficulties in considering the opposite gender of an assailant as a cue to regulate reactive aggression responses. Earlier studies have shown that neurotypical children attenuate their responses to provocations that are delivered by assailants whose physical and/or social status differ from their own, probably in order to secure personal fitness or avoid punishment from caregivers or other figures of authority (e.g. Juujärvi et al., 2001; Pellegrini et al., 2007). A larger assailant seems to attenuate children's aggressive responses less than a smaller one or the assailant having the status of a parent (Juujärvi et al., 2001). Already little children with typical development seem to engage more often in aggressive confrontation with a same-gender peer rather than an opposite-gender peer, and at school-age typically developing boys and girls consider the opposite gender of an assailant as an inhibitory cue; they show the highest level of aggression toward assailants of the same gender and physical strength in the PAM task (Juujärvi et al., 2001; Pellegrini et al., 2007).

The possible mechanisms relating to the less regulated responses to attacks from an assailant of the opposite gender among the boys with ASD can only be speculated on. It is possible that the boys with ASD were able to estimate the immediate physical threat in conflicts according to varying attack intensity and the strength of the assailant, but were impaired in estimating the delayed consequences of aggressive behavior toward an opposite-gender peer, e.g. reprimands or sanctions from caregivers or other figures of authority (Shantz, 1987). Social norms may also define children's aggressive confrontations with

opposite-gender peers as even more inappropriate than conflicts with same-gender peers, which the boys with ASD were possibly not aware of or were unable to apply to the present conflict. The boys with ASD might also have been impaired in estimating how gender, in general, affects physical strength and the imminence of an assailant, and thus they considered assailants of the opposite gender more as threatening than the boys without ASD in the PAM task. The male gender has represented greater physical strength and risk for physical confrontation than the female gender through human evolution, and even today neurotypical individuals have a tendency to rate an angry male face with an out-group status as more threatening than other facial expressions, the facial expressions of females, or faces of in-group members (Boyer and Bergström, 2011; Geary et al., 2003; Neuberg et al., 2011).

As the number of the girl participants was very low in this study, the findings concerning the girls with ASD must be regarded with caution and considered as preliminary. According to the results of Study IV, boys with ASD – but not girls with ASD – seem to have impairments in using situational cues, i.e. the strength of the attack and the gender of an assailant, to regulate their aggressive responses compared to their typically developing peers. As the earlier observations have also shown divergent reinforcements behind maladaptive behavior among boys and girls with ASD, it is possible that the processes that yield maladaptive aggressive behavior hold a gender difference in ASD (Farmer et al., 2015; Hartley et al., 2008; Kanne and Mazurek, 2011; Kopp and Gillberg, 1992; Reese et al., 2005).

Previous evidence has shown a positive association between maladaptive aggressive behavior and autism-related impairments in social behavior, communication, and repetitive behavior among children with ASD (De Giacomo et al., 2016; Dominick et al., 2007; Hartley et al., 2008; Kanne and Mazurek, 2011; Mazurek et al., 2013; see Hill et al., 2014 for contradictory results). However, the present study did not find that an ability to regulate the responses of reactive aggression in the PAM task were related to autism severity among the boys with ASD. The processes that yield impairments in regulation of aggressive responses among children with ASD are most likely complex, and may relate, e.g. to impairments in perceiving and processing information accurately, a limited range of constructive coping strategies in frustrating situations, and a lowered threshold for action (Ashwin et al., 2007; Boraston et

al., 2007; Bradley et al., 2006; Corden et al., 2008; Howard et al., 2000; Konstantareas and Steward 2006; Krysko et al., 2009; Lane et al., 2010; Pouw et al., 2013; Robinson et al., 2009; Serra et al., 2002; Yirmiya et al., 1998).

Study III showed that cooperativeness is associated with higher levels of reactive aggression regulation among the boys with ASD in behavioral tasks. As neither the PD nor the PAM task is validated in ASD, it is not known how this finding resembles behavior patterns in real life. If future studies observe an association between enhanced cooperativeness and decreased aggressive behavior also in naturalistic situations among children with ASD, interventions that strengthen the regulation of both reactive aggression and the preference for mutual gain over self-interest in cooperation might have the potential to enhance pro-social behavior in ASD. However, associations between altruistic cooperation and aggressive behavior are most likely complex, as a readiness to act violently against out-group members possibly co-evolved with cooperation with in-group members (Rusch, 2014; Van Vugt et al., 2007). Even today, strong theory of mind skills not only serve altruistic cooperation, but also enable maximizing individual gain when engaging in cooperative dilemmas with strangers (DeAngelo and McCannon, 2015). In addition, although nasally administered oxytocin seems to increase sociability and cooperativeness toward familiar people, it at the same time enhances withdrawal and defensive aggressive behavior toward strangers (De Dreu and Kret, 2016).

It should also be noted that the findings concerning the positive association between cooperativeness and decreased reactive aggression were observed among the boys with ASD and may thus not concern girls with ASD. Ancient cooperative roles differed remarkably between the male and female genders, especially in the field of aggressive behavior, and even today the association between cooperative and aggressive behavior seems to hold a gender difference among typically developed individuals (Bailey et al., 2012; Gallup et al., 2010; Geary et al., 2003; Van Vugt et al., 2007). Future studies are needed to investigate whether cooperativeness and reactive aggression are related with ASD also among girls.

## 6.4 Strengths and limitations

### 6.4.1 Study design

Cross-sectional and case-control study designs were used in the studies that make up this dissertation. A case-control study allows the investigation of a phenomenon of interest simultaneously in clinical and control groups, and thus aids to clarify whether the observations made are specific to the individuals belonging to the studied clinical group or are more widely manifested in the population. Even though case-control studies carried out with small groups can mainly yield only preliminary evidence for future studies, they offer an inexpensive way to accomplish studies that face recruitment difficulties and offer an opportunity to implement studies in small research centers.

The matched case-control study design used in Studies III and IV provided clear benefits: it minimized the possible confounding effects from a small sample, but also set restrictions on the statistical analysis. Some late-arising research questions of the associations between cooperation and reactive aggression had to be limited to concern only the children with ASD, as the participants without ASD who were selected individually to match the gender, age and total IQ of the children with ASD could not be considered representative of the population of typically developing children.

Cross-sectional studies offer an inexpensive way to provide beneficial preliminary information in a short period of time for future prospective studies. However, cross-sectional studies do not yield any information on causal relationships. For example, according to the results of the present dissertation, it would be tempting conclude that attenuated response habituation to a direct gaze is one mechanism that leads to social impairments in ASD. While this might be the case, it is also possible that social impairments cause attenuated response habituation to a direct gaze, e.g. via enhanced social anxiety, or that causality between social impairments and attenuated response habituation to a direct gaze is bi-directional in ASD.

The study design was not blinded in the present studies, and it is possible that knowledge of the clinical status of the participants may have affected the behavior of the examiners who recruited the participants, and thus they were

aware of the clinical status of the participants. The problem relating to a non-blind approach was recognized beforehand and the examiners tried to avoid alteration in their behavior according to the clinical status of the participant. As the data were not observational, it is unlikely that awareness of the clinical status of the participants affected the main results of the studies.

The study design used in Studies I and II failed to investigate autonomic arousal and response habituation to control stimuli, as the duration of the block including facial stimuli and the block including the control stimulus was not the same. A control block including 18 trials would have been optimal. However, this flaw in the study design did not jeopardize the main objective of the study, which was to investigate autonomic arousal to a direct gaze among children with ASD.

### 6.4.2 Sample

First of all, it should be noted that the findings of the present studies only represent children with ASD aged seven to 17 years old with a total IQ over 70. Future studies with larger samples are needed to investigate whether the observed findings are also observable when ASD is accompanied with intellectual impairment, and among younger and older individuals with ASD.

As is often the case when investigating clinical groups, the sample sizes were small in the present studies. A power analysis was not performed to estimate the sample sizes needed, as the effect sizes of measured variables could not be estimated reliably. Thus, decisions on the sample sizes in the present studies had to be based on sample sizes used in previous studies with similar study questions, and the available time, laboratory and financial resources. Even though studies with small sample sizes have the potential to yield preliminary advantageous evidence for future studies, some issues need to be considered. Studies with small sample sizes are prone to sampling error, and especially when measured variables show a large variation in the population, there is a risk of the results being false-positive due to effect of change (Button et al., 2013). However, it is more likely that in small samples, expected true effects are harder to detect due to the lowered power of the statistical tests. Due to recruiting problems, the number of girl participants in particular was very low in the present studies. This was problematic especially in Studies III and IV, which

failed to investigate cooperation and the associations between cooperation and impairments in the social skills of the girls with ASD due to the small sample size. An uneven male/female ratio in ASD has the potential to yield difficulties in recruiting an adequate number of girl participants, and this should be considered when investigating phenomena that might show gender-related differences (Kirkovski et al., 2013; Schaafsma and Pfaff, 2014). It is likely that when studying clinical groups, multicenter research trials can more easily meet the need for large samples sizes than single center trials.

The samples in the studies consisted of children who were randomly selected from patients diagnosed with ASD at the Department of Child Psychiatry, Tampere University Hospital. The clinical status of the children with ASD was not verified in the studies, as they had been previously diagnosed by experienced physicians. Verifying the ASD diagnosis with an observational measurement, the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2000), would have been elegant and yielded useful information on the participants' current social impairments, e.g. eye contact behavior, in natural situations.

The ASD groups were heterogenic in age, IQ measures and the level of social impairments. The present work used 3Di to measure dimensions of social impairments, which allowed heterogeneity in ASD symptom range and severity among the children with ASD to be taken into account. This should be considered a strength, as previous evidence has shown that ASD includes a broad range of symptoms of varying severity and possibly separate subgroups with distinct early developmental profiles and prognoses (Bedford et al., 2014; Bryson et al., 2007; Campbell et al., 2014; Elsabbagh and Johnson, 2010; Jones and Klin, 2013; Rice et al., 2012; Viding and Blakemore, 2006). If these subgroups exist, it is possible that they differ from each other also in the field of gaze behavior and social development (Bryson et al., 2007; Jones and Klin, 2013; Wass et al., 2015). The small sample size did not allow the division of the ASD groups into subgroups, e.g. by cognitive profiles. Furthermore, due to the small sample size, the possible effects of the participants' age and cognitive profiles on the observed findings could not be investigated in Studies I and II. For example, the direct gaze of the female model may elicit a different level of autonomic arousal in pre-adolescence and in adolescence, and this phenomenon might be different in ASD than in typical development. In Studies III and IV,

children with and without ASD were matched by pairs in order to minimize the possible confounding effects of gender, age and total IQ on the results of cooperative behavior and reactive aggression. The possible effects of age, cognitive profiles and social impairments on the results could be more easily controlled in future studies if larger and more homogenous study samples than those used in the present studies are selected.

Observations from the present work imply that electrophysiological measurement data collected from children with ASD might be more prone to be excluded from the final analysis than data collected from children without ASD. In the present dissertation, 21.7% (5/23) of children with ASD and 14.2% (3/21) of children without ASD were excluded from SCR analysis. Restlessness and sleepiness were only observed only among children with ASD to such a degree that the data could not be included in the analysis. In addition, 9% of trials in the group of children with ASD – in comparison to 5% of trials in the control group – were rejected from the SCR analysis due to contaminated data by the child's body movements or technical problems with the measurements. Thus, even though the sample sizes of the children with and without ASD in the final analysis were quite similar in Studies I and II, the results were based on fewer observations of SCR to gaze stimuli in the group of children with ASD than in the group of neurotypical children. Due to the small number of participants, dropout analysis could not be performed. When the characteristics of dropouts and attending participants were viewed, it seemed that participant dropout did not have major effect on mean age and total IQ in either of the groups. However, it seemed that relatively more dropouts had a diagnosis of childhood autism (F84.0) than atypical autism (F84.1) or Asperger's syndrome (F84.5). In Studies I and II, the dropout rates of above-mentioned diagnoses were 50% (3/6), 25% (1/4) and 31% (4/13) respectively, and in Studies III and IV, they were 29% (2/7), 0% (0/6) and 5% (1/22), respectively. Thus, it is possible that children with more severe ASD symptoms had a tendency to be excluded from the final analysis.

It should be noted that despite of pursuit of a random selection, there might have been also some selection bias regarding the participants with ASD. It is possible that those children with ASD who agreed to participate might have been more familiar with novel experiences, had better functional skills and had less hypersensitivity (slightly uncomfortable physiological measurements in Studies

I and II) than those who did not agree to participate. On the other hand, this kind of selection bias might be true also for the control participants, and might be hard to totally avoid in any kind of study including participants with ASD.

As current anxiety and depressive symptoms might have confounding effects, especially on the results of autonomic arousal (Gold, 2015; Myllyneva et al., 2015; Siess et al., 2014), the existence of these internalizing symptoms among the participants was ruled out by using the parents of the participants as informants. There seems to be a discrepancy between children's and parents' reports of the children's intrinsic symptoms, of which anxiety in particular is a common comorbidity among children with ASD (Hollocks et al., 2016; Sawyer et al., 1993). Measuring children's current internalizing symptoms with scales would have provided more reliable information of the participants' current anxiety or depression symptoms than the information received from the parents. An evaluation of another comorbidity of interest, ADHD symptomatology among children with ASD, would also have given additional information of the mechanisms, e.g. impulsivity, that may relate to the ability to inhibit reactive aggression responses to provocations (Study IV).

The participants' possible medication was not monitored in the studies. This should be considered as a limitation, as earlier studies have shown that, e.g. antipsychotic and methylphenidate medication might have an attenuating and enhancing impact on skin conductance responses, and an attenuating impact on aggressive behavior, respectively (Conzelmann et al., 2014; Green et al., 1989; Negrao et al., 2011; Parikh et al., 2008; Pringsheim et al., 2015; Spohn et al., 1971). However, it is unlikely that possible medication had a role in the main findings of Studies I and II, since if the medication had influenced the SCR, the influence would have been similar for all stimuli in the four different stimulus categories. In addition, it is likely that the effect of the possible medication on the responses of reactive aggression in Study III would have been similar throughout the whole PAM task, and thus may not have influenced the main findings.

### 6.4.3 Methods

The methods used in the present studies are highly structured in nature, and thus yield precise and consistent data, which increases the reliability of the obtained

results, and permits the studies to be easily replicated in future. Even though the used methods are time- and resource-consuming, they are able to provide information that could not be obtained, e.g. with more inexpensive and less time-consuming self-evaluations.

In all the studies, the children's social impairments were determined by interviewing the parents with 3di (Skuse, 2004). The strength of 3di is that it allows social impairments to be dimensionally categorized. Like other measurements using parents as informants, 3di is also influenced by the parents' ability to objectively evaluate their child's skills, and depends, e.g. on the parents' own social perception skills. Thus, the present study would have benefited from the parallel measurement of children's social behavior, e.g. in naturalistic situations.

The use of physiological measurements to measure autonomic arousal should be considered a strength (Studies I and II). Measuring skin conductance responses (SCR) offers a noninvasive way to study autonomic arousal by measuring involuntary alterations in an activity of the sympathetic nervous system (Büchel et al., 1998; Critchley, 2002, 2009; Knight et al., 2005; LaBar et al., 2008; Sequeira et al., 2009). As the sympathetic nervous system functions largely without voluntary control, measurement of SCR is markedly less vulnerable to interpersonal interpretations than self-evaluations. Another strength is that facial stimuli were presented live instead of as pictures, because there is preliminary evidence that only live stimuli are able to activate an observer's mental attribution that she/he is actually being looked at, which, in turn, seems to modulate the neural processing of socially relevant information (Myllyneva and Hietanen, 2015; Pönkänen et al., 2011). The participants' gaze behavior during the gaze stimuli was investigated with video recordings. However, mainly due to technical reasons, the recordings failed to provide satisfactory data, and observations of gaze behavior were scarce, especially among the children with ASD. Thus, the present study cannot provide reliable information on the participants' fixation duration to gaze stimuli. This should be considered as a limitation, as alteration in fixation duration to the eye region of the model has the potential to affect the level of SCR. In future studies, eye tracking measurements to control the possible effects of fixation duration to autonomic arousal and response habituation to a direct gaze are recommended. Information on the possible associations between neurophysiological measurements to a

direct gaze in the laboratory and eye contact behavior in real life is also lacking. This information is essential when considering the possible roles of enhanced autonomic arousal and attenuated response habituation to a direct gaze in atypical gaze behavior in ASD.

The Pulkkinen Aggression Machine (PAM; Juujärvi et al., 2001) was used in Studies III and IV to study the effects of situational cues in the inhibition of reactive aggression responses among children with ASD. In relation to the small sample size, quite a large number of variables were included in the statistical analysis, leading to a situation of multiple comparisons. As in a situation of multiple comparisons the risk for sampling error increases, studying smaller amount of variables would have lessened this risk. However, it was considered appropriate to include all studied variables in the analysis, as the variables were related to each other. There are no previous studies with the PAM task among individuals with ASD. Future studies are needed to investigate how observations in the PAM task resemble behavior in real life among children with ASD. The PAM task seems to be usable among children with ASD, and would therefore be worth validating for this group. A limitation of the PAM task is that the responses do not include proactive acts, which might bias results toward enhanced aggression. On the other hand, even though the responses in the PAM task include an "I do nothing" option, the participants often choose to respond to attacks with aggressive acts. If the PAM task could be developed further, it might be beneficial to add proactive acts to responses, and to estimate whether other social cues than strength, gender and the social status of assailants, e.g. facial expressions, could be also altered in the task.

Cooperation was studied with the PD task, which is one of the mostly widely used game-theoretic dilemmas in cooperative studies, and it has also been used in earlier cooperative studies conducted among children with ASD (Dows and Smith, 2004; Hill and Sally, 2003; Li et al., 2014; Sally, 1995). A limitation of Study II is that the strategies exploited in the PD task were not studied. There are observations that strategies exploited in game-theoretic dilemmas may vary between individuals and even within the same individual according to the demands of the task, and they may differ between individuals with and without ASD (Batson and Ahmad, 2001; Pantelis and Kennedy, 2017; Tayama et al., 2012). Study III also lacked information on the participants' cooperative behavior in real life. Future studies are needed to investigate whether an ability

to make basic cooperative choices in game-theoretic dilemmas is associated with cooperative behavior in naturalistic situations among children with ASD.

The original design of the present studies included measurements of autonomic arousal to a direct gaze, cooperation and reactive aggression within the same study participants. Due to recruiting problems, this was not possible. As evolutionary theory suggests that cooperation and the regulation of aggressive behavior may have co-evolved with mechanisms that regulate autonomic arousal to a direct gaze, combining neurophysiological measurements with measurements of cooperation and reactive aggression might yield novel information on the mechanisms that relate to prosocial behavior among children with and without ASD (Bickham, 2008; Emery, 2000; Skuse, 2003).

# 7 CONCLUSIONS

The present dissertation aimed to investigate autonomic arousal and arousal response habituation to a direct gaze, cooperation and reactive aggression, and their associations with social impairments among children with ASD. According to the conducted experiments, several conclusions can be drawn:

1. The children with ASD did not significantly differ from the neurotypical children in the level of autonomic arousal or response habituation to a direct gaze. At least two explanations are possible:
    a. Not all children with ASD show enhanced autonomic arousal or attenuated response habituation to a direct gaze;
    b. The children with ASD regulated the level of autonomic arousal and response habituation to a direct gaze by modifying eye fixation duration, which was not controlled in the present study.

2. ASD-related impairments in social skills were positively associated with the probability that a direct gaze evokes stronger skin conductance responses (SCR) than the other gaze conditions, and with attenuated autonomic response habituation to a direct gaze.
    a. These results imply that enhanced autonomic arousal and attenuated response habituation to a direct gaze might be mechanisms that are related to atypical social behavior in ASD.

3. The boys with ASD did not have a general impairment in engaging in cooperation when compared to the typically developing boys.
    a. The boys with ASD showed an ability to prefer a maximal mutual payoff over maximal individual gain and were able to refuse to cooperate with a non-cooperative opponent instead of self-sacrificing in a game-theoretic dilemma. These results imply that similarly to typically developing boys, boys with

ASD are able to follow the rule of equity when inequity represents harm to the self or another.

b. Cooperativeness among the boys with ASD seemed to relate to higher age, but not to the level of total IQ.
c. The cooperative choices in the gender-, age- and total IQ-matched pairs had a tendency to be opposite, especially when cooperating with an unknown cooperator. The result implies that same-aged boys with and without ASD may exploit different strategies in cooperative choices.

4. The boys and the girls with ASD did not to show a general enhancement in reactive aggression in the PAM task.

    a. The boys with ASD had a tendency to respond with enhanced reactive aggression to minor attacks and attacks from an assailant of the opposite gender when compared to the boys without ASD. The results imply that boys with ASD may not follow typical development in the cognitive regulation of reactive aggression.
    b. The girls with ASD used inhibitory cues – i.e. intensity of the attack, the size and gender of assailants – similarly as the typically developing children in order to regulate their aggressive responses.
    c. As the number of the girl participants was small, the results yield only preliminary evidence that cognitive regulation of reactive aggression might show gender-related differences in ASD.

5. The severity of ASD was not related to cooperative choices in the Prisoner's dilemma task or to the level of reactive aggression in the PAM task among the boys with ASD.
    a. However, the non-cooperative boys with ASD showed a higher level of reactive aggression in the PAM task than the cooperative boys with ASD.
    b. Age might at least partly explain the observed association between cooperative choices and the regulation of reactive aggression among boys with ASD.

# 8 IMPLICATIONS FOR CLINICAL PRACTICE AND FUTURE RESEARCH

Children with autism spectrum disorders (ASD) show persistent impairments in social communication and social interaction besides restricted, repetitive patterns of behavior, interests, or activities. Gaze avoidance represents one of the most conspicuous atypical behavior patterns in social interaction among children with ASD. Taking into account that the eye region conveys essential information on, e.g. feelings, identity and the intentions of another in social interaction, interventions that aim to enhance gaze orientation to another's eye region might benefit the social functioning of children with ASD. However, Studies I and II showed that the level of autonomic arousal to a direct gaze in relation to autonomic arousal to other gaze conditions might be enhanced and the habituation of autonomic arousal to a direct gaze might be attenuated, especially among those children with ASD who show the most severe ASD symptoms. As it is possible that attenuated autonomic response habituation to a direct gaze results in unpleasant feelings in eye contact, interventions aiming to enhance eye contact engagement among children with ASD should take this possibility into consideration. For example, increasing the duration of eye contact engagement gradually might aid children in tolerating unpleasant arousal. However, it is also possible that regardless of the interventions, some children with ASD may not overcome enhanced arousal to eye contact, and for those children other approaches to normalize gaze behavior in social interaction might serve as a solution, e.g. advice to focus one's gaze on the bridge of the other's nose rather than on the eyes. Future studies are needed to investigate whether the familiarity of the gazer has an effect on autonomic arousal and response habituation to a direct gaze in ASD. In addition, there is no knowledge of whether autonomic response habituation to a direct gaze, when achieved, is maintained over a short or long period or if the gazer changes (Harding & Rundle, 1969; Kimmel & Golstein, 1967).

Social anxiety, which has been observed to be a common comorbid symptom in ASD and relate to diminished eye fixation among individuals with ASD, might

mediate the observed association between impairments in social skills and enhanced autonomic arousal and attenuated response habituation to a direct gaze among children with ASD (Corden et al., 2008; Kuusikko et al., 2008; Mattila et al., 2010). Social anxiety seems to relate positively to social and communicational difficulties among neurotypical children (Pickard et al., 2017). It would be beneficial to include measurements of social anxiety in future studies that aim to study associations between autonomic arousal and response habituation to a direct gaze and social impairments in ASD.

Even though boys with ASD have extensive social impairments, they seem to be able to follow simple rules of cooperation in a game-theoretic dilemma. Game-theoretic cooperative dilemmas might offer them opportunities to learn simple rules of social interaction. Future studies are needed to investigate whether strengthening cooperative abilities could be beneficial for forming and maintaining friendships among children with ASD.

According to the findings of the present dissertation, several issues should be kept in mind when children with ASD show maladaptive aggressive behavior. First, there might be a gender difference in mechanisms that yield maladaptive aggression in ASD; this should be considered in future studies and in interventions targeted to decrease aggressive behavior among children with ASD. The results of the present dissertation also imply that boys with ASD might benefit from interventions that are targeted to increase constructive coping strategies to manage situations with minor provocations. Boys with ASD might benefit from learning to recognize the varying intensity in different types of minor provocations and how to respond to them with constructive behavior models. In addition, if boys with ASD have difficulties in estimating the strength and imminence of an assailant from situational cues (e.g. size and gender of an assailant), strengthening their ability to estimate actual threat in provocative situations might lessen their aggressive behavior in conflicts. Future studies are needed to investigate the issue.

If the finding of an inverse association between cooperative behavior and reactive aggression is confirmed in future studies, a combination of techniques that enhance cooperative behavior and reduce reactive aggression might have the potential to increase prosocial behavior in ASD.

Due to the small sample size, all the results from the present study should be regarded as preliminary and need to be confirmed in the future. The possibility that ASD consists of distinct subgroups should be considered in future studies. Furthermore, it is important to recognize that at least some ASD-related symptomatology might have a gender difference, and this should be taken into account in future studies.

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# 11 ORIGINAL COMMUNICATIONS

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ORIGINAL PAPER

# Autonomic Arousal to Direct Gaze Correlates with Social Impairments Among Children with ASD

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**Abstract** The present study investigated whether autonomic arousal to direct gaze is related to social impairments among children with autism spectrum disorder (ASD). Arousal was measured through skin conductance responses (SCR) while the participants (15 children with ASD and 16 control children) viewed a live face of another person. Impairments in social skills was assessed with the Developmental, Dimensional and Diagnostic Interview. The level of arousal enhancement to direct gaze in comparison to arousal to faces with averted gaze or closed eyes was positively associated with impairments in social skills (use of language and other social communication skills and use of gesture and non-verbal play) among children with ASD. There was no similar association among children without ASD. The role of arousal-related factors in influencing eye contact behaviour in ASD is discussed.

**Keywords** Autism · Eye contact · Gaze · Skin conductance · Social skills

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## Introduction

Human gaze has several socially important functions; it provides information, regulates interaction, expresses intimacy, mediates social control, and enhances communication and cooperation (Kleinke 1986). A human baby is born with an ability to orient to faces and a human face effectively attracts a newborn's attention (Morton and Johnson 1991). The eyes of a face seem to be especially attractive to a newborn baby. Newborns look longer at faces with eyes open than eyes closed (Batki et al. 2000). They also look longer and more frequently at faces with direct gaze than at faces with averted gaze (Farroni et al. 2006). Interestingly, looking at another person's direct gaze, i.e., establishing a mutual gaze or an eye contact with another person, has been associated with one's social skills. Babies who show gaze avoidance at the age of 2 months will need more time to establish interaction with other people at the age of 2 years (Keller and Zach 1993). Also, especially among the boys, the frequency of face-to-face interaction between an infant and a mother during the first 3 months of age has been found to correlate positively with an infant's gazing and approach-behaviour towards a stranger 6 months later, at the age 8–9.5 months (Robson et al. 1969). The association between gaze behaviour and social functioning is also seen later in life. In adults, the amount of eye contact in real life situations has been found to correlate positively with their social skills (Cherulnik et al. 1978). Such

findings are compatible with results showing that both structural (white matter volume) and functional (BOLD response) properties of the posterior superior temporal sulcus (pSTS), an area heavily implicated in gaze perception (George and Conty 2008; Nummenmaa et al. 2010; Senju and Johnson 2009), correlate with social-communicative skills in neuro-typical population (von dem Hagen et al. 2011).

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by qualitative impairments in social interaction and communication, repetitive and stereotyped patterns of behaviour, and restricted interests and activities (DSM IV 2000). Individuals with autism have been found to process information from faces in an atypical way. They seem to rely more on the detailed information on the faces (Behrmann et al. 2006; Deruelle et al. 2004), fixate less on other people's eyes (Dalton et al. 2005), and pay more attention to lower parts of people's faces than individuals without ASD (Joseph and Tanaka 2003; Klin et al. 2002; Spezio et al. 2007). Poor eye contact is commonly associated with autism and diminished orienting to faces (Osterling and Dawson 1994) and eyes (Zwaigenbaum et al. 2005) is already seen during the first year of life among infants with ASD. Two- to three-year-old children with ASD use gaze in communication less than children without ASD, and they use gaze more to obtain a desired goal than to direct others' attention to their own focus of interest. They also show significantly less complex-level communication where the eye gaze is combined with gestures and verbalizations than do children without ASD (Stone et al. 1997).

Earlier studies among individuals with ASD have found associations between reduced eye fixations and impairments in social skills. Jones et al. (2008) reported that 2-year-old children with ASD showed significantly reduced fixation times to the eye region than did children with typical development and non-autistic children with developmental delays. Moreover, their fixation times to the eye region correlated positively with the level of their social abilities. Jones et al. (2008) suggested that inattentiveness to the eyes could disrupt such children's social learning in interaction with other people and lead to atypical development of social mind and brain. Nacewicz et al. (2006) reported that 10- to 24-year-old individuals with ASD who had the smallest amygdalae spent less time fixating on the eyes and also exhibited the most severe social impairments in early childhood. As Nacewicz et al. did not study the correlations between eye fixation time and social impairments it is unclear whether this correlation persisted after early childhood. Support for this possibility comes from the study by Corden et al. (2008) reporting that among adults with ASD diminished fixation on the eyes was related to impaired recognition of facial expressions of fear and greater levels of social anxiety. On the other hand Klin et al. (2002) did not find an association between diminished eye fixation and impairments in social skills among adolescents and adults with ASD, but in their study greater fixation times on the mouth region were associated with less social impairment.

Skuse (2003) has suggested that among individuals with autism, eye contact may serve as a threatening stimulus leading to avoidance behaviour. According to Skuse (2003), amygdala-mediated arousal enhancement provoked in eye contact is normally diminished by neocortical top-down regulation. As the neocortical and amygdalar networks may function abnormally among individuals with autism, this regulation is ineffective and leads to elevated levels of arousal during eye contact. Recent direct evidence has supported the view of Skuse (2003) that eye contact may elicit hyperarousal among individuals with ASD, and that this hyperarousal may be linked to impairments in face perception/recognition skills. Kylliäinen and Hietanen (2006) measured skin conductance responses (SCR) to investigate the autonomic arousal to direct and averted gaze among children with ASD. The results showed that direct gaze elicited higher arousal than averted gaze among children with ASD, whereas there was no effect of gaze direction on the SCR in children without ASD. In an fMRI study, Dalton et al. (2005) reported hyperactivation of the orbitofrontal cortex and amygdala during eye contact in adults with ASD. The duration of the sustained eye contact also correlated with the level of amygdalar activation. Interestingly, they also found that the eye contact influenced the cognitive processing of faces among individuals with ASD: their ability to discriminate neutral and emotional faces with a direct gaze was slower than that among individuals without ASD. Recently, Joseph et al. (2008) found that SCR to direct gaze was negatively correlated with performance in a face recognition task in children with ASD. No such correlation was found between SCR to averted gaze and face recognition accuracy in children with ASD or between SCR to gaze stimuli (direct and averted) and face recognition accuracy in control children. The authors concluded that the results could be seen as providing support for the view that psychophysiological aversion to eye contact impairs face recognition in at least some individuals with ASD, although it is possible that the causality could also have worked in the opposite direction, i.e., that the heightened autonomic reactivity could have reflected the consequences of experienced deficits in face recognition skills.

In the present study, we aimed to extend the previous findings by investigating whether autonomic arousal during eye contact, as measured by skin conductance responses (SCR), is associated with the level of social skills among children with ASD. A special feature of the present study

was that, in contrast to most other recent studies presenting the facial stimuli as pictures on a computer screen, the participants viewed the live face of another person. We anticipated that facing another person instead of a picture of a face would bring the experiment closer to a natural social encounter and could, perhaps, result in more robust experimental effects. Recent results from our laboratory have shown that electrophysiological brain responses as well as SCRs to face and gaze stimuli are more sensitive to real faces than to pictures of faces (Hietanen et al. 2008; Pönkänen et al. 2008, 2011).

Because research has suggested that ASD is associated with enhanced autonomic arousal to direct gaze and that the level of arousal to direct gaze is correlated with performance in a social recognition task in children with ASD (Dalton et al. 2005; Joseph et al. 2008; Kylliäinen and Hietanen 2006; Skuse 2003) we expected that among children with ASD the level of impairments in social skills would be positively correlated with arousal specifically to faces with a direct gaze. A similar correlation between social skills and SCR to averted gaze and closed eyes was not expected either in the group of children with ASD or between any gaze condition and social skills in the group of normally developing control children. We also investigated whether SCR was higher to a direct gaze versus averted gaze/closed eyes, and whether subjectively estimated arousal during eye contact would correlate with the SCR measurements. We were, of course, also interested in identifying possible differences between the clinical and control group in these results.

## Methods

### Participants

The participants were 23 children with ASD and 21 control children. The children with ASD were aged from 8 to 16 years and were recruited from the Department of Child Psychiatry, Tampere University Hospital. They had been diagnosed with autism spectrum disorder (ASD) by experienced physicians using clinical procedures and according to the ICD-10 classification. Six of them had a diagnosis of childhood autism (F84.0), four had a diagnosis of atypical autism (F84.1), and thirteen were diagnosed as having Asperger's syndrome (F84.5). Exclusion criteria included the presence of depression or anxiety diagnosis or total IQ lower than 70. The children in the control group were recruited from local schools, and according to their parents had no history of mental or neurological disorders or learning disabilities.

SCR data from five autistic and three control children were excluded from the main analyses of the study. Data from one autistic child and two control children were excluded due to technical problems in SCR data acquisition. Furthermore, two autistic children were excluded due to sleepiness, one autistic child due to a migraine attack, and one autistic child due to restlessness during SCR recordings. One control child was excluded due to joking during direct gaze stimuli. After exclusions, SCR data were available from 14 boys and 4 girls in both groups. Interview data were missing from 3 children with ASD and 2 children without ASD as their parents did not participate in the Developmental, Dimensional and Diagnostic Interview (3di; Skuse et al. 2004) used to measure impairments in social skills. After all exclusions, we had both the SCR and the 3di data from 15 children with ASD (11 boys and 4 girls) and 16 children without ASD (12 boys and 4 girls).

Before the data collection, all the children and their parents received a letter including information about the methods and the aims of the study, and all the children and their parents gave their written informed consent. After participating the children received two cinema tickets as a reward for participating. The study was approved by the Ethical Committee of the Pirkanmaa Hospital District.

### Pre-experiment Methods

The parents of the children were interviewed according to the Developmental, Dimensional and Diagnostic Interview (3di; Skuse et al. 2004). The 3di is a standardized interview that was primarily developed to assess autistic symptoms, but it also covers other ICD-10 child psychiatric diagnostic categories to assess a full range of comorbidity. The interview includes 183 questions concerning demography, family background, developmental history, and motor skills, 266 questions concerning directly or indirectly disorders of the autistic spectrum, and 291 questions that relate to current mental states and are relevant to other child psychiatric diagnoses. The interview is computerized and besides answering structured questions, an interviewer can enter unstructured information as written text. After the assessment, a structured, computer-generated report is immediately available, and all the unstructured answers appear in the report as well. Skuse et al. (2004) found that the 3di's concurrent validity (agreement with independent clinician formulation) is very good, criterion validity (a comparison with the Autism Diagnostic Interview) is excellent and sensitivity 1.0 and specificity >0.97. The Finnish version of the interview was created by one of the authors (KP) together with the original developer of the instrument (David Skuse). For the study, five subscales from the interview's PDD (Pervasive Developmental Disorder) Scale were used to measure impairments in social skills. These subscales (scale range in parentheses) are Reciprocal Social Interaction (0–30), Use of Language and

Other Social Communication Skills (0–26), Use of Gesture and Non-verbal Play (0–14), Repetitive and Stereotyped Behaviour (0–12), and Social Expressiveness (0–4).

The participants were also tested with WISC-III (Wechsler Intelligence Scale for Children, Third Edition) as a normal part of the clinical evaluation. The controls had their IQ estimated by WISC-III during the same session when the physiological data collection took place or at another session, depending on the schedules of their families.

### Skin Conductance Measurements

Skin conductance data were recorded from the left hand with two Ag–AgCl electrodes (diameter 8 mm) filled with paste attached to the palmar surface of the medial phalanxes of the index and middle fingers (Lykken and Venables 1971). The signal was acquired with a GSR amplifier supplying constant-voltage AC excitation (22 mV; AD Instruments). Skin conductance was measured by Power Lab 400 equipment and data collection was controlled by Power Lab Chart v3.6 computer program running on a Power Macintosh 7100/80 computer.

The system was calibrated prior to each session to detect activity in the range of 0–40 microSiemens (μS). The data sampling rate was 100 Hz. After attachment of the finger electrodes, two nurses prepared the participant for electroencephalography (EEG) and electrocardiography (ECG) measurements which are not, however, reported here.

The skin conductance response (SCR) was defined as the maximum amplitude change from baseline (the level of EDA at stimulus onset) between 1 and 3.5 s from the stimulus onset. SCR data contaminated by the children's body movements or technical problems with the measurements were excluded from the following analysis. Due to this 9% of trials in the group of children with ASD and 5% of trials in the control group were discarded. Changes of 0.05 μS or more were considered as responses, whereas smaller changes were not considered as responses and were marked as a zero response (Andreassi 2006). There was no significant difference between the groups in the percentage of eliminated trials (Mann–Whitney, $p = 0.44$). After exclusions, the mean values of SCR for a direct gaze, an averted gaze and closed eyes were computed separately for each individual.

### Stimuli

Two women modeled for the facial stimuli. The models were young adults and of the same ethnicity as the participants. The direction of the gaze was either direct, averted (left and right), or the eyes were closed. In every condition, the face was oriented directly towards the participant and bore a neutral expression. The model tried to avoid blinking. In the averted gaze condition, the gaze was 30° to the left or right. Stimuli were presented through a voltage sensitive liquid crystal shutter (LC-TEC Displays AB; 30 cm width × 40 cm height) attached to a white panel between the participant and the model. The shutter could be changed between opaque and transparent state within a millisecond range. The participant sat in a chair 70 cm away from the shutter and the model sat in a chair 40 cm away from the shutter. Thus the distance between the participant and the model was 110 cm. The model was positioned on the same level as the participant.

### Design and Procedure

In the experiment each of the three conditions (direct gaze, averted gaze and eyes closed) was presented six times in random order as 18 trials. The model for the facial stimuli was the same throughout the whole experiment. Each trial lasted 5 s and the inter-stimulus interval (ISI) varied from 20 to 35 s. During the ISI, the voltage sensitive liquid crystal shutter was opaque. The presentation of the stimuli was controlled with NeuroScan Stim software. The experimenter initiated every trial by pressing a button on the remote controller. In this way, the experimenter was able to stop the experiment at any time without causing the experiment to fail. Before each trial, the experimenter said "Let's look at the next one" [in Finnish "Katsotaan seuraava!"]. This was done in order to ensure that the participant directed his or her attention towards the shutter and that the model could be prepared for the opening of the shutter. After each facial stimulus, the experimenter asked the participant whether the model gazed directly ahead, sideways or whether the eyes were shut. The question was asked in order to be sure that the participant was looking at the facial stimuli and that he or she distinguished the different stimulus conditions.

Before the experiment, the laboratory was introduced to the participant with picture cards and together with their parent they signed a written consent form. The participants were told that during the experiment physiological signals would be measured while the participant performed a task in which they would view the face of the model through the window. The participant was asked to avoid large-scale movements during the experiment and to look at the stimuli when presented.

During the experiment the experimenter sat behind the participant so that she was able to make observations of the participant's possible movements and also the possible movements and blinks of the model. The eye movements of ten children with ASD and 17 control children were recorded and saved using a video camcorder. The recordings were used to ascertain whether the time children

viewed the stimuli differed between the children with ASD and the control children. However, because some of the children changed their body position during the recordings, their eyes were not visible enough on the videotape, and data from their videos had to be excluded. Satisfactory video data were collected from five children with autism and from 13 control children. The gazing behaviour during stimulus presentation was coded with the Queen's video coder program (Baron et al. 2001). Each stimulus trial was analysed frame by frame (0 = not looking at the stimulus, 1 = looking towards the stimulus). Thirty percent of the videos were rated by two independent researchers in order to calculate interrater reliability.

Before each stage of the experiment, verbal instructions were given to the participant with the assistance of picture cards and an example of each stimulus was shown by opening the shutter. The data collection started with a 4-min period of baseline EEG activity recording during which the participant was asked to keep his or her eyes closed and open in 30-s turns. After that, the participant saw the facial stimuli. After the experiment, the electrodes were removed and a short break was held.

### Self-Assessment Manikin, SAM

After the break, the participants evaluated how pleasant or unpleasant and how calm or aroused they felt during each stimulus condition by filling a self evaluation form (Self-Assessment Manikin, SAM; Bradley and Lang 1994). For both ratings, the scales ranged between 1 (unpleasant/calm) and 9 (pleasant/aroused). The participants were given three pieces of paper on which they separately evaluated their feelings toward different stimulus conditions (direct gaze, averted gaze and closed eyes). In total, the experiment lasted about an hour per participant.

### Statistical Analysis

The statistical analysis was originally planned to be based on participants' mean SCR values in each gaze condition. However, due to the markedly positive skewing of the SCR distributions and the fact that in many cases the participants' responses within a condition showed wide intra-individual variation, we decided also to analyse the data based on the probability that, for each participant, SCRs to a given gaze condition would be greater than SCRs to the other two conditions.

To study how often direct gaze (D) elicited greater SCR than averted (A) and closed eyes (C), a new measure $P_D$ was created. For each participant, consider all possible triples of measurements D, A and C. (Most often the number of triples is $6 * 6 * 6 = 216$.) Then $P_D$ = the proportion of triples for which D > max(A, C). Similarly $P_A$ = the proportion of triples for which A > max(D, C), and $P_C$ = the proportion of triples for which C > max(D, A). Note that new variables are assigned values between 0 and 1, and depend on original measurements only through their ranks.

The limit for statistical significance was set equal to 0.05. The association of the social skills with arousal to different gaze directions was analysed in two different ways. First, correlation analyses (Kendall's tau b) were separately conducted between the individual mean SCR values for direct gaze, averted gaze, and closed eyes and the variables of social skills. Secondly, similar analyses were run between the $P_D$, $P_A$, $P_C$ and the variables of social skills. The T-test was used to analyse differences between the groups in age and IQ. The individual mean SCR values were not normally distributed even after square root or logarithmic transformations. Likewise the variables of social skills, $P_D$ values, data from S.A.M ratings and video recordings were not normally distributed. We therefore used non-parametric tests (Friedmann, Kruskall–Wallis, Mann–Whitney and $\chi^2$-test) in these analyses.

Analyses were carried out by R (Software environment for statistical computing and graphics, version 2.5.1, The R Foundation for Statistical Computing, Vienna, Austria) and SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA).

## Results

There were no significant differences between the children with ASD and the children without ASD in chronological age, full scale IQ, verbal IQ, and performance IQ, however, as expected, the children with ASD showed significantly more impairments in social skills than the children without ASD (Table 1). The respective median looking times for direct gaze, averted gaze, and closed eyes conditions were among children with ASD (n = 5) 4.32, 4.38, and 4.62 s, and among the children without ASD (n = 13) 4.86, 4.78, and 4.74 s. There were no statistically significant differences between the groups in the stimulus looking times in any stimulus condition (Mann–Whitney test) or in looking times between the three different stimulus conditions within either of the groups (Friedman test) although it should be noted that the analysis might be underpowered to show statistically significant differences between the groups due to small sample size, especially in the group of children with ASD. The inter-rater reliability in video coding was analysed with bivariate correlations. There was a significant correlation between the ratings of the two independent researchers ($r = 0.96$, $p < 0.05$). In both groups, the children's performance in identifying the gaze direction was practically faultless. In the total data set, there was only one wrong answer to a question regarding

**Table 1** Subject characteristics

| | ASD | | | | | Controls | | | | | *p* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean/Median | SD | Min/$Q_1^a$ | Max/$Q_3^b$ | n | Mean/Median | SD | Min/$Q_1^a$ | Max/$Q_3^b$ | |
| Gender | | | | | | | | | | | ns |
| Boys | 11 | | | | | 12 | | | | | |
| Girls | 4 | | | | | 4 | | | | | |
| Age | | 12.7 | | 8.6 | 15.1 | | 12.3 | | 8.5 | 15.9 | ns |
| IQ | | | | | | | | | | | |
| Verbal | | 102 | 20 | 69 | 145 | | 107 | 20 | 71 | 137 | ns |
| Performance | | 94 | 17 | 56 | 119 | | 101 | 11 | 81 | 122 | ns |
| Total | | 97 | 13 | 74 | 118 | | 104 | 15 | 77 | 127 | ns |
| Impairments in social skills | | | | | | | | | | | |
| Reciprocal social interaction | | 10.8 | | 8.5 | 17.6 | | 3.7 | | 3.2 | 4.3 | <0.001 |
| Use of language and other social communication skills | | 10.4 | | 8.0 | 14.1 | | 1.4 | | 1.1 | 3.0 | <0.001 |
| Use of gesture and non-verbal play | | 6.1 | | 4.7 | 8.8 | | 1.1 | | 0.6 | 2.2 | <0.001 |
| Repetitive and stereotyped behaviour | | 4.0 | | 1.0 | 5.7 | | 0.0 | | 0.0 | 0.2 | <0.001 |
| Social expressiveness | | 1.8 | | 1.5 | 2.8 | | 1.1 | | 0.9 | 1.6 | 0.025 |

[a] Minimum/lower quartile

[b] Maximum/upper quartile

the model's gaze direction. This was given by a child with ASD.

The correlation analyses based on the mean SCR values for direct gaze, averted gaze and closed eyes conditions and the variables measuring the level of impairments in social skills did not result in significant correlations. However, when the correlation analyses were based on the frequency of how often a given gaze elicited greater SCR than the other two gaze conditions significant correlations were observed. Importantly, as expected, significant positive correlations were found only between $P_D$ and the social skill impairment variables and only among the children with ASD (Table 2). Among children with ASD, $P_D$ correlated significantly with two social skill impairment variables: Use of Language and Other Social Communication Skills and Use of Gesture and Non-verbal Play (identical correlations with both variables: 0.55, $p = 0.005$). In addition, the correlations between $P_D$ and the variables measuring impairments in other types of social skills also approached statistical significance among the children with ASD ($r = 0.35$–$0.36$, $p = 0.066$–$0.074$). None of the correlations between $P_A$ or $P_C$ and the social skills impairment variables were significant ($r = -0.29$ to 0.23, $p = 0.137$–$0.960$). In the group of control children there were no even marginally significant associations between any of the gaze direction conditions ($P_D$, $P_A$, $P_C$) and the social skill impairment variables.

**Table 2** Correlations (Kendall's tau-b) between the probability that direct gaze evokes stronger skin conductance responses (SCR) than averted gaze and closed eyes ($P_D$) and Impairments in social skills

| | ASD tau-b | Controls tau-b |
|---|---|---|
| Reciprocal social interaction | 0.36 | 0.17 |
| Use of language and other social communication skills | 0.55 | 0.14 |
| Use of gesture and non-verbal play | 0.55 | 0.10 |
| Repetitive and stereotyped behaviour | 0.35 | −0.01 |
| Social expressiveness | 0.36 | −0.03 |

The children without ASD seemed to show a higher level of overall autonomic arousal (SCR averaged over all stimuli) than the children with ASD, but this difference was only marginally statistically significant (Mann–Whitney, $p = 0.072$). Mean SCRs to direct gaze, averted gaze and closed eyes and the distribution of $P_D$ in tithes of 1 are presented in Figs. 1 and 2 respectively. The stimulus type did not have an effect on the mean SCRs, neither when analysed across all participants (Kruskall–Wallis, $p = 0.111$), nor among the children with ($p = 0.288$) or without ASD ($p = 0.167$). As seen in Fig. 2, the distribution of $P_D$ does not seem to be normal but is rather bi-modal among children without ASD, with 4 children actually displaying greater arousal to direct gaze compared to averted gaze and closed eyes than any of the ASD children. There was no difference between the children with and without ASD in the probability for SCRs to direct gaze being greater than SCRs to averted gaze and closed eyes ($P_D$; Mann–Whitney test).

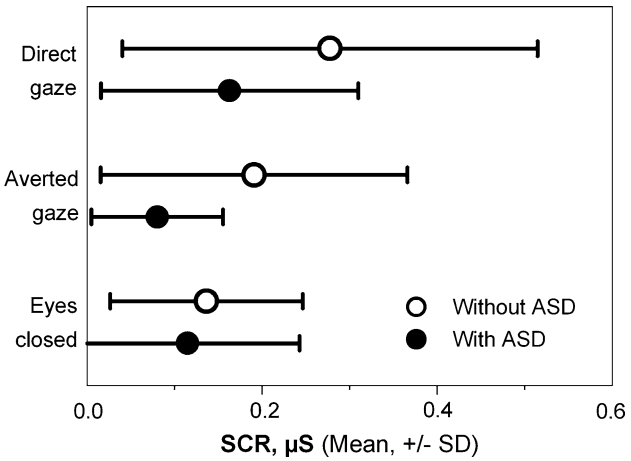


**Fig. 1** Arousal (mean SCR) to direct gaze, averted gaze and closed eyes

The median values of emotional valence (pleasant/unpleasant) and arousal (aroused/calm) ratings for stimuli are shown in Fig. 3. In general, stimulus type had an effect on valence (Kruskall–Wallis, $p = 0.001$) and arousal (Kruskall–Wallis, $p = 0.010$) when the effect was analysed across all participants. A face with direct gaze was rated as less pleasant and more arousing than a face with closed eyes (Kruskall–Wallis post hoc test, Langley 1979, $p < 0.01$ and $p < 0.05$ respectively). When the analysis was conducted separately in the two groups, stimulus type had an effect on valence ratings, both among the children with and without ASD (Kruskall–Wallis, $p = 0.031$ and $p = 0.041$ respectively), but for arousal ratings there was only a non-significant tendency among the children in the ASD and control groups (Kruskall–Wallis, $p = 0.109$ and $p = 0.096$ respectively). The children with and without ASD rated a face with closed eyes as more pleasant than a face with direct gaze (Kruskall–Wallis post hoc test, Langley 1979, $p < 0.05$ in both groups). No other statistically significant differences in subjectively experienced valence ratings between different stimulus conditions were found in either of the groups. Correlation analyses were conducted between the valence/arousal ratings and autonomic response indices to direct gaze. Arousal and valence ratings to direct gaze did not correlate either with the mean SCR to direct gaze or with $P_D$ among the children with or without ASD.

## Discussion

Our aim was to study whether autonomic arousal evoked by seeing another person with a direct gaze is related to the level of social skills among children with ASD. The results indicated that autonomic arousal to direct gaze was indeed positively related to impairments in two dimensions of social skills: (1) use of language and other social communication skills and (2) use of gesture and non-verbal play. Interestingly, this association was not evident when each participant's arousal responses to direct gaze (averaged across trials) were directly correlated with social skill measurements. Instead, the association became evident when participants' autonomic responsiveness to direct gaze was inspected in relation to responsiveness to faces not looking directly ahead, i.e. faces with averted gaze and faces with closed eyes. More specifically, we defined, for each participant, the probability that direct gaze would evoke stronger skin conductance responses (SCR) than averted gaze and closed eyes, and this probability was significantly correlated with the level of impairments in the two social skill measures mentioned above. This correlation was observed only among the children with ASD; there was no indication of such an association among the children without ASD. The results therefore suggest that it is not the absolute level of arousal to direct gaze which is critical in relation to social skills in ASD children, but the

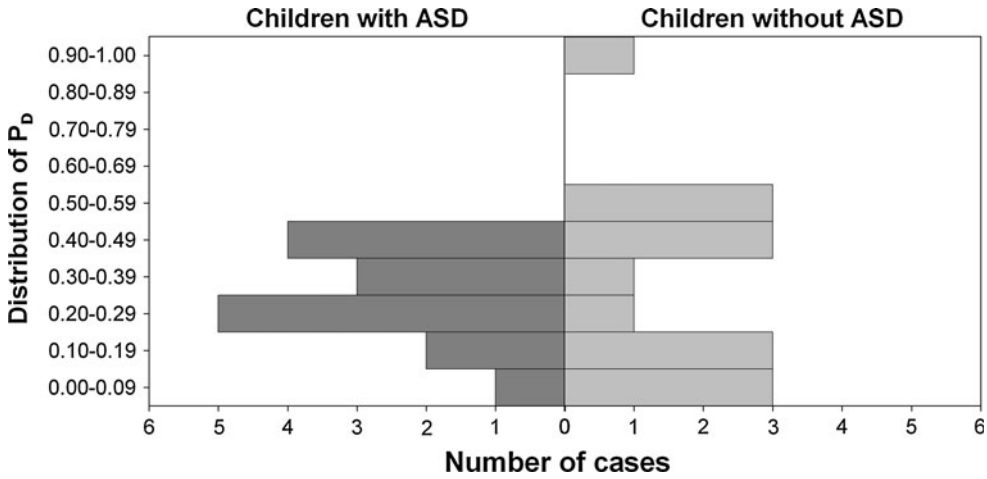


**Fig. 2** Distribution of the probability that direct gaze evokes stronger skin conductance responses (SCR) than averted gaze and closed eyes ($P_D$) in tithes of 1

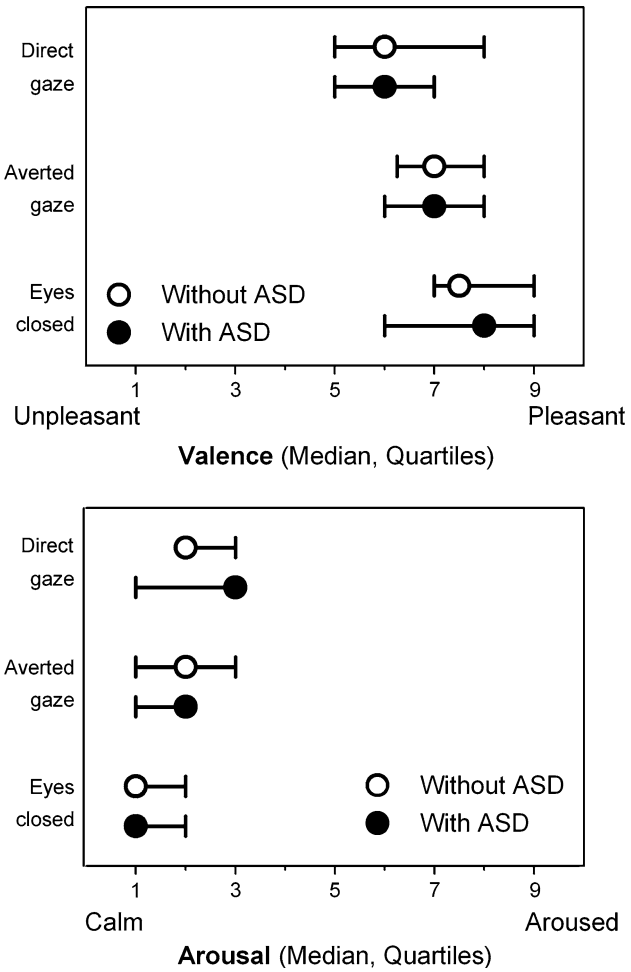


**Fig. 3** Self-evaluation ratings of arousal and valence to different gaze conditions in children with and without ASD

level of arousal enhancement to direct gaze in comparison to arousal to faces with averted gaze or closed eyes.

The heightened arousal to direct gaze was associated with impairments in use of language and other social communication skills and with impairments in use of gesture and non-verbal play among children with ASD. In addition, the associations between heightened arousal to direct gaze and impairments in reciprocal social interaction, repetitive and stereotyped behavior, and social expressiveness approached significance. The present results permit no conclusions regarding the causality between heightened arousal to direct gaze and impairments in social skills. The causality may work in either direction. It is possible that heightened arousal to direct gaze experienced early in life leads via avoidance of eye contact to more general impairments in social skills among children with ASD. More specifically, the correlation between heightened arousal to direct gaze and general impairments in social skills may be mediated by diminished engagement in joint attention. In joint attention, other people's gaze and gestures are spontaneously used to direct one's own attention to the same object, person, or event, and it has been shown that joint attention skills in infancy have an impact on subsequent social development, e.g. development of language (Mundy et al. 2007; Strid et al. 2006), social and behavioural competence (Vaughan Van Hecke et al. 2007), and general cognitive performance (Strid et al. 2006). Taking into account the fundamental role of gaze in joint attention and the importance of joint attention to further social development, one could assume that any disturbances in gaze behaviour will have a negative effect on the development of social skills. It is also possible that impairments in social skills elicit enhanced arousal to direct gaze, e.g., via social anxiety, which has indeed been reported to be associated with diminished eye fixation among individuals with ASD (Corden et al. 2008). The direction of causality in the association between heightened arousal to direct gaze and level of social skills needs to be investigated in future studies to fully understand how this association fits into the pathogenesis of autism.

It has been suggested that avoidance of eye contact results from enhanced arousal to direct gaze among individuals with ASD (e.g., Hutt and Ounsted 1966; Skuse 2003; Tinbergen 1974). However, the present study together with the study by Joseph et al. (2008) did not find evidence that either children with ASD or without ASD exhibit pronounced autonomic responses to direct gaze in relation to averted gaze or to closed eyes. In this respect, the present results contradict previous results from our own laboratory (Kylliäinen and Hietanen 2006) showing enhanced autonomic responses to direct gaze in comparison to averted gaze in children with ASD. These discrepancies may be explained by some differences in methodology and in samples of participants. In the study by Kylliäinen and Hietanen the facial stimuli were pictures of faces which were looming, i.e., appearing to be moving towards the participants. It is possible that perception of direct gaze combined with an impression of an approaching person could have been an especially arousing stimulus for children with ASD. Also, in the study by Kylliäinen and Hietanen the children were younger than in the present study, and the children in the control group had higher verbal and full scale IQ than the children in the clinical group. In the present stud as well as in that by Joseph et al. (2008), the clinical and control groups were also matched for verbal and full scale IQ. In addition, earlier studies have suggested that there may be subgroups of ASD with different developmental courses showing differential autonomic reactivity and amount of gaze fixation to facial stimuli (Joseph et al. 2008; Nacewicz et al. 2006). Thus it is possible that the differences in the results between

studies could also reflect, at least in part, differences in the selection of participants from different subgroups of ASD.

Our results suggest that the gaze aversion of children with and without ASD may not be solely explained by enhanced arousal to direct gaze. Instead, there may be other concomitant factors influencing eye contact avoidance and affecting the development of social skills. One such factor could be related to motivation to engage in eye contact. Dawson et al. (2005) have suggested in their social motivational/affective theory regarding face processing impairments that, in typical development, the reward value of social stimuli is an important precursor for social interaction from the second half of the first year of life onwards. They also suggest that infants and children with ASD may have impairments in this reward system or in neural systems important for perception of social reward. These impairments would lead to decreased motivation to engage in social interaction and, for example, to make eye contact with other people. Mundy (1995) has also suggested that social motivation is important for the development of social cognition skills and may play a role in the pathogenesis of autism. According to his theory, typically developing infants are motivated to engage in social-emotional approach behaviours that include affectively positive content, e.g., joint attention leading to rewarding outcome such as laughter, exchange of affect etc. He argues that infants with ASD may have inherent neurological disturbances in brain areas involved in social-approach and reward. This, in turn, will lead to diminished motivation to engage in social interaction and, particularly, in actions that initiate social interaction, ultimately resulting in maladaptive social-cognitive development.

Now, the present results nevertheless indicated that enhanced arousal to direct gaze was associated with impairments in social skills in children with ASD. So what kind of a role does autonomic arousal to eye contact then play in modulating gaze behaviour in ASD? Even though the present study only investigated autonomic arousal, we would like to argue, here, that both motivational (cf. Dawson et al.; 2005; Mundy 1995) and arousal-related factors (cf., Skuse 2003) may play a role in influencing eye contact behaviour in social interaction. For typically developing children, establishing eye contact with another person is rewarding to such an extent that they are motivated to engage in eye contact, even if it is sometimes experienced as too arousing. Instead, due to disturbances in the social reward system, children with ASD do not find eye contact rewarding, and are more likely to display gaze avoidance behaviour when autonomic arousal to direct gaze increases. This motivation-arousal model could accommodate, on the one hand, findings not showing enhanced arousal to direct gaze in ASD versus control children thereby suggesting that gaze aversion in ASD is not likely to be critically dependent on atypically high arousal to direct gaze, and, on the other hand, findings showing that the arousal to direct gaze correlates with the level of social skills, as in the present study, or with face recognition skills as in the study by Joseph et al. (2008).

Gaze condition had a weak effect on subjective experiences of arousal and valence. When analysed across children with and without ASD, direct gaze was rated more arousing and less pleasant than closed eyes, but when the groups were analysed separately, similar statistical significance was only seen in experienced valence and effect of gaze on arousal was only marginally significant in both groups. Importantly, there were no differences between the groups in arousal or valence ratings. The ratings of arousal or valence did not correlate with autonomic arousal in either of the groups. This may be explained, at least in part, by the fact that the ratings were done after the block of stimuli presentation during which the autonomic responses had been measured. Therefore the participants' ratings were based on their memory of how they had felt during each stimulus presentation. This procedure may have impaired the accuracy of the ratings. At the same time, our results highlight the importance of physiological measurements in addition to subjective evaluations in the investigation of arousal responses to sensory stimuli.

## Limitations of the Study

As often when investigating clinical groups, our sample of participants with ASD was small. Even though individuals with ASD commonly have impairments in social interaction and communication, repetitive and stereotyped patterns of behaviour, and restricted interests and activities (DSM IV 2000), there seem to be different cognitive phenotypes of ASD, possibly due to different aetiological factors (Charman et al. 2011). Studies with larger sample sizes would shed light upon whether arousal to direct gaze is associated with certain cognitive phenotypes more strongly than with others. Also, as our study only included participants with normal intelligence, it is not possible to draw any conclusions about whether arousal to direct gaze is associated with social skills among autistic individuals with low IQ.

In our study the gazer was unfamiliar to the children. As social development evolves mostly in parent–child interaction, it would be interesting to know whether arousal elicited by direct gaze is varied by the familiarity of a gazer and whether arousal elicited by the direct gaze of a primary caregiver and of a stranger would be differently associated with social skills. Social skills in our study were measured by interviews with parents using 3di (Skuse et al. 2004), as it allows broader dimensional categorization of social skills

than, for example, the autism diagnostic observation schedule (ADOS, Lord et al. 2000). Of course, interviews with parents are always subject to the parents' ability to objectively evaluate their child's social skills, depending e.g. on parent's own social perception skills, parent's awareness of their child's social functioning in peer groups, and the quality of the parent–child relationship. Studies are needed in future to ascertain whether arousal to direct gaze is similarly associated with observed social behaviour in naturalistic situations, e.g. in peer groups.

In the present study, we also recorded the eye movements of the children. However, due to technical problems we had video data from only a small group of children with and without ASD. Therefore we were unable to study correlations between looking time for direct gaze, arousal of direct gaze and impairments in social skills. It is possible that some participants regulated their arousal to direct gaze by fixating their gaze instead of the eye region on other parts of the face, e.g. the mouth region and even away from a face of a model. However, even in that case the participants were aware of the gaze condition targeted towards them as they were able to correctly discriminate the stimulus conditions. Future studies with an eye tracking camera would make it possible to ascertain how the duration of maintained eye contact is associated with autonomic arousal among children with autism.

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ORIGINAL PAPER

# Autonomic Arousal Response Habituation to Social Stimuli Among Children with Asd

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**Abstract** Sustained autonomic arousal during eye contact could cause the impairments in eye contact behavior commonly seen in autism. The aim of the present study was to re-analyze the data from a study by Kaartinen et al. (J Autism Develop Disord 42(9):1917–1927, 2012) to investigate the habituation of autonomic arousal responses to repeated facial stimuli and the correlations between response habituation and social impairments among children with and without ASD. The results showed that among children with ASD, the smaller the habituation was, specifically in responses to a direct gaze, the more the child showed social impairments. The results imply that decreased autonomic arousal habituation to a direct gaze might play a role in the development of social impairments in autism.

**Keywords** Autism · ASD · Autonomic arousal · Habituation · Gaze

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## Introduction

Autism spectrum disorders (ASD) are characterized by persistent deficits in social communication and interaction, and restricted, repetitive patterns of behavior, interests, or activities (DSMV, American Psychiatric Association 2013). Diminished orientation to faces and especially to eyes has been frequently observed in children and adults with ASD (e.g., Dalton et al. 2005; Joseph and Tanaka 2003; Kliemann et al. 2012; Klin et al. 2002; Rice et al. 2012; Spezio et al. 2007; Tottenham et al. 2014; for negative results, see; Falck-Ytter et al. 2015; Webb et al. 2010). However, studies conducted among infants with ASD or among siblings with a high risk for autism have not provided unambiguous results on gaze and face avoidance in early development; rather, they have shown evidence of typical orientation to faces and gaze directions during the first months of life (Cassel et al. 2007; Jones and Klin 2013; Osterling and Dawson 1994; Yirmiya et al. 2006; Zwaigenbaum et al. 2005). This could be explained by supposing that, in autism, there are separate subgroups that can be distinguished by their early developmental profiles; these subgroups may show a divergence in when gaze and face avoidance emerge (Bedford et al. 2014; Bryson et al. 2007; Campbell et al. 2014; Jones and Klin 2013; Rice et al. 2012; for a review, see; Elsabbagh and Johnson 2010).

As information perceived from the face and eyes seems to have a fundamental importance in social interaction and socio-emotional development (Cherulnik et al. 1978; Keller and Zach 1993; Kleinke 1986; Robson et al. 1969), the appearance of gaze and face avoidance early in development might interrupt socio-emotional development in autism (Corden et al. 2008; Jones et al. 2008; Nacewicz et al. 2006; Rice et al. 2012; for contradictory results, see; Klin et al. 2002). If this suggestion proved to be true and the

mechanisms behind the gaze and face avoidance behavior could be revealed, tailored interventions targeted at enhancing orientation to faces and eyes might be beneficial in supporting socio-emotional development in children with a high risk for autism and children with ASD. Even though many studies (e.g., Dalton et al. 2005, 2007; Joseph et al. 2008; Kaartinen et al. 2012; Kliemann et al. 2012; Kylliäinen et al. 2012; Kylliäinen and Hietanen 2006; Tottenham et al. 2014; for reviews, see e.g.; Dawson et al. 2005; Gaigg 2012; Skuse 2003; Tanaka and Sung 2016; Zalla and Sperduti 2013) have tried to ascertain the mechanisms that account for the failure to orient towards faces and eyes in autism, conclusive evidence is still lacking.

It has been debated whether hyperarousal to facial stimuli, especially to a direct gaze (Hutt and Ounsted 1966; Dalton et al. 2005, 2007; Kliemann et al. 2012; Kylliäinen and Hietanen 2006; Skuse 2003; Tanaka and Sung 2016; Tottenham et al. 2014), could cause the failure to orient to facial stimuli among individuals with ASD. However, studies investigating physiological arousal to a direct gaze versus an averted gaze and closed eyes have yielded mixed results (Joseph et al. 2008; Kaartinen et al. 2012; Kylliäinen and Hietanen 2006; Kylliäinen et al. 2012). Studies by Kylliäinen and Hietanen (2006) and Kylliäinen et al. (2012) showed that a direct gaze elicits greater skin conductance responses than an averted gaze or closed eyes among children with ASD but not among children without ASD. Studies by Kaartinen et al. (2012) and Joseph et al. (2008) did not find enhancement in autonomic arousal responses to a direct gaze compared to other facial stimuli among children with ASD. Even though the studies by Kaartinen et al. (2012) and Joseph et al. (2008) did not show enhanced arousal to a direct vs. an averted gaze, they found that the strength of the arousal response to a direct gaze was positively associated with social impairments and reduced facial recognition skills, respectively. Kaartinen et al. (2012) showed that autonomic arousal to a direct gaze was positively related to impairments in use of language and other social communication skills and in the use of gesture and non-verbal play. Interestingly, this association was not evident when each participant's arousal responses to a direct gaze were averaged across trials; the association was revealed when arousal was measured as the probability that a direct gaze would evoke stronger skin conductance responses (SCR) than an averted gaze and closed eyes over stimulus repetitions.

Since the study by Kaartinen et al. (2012) was published, Swartz et al. (2013) showed that the amygdala activation response to sad and neutral faces habituated less across the stimulus presentation trials among children and adolescents with ASD compared to typically developing peers. Interestingly, the reduced amygdala response habituation to neutral faces correlated with autism severity. In fact, similar results had been reported even earlier by Kleinhans et al. (2009), who showed that adult individuals with ASD did not show a typical amygdala response habituation to neutral faces, and that the more severe an individual's social impairment was, the less the amygdala response habituated to faces. Kleinhans et al. (2009) suggested that individuals with ASD may have amygdalar dysfunctions, leading to stimulus overload and impairments in discrimination between salient and non-salient social information, both having fundamental effects on the development of social cognition.

Habituation is a process wherein a frequent or sustained exposure to a stimulus reduces behavioral and neural responses over time (Thompson and Spencer 1966). For example, an exposure to repeated affective or facial stimuli leads to habituation in neural responses, which, however, is modulated by emotional valence and the behavioral relevance of the stimuli (Breiter et al. 1996; Ishai et al. 2004; Klorman and Ryan 1980; Klorman et al. 1975, 1977). Atypical habituation to sensory input might result in hypo- or hypersensitivity to stimuli, leading to maladaptive sensory-seeking or sensory-avoiding behavior, respectively (McIntosh et al. 1999).

Unusual sensory responses are over-represented among individuals with ASD (for reviews, see Iarocci and McDonald 2006; O'Neill and Jones 1997). Even though habituation to sensory stimuli among individuals with ASD has been investigated for decades, it is not clear whether abnormal habituation has a role in autism pathogenesis or not. In their review of sensory theories in autism, Rogers and Ozonoff (2005) concluded that the evidence in existence did not provide support for abnormal levels of arousal or atypical habituation to visual or other sensory stimuli in autism, although they highlighted that the evidence was not consistent among the studies reviewed. Since the review by Rogers and Ozonoff (2005) was published, several studies (Bruno et al. 2014; Ewbank 2015; Guiraud et al. 2011; Kleinhans et al. 2009; Swartz et al. 2013; Webb et al. 2010) have yielded more findings related to adaptation and habituation to sensory stimuli among individuals with ASD or with autistic traits. Infants with a high risk for autism seem to show reduced neural habituation to auditory stimuli (Guiraud et al. 2011) and toddlers with ASD or autistic traits seem to habituate their looking times to familiarized faces more slowly than typically developing toddlers (Webb et al. 2010). The study by Webb et al. (2010) also showed a negative correlation between the habituation of looking times to repeated pictures of faces (familiarization) and autistic symptoms. A recent study (Ewbank 2015) showed that also later in life, autistic traits among typically developing individuals seem to be associated with an attenuated reduction in neural responses to repeated social (face) and nonsocial (e.g., scenes) stimuli. In addition, individuals with Fragile X syndrome, who often exhibit characteristics of autism spectrum disorder, show a diminished habituation of cortical

activation to faces, with no difference in habituation to a direct versus an averted gaze (Bruno et al. 2014). These results support the above-mentioned findings of atypical amygdala response habituation by Kleinhans et al. (2009) and Swartz et al. (2013), and together, these findings imply that autistic traits might indeed be related to atypical brain response habituation to the repetition of sensory stimuli.

Attenuated arousal response habituation limited to a direct gaze could explain the earlier findings by Kylliäinen and Hietanen (2006) and Kylliäinen et al. (2012), which show enhanced skin conductance responses to a direct gaze in comparison to responses to an averted gaze or closed eyes among children with ASD. This could mediate the observed relation between social impairments and enhanced autonomic arousal to a direct gaze in earlier studies by Joseph et al. (2008) and Kaartinen et al. (2012). In the study by Kaartinen et al. (2012), autism severity was associated with arousal responses only when the analysis was based on the *probability* that over the sequence of stimulus trials, a direct gaze would evoke stronger autonomic arousal than an averted gaze and closed eyes.

The aim of the present study was to re-analyze the data from the study by Kaartinen et al. (2012) to investigate (a) habituation of autonomic arousal responses to repeated facial stimuli among children with and without ASD, (b) differences in the habituation of autonomic responses between a direct gaze, an averted gaze, and closed eyes among children with and without ASD, and (c) correlations between response habituation and social impairments among children with and without ASD. The data were the same as in the study by Kaartinen et al. (2012), where the skin conductance responses of participants were measured while they saw a live neutral face with three different gaze conditions: a direct gaze, an averted gaze, and eyes shut. We expected that the children with ASD would show diminished habituation of autonomic responses, especially to a direct gaze, and that the level of response habituation to a direct gaze would correlate negatively with social impairments, i.e. diminished habituation of autonomic responses to a direct gaze would be associated with greater social impairments. We expected that children without ASD would show autonomic response habituation to all three facial stimuli and not show correlations between levels of autonomic response habituation and social skills.

## Methods

The participants, the experimental setup, and the methods of measurement used in this study are based on and described in more detail in the study by Kaartinen et al. (2012); as a result, they are presented here only briefly.

The participants were twenty-three children with ASD and 21 control children aged from 8 to 16 years. Children with ASD were recruited from the Department of Child Psychiatry, Tampere University Hospital, where they had been diagnosed with autism spectrum disorder (ASD) by experienced physicians using clinical procedures and according to the ICD-10 classification. The exclusion criteria for children with ASD included a total IQ lower than 70 or the presence of a depression or anxiety diagnosis. The control children without a previous history of mental or neurological disorders or learning disabilities were recruited from local schools. The exclusion criteria for children without ASD also included a total IQ lower than 70.

The participants were studied with skin conductance measurements while they saw the "live" face of a female model or a vase (a control stimulus) through a voltage-sensitive liquid crystal shutter window. The facial stimuli and the control stimulus were presented in separate blocks, and half of each group (ASD and control) were first presented the facial stimuli followed by the control stimulus, whereas the other half saw the stimuli in the reverse order. The face was oriented directly towards the participant and bore a neutral expression with a direct gaze, an averted gaze, or closed eyes. The control stimulus was a 30 cm-high curved vase with two round handles on both sides. The control stimulus was located at the same height as the facial stimulus. Each facial condition (direct gaze, averted gaze, and eyes closed) was presented six times in random order (18 trials in total). The control stimulus was presented six times in a separate block. In total, each participant underwent 24 trials in the experiment. A single trial lasted for 5 s and the inter-stimulus interval (ISI) varied from 20 to 35 s. Before each trial, the individual conducting the experiment said, "Let's look at the next one" to ensure that the participant directed his or her attention towards the shutter. After each facial stimulus, to ensure that the participant was looking at the facial stimuli and that he or she distinguished the different stimulus conditions, the individual conducting the experiment asked the participant whether the model gazed directly ahead, sideways, or whether the eyes were shut. During the control stimulus block, the participants simply viewed the stimuli.

During stimulus presentation, skin conductance was recorded from the participants' left hand using standard procedures. The skin conductance response (SCR) was defined as the maximum amplitude change from baseline (stimulus onset) between 1 and 3.5 s from the stimulus onset. The parents of the participants were interviewed with the Developmental, Dimensional and Diagnostic Interview (3di) (Skuse et al. 2004) to measure impairments in social skills. The following five subscales from the interview's Pervasive Developmental Disorder (PDD) Scale were used to measure impairments in social skills (scale range in parentheses): reciprocal social interaction (0–30), use of language and other social communication skills (0–26), use of gesture and non-verbal play (0–14), repetitive and stereotyped behavior (0–12), and social expressiveness (0–4).

**Table 1** Participant characteristics

| | ASD | | | | | Controls | | | | | *p* value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | SD | Min | Max | n | Mean | SD | Min | Max | |
| Gender | | | | | | | | | | | |
| Boys | 14 | | | | | 14 | | | | | |
| Girls | 4 | | | | | 4 | | | | | |
| Age | | 12.7 | | 8.6 | 15.1 | | 12.3 | | 8.5 | 15.9 | 0.605 |
| IQ | | | | | | | | | | | |
| Verbal | | 102 | 20 | 69 | 145 | | 107 | 20 | 71 | 137 | 0.443 |
| Performance | | 94 | 17 | 56 | 119 | | 101 | 11 | 81 | 122 | 0.035 |
| Total | | 97 | 13 | 74 | 118 | | 104 | 15 | 77 | 127 | 0.109 |

After exclusions, e.g., due to missing data and technical reasons (reasons for exclusions are described more in detail in Kaartinen et al. (2012)), SCR data were available for 18 children with ASD (14 boys and 4 girls) and for 18 controls (14 boys and 4 girls). Participant characteristics are presented in Table 1. SCR and 3di data were available for 11 boys and 4 girls in the group of children with ASD and for 12 boys and 4 girls in the control group. Social impairment scores among participants are presented in Table 2. The complete participant characteristics are presented in Kaartinen et al. (2012). The study was approved by the Ethical Committee of the Pirkanmaa Hospital District.

### Statistical Analysis

The *t* test was used to analyze differences between the groups in terms of age and IQ. To address habituation, we fitted a random coefficients linear mixed model on the logarithmically transformed SCR data assuming child-specific random intercepts and slopes. The random coefficients model enables a flexible and realistic modelling of the mean and covariance structure across repeated measurements. Overall habituation to the facial stimuli was investigated in a time-ordered sequence of 18 measurements, with time and stimulus as fixed effects. Habituation to the control stimulus and for each facial stimulus separately was investigated in a sequence of six measurements with time as a fixed effect. Differences between the ASD and control children were investigated by testing equalities in group-wise slopes, using appropriate Wald tests accompanied with the Kenward-Roger adjustment. Participant-specific slopes (best linear unbiased prediction; BLUP) were extracted from the models as quantities measuring the tendency for habituation, and those were then correlated with the social skill measures of the child. Spearman's correlation coefficient was used for studying the associations. The level of statistical significance was set at 0.05.

## Results

### Habituation of Autonomic Arousal Responses to Facial Stimuli

In the control group, there was an overall habituation of the SCRs to repeated presentations of facial stimuli ($p = 0.048$),

**Table 2** Social impairment scores among participants

| | ASD (n = 15) | | | Controls (n = 16) | | | p |
|---|---|---|---|---|---|---|---|
| | Median | Min | Max | Median | Min | Max | |
| Impairments in social skills | | | | | | | |
| Reciprocal social interaction | 10.8 | 3.3 | 19.4 | 3.7 | 1.8 | 5.5 | <0.001 |
| Use of language and other social communication skills | 10.4 | 4.4 | 17.4 | 1.4 | 0.2 | 9.4 | <0.001 |
| Use of gesture and non-verbal play | 6.1 | 2.0 | 11.0 | 1.1 | 0.0 | 8.0 | <0.001 |
| Repetitive and stereotyped behavior | 4.0 | 0.3 | 6.7 | 0.0 | 0.0 | 1.0 | <0.001 |
| Social expressiveness | 1.8 | 0.0 | 3.5 | 1.1 | 0.8 | 2.1 | 0.025 |

i.e. habituation analyzed across all 18 experimental trials of faces with three different gaze directions. A further analysis of the SCR habituation to different gaze directions separately showed that there was a statistically significant SCR habituation to repetitions of direct gaze stimuli ($p=0.025$), no habituation to repetitions of averted gaze stimuli ($p=0.727$), and a trend of SCR habituation to closed eyes stimuli ($p=0.061$). There was only a marginal difference between the SCR habituation to direct gaze, averted gaze, and closed eyes stimuli ($p=0.097$). This finding suggests that, in the control group, SCR habituation tends to be more evident in repetitions of direct gaze stimuli than in repetitions of averted gaze or closed eyes stimuli (Fig. 1).

In the ASD group, there was no indication of an overall SCR habituation to repeated presentations of facial stimuli ($p=0.590$). An analysis for each gaze direction separately confirmed that the SCRs did not habituate in response to repetitions of direct and averted gaze stimuli ($p=0.483$ and $p=0.927$, respectively). Response habituation to repetitions of closed eyes stimuli was marginally significant ($p=0.080$) (Fig. 1).

Even though the analyses presented above revealed SCR habituation only in the control group and not in the ASD group, a direct comparison of the group-wise habituation slopes showed that the overall SCR habituation was not significantly different between the groups ($p=0.209$) (see Fig. 1). Between-group comparisons for habituation to each gaze direction separately did not show statistically significant differences either in SCR habituation to direct gaze, averted gaze or closed eyes stimuli ($p=0.136$, $p=0.726$, and $p=0.758$, respectively).

### Habituation of Autonomic Arousal Responses to a Control Stimulus (a Vase)

SCR habituation to a control stimulus was not observed when the analysis was conducted on the groups ($p=0.515$), and habituation did not differ between the groups ($p=0.532$).

### Correlations Between SCR Habituation and Social Impairments

To study whether social impairments were associated with SCR habituation, the slopes of the SCRs to repetitions of facial stimuli were correlated (Spearman's correlation) with social impairment scores individually for each participant. It should be noted that a negative slope represents habituation, whereas a positive slope represents enhancement of the SCRs over stimulus repetitions (Fig. 2).

Analyses conducted for each gaze direction separately indicated that in the control group, the habituation of the SCRs to direct gaze or eyes closed stimuli did not correlate with any social skill impairment. Instead, the SCR habituation to an averted gaze appeared to correlate positively with impairments in use of language and other social communication skills ($p=0.016$), use of gesture and non-verbal play ($p=0.019$), in social expressiveness ($p=0.012$), and marginally in reciprocal interaction ($p=0.076$), i.e. a greater habituation to an averted gaze was associated with more social impairments among the participants in the control group (Table 3).

In the ASD group, the gaze direction analyses revealed that an association was found only in the SCR habituation

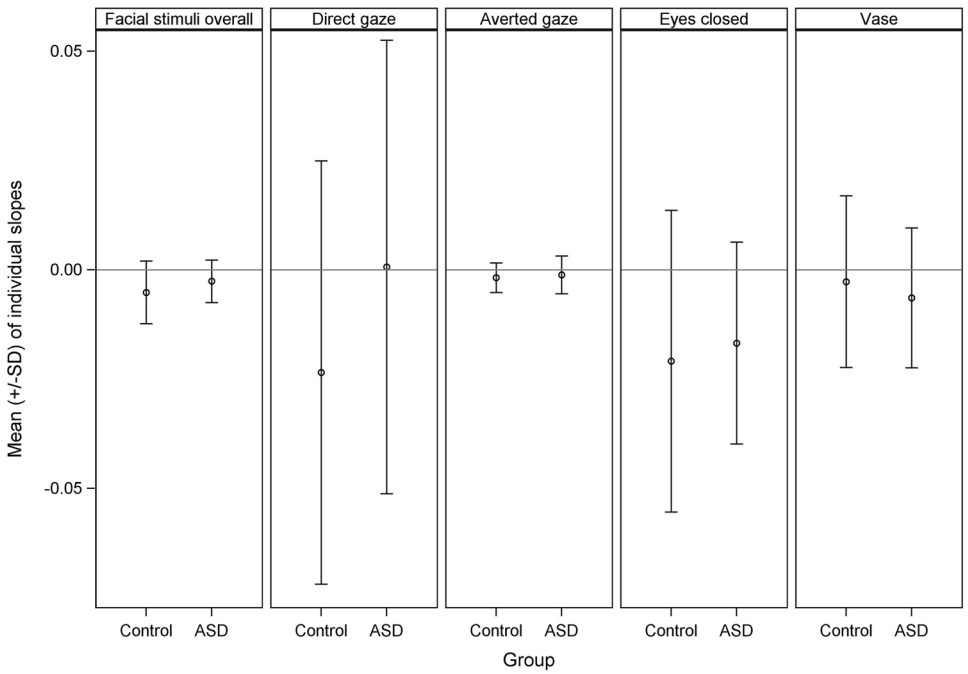


**Fig. 1** Mean values of the SCR habituation slopes

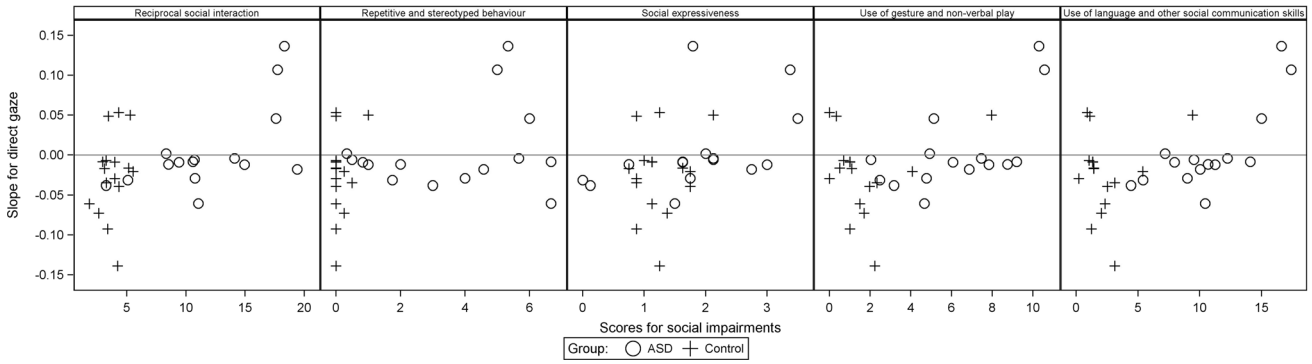


**Fig. 2** Correlations between the social impairment scores and the SCR habituation slopes

to a direct gaze; habituation of the SCRs in response to a direct gaze was inversely associated with impairments in use of language and other social communication skills ($p = 0.010$), use of gesture and non-verbal play ($p = 0.032$), and social expressiveness ($p = 0.015$). The slopes of the SCR for an averted gaze or closed eyes were not associated with impairments in social skills among the children in the ASD group (Table 3).

Autonomic response habituation to a control stimulus did not correlate with any social impairment in either of the groups.

**Table 3** (Spearman's) correlations between the slopes of the SCRs to repetitions of stimuli and impairments in social skills among children with ASD and without ASDTable 3 (Spearman's) correlations between the slopes of the SCRs to repetitions of stimuli and impairments in social skills among children with ASD and without ASD

| | All stimuli | Direct gaze | Averted gaze | Closed eyes | A control stimulus |
|---|---|---|---|---|---|
| Reciprocal social interaction | | | | | |
| Control group | 0.02 | 0.25 | −0.46 | −0.02 | −0.16 |
| ASD group | 0.42 | 0.41 | 0.08 | −0.41 | −0.09 |
| Use of language and other social communication skills | | | | | |
| Control group | −0.07 | −0.34 | **−0.59** | 0.04 | −0.14 |
| ASD group | **0.70** | **0.64** | −0.05 | −0.21 | 0.26 |
| Use of gesture and non-verbal play | | | | | |
| Control group | −0.05 | −0.38 | **−0.58** | 0.08 | −0.11 |
| ASD group | **0.63** | **0.55** | −0.03 | −0.23 | 0.35 |
| Repetitive and stereotyped behavior | | | | | |
| Control group | 0.04 | −0.01 | −0.41 | 0.01 | −0.36 |
| ASD group | 0.38 | 0.07 | 0.11 | −0.02 | 0.18 |
| Social expressiveness | | | | | |
| Control group | −0.06 | 0.06 | **−0.61** | −0.05 | 0.06 |
| ASD group | 0.22 | **0.61** | −0.42 | −0.32 | −0.17 |

Statistically significant correlations are bolded

## Discussion

The aim of the present study was to ascertain whether children with ASD show atypical habituation of autonomic arousal responses to facial stimuli and whether autonomic response habituation to seeing a face with a direct gaze would be associated with the level of social impairments among children with ASD. The results showed that, among the children with ASD, the smaller the habituation was, specifically in responses to a direct gaze, the more a child showed social impairments in the fields of use of language and other social communication skills, use of gesture and non-verbal play, and social expressiveness. Autonomic response habituation to faces with an averted gaze or closed eyes or to a control stimulus (a vase) was not related to autism severity.

Although the results showed that the autonomic responses to facial stimuli, in general, habituated in the group of control children but not among the ASD children, direct comparisons between the groups failed to show statistically significant differences in the slopes of response habituation to facial stimuli overall or to any category of gaze stimuli (direct gaze, averted gaze, closed eyes) separately. The SCR response habituation to facial stimuli varied among the children with ASD and there seemed to be children with ASD who actually showed enhancement of autonomic arousal over repetitions of facial stimuli (positive slopes of the SCRs to repetitions of facial stimuli) (see Fig. 1). Swartz et al. (2013) also reported amygdala response sensitization, i.e. enhancement in amygdala responsivity, over repetitions of facial stimuli in children and adolescents with ASD. According to the present results,

attenuated response habituation to facial stimuli and to a direct gaze specifically is not a core symptom in autism, but might be an important element in determining how severe autism symptoms develop. Considering that the amygdala has been suggested to play a central role in mediating the affective arousal elicited by eye contact (Senju and Johnson 2009), the present results are also in line with the previous studies by Kleinhans et al. (2009) and Swartz et al. (2013), which showed that, among children and adults with ASD, amygdala activation seems to be attenuated to facial stimuli, and that reduced amygdala habituation is related to greater autism severity. As in the present study, autism severity was related to the response habituation to a direct gaze only; it is also possible that in the studies by Kleinhans et al. (2009) and Swartz et al. (2013), social impairments were actually related to habituation of amygdala activation in response to neutral face stimuli with a direct gaze.

The present study cannot answer how attenuated response habituation and autism severity are causally related to each other from a developmental perspective. It has been suggested that in autism, facial stimuli, especially faces with a direct gaze, elicit hyperarousal that is experienced as unpleasantness and yields eye contact avoidance, and this, in turn, has negative impacts on social development (Hutt and Ounsted 1966; Skuse 2003). Attenuated response habituation to a direct gaze could be one mechanism that might reduce experienced pleasure in social interaction and lead to the active avoidance of eye contact. It seems that infants who later develop ASD orient to social stimuli during the first months of life and also show typical eye contact behavior (Bryson et al. 2007; Jones and Klin 2013; Rozga et al. 2011). A decline in the amount of looking at other people's eyes seems to occur from 2 months until 24 months of age, and a steeper decline in looking at the eyes is associated with more severe social disability (Jones and Klin 2013). As eye contact seems to be intact after birth among infants who later develop ASD, one could speculate that diminished response habituation to eye contact does not seem to be present immediately after the birth or have an impact on orienting to other people's eyes in early life. It is also possible that the importance of autonomic arousal response habituation increases after the second half of the first year of life. This view is supported by Peltola et al. (2009), who observed an emergence of an attention allocation bias towards fearful emotional expressions between the ages of 5 and 7 months. Peltola et al. (2009) suggested that their finding might reflect a developmental change in brain function, e.g., enhancement of amygdala responses to stimuli that signal potential threats. Following this line of reasoning, one could speculate that if social stimuli (e.g., a direct gaze) also start to elicit amygdala-mediated enhanced arousal responses at this developmental stage, and if these responses show atypical (diminished) habituation, it is possible that this mechanism plays a role in the development of autismrelated abnormalities in eye contact behavior. However, it is unlikely that attenuated autonomic habituation would exclusively explain diminished eye contact behavior in autism, and it is more likely that multiple factors are working side by side to cause the typical impairments in gaze behavior in ASD (Dalton et al. 2005; Joseph and Tanaka 2003; Klin et al. 2002; Osterling and Dawson 1994; Rice et al. 2012; Spezio et al. 2007; Zwaigenbaum et al. 2005; for negative results, see Falck-Ytter et al. 2015).

Amygdala seems to have an important role, especially in autonomic arousal responses and arousal habituation to repeated fearful and threatening stimuli (Critchley 2002; LaBar et al. 2008; Sequeira et al. 2009). Besides amygdala-mediated emotional processes, attentional and cognitive processes also participate in the generation of autonomic arousal responses controlled by activation in prefrontal and parietal cortices and limbic areas (e.g., the hippocampus) (Büchel et al. 1998; Critchley 2002, 2009; Knight et al. 2005; LaBar et al. 2008; Sequeira et al. 2009). Keehn et al. (2013) suggested that early emerging impairments in disengaging attention from a direct gaze might lead to hyperarousal during eye contact in autism, and this, in turn, could have a negative impact on development in a broad array of domains associated with ASD, including novelty processing, social attention, over-focused attention, restricted and repetitive behaviors, and executive functions. Autistic infants might involuntarily maintain engaged eye contact longer than typically developing infants due to e.g., impairments in flexible visual orienting and face processing (Bryson et al. 2007; Elison et al. 2013; Elsabbagh et al. 2013; Webb et al. 2010; for a review, see Jones et al. 2014; Keehn et al. 2013). While typically developing toddlers increase the speed and flexibility of their visual orienting between the ages of 7 and 14 months, infants later diagnosed with ASD do not show similar advantages in the speed and flexibility of their visual orienting skills, and indeed, a significant number of the infants later diagnosed with ASD might even show increasing disengagement latencies during the abovementioned time frame (Elsabbagh et al. 2013). Toddlers with ASD and with the most severe autistic symptoms habituate more slowly to familiarized faces, i.e., they do not show a similar decline in looking times to repeated presentations of facial photographs, as compared to toddlers with milder ASD symptoms or to their typically developing peers at the age of 18–30 months (Webb et al. 2010). If autistic infants are prone to maintain eye contact for atypically long times due to their impairments in flexible orienting and face processing while simultaneously experiencing enhanced arousal due to impairments in arousal habituation, gaze aversion would offer a natural solution to avoid these unpleasant experiences.

Typically, besides rapid autonomic response habituation, presentation of a sensory stimulus leads to longer lasting enhancement in autonomic arousal response habituation to similar stimuli, and this habituation enhancement can be maintained at least for several days (Harding and Rundle 1969; Kimmel & Golstein 1967). It is possible that the level of short-term autonomic response habituation to facial stimuli measured in the present study was affected by long-term habituation caused by earlier exposure to these stimuli. Importantly, it is also possible that this long-term habituation differs between the groups. For example, in ASD, avoidance of eye contact might have led to diminished exposure to faces and to attenuated long-term and short-term autonomic response habituation to direct gaze. Inclusion of a novel control stimulus (a vase) allowed investigation of short-term autonomic response habituation to a non-social stimulus without long-term habituation (previous exposure to this stimulus) influencing the results. The results showed no autonomic response habituation in either of the groups, possibly reflecting arousal induced by novelty processing. Unfortunately, it was impossible to draw definitive conclusions on the possible differences in autonomic arousal habituation in response to social and non-social stimuli, as the facial stimuli were presented in a block of three different stimuli (three gaze directions), whereas the control stimulus block contained six consecutive presentations of a single control stimulus: it is entirely possible that this might have biased the habituation process. Future studies are needed to ascertain whether the observed association between autonomic arousal to direct gaze and autism severity reflects direct gaze-specific or generalized impairment in arousal regulation, e.g., due to impairments in attentional processes.

In the present study, only faces with a neutral expression were shown. Thus, it is not clear whether the observed association between autism severity and diminished arousal response habituation to a direct gaze is actually limited to neutral faces. Indeed, abnormal habituation of amygdala activation in response to repetition of facial stimuli is not evident for all emotional facial expressions. Swartz et al. (2013) reported that amygdala activation habituation was diminished only to sad and neutral faces but not to happy or fearful faces, and autism severity correlated only with diminished habituation of amygdala activation to neutral faces. Swartz et al. (2013) speculated that sad and neutral faces could be more aversive or ambiguous stimuli than happy and fearful faces to children with ASD. Support for this speculation comes from a study by Tottenham et al. (2014). In their study, individuals with ASD attended less to the eye region of a neutral face but attended similarly to the eye region of an angry face in a comparison with controls. When Tottenham et al. experimentally manipulated the participants' focus of gaze to the eye region of the stimuli, enhanced amygdala activation was seen only among the autistic individuals, and only when they were viewing neutral faces. The amount of potentiation in amygdala activation was positively associated with inattentiveness to the eye region in naturalistic situations. In addition, autistic individuals seemed to have a bias to mislabel neutral faces as negative facial expressions, and those individuals with ASD who estimated the neutral faces as most threatening attended less spontaneously to the eye region of the neutral faces. Taken together, these results might suggest that interventions that aim to support eye contact behavior among autistic individuals could be facilitated by combining a direct gaze with a positive, happy facial expression in order to reduce direct, gaze-induced autonomic arousal.

The present results suggest that among typically developing children, autonomic arousal response habituation to a direct gaze marginally differed from that to an averted gaze and closed eyes; while repetition of direct gaze stimuli resulted in a decline in arousal response, repetition of averted gaze stimuli did not (see Fig. 1). There are several explanations for this finding. First of all, these two gaze directions have distinct meanings in social interaction. Already from birth, human individuals tend to look longer and more frequently at faces with open eyes and direct gazes than at faces with averted gazes or closed eyes (Batki et al. 2000; Farroni et al. 2006). Direct and averted gazes seem to trigger distinct neural processes in the so-called social brain, confirming their different roles in social interaction (Hadjikhani et al. 2008; for reviews, see e.g.; George and Conty 2008; Nummenmaa and Calder 2009; Senju & Johnsson 2009). While another individual's averted gaze triggers shifts of attention orienting and has an important role in joint attention (Frischen et al. 2007), a direct gaze enhances engagement in social interaction and boosts socially important information processing, as it captures and holds attention, and facilitates other facial information processing (Macrae et al. 2002; Pellicano and Macrae 2009; Senju et al. 2003; Senju and Hasegawa 2006; for a review, see Senju and Johnson 2009). Considering the important role of the averted gaze in shifting attention and signaling environmental danger, it is understandable why averted gaze stimuli resist arousal response habituation (Hadjikhani et al. 2008). In addition, sustained eye contact plays an important role in social development and, therefore, it might be important to be able to regulate arousal to a direct gaze (Cherulnik et al. 1978; Keller and Zach 1993; Robson et al. 1969). It is also possible that autonomic response habituation to an averted gaze was not found because, in the sequence of stimulus presentation, averted gaze stimuli contained two different gaze directions, an averted gaze to the left and an averted gaze to the right. These two gaze directions might be processed as two different stimuli at the neural level; neurons in the human brain code specific

gaze directions (left vs. right) instead of distinguishing just between a direct and averted gaze (Frischen et al. 2007). As it was shown that, among the control children, enhanced response habituation to an averted gaze was associated with autistic traits, the first explanation seems more likely. For social development, it might be important that the arousal response triggered by an averted gaze does not habituate. More studies are needed to confirm these findings and to ascertain why children with ASD did not show an association between habituation of autonomic arousal responses to an averted gaze and social impairments similarly to their typically developing peers.

### Limitations

The sample size in the present study was relatively small and included only children with ASD and an IQ over 70. For example, it is possible that statistically significant differences between autistic and typically developing children in response habituation to a direct gaze could be observed if the study was replicated among children with more severe ASD than those in the present study. The present study did not include eye-tracking measurements and, therefore, we cannot know whether attenuated arousal response habituation to a direct gaze was related to the participants' gaze behavior during the task. For example, the duration of looking at the stimuli could have been shortened due to avoidance of the eye region or extended due to impairments in disengaging from the visual stimuli (e.g., Elsabbagh et al. 2013; Klin et al. 2002; Rice et al. 2012; Webb et al. 2010). Future studies with eye tracking measurements are needed to ascertain whether attenuated arousal response habituation is related to fixation patterns and fixation times to facial stimuli and different facial regions (e.g., the eyes and mouth). As the participants' gaze was directed at the stimulus person's eye region by asking them to discriminate the gaze direction after each trial, it might have resulted in an atypical situation among those children who avoid looking at other people's eyes, and thus could have exerted an impact on arousal response habituation. Measurements of the participants' gaze behavior in more naturalistic settings would yield additional information about the association of arousal response habituation in conditions of non-manipulated gaze behavior. It should also be noted that as the scores for social impairments were very low in the group of children without ASD, it is possible that associations between autonomic arousal response habituation to a direct gaze and social impairments were not revealed because of the small variance in the social impairment scores. On the other hand, despite the small variance in these scores, the present study was able to detect an association between social impairments and enhanced habituation of autonomic arousal responses to an averted gaze among these same children. Due to the different number of trials in the block of the facial stimuli and the block containing the control stimulus, it was not appropriate to make direct statistical comparisons between the data to ascertain whether autonomic response habituation to social and non-social stimuli differed among children with ASD. Future studies are needed to clarify this issue. The participants' possible medication was not monitored in the present study. This should be considered as a limitation, as earlier studies have shown that, e.g., antipsychotic and methylphenidate medication might have an attenuating and enhancing impact, respectively, on skin conductance responses (Conzelmann et al. 2014; Green et al. 1989; Negrao et al. 2011; Spohn et al. 1971). However, if medication had influenced the SCRs in the present study, the influence would have been similar for all stimuli in the four different categories, and thus the medication is unlikely to have had a role in the main findings.

## Conclusions

Diminished autonomic arousal response habituation to repetitions of direct gaze stimuli seems to be associated with increased social impairments among children with ASD. Even though the present study cannot answer how attenuated response habituation and autism severity are causally related to each other, it is possible that, in autism, attenuated response habituation to a direct gaze reduces experienced pleasure in social interaction. The avoidance of eye contact might serve as a solution in avoiding unpleasant social experiences, which, in turn, might narrow a child's opportunities to proceed in social development, as engaging in eye contact seems to be essential for social development (Corden et al. 2008; Jones et al. 2008). If this suggestion is proved true, interventions that increase experienced pleasure in social interaction, e.g., by combining a direct gaze with a positive, happy facial expression to reduce direct gaze-induced autonomic arousal, might be useful in the rehabilitation of eye gaze behavior in autism. An interesting question for future studies is whether abnormalities in gaze behavior could even be prevented, or at least alleviated to some degree, if these kinds of interventions could be targeted at infants at risk for ASD from the very early stages, before the first signs of gaze avoidance behavior.

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**Author Contributions** Miia Kaartinen has made substantial contributions to conception and design, and acquisition of data, and analysis and interpretation of data, participated in drafting and revising the article and has given final approval of the version to be submitted. Kaija Puura has made substantial contributions to conception and design, and analysis and interpretation of data, participated in drafting and revising the article and has given final approval of the version to be submitted. Sari-Leena Himanen has made substantial contributions to acquisition of data, has participated in revising the article and has given final approval of the version to be submitted. Jaakko Nevalainen has made substantial contributions to analysis and interpretation of data, participated in drafting and revising the article and has given final approval of the version to be submitted. Jari Hietanen has made substantial contributions to conception and design, and acquisition of data, and analysis and interpretation of data, participated in drafting and revising the article and has given final approval of the version to be submitted.

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**Compliance with Ethical Standards**

**Conflict of Interest** Author Miia Kaartinen has received research grants from the European Union (The GEBACO Project), the Medical Research Fund of Tampere University Hospital, The Child Psychiatric Research Foundation (Finland), The Emil Aaltonen Foundation and The Finnish Medical Foundation, but declares that she has no other conflict of interest. Author Kaija Puura declares that she has no conflict of interest. Author Sari-Leena Himanen is a shareholder in the company Neurotest and has received research grant from Competitive Research financing of the Expert Responsibility area of Tampere University Hospital (Grant No. 9R007 and 9PO13), but declares that she has no other conflict of interest. Author Jaakko Nevalainen declares that he has no conflict of interest. Author Jari Hietanen has received research grant from the Academy of Finland (MIND program Grant, Project No. #266187), but declares that he has no other conflict of interest.

**Ethical Approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed Consent** Informed consent was obtained from all individual participants included in the study.

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ORIGINAL PAPER

# Reactive aggression among children with and without autism spectrum disorder

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**Abstract** Twenty-seven boys and eight girls with ASD and thirty-five controls matched for gender, age and total score intelligence were studied to ascertain whether boys and girls with ASD display stronger reactive aggression than boys and girls without ASD. Participants performed a computerized version of the Pulkkinen aggression machine that examines the intensity of reactive aggression against attackers of varying gender and age. Relative to the control group boys, the boys with ASD reacted with more serious forms of aggression when subjected to mild aggressive attacks and did not consider a child attacker's opposite sex an inhibitory factor. The girls with ASD, on the other hand, reacted less aggressively than the girls without ASD. According to the results boys with ASD may not follow the typical development in cognitive regulation of reactive aggression.

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## Introduction

Aggression may be adaptive, occurring for purposes of ensuring the integrity or survival of the individual, or maladaptive. Maladaptive aggression includes aggression that appears to occur independently of a usual, definable social context; aggression that occurs in the absence of antecedent social cues; aggression that is disproportionate to its apparent causes in intensity, frequency, duration, and/or severity; and aggression that does not terminate appropriately. (Connor 2002). Every human being is born with certain aggressive traits. Typically, frequency of aggressive behavior peaks during the first 3 years of life (Connor 2002). Following the normal course of development, children's ability to regulate emotion and associated aggressive behavior improves, leading to enhanced cognitive abilities to handle social situations with other, more constructive forms of behavior (Calkins and Fox 2002). In normal development, after toddlerhood boys exhibit more aggressive behavior than girls (Card et al. 2008; Loeber and Hay 1997). According to Connor (2002), among preverbal infants aggressive acts are reactive (also called impulsive, affective or hostile aggression), and are provoked and reinforced by frustration, anger or immediate intention and need. Between 2 and 4 years the use of direct physical aggression is gradually replaced by verbal aggression, and this development continues throughout childhood. The most complex, proactive forms of aggression (also called predatory, instrumental, premeditated aggression), become more typical later in childhood. They are based on complex social learning and reinforced by

planned, goal oriented action to obtain objects from others or to establish social dominance over others.

Autism spectrum disorders (ASD) are characterized by impairments in social interaction and communication and restricted, repetitive and stereotyped patterns of behavior, interests, and activities (DSM IV, 2000) and seem to be associated with too frequent or intense aggression. Individuals with ASD show significantly more self-injurious and aggressive behavior than individuals without ASD, at least when combined with intellectual disability (Green et al. 2000; McClintock et al. 2003; Tsakanikos et al. 2007). Significant aggressive behavior towards either a caregiver or another person seems to complicate the lives of thirty-five percent of the children and adolescents with ASD (Kanne and Mazurek 2011). Aggressive behavior may impair the developmental outcomes of individuals with ASD, as ASD combined with aggressive behavior is associated with an elevated risk for mood disorder during childhood (Ming et al. 2008), and predicts use of anti-psychotic medication and psychiatric services in adulthood (Tsakanikos et al. 2007). Also, individuals with ASD may be overrepresented among adult violent offenders (Soderstrom et al. 2004).

Among children with ASD, the typical age for the onset of aggressive behavior seems to be during toddlerhood, but in some cases onset considerably later at 5 years has also been reported (Dominick et al. 2007). There seems not to be a difference in the frequency of aggressive behavior between boys and girls with ASD in infancy and toddlerhood (Hartley et al. 2008), but little is known about possible differences between boys and girls with ASD in aggressive behavior after toddlerhood. Kopp and Gillberg (1992) have proposed that whereas boys show excessive aggression girls do not, and thereby conceal their autistic symptoms, which may lead to undiagnosed cases of ASD among girls. Even though there do not seem to be gender differences in the presence of definite aggression towards either a caregiver or non-caregiver among children and adolescents with ASD (Kanne and Mazurek 2011), the phenotype of aggressive behavior may still be different among boys and girls with ASD. Reese et al. (2005) found that disruptive behavior may serve different functions among boys and girls with ASD in childhood; while the boys with ASD in their study used disruptive behavior to gain or maintain access to items with which to engage in repetitive behavior, or to avoid unpleasant sensory stimulation, girls with ASD used disruptive behavior to gain attention or items, or to escape demands in general, as also did girls and boys without ASD.

Research in developmental psychology has yielded a vast amount of information on individual differences in aggression by using observations and questionnaires to measure the forms and frequency of intentional and harmful behavior (Coie and Dodge 1997). However, in order to devise effective help for aggressive children (and their families) at the earliest possible time, experimental studies are needed to yield insight into the process of aggression, that includes both the situational elements that conducive to aggression and the emotional, cognitive, and behavioral processes that antecede aggression (Berkowitz 1993; Connor 2002). One method to study individual differences in the developmentally earliest form of reactive aggression is the computerized Pulkkinen aggression machine (PAM; Juujärvi et al. 2001), designed to study the inhibition of reactive aggression in the absence and presence of situational cues. Juujärvi et al. (2001) noted that without situational cues typically developing school-aged boys' and girls' response intensity is associated with attack intensity; the stronger the attack, the stronger the response, and that situational cues (a specified assailant of a certain age and sex) serve as inhibitory cues and attenuate children's responses to attacks. An assailant of the same sex and physical strength was observed to signal the weakest inhibitory cues among boys and girls, and a bigger child signalled weaker inhibitory cues than a smaller child or a parent. Both boys and girls seemed to inhibit their responses when faced with an assailant of the opposite sex. (For further details, see Juujärvi et al. 2001). Earlier studies conducted with the PAM task have shown that male gender, low self-control of emotions (enhanced aggressive and anxiety/depressive symptoms) and low-levels of emotion reactivity are associated with more intense responses to attacks with minor intensity and that poor cognitive regulation of emotion is associated with more intense responses to all attacks among typically developing children (Juujärvi et al. 2001, 2006a, b). The aim of this study was to ascertain whether boys and girls with ASD and total IQ over 70 showed more intense reactive aggression than boys and girls without ASD, and whether boys and girls with ASD failed to use the situational cues (gender and age) of an assailant as inhibitory cues to attenuate their reactions to attacks in the PAM (Juujärvi et al. 2001). The first hypothesis was that boys with ASD would show more intense reactive aggression than boys without ASD, but that there would be no differences in reactive aggression between girls with and without ASD because of the different phenotypes of aggression among boys and girls with ASD (Kopp and Gillberg 1992). It was also expected that the different phenotype in aggression among the boys and girls with ASD would also be seen in the ability to inhibit reactions to aggressive acts according to situational cues of assailants. The second hypothesis was, therefore, that in the controlled aggressive condition the situational cues (gender and age) of an assailant would not attenuate reactions of boys with ASD similarly than reactions of boys with ASD but girls with ASD would be able to use the situational cues of an assailant to inhibit their responses similarly than girls without ASD.

## Participants and procedure

The participants were 27 boys and eight girls with ASD and their 35 controls matched for gender, age and total score intelligence. Seven- to 17-year-old children with ASD were recruited from the Department of Child Psychiatry of Tampere University Hospital, where they had been diagnosed by experienced physicians using standard clinical procedures to have an autism spectrum disorder according to ICD-10. Seven children had a diagnosis of childhood autism (F84.0), six that of atypical autism (F84.1) and 22 Asperger's syndrome (F84.5). Exclusion criteria included the presence of depression or anxiety diagnosis or total IQ lower than 70. The children in the control group were recruited from local schools, and according to their parents had no history of mental or neurological disorders or learning disabilities.

All the children and their parents received information concerning the methods and aims of the study before they were interviewed and tested. Afterwards the children received a movie ticket as a reward for their participation. The study was approved by the Ethics Committee of the Pirkanmaa Hospital District, and all participants and their parents gave their written informed consent.

## Methods

Aggressive behavior was tested using the Pulkkinen Aggression Machine (PAM; Juujärvi et al. 2001). The children were seated in front of a laptop computer in a silent room, with only a researcher present. She seated herself so that she could not see the responses chosen by the child to minimize any possible effect of her presence on the children's choices in the task. Girls especially might have been inclined to follow social norms in such situations if they believed the experimenter was aware of their responses (Pepler and Craig 1995). The duration of the PAM task was approximately 20 min. The method used in the clinic to test the IQ of the ASD subjects at the time of the study as WISC-III (Wechsler Intelligence Scale for Children, Third edition). Consequently, the controls also had their IQ tested by WISC-III.

### The pulkkinen aggression machine (PAM)

The PAM is a computerized task designed to study reactive aggression (Juujärvi et al. 2001). The total PAM task comprises three conditions administered in the following fixed order: (1) the arbitrary condition (2) the impulsive aggression condition, and (3) the controlled aggression condition. In every condition two parallel columns of eight stimulus and nine response icons are presented respectively on the left and the right sides of the computer screen. The stimulus icons depict offensive aggressive acts of increasing degrees of intensity, while the response icons depict retaliatory acts of aggression, also of increasing intensity. A rectangle which appears around one of the stimulus icons marks the delivery of an attack and requires a participant to determine his or hers response in retaliation. Stimulus presentation is self-paced, with the generation of a response triggering the next stimulus at a constant interval of 3 s after the last response. Responses are given by clicking a mouse over one of the response icons on the right side of the screen.

1. The arbitrary condition serves as a training condition. No aggressive content is assigned either to the stimulus or to the response icons. Instead, the icons contain neutral black dots. Each stimulus icon is delivered once in the following predetermined order: 3–5–4–2–7–0–1–6.
2. In the impulsive aggression condition the rows of stimulus and response icons depict aggression of varying degrees of intensity. The levels of intensity are scaled as follows: row 0 = a harmless interaction, row 1 = you are slightly pushed, row 2 = you are pinched, row 3 = you are slapped, row 4 = you are knocked to the ground, row 5 = your hair is being pulled, row 6 = you are hit with a stick, and row 7 = you are punched in the face. Participants are instructed to defend themselves against offensive attacks, as follows: "You are having a quarrel with somebody. A black rectangle around one of the icons on the left shows what the other person does to you. You may do to him or to her what you wish by touching one of the icons on the right and you do not need to worry about the consequences of your choice." Participants are free to imagine the assailant. Each stimulus icon is delivered twice in a predetermined order: 3–5–4–2–7–0–1–6–2–5–7–3–1–4–6–0. Note that the stimulus and response icons parallel each other with one exception: one icon is added on to the extreme end of the response scale to deal with possible ceiling effects (i.e., row 8 = the assailant is kicked while lying on the ground).
3. The controlled aggression condition is similar to the preceding one except that the assailant is now specified. In the center of the screen, a picture displays the identity of the assailant, and participants are told to imagine how they would behave in a real confrontation with the assailant. There are eight categories of assailants— a boy of the same size, a girl of the same size, a smaller boy, a smaller girl, a bigger boy, a bigger girl, a father and a mother. For each assailant, all 8 stimuli are presented twice in a fixed order:

3–5–4–2–7–0–1–6–2–5–7–3–1–4–6–0. The assailants are administered in random order. In addition to a picture of an assailant in the computer screen, the participants are instructed verbally about the size and the sex of assailants.

### Variables

For each stimulus–response pair a difference score was calculated by subtracting the stimulus intensity score from the respective response intensity score. The arithmetic mean of the differences of the two presentations of the pair (called response intensity from here on) was then used as the representative of the respective stimulus–response pair in further calculations.

To study whether the boys and girls with ASD showed more intense reactive aggression than the boys and girls without ASD either in the impulsive aggression condition or in the controlled aggression condition, one variable for the overall response intensity of impulsive and another for overall controlled reactive aggression were generated. The variable for impulsive aggression was calculated as an arithmetic mean of response intensities across all the eight stimulus levels (i.e., from 0 to 7). The variable for controlled aggression was calculated similarly across both all the eight stimuli and eight assailants.

To examine how the specification of an assailant influences the response intensity in the controlled aggression condition, one variable for each of the eight different assailants was generated: the arithmetic mean of the response intensities across the eight stimulus levels (i.e. for 0 to 7). The differences between the groups for low and high levels of attack intensity separately were also of interest. The abovementioned variables were recalculated for minor attacks (i.e., stimulus levels 0 to 3: 0 = no provocation, 1 = a slight push, 2 = pinching, and 3 = slapping) and for major attacks (i.e. levels 4 to 7; 4 = knock to the ground, 5 = pulling hair, 6 = hitting with a stick, and 7 = punching in the face).

Due to recruitment difficulties only eight girls with ASD participated in the study. Therefore their results are presented here as preliminary results and should be regarded with caution as the sample size may be insufficient to show statistically significant differences.

The limit for statistical significance was set equal to 0.05, but values up to 0.10 were considered to show a tendency towards significance. The paired $t$ test as well as the Wilcoxon signed rank test were used to analyze differences between the groups in age and IQ. A preliminary analysis of the data revealed that the variables for the reactive aggression specified above had a tendency to be skewed to the right and could not be normalized even by a square root or logarithmic transformation. Our sample being paired as well, the nonparametric Wilcoxon test was employed to ascertain the significance of possible differences in the intensity of reactive aggression between the groups.

## Results

There were no statistically significant differences between the boys with ASD and without ASD or the girls with and without ASD in chronological age, full scale IQ, verbal IQ or performance IQ (Table 1).

### Intensity of reactive aggression to the attacks in the impulsive aggression condition

The full range of attacks in the impulsive aggression condition elicited equally intense levels of reactive aggression among the boys with and without ASD (Fig. 1). However, in the responses to the minor attacks there was a trend towards statistically significant difference between the boys with and without ASD as the boys with ASD seemed to respond with slightly more intense reactive aggression than the boys without ASD ($p = 0.060$). The major provocations evoked similar levels of retaliation among the boys with or without ASD.

There were no statistically significant differences in the intensities of responses in the impulsive aggression condition between girls with and without ASD, not even when the responses were separately examined against minor and major attacks (Fig. 1).

As the minor attacks elicited differences between the groups in response intensity, the influence of the varying minor attack intensity on reactive aggression was studied. While harmless interactions and slight pushes provoked equal reactions among all the boys, the boys with ASD reacted significantly more intensively to slaps than the boys without ASD ($p = 0.013$). There was also a trend towards statistically significant difference in responses to pinches between the boys with and without ASD ($p = 0.084$). In light of the previously mentioned analyses, no attack level dependent differences in the intensity of reactive aggression between the two groups of girls were expected, but, as Fig. 2 illustrates, the girls with ASD showed *lower* intensity of reactive aggression to slight pushes than their non-diagnosed counterparts ($p = 0.039$) (Fig. 2).

### Intensity of reactive aggression to the attacks in the controlled aggression condition

In the controlled aggression condition, the results regarding overall aggression (i.e. aggression across all assailants)

**Table 1** Subject characteristics

| | Girls | | | | | | | Boys | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | With ASD (n = 8) | | | Without ASD (n = 8) | | | *p* | With ASD (n = 27) | | | Without ASD (n = 27) | | | *p* |
| | Mean | SD | Range | Mean | SD | Range | | Mean | SD | Range | Mean | SD | Range | |
| Age | 12.4 | 2.9 | 8.7–16.0 | 12.5 | 2.7 | 9.3–15.9 | 0.575 | 12.9 | 2.2 | 8.1–16.9 | 12.9 | 2.3 | 7.7–16.6 | 0.486 |
| IQ | | | | | | | | | | | | | | |
| Total | 104 | 13 | 80–120 | 104 | 4 | 96–108 | 0.389 | 102 | 15 | 77–138 | 105 | 15 | 75–127 | 0.303 |
| Verbal | 105 | 9 | 95–120 | 99 | 8 | 89–111 | 0.172 | 105 | 14 | 83–140 | 110 | 20 | 71–150 | 0.082 |
| Performance | 104 | 23 | 58–122 | 109 | 9 | 100–125 | 0.674 | 100 | 19 | 65–141 | 100 | 13 | 66–122 | 0.892 |

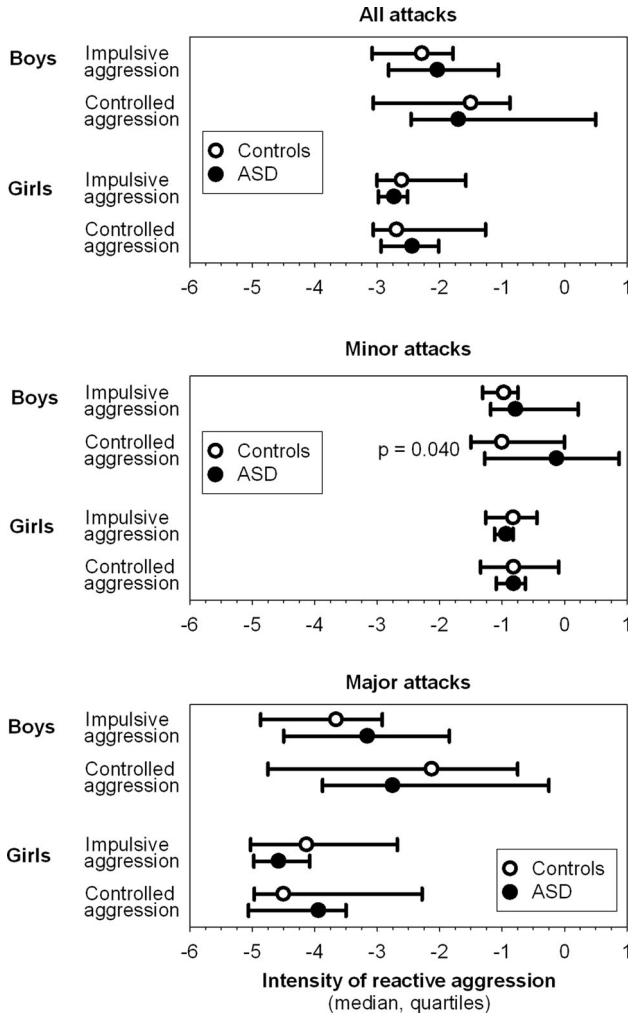


**Fig. 1** Intensity of reactive aggression among participants

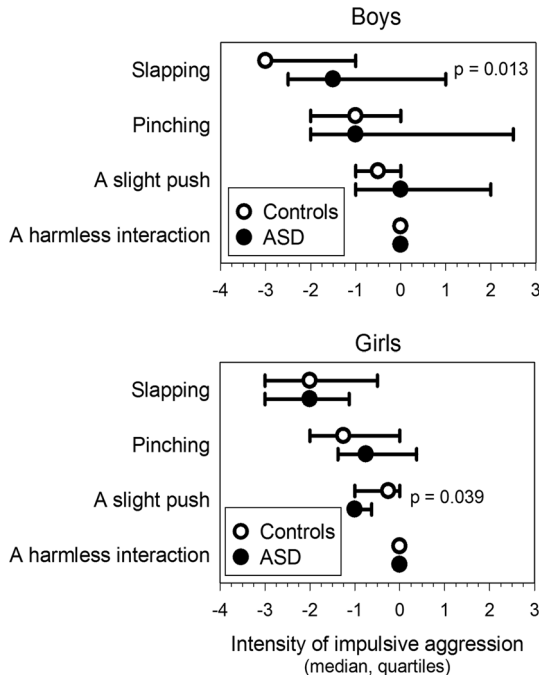


**Fig. 2** Intensity of reactive aggression to the minor attack stimuli in the impulsive aggression condition

results resembled those in the impulsive aggression condition. The boys with and without ASD responded with equally intense levels of reactive aggression in general and also when they were confronted with major attacks. When their responses to the minor attacks were analyzed, it was observed that they responded with more intense reactive aggression than the boys without ASD ($p = 0.040$). The girls with ASD did not differ significantly from their matched controls in their response intensity against all, minor or major attacks (Fig. 1).

Analyzed by assailant, it was seen that the boys with ASD reacted with more intense aggression than the boys without ASD to attacks from a same-sized, a smaller and a bigger child of the opposite sex ($p = 0.004$, $p = 0.007$ and $p = 0.012$, respectively; Fig. 3). In case of minor attacks of three opposite-sex children there were statistically significant differences in the intensity of reactive aggression between the boys with and without ASD ($p = 0.009$, $p = 0.001$ and $p = 0.002$ respectively). Significantly more intense responses were also observed among the boys with ASD than among the boys without ASD to major attacks delivered by a same-sized and a smaller child of the

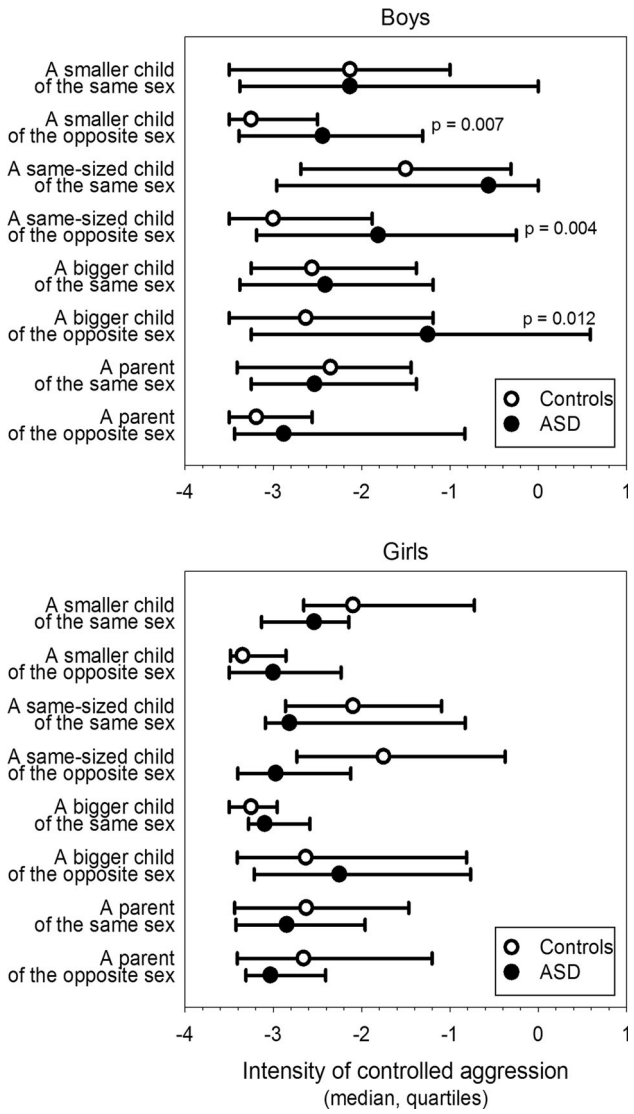


**Fig. 3** Intensity of reactive aggression to the attacks in the controlled aggression condition

opposite sex ($p = 0.008$ and $p = 0.019$ respectively). Even the difference between the two groups of boys in the intensity of reactive aggression against major provocations by a bigger child of the opposite sex was almost statistically significant ($p = 0.058$). (Fig. 3)

The comparisons between the girls with and without ASD in the intensity of reactive aggression against the different assailants confirmed the general finding that the alteration in physical strength and gender of the assailant resulted in similar changes in the intensity of aggression in both groups of girls (Fig. 3). The only difference in the intensity of retaliations was that the girls with ASD responded with lower intensity than girls without ASD to major attacks from a same-sized child of the opposite sex ($p = 0.043$).

## Discussion

The aim of the present study was to investigate whether boys and girls with ASD showed more intense reactive aggression than boys and girls without ASD in the PAM that varies the intensity of proactive aggression, the physical strength and gender of the assailant (Juujärvi et al. 2001). The present study also sought to ascertain whether boys and girls with ASD utilized the social, inhibitory cues to regulate their aggression the same way as boys and girls without the disorder were expected to do. Earlier findings demonstrate that normally developing children attenuate their retaliations to provocations delivered by assailants whose physical and/or social status differs from their own, presumably to avoid physical injury or other punishment from figures of authority (e.g., Pellegrini et al. 2007).

Since ASD is associated with frequent and seriously disturbing aggression (Dominick et al. 2007; Green et al. 2000; Ming et al. 2008), it was anticipated that at least boys with ASD might have a general deficit in controlling their reactive aggression. The hypothesis was clearly refuted by the data, as without considering the intensity of proactive aggression and identity of the assailant, no significant differences were observed in the intensity of reactive aggression between boys with and without ASD or girls with and without ASD.

The answer to the question how children with ASD benefited from the social cues to accommodate their own behavior relative to children without ASD, proved to be complex. Even though the minor attacks (slight pushes, pinches, and slaps) evoked more intense retaliation from the boys with ASD than from the boys without it, the major attacks (being knocked down, pulled by the hair, hit with a stick, or punched in the face) induced similarly intense retaliation among all the boys in both conditions of PAM. However, the girls with ASD responded with as intense levels of reactive aggression as the girls without ASD to minor attacks, except that they showed *lower* intensity of reactive aggression to slight pushes than their controls. Thus, these results imply that the escalating intensity of proactive aggression alone contains some information that enables mitigation of reactive aggression, even among children who otherwise have difficulties in social information processing due to autism.

When the physical strength and gender of the assailant was manipulated, we found that even though the boys with ASD were not able to consider the child assailants' opposite sex as a signal to mitigate the intensity of reactive aggression, they did regulate their behavior along with increasing physical strength of an assailant in the same way as the boys without ASD. By contrast, the girls with ASD displayed similarly intense reactions to the girls without ASD regardless of the intensity of attacks or identity of the

assailant. Together these findings provide support for the hypothesis that boys with and without ASD do not utilize inhibitory cues similarly but girls with ASD are able to use the situational cues of an assailant to inhibit their responses similarly to girls without ASD. This finding indicates that the phenotype of aggression (Kopp and Gillberg 1992) might indeed be different among boys and girls with ASD. As the number of the girl participants was very low in this study, this finding has to be regarded with caution and needs to be confirmed in future studies with a larger sample.

One explanation why boys did not mitigate their responses when faced with assailants of opposite sex might be that they are impaired in evaluating delayed consequences of their behavior. Since greater intensity of attacks and increasing physical strength of assailants induced the boys with ASD to regulate the intensity of their retaliations, it is plausible to argue that in the midst of conflict with a same-sex opponent they at least evaluate the risk for immediate, physical harm to themselves in case their own behavior escalates the situation into a more dangerous encounter (McGrew 1972). By contrast, the negative consequences of excessively intense reactive aggression towards an opposite-sex assailant are probably delayed and come in the form of reprimands or sanctions from figures of authority (Shantz 1987). It is possible that the evaluation of a physical threat may be done by different emotional and cognitive processes than the assessment of social punishment which, for example, entails knowing and applying relevant social norms to the conflict at hand. It may be that boys with ASD are less aware than boys without ASD that in general it is not socially appropriate to react aggressively towards an assailant of the opposite sex. If this hypothesis is confirmed in the future, then the shortening of time between unwanted behavior (aggression) and consequence (reprimands) may prove to be crucial for successful behavioral modification schemes in the treatment of aggression problems. Another explanation is that the boys with ASD considered assailants of the opposite sex more threatening than did the boys without ASD and therefore responded with enhanced reactive aggression. Even though the boys with ASD seemed to be able to use obvious cues, e.g. the size of an assailant, to estimate assailants' physical strength and imminence, they might be impaired to take into account an effect of a gender of an assailant. Consequently, a clinician working with a school-aged boy with ASD and problematic reactive aggression might focus on enhancing the development of the skills needed to cope in situations with minor provocations. These skills include e.g. recognition of how intensity varies between different types of minor provocations, identifying cues that besides the size of an assailant affect the strength and imminence of an assailant (e.g. gender of an assailant), matching the response to the level of provocation and instructing non-aggressive behavior models to cope in situations that are provocative.

A significantly more detailed analysis of the process of provocation and retaliation using the PAM and other experimental paradigms will be necessary to understand why the girls with ASD did not differ from their same aged peers in their intensity of reactive aggression behavior, but instead seemed to show less aggression than the girls without ASD. If this finding is replicated in a new sample of children, for girls with ASD the focus of clinicians and parents will be on modeling and teaching more effective reactions to better self-advocate and counter provocations, to reduce the risk for physical harm or exclusion from the psychological or concrete benefits of peer interactions.

Impairments in social skills and the presence of repetitive behaviors seem to be related to aggressive behavior among children, adolescents and adults with ASD (Kanne and Mazurek 2011; Matson and Rivet 2008). Only further research will show to what extent the poor regulation of emotion and resulting reactive aggression (Juujärvi et al. 2001) among boys with ASD is in fact due to already known biases in sensory-level stimulus processing (Lane et al. 2010), recognizing facially expressed emotions, especially sadness and fear (Ashwin et al. 2007; Boraston et al. 2007; Corden et al. 2008; Howard et al. 2000), or detecting threat in big crowds (Krysko and Rutherford 2009). It is also possible that a temporally consecutive, more complex level of processing such as a lowered threshold for action (Bradley and Isaacs 2006; Konstantareas and Stewart 2006; Robinson et al. 2009), an impaired assessment of others' intentions (Serra et al. 2002; Yirmiya et al. 1998) or simply a more limited range of constructive coping strategies for frustrating situations (Konstantareas and Stewart 2006) make boys with ASD more likely to act aggressively than normally developing children (Dominick et al. 2007; Green et al. 2000; Ming et al. 2008).

This study shows that boys with ASD have impairments in the regulation of the developmentally earliest, reactive form of aggressive behavior. It would be interesting to know how their development unfolds with regard to subsequently emerging proactive aggression and assertiveness, which serve to enhance goal pursuit and influencing other people. Even the question whether adult males with ASD may still show a tendency to use escalated force in their aggressive responses when faced with mild aggressive attacks becomes relevant, because if so, it might partly explain why individuals with ASD seem to be overrepresented in prison populations (Soderstrom et al. 2004). Finally, this study highlights the importance of behavioral and cognitive interventions targeted especially at boys with ASD to promote assertive rather than aggressive responses when they are involved in conflicts or confronted with acts of aggression. *Limitations:*

Our study sample size was small, and especially results concerning girls with ASD should be regarded with caution as only eight girls with ASD were included in the study. The small sample size was taken into account in the study design and statistical analyses by matching the subjects in the groups individually for gender, age and total IQ and conducting statistical analyses in pairs, with non-parametric methods. The findings of the present study only represent children with ASD and total IQ over 70, and more studies are needed to confirm whether these findings also occur among children with ASD and intellectual impairment and among older individuals with ASD. Our study sample was heterogeneous as regards overall, verbal and performance IQs. It is possible that the different cognitive profiles of the participants, e.g. whether they preferred verbal or non-verbal processing, might have influenced their responses in the PAM task. However, the possible impact of cognitive profiles on reactive aggression can be expected to be similar in children with and without ASD as the pairs were matched for total IQ and there were no statistically significant differences in verbal and performance IQs. Future studies with larger samples would make it possible to ascertain how different cognitive profiles are associated with reactive aggression among children with ASD. As our study did not include the measurement of children's current comorbid aggressive behavior, it is impossible to say whether children with ASD and aggressive behavior differ in their responses to aggressive acts in PAM from those who do not show aggressive behavior in daily life. However, enhanced intensity of responses to minor attacks in the PAM task has been found to be associated with maladaptive aggressive behavior in daily life among typically developing school-aged children (Juujärvi et al. 2001). In future, studies on separate groups for participants with and without aggressive behavior are needed to investigate what distinguishes the approximately 30 % children and adolescents with ASD who display clinically significant aggressive behavior (Dominick et al. 2007; Green et al. 2000; Kanne and Mazurek 2011; Ming et al. 2008) from those who are less aggressive.

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**Conflict of interest** The authors report no conflicts of interest.

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ERRATUM

# Erratum to: Reactive aggression among children with and without autism spectrum disorder

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In the original publication, the data in Fig. 1 were accidently switched between impulsive aggression and controlled aggression. The figure has been corrected with this erratum.

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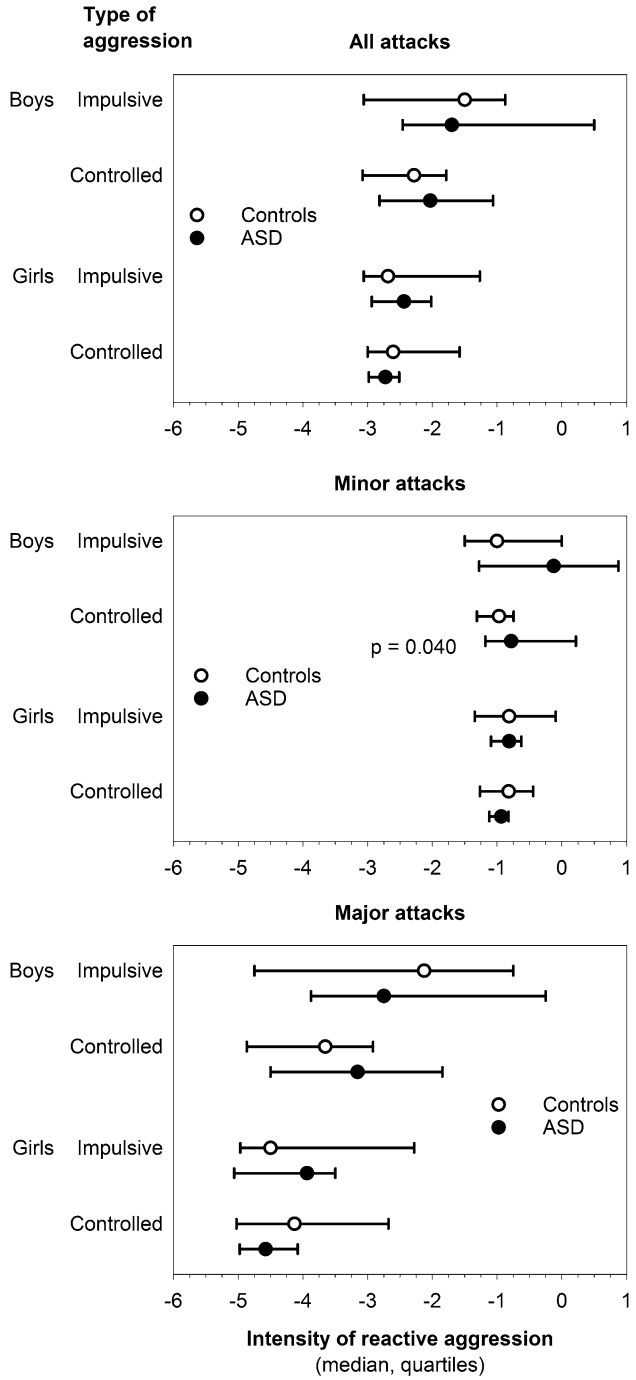


**Fig. 1** Intensity of reactive aggression among participants