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Implicit and Explicit Facial Emotion Recognition in Autism Spectrum Disorder: Insights from Behavior, Gaze and Functional Magnetic Resonance Imaging

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Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt,

- dass ich die vorliegende Arbeit selbstständig und ohne unerlaubte Hilfe verfassthabe,
- dass ich mich nicht bereits anderwärts um einen Doktorgrad beworben habe undkeinen Doktorgrad in dem Promotionsfach Psychologie besitze und
- dass ich die zugrunde liegende Promotionsordnung vom 02.12.2008 kenne.

Berlin, den 10.12.2012 Dorit Kliemann

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To Marc

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Summary

Our social environment challenges us with a richness of social cues that need to be effectively recognized and processed in order to initiate adequate behavioral responses. Importantly, social information is not always presented obviously and respective demands towards the social agents are seldomly stated explicitly. Instead, a great portion of social information is reflected in subtle social signs. To successfully interact with others, we thus need to both implicitly and explicitly process aspects of our social world.

Impairments in social cognitive functioning can have a severe impacton individual well-being and integration in society, such as in autism spectrum disorder (ASD). Individuals on the autism spectrum show impairments in implicit and explicit social functioning starting early in development, persisting into adulthood, crucially impactinglife of affected individuals, even of those at the higher functioning end of the spectrum. The relations of implicit and explicit socio-cognitive impairments, as well as specific mechanisms behind respective altered processes, remain unclear to date.

This dissertation represents an empirical attempt towards advancing our understanding of implicit and explicit social cognitive impairments in ASD with a multi-method approach including behavioral, eye-tracking and neuroimaging methods. To this end, in this dissertation I focus on the behavioral and neural mechanisms underlying one of the most prominent features within the autistic social symptomatology: impairments in recognizing emotions from faces.

In Study I we developed and evaluated two new video-based tasks for implicit and explicit facial emotion recognition to identify and define relations between respective impairments in ASD. In Study II, we assessed gaze on emotional faces in ASD, specifically the puzzle of reduced focus on the eye region, as an important aspect during implicit social processing. In Study III, we investigated the underlying neural basis of atypical reflexive gaze patterns in ASD as identified in Study II, in particular the role of the amygdala.

Results of Study I of this dissertation underline the previously suggested greater impairments in implicit as compared to explicit processing in ASD, as shown by respective group differences and interaction regarding performance in the new Face Puzzle tasks. In fact, implicit and explicit aspects of facial emotion recognition processes seem to be more closely related in ASD as compared to healthy controls. In the context of impaired implicit processing, I show in Study II and III that a reduced eye focus in ASD seems to be characterized by an interaction of avoidance and reduced orientation related gaze,which is accompanied by specific blood-oxygen level dependent signal (BOLD) response patterns in the amygdala. Taking gaze behavior and brain function together, the findings suggest altered avoidance processing and impaired implicit reflexive orientation to salient social cues in ASD. Distinct increases and decreases in amygdala activity in response to emotional faces imply that the amygdala is a dysfunctional node in the neural network underlying emotional face

recognition, leading to alterations in function and structure of the facial emotion recognition network, ultimately affecting effective social processing and thus the behavioral phenotype of ASD.

Zusammenfassung

Unsere soziale Umwelt enthält eine Vielzahl von sozialen Hinweisreizen, die erkannt und verarbeitet werden müssen damit eine adäquate Verhaltensantwort erfolgen kann. Soziale Information ist jedoch nicht immer offensichtlich erkennbar dargeboten und selten sind Interpretationsanforderungen explizit ausgewiesen. Im Gegenteil, ein Großteil der sozialen Information in unserer Umwelt ist in subtilen sozialen Hinweisen versteckt. Um erfolgreich mit unseren Mitmenschen zu interagieren müssen wir demnach sowohl implizite als auch explizite Aspekte unserer sozialen Umwelt verarbeiten.

Beeinträchtigungen sozial-kognitiver Funktionen können zu schwerwiegenden Einschnitten im individuellen Wohlbefinden und mangelnder Integration in die Gesellschaft führen. Ein Beispiel dafür sind Autismus Spektrumsstörungen (ASD): Individuen aus dem Autismusspektrum weisen schon in früher Kindheit Beeinträchtigungen in impliziten und expliziten sozial-kognitiven Funktionen auf, die bis ins hohe Lebensalter bestehen bleiben und somit das Leben der Betroffenen stark beeinflussen. Diese Beeinträchtigungen betreffen auch Individuen, die am hoch-funktionalen Ende des Spektrums eingeordnet werden, also keine Intelligenzminderung aufweisen. Die Beziehung zwischen impliziten und expliziten sozialen Beeinträchtigungen, als auch die Mechanismen die zu diesen Beeinträchtigungen führen, bleiben jedoch bis heute unerklärt.

Diese Dissertation stellt einen empirischen Versuch dar, unser Verständnis von impliziten und expliziten sozial-kognitiven Beeinträchtigungen in ASD mit einem multi-methodalen Ansatz, der Verhaltens-, Blickbewegungs- und Bildgebungsverfahren umfasst,voranzutreiben. Der Fokus liegt hierbei auf einem der prominentesten Merkmale autistischer sozialer Symptomatologie, sowie deren zugrunde liegenden Prozesse und Mechanismen auf Verhaltens- und neuronaler Ebene: Beeinträchtigungen im Erkennen von Emotionen anhand von Gesichtsausdrücken.

In Studie I haben wir zwei neue Video-basierte Verhaltenstests für implizite und explizite faziale Emotionserkennung entwickelt und evaluiert um entsprechende Beeinträchtigungen in ASD besser zu definieren. Studie II untersuchte einen wichtigen Aspekt impliziter sozialer Kognition: Blickbewegungen auf emotionalen Gesichtern, im Besonderen den reduzierten Fokus auf die Augenregion in ASD. Darauf aufbauend haben wir in Studie III die zugrunde liegende neuronale Basis atypischen Blickverhaltens in ASD untersucht, wobei besonders die Rolle der Amygdala im Vordergrund stand.

Die speziellen Gruppenunterschiede und Interaktionen der Performanzergebnisse von Studie I unterstreichen die Annahme dass implizite soziale Beeinträchtigungen in ASD prominenter sindals explizite. Die Ergebnisse legen nahe, dass implizite und explizite Prozesse in ASD stärker miteinander assoziiert sind als bei gesunden Kontrollprobanden. Ergebnisse von Studie II zeigen weiterhin, dass der reduzierte Fokus auf die Augenregion in ASD durch eine Interaktion von Vermeidungs- und verminderten Orientierungsprozessen auf Blickbewegungs- und auf neuronaler Ebene charakterisiert

wird. Hypo- und hyperaktivierungen in der Amygdala implizieren weiterhin, dass diese Region einen wichtigen Knotenpunkt innerhalb des neuronalen Netzwerkes zur Verarbeitung sozialer Reize darstellt. Entsprechende Beeinträchtigungen führen demnach zu Veränderungen in der Funktion und Struktur des neuronalen Netzwerkes zur fazialen Emotionserkennung, die letzendlich effektive sozial-kognitive Funktionen beeinträchtigen und somit zum Phänotyp von ASD führen.

List of Original Publications

This dissertation is based on the following original research articles:

Study I

Kliemann, D., Rosenblau, G., Boelte, S., Heekeren, H.R., Dziobek, I. (in revision) Face Puzzle – Two new video-based tasks for measuring implicit and explicit aspects of facial emotion recognition. Frontiers in Psychology.

Study II

Kliemann, D., Dziobek, I., Hatri, A., Steimke, R., Heekeren, H.R. (2010): Atypical reflexive gaze patterns on emotional faces in autism spectrum disorders. Journal of Neuroscience. 30:12281-12287.

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Study III

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

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List of Abbreviations

ADOS	Autism Diagnostic Observation Scale
ADI-R	Autism Diagnostic Interview - Revised
AQ	Autism Spectrum Quotient
ASD	Autism Spectrum Disorder
ASDI	Asperger Syndrome Diagnostic Interview
BOLD	Blood Oxygen Level Dependent
DTI	Diffusion Tensor Imaging
DSM	Diagnostic and Statistical Manual of Mental Disorders
EOT	Externally Oriented Thinking Style
EPI	Echo Planar Images
FFA	Fusiform face Area
FG	Fusiform Gyrus
FWHM	Full Width at Half Maximum
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
HFA	High-Functioning Autism
ICD-10	International Classification of Diseases and Related Health Problems -10
IQ	Intelligence Quotient
LPS	Leistungsprüfsystem
MPFC	Medial Prefrontal Cortex
MRI	Magnetic Resonance Imaging
MWT	Mehrfach-Wortschatz Test
NT	Neurotypically-Developed
PDD	Pervasive Developmental Disorders
PDD-NOS	Pervasive Developmental Disorder Not Otherwise Specified
PET	Positron Emission Tomography
RMET	Reading the Mind in The Eyes Test
SCR	Skin Conductance Response
SD	Standard Deviation
SE	Standard Error of the Mean
STS	Superior Temporal Sulcus
TAS	Toronto Alexithymia Scale
TE	EchoTime
ToM	Theory of Mind
TR	Time of Repetition

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1. Theoretical and Empirical Foundations

1.1. Autism Spectrum Disorder

Autism Spectrum Disorders (ASD) is a pervasive neurodevelopmental condition, characterized by a triad of symptoms: impaired social interactions, stereotypical behavior, and deficient communicative capacities(Levy, Mandell, & Schultz, 2009).

1.1.1. Diagnosis

Leo Kanner first described the core symptoms of the autistic phenotype in 1943 in his seminal publication the "early infantile autism"(Kanner, 1943, 1944). One year later, in 1944, Hans Asperger described a condition of stable personality disorder characterized by social isolation as "autistic psychopathy" (impairments in nonverbal communication, tendency to intellectualize emotions but preserved intellectual skills)(Asperger, 1944), which represents the basis for the "High-functioning Autism" (HFA) and "Asperger Syndrome" (AS) diagnosis in its current diagnostic format as described in the Diagnostic and Statistical Manual of Mental Disorders (4th ed., text rev.; DSM-IV-TR; American Psychiatric Association, 2000)and the International Classification of Diseases (10^{th} ed., World Health Organization, 1992). In fact, the umbrella term "autism" comprises several subcategories of pervasive developmental disorders (PDDs): autistic disorders, childhood disintegrative disorder, Rett's disorder, Asperger Syndrome and pervasive developmental disorder not otherwise specified (PDD-NOS).

Within clinical practice and research context, the term *autism spectrum disorder* has become a commonly accepted yet unofficial term. There have been several attempts to further define consistent and homogenous subtypes of autism within its heterogeneous expression of behavioral symptoms. Despite numerous studies that aimed at defining subtypes based on behavioral or neural data, the only accepted and widely used classification is rooted in clinical descriptions of "low-functioning" and "high-functioning" describing individuals with and without intellectual disabilities, respectively.

The current development of DSM-V suggests a new perspective on the disorder in general, merging all previous sub-categories into the diagnosis of autism spectrum disorder (ASD). The suggested diagnostic structure and composition of criteria aim at creating a diagnostic basis with greateraccuracy, which should in turn leadto promotion of individual service. At the same time, changes in the diagnostic basis of ASD aims at improving specificity in research. In particular,the new criteria for ASD and its subtypes (e.g., with or without language delay) are expected to result in a more precise empirical foundation of respective behavioral as well as biological basis, while reducing diagnostic confusion(Huerta, Bishop, Duncan, Hus, & Lord, 2012; Huerta & Lord, 2012).

In this dissertation's studies we only included individuals on the autism spectrum with no intellectual impairments or language delay, comprisingthe high-functioning end of the spectrum, i.e., AS or HFA. With regard to the new DSM-V categorization of ASD, possible differences between AS and HFA (Klin, 2000; Klin, Jones, Schultz, Volkmar, & Cohen, 2002a), will not be discussed in this dissertation. For simplicity, I will use the term ASD to refer to the participants on the autism spectrum in the following sections of this dissertation.

1.1.2. Diagnostic Instruments

Although it is by now undisputed that ASD has a biological basis, there are no biological tests or medical standard protocols for the diagnoses of ASD. Instead, diagnoses are based on observations of behaviors, as witnessed by clinicians and reported by parents or caregivers. Diagnostic signs appear usually around the age of three, with low-functioning related symptomatology (e.g.severe intellectual disabilities) appearing often earlier. Language development might further result in a delay of identification. Because this dissertation includes only adults withASD, which have mostly been diagnosed in their adulthood or late adolescence, I will only report diagnostic assessment and related instruments, which are aimed at diagnosing high-functioning ASD in adulthood/adolescence. Thus, further detailed description of early detection signs and clinical procedures for children are excluded as well.

To aid an accurate diagnosis with respect to the DSM-IV and the ICD-10, the most widely accepted and often used instruments in diagnosing individuals on the high-functioning end of the autism spectrum are the Autism Diagnostic Interview-Revised (ADI-R, Lord, Rutter, & Le Couteur, 1994) and the Autism Diagnostic Observation Schedule (ADOS, Lord, et al., 2000). These instruments are known as the current "gold standard" for diagnosing ASD(Levy, et al., 2009).

The ADI-R is a semi-structured interview conducted with the individual's parent or caregiver. It covers qualitative assessment of reciprocal social interaction, communication and language, as well as restricted and repetitive, stereotyped interests and behaviors. The ADI-R has been shown to differentiate between autism, mental retardation and language impairments(Lord, et al., 1994). The ADOS is a semi-structured standardized observational assessment of social communication, social behavior and imaginative play. Both the ADOS and the ADI-R provide diagnostic algorithms as described in DSM-IV and ICD-10, and have been shown to represent reliable diagnosis of autism. In addition, the Asperger Syndrome Diagnostic Interview(ASDI, Gillberg, Rastam, & Wentz, 2001) provides sensitivity in the diagnosis of higher-functioning individuals, including AS and HFA.

All three described instruments have been used in the clinical process to aid the diagnostic procedure according to DSM-IV and ICD-10 in the studies included in this dissertation. In addition, we applied the Autism Spectrum Quotient (AQ, Baron-Cohen, Wheelwright, Skinner, Martin,

&Clubley, 2001)to both individuals with ASD and all healthy control participants. Importantly, the AQ is a self-referential questionnaire, which shares no diagnostic validity for ASD per se, but serves as a measure to quantify autistic symptomatology in the general population.

1.1.3. Epidemiology

This dissertation has no clinical focus in general, but on the behavioral and biological factors underlying the autistic phenotype. Therefore within the description of epidemiological factors of ASD I will focus on prevalence (excluding, e.g., incidence or a detailed description of comorbidities).

Despite the fact that estimates vary greatly between studies (for a review on prevalence in ASD see, Williams, Higgins, & Brayne, 2006), there is a general agreement on an increase of prevalence since the 1960s. For instance, prevalence rates increased in the USA and Europe from 5 to 72 of 10,000 children over 20 years(Kadesjo, Gillberg, & Hagberg, 1999; Sponheim & Skjeldal, 1998). Several reasons for this have been discussed, such as changes in diagnostic routines, an increase in general public awareness, public heath concerns, the need for individual care, age of diagnosis, close comorbidity to other highly prevalent disorders, such as ADHD, or even a hypothesis regarding an epidemic of ASD (see, e.g., Boelte, 2009, for a discussion). Importantly, differences in study design (e.g., cross-sectional versus longitudinal) and study characteristics (e.g., screenings, diagnostic instruments, sample sizes) may have influenced the variance in reported prevalence rates. Levy et al (2009), however, suggested that the increase in prevalence might be mostly due to policy and practice changes, as opposed to true changes community prevalence (but, see Weintraub, 2011, for further discussion of the prevalence increase). As of 2009, it is assumed that 1 out of 110children is on the autism spectrum (Weintraub, 2011).

1.1.4. Causes

Causes of ASD are largely unknown. However, there is strong empirical evidence for a high genetic contribution to its occurrence and variability in the expression of individual symptoms(for a review, see Freitag, 2007). For example, the relative risk of a second child with an ASD diagnosis is 20-50 times higher than the population base. Estimated heritability from twin and family studies suggests about 90% in variance can be attributed to genetic factors (O'Roak & State, 2008). Along the same lines, ASD seems to be more frequent in male individuals as compared to females (2:1). In addition, ASD is associated with known genetic causes, e.g. deletion and duplication of 16p11 or fragile X syndrome(Kumar & Christian, 2009). None of these causes, however, have been classified as specific to ASD. Instead, these causes are rather specific to different autistic phenotypes.

Despite a general agreement on the existence of several interacting risk factors (e.g., gene-gene or gene-environment) there is no consensus on causal environmental and epigenetic factors (e.g., virus infections during pregnancy, toxic/biochemical environmental, immunization, psychological factors) on the autistic phenotype to date (Abrahams & Geschwind, 2008; Anney, et al., 2010; Bucan, et al., 2009; Chess, Fernandez, & Korn, 1978; Freitag, 2007; Ylisaukko-oja, et al., 2006).Many of the potential candidate genes or common variants for ASD could not be replicated and verified in subsequent independent samples(Abrahams & Geschwind, 2010).

In contrast to defining and identifying risk factors for the cause of ASD, investigations of neurobiological (i.e., neuroanatomical, -chemical, -physiological) causes are rather aimed to illuminate underlying mechanisms of functional and structural aberrations (Boelte, 2009).It is important to mention, that despite the extensive growth in studies investigating the biological basis and biological factors leading to ASD, there is no generally accepted unified theory explaining the behavioral symptomatology, crediting its heterogeneous expression. Neurochemical findings based on animal models or drug studies remain inconclusive (see, Boelte, 2009). The most consistent results suggest some disturbances in the serotonin system and general genetic differences in serotonin transport (Cook & Leventhal, 1996; Klauck, Poustka, Benner, Lesch, & Poustka, 1997; Yirmiya, et al., 2001). Recent promising, yet not sufficiently investigated and replicated, attempts have shown first hints towards the pro-social effect of oxytocin administration in ASD (Guastella, Mitchell, & Dadds, 2008; Guastella, Mitchell, & Mathews, 2008).However, definition of biological mechanisms and potential genetic aberrations in oxytocin production or transport are missing to further inform causes of ASD related to oxytocin.

Neuroanatomical findings in ASD comprise, e.g., increased brain growth (see, e.g., Minshew & Williams, 2007), macroencephaly(see, e.g., Fidler, Bailey, & Smalley, 2000), overgrowth in cortical white matter(see, e.g., Barnea-Goraly, et al., 2004), and abnormal patterns of growth in particular brain lobes or limbic structures(see, e.g., Courchesne, 1997). Post-mortem studies added some findings of cytoarchitectonical aberrations, e.g., in cortical minicolumns (Buxhoeveden, et al., 2006; Casanova, Buxhoeveden, & Brown, 2002; Casanova, Buxhoeveden, Switala, & Roy, 2002). Neuroimaging studies revealed specific functional abnormalities in brain function in response to social stimuli, such as decreased blood-oxygen-level (BOLD) signal as compared to control samples. The most replicated finding regarding differences in brain function is decreased activity in the fusiform gyrus (FG) in response to human faces as compared to healthy control subjects(see, e.g., Schultz, et al., 2003). Among the regions of the brain showing altered BOLD response profiles in ASD, the amygdala has caught particular attention. In fact, the proposed ‘amygdala theory of autism’ suggest a crucial role of this region in the neurobiological basis of ASD(Baron-Cohen, et al., 2000; Dziobek, Fleck, Rogers, Wolf, & Convit, 2006). Neuroimaging findings in ASD related to social stimuli, in particular to faces, will be further discussed in section 1.2.3 of this dissertation.

Despite the apparent inconsistency in biological findings and the lack of resulting unified theories, there are, however, a number of cognitive theories based on neurobiological findings, such as impairments in mental state inferences (Baron-Cohen, Jolliffe, Mortimore, & Robertson, 1997; Beaumont & Newcombe, 2006; Dziobek, Fleck, Kalbe, et al., 2006; Moran, et al., 2011; Senju, 2012b; Yoshida, et al., 2010), weak central coherence (Gauthier, Klaiman, & Schultz, 2009; Grelotti, Gauthier, & Schultz, 2002; López, Donnelly, Hadwin, & Leekam, 2004), impaired executive functions(Hill, 2004; Hughes, Russell, & Robbins, 1994; Kleinhans, Akshoomoff, & Delis, 2005)and deficits in general connectivity between brain regions (Alexander, et al., 2007; Belmonte, et al., 2004; Cherkassky, Kana, Keller, & Just, 2006; but see, Deen & Pelphrey, 2012). These findings and theories significantly contributed to the general understanding of biological mechanisms underlying the autistic phenotype. Nevertheless, because this dissertation focuses on one particular aspect of impaired social functioning of ASD, namely facial emotion recognition impairments, further description of cognitive theories and concepts regarding ASD will be spared.

In sum, ASD is a multifactorial condition with heterogeneous expression of symptoms and high genetic heritability. Neurobiological changes, such as neuronal-cortical organization over development, lead to deficits in information processing from synapses to brain structures affected by environmental and genetic contributions over the neurodevelopmental trajectory altering social functioning.

1.2. Social Cognition

Humans are uniquely social beings. The biological development and maturation of the self as independent social agents are crucially dependent on our interaction with others. For effective social functioning, humans have to process the present information on line and integrate relevant portions into the respective context to initiate adequate behavioral responses. Thereby, social cognition comprises the cognitive mechanisms that underlie social behavior(Frith & Frith, 2007).Probably the most fundamental source of social information in addition to language/speech is provided in facial expressions. Facial expressions offer cues about the environment (e.g., if danger is approaching indicated via gaze directions) as well as about the internal emotional states of the counterpart (e.g., if she feels happy or sad).

According to Fehr and Russel *"Everyone knows what an emotion is until asked to give a definition." (1984, p. 464).*In fact, there is no consensus to date on what an emotion exactly is or represents. There are long-standing debates in psychology and philosophy about the definition of emotion and several theories have been debated (for a review, see, Scherer, 2005). Most theories agree, however, that emotion processing in general involves some automatic, rather unconscious processing, followed by conscious processing(Fellous, 2002). Within the context of this dissertation I

will follow Adolphs' operationalization of emotion: emotions represent complex physiological and psychological states with an onset, a finite duration and an offset(Adolphs, 2002). Thereby, an emotion is a *"concerted, generally adaptive, phasic change in multiple systems in response to the value of a stimulus"* (Adolphs, 2002, p. 24).

1.2.1. Implicit and Explicit Social Cognition

Social information is of highly complex nature and not always obviously present. Consider the following example: within a conversation, you make a joke and your counterpart smiles. A smile is amongst the most universal emotional cues about the emotional states of others; the person is very likely expressing some kind of happiness. In everyday life, we do not need the explicit prompt what the person is feeling ("Is this person happy?"). Instead we infer the emotional state rather automatically without conscious awareness of doing so. In addition to the obviously present information that the person is smiling and thus probably happy, there can, however, be a deeper social meaning to a smile. For instance, a smile can be genuine ("Duchenne", involving contraction of both the zygomatic major muscle, which raises the corner of the mouth *and* the orbicularis oculi muscle, which raises the cheeks) or fake ("non-Duchenne", only contracting the zygomatic major muscle). Again, rarely we are presented with explicit prompts whether someone is smiling genuinely or not. We are thus confronted with a large fraction of subtle social information that is not obviously emphasized, and we are rarely explicitly triggered to process particular aspects of information in our everyday lives. Instead, we have to automatically recognize and integrate implicit aspects of social information (such as the fine differences between a genuine and a fake smile) in addition to obviously present information (such as a smile) to successfully and effectively read the emotional states and interact with others. Taken together, social cognition in its explicit form is usually concerned with conscious and controlled processes and representations, which are rather flexible but, at the same time, also demanding many cognitive resources(Frith & Frith, 2008). In contrast, implicit social cognition comprises more automatic, unconscious reflexive processes that are time efficient but inflexible and limited in terms of cognitive resources (for further reading on the definition of implicit versus explicit, see, e.g., Moors, Spruyt, & DeHouwer, 2010).

The question remains, whether and to which extent implicit and explicit processes are interacting or operating independently. In the context of general knowledge within cognition, it is assumed that implicit knowledge is a precursor to explicit knowledge in development (Dienes & Perner, 1999; Perner & Dienes, 2003), e.g., by the re-description of implicit representations to explicit knowledge (Karmiloff-Smith, 1992). Within memory, for instance, distinctions between an implicit and an explicit memory system, analogous to the distinction of declarative and procedural knowledge have been proposed(Graf & Schacter, 1985; Graf, Squire, & Mandler, 1984). In social cognition and

social neuroscience, there have been similar recent attempts to dissociate implicit and explicit processes focusing on particular socio-cognitive concepts, e.g., the ability to infer the mental states of others (Theory of Mind, ToM). Several colleagues suggested a dissociation of implicit and explicit ToM processes according to dual-process or two-systems perspectives(Frith & Frith, 2008; Rosenblau, Kliemann, Heekeren, & Dziobek, under review). Thereby an earlier developing implicit and a later developing explicit ToM system evolve and can independently be at work in adulthood (Apperly & Butterfill, 2009; Low & Perner, 2012).

A comprehensive empirical validation of this theoretical hypothesis, however, demands an empirical comparison of individual performance in implicit and explicit processing and is challenged by methodological incomparability in study and task designs. In addition, it remains unclear, whether and to what extent respective postulated distinctions within higher-level social cognitive constructs, as outlined with the ToM example, could be applied to more basic types of social stimuli, such as emotional faces.

In the following sections of this chapter, I first introduce facial emotion recognition and illustrate standard explicit behavioral tasks. Second, gaze on emotional faces will be elaborated as one example of implicit processing within facial emotion recognition. Third, I describe neural correlates of facial emotion recognition to outline the biological basis in terms of brain function. The last section of this chapter focuses on the social impairments of ASD in explicit facial emotion recognition, atypical gaze on emotional faces and underlying alterations in brain function. Based on the outline of the relevant theoretical and empirical background, I then develop the research aims of this dissertation.

1.2.2. Social Cognition in Typical Development

1.2.2.1. Explicit Facial Emotion Recognition

As aforementioned, faces provide crucial information about the internal emotional states of others via facial mimic. Thereby, the ability to discern emotional information from faces is crucial to our development as social individuals and serves as a precursor to effective social interactions and communication. There are certain emotions, which appear to be universal, thus respective expression and recognition is regarded as independent from cultural background (Ekman, 1992; Ekman, et al., 1987). These emotions are considered basic emotions, including happy, fear, sad, angry, surprise and disgust(but, see Panksepp, 1992).

By the age of 3-4 months infants are able to discriminate among some emotional expressions, indicated by longer viewing times on happy as compared to, e.g., neutral or angry faces (Nelson & Ludeman, 1986). By 4 month, infants develop sensitivity to intensity levels of depicted expressions (Baldwin & Moses, 1996; Serrano, Iglesias, & Loeches, 1992)and only one month later category formation to happy faces, subsequently followed by other expressions, can be observed (Oster, 1981).

Experiments using social referencing revealed that around the end of the first year, infants use information from other social agents' facial emotion expression to guide their own behavior in uncertain situations(Baldwin & Moses, 1996). In sum, infants develop the ability to discriminate and categorize emotional expressions over the first few months of life and start to use information from facial expressions to interpret external events and guide their own behavior(Nelson & Dolgin, 1985; Nelson, Morse, & Leavitt, 1979). The innate and implicit saliency of human faces may serve as the crucial prerequisite for later explicit and conscious processing of emotions from facial expressions (Leppanen & Nelson, 2006). There are important considerations to take into account when assessing emotion discrimination abilities in infants. First, it has been shown that some findings on emotional categorization and discrimination are confounded by order of presenting stimulus conditions (Young-Browne, Rosenfeld, & Horowitz, 1977). Second, assessing emotion discrimination in infants prior to the maturation of speech differs significantly from studies in children and adults with specific concepts of emotions, which can be verbally described and expressed. In fact, studies using emotional labeling paradigms revealed that the actual discrimination and categorization of facial expressions to verbal emotional labels improves by 40% between 2 and 5 years to adult-like performance(Barrera & Maurer, 1981; Camras & Allison, 1985; Reichenbach & Masters, 1983). At the age of 4 to 5 children are able to recognize happy, sad and angry faces with adult-like accuracy, whereas the recognition of fearful, neutral and surprised expressions remains affected by misjudgments until the age of 6 (Pollak & Kistler, 2002; Russell & Bullock, 1986). Typically developed individuals are then able to discriminate basic and more complex emotions in various task designs and manipulations of facial stimuli, including, e.g., inverted faces (McKelvie, 2011), face parts(Spezio, Adolphs, Hurley, & Piven, 2007), backward-masked faces(Pessoa, 2005).

To date, facial emotion recognition in adulthood has been investigated by a tremendous number of behavioral, electrophysiological and neuroimaging studies in healthy controls, psychiatric patients and over development. Mostly used standard emotion recognition tasks use some type of visual facial stimulus (pictures of faces, or parts of faces, and recently videos of faces) and provide additional verbal labels(see, e.g., Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001; Kennedy & Adolphs, 2012; Kessler, Bayerl, Deighton, & Traue, 2002). Participants then have to match the emotional expression depicted in the target face with the correct emotional label, usually from at least one or more distractor labels. Standard tasks, thus ask participants to consciously process and choose between different emotional concepts (e.g. happy versus fearful) by explicitly comparing the emotional information in the mimic with verbal concepts of particular emotional concepts. Depending on specific experimental manipulations (e.g. presentation duration of faces, degree of noise) typical participants show high recognition rates for basic emotions.

As outlined in this section, facial emotion recognition abilities develop early in development and is usually tested explicitly with verbal labeling. Facial emotion recognition, however, comprises

implicit processes as well. One example of these implicit processes will be introduced in the next section.

1.2.2.2. Implicit Gaze on Emotional Faces

One important aspect of implicit social cognition is the immediate orientation towards salient social cues. Infants and even newborns showgreater attention to social stimuli and in particular to faces. Findings of increased focus on face-like stimuli, as compared to other stimuli (Goren, Sarty, & Wu, 1975)suggest that faces are special to humans in the sense of triggering an innate orientation measured as early as in 36 hours old newborns(Johnson, Dziurawiec, Ellis, & Morton, 1991).

Among the facial features, the eye region seems to be particularly important and salient (Haith, Bergman, & Moore, 1977; Robson, 1967).In fact, the eyes carry important information about the internal state of others (e.g. wide-opened eyes suggest fear). Underlining the importance of the eye region for social communication and interaction, typically developed individuals show a preference for the eye region when looking at faces starting early in development (see, e.g., Argyle & Cook, 1976; Gibson & Pick, 1963). In addition, negative emotions, such as fear or anger, are experienced considerably more intensely when combined with direct as compared to averted gaze (Adams Jr & Kleck, 2003). Along the same lines, categorization of emotional expressions seems to be enhanced by direct gaze(Bindemann, Burton, & Langton, 2008), indicating a behavioral benefit when focusing on the eyes.

Two recent multimethodal studies, combining neuroimaging with eye-tracking methods (Gamer & Buchel, 2009; Gamer, Zurowski, & Buchel, 2010)further suggest that the eye preference in healthy participants is mediated by emotional expression.The eye preference was reduced for happy as compared to fearful and neutral faces. In fact, for happy faces, the mouth is more informative than the eyes, indicating that the initial eye preference and respective orientation towards the eyes can be modulated by specific task demands and is adaptive to the emotional expression. In addition, gaze on emotional faces and the implicit orientation towards the eyes seems to be accompanied by distinct neural responses in the amygdala (see next section).

1.2.2.3. Neural Correlates of Facial Emotion Recognition

A prerequisite to recognizing emotions from faces is adequate face perception. According to Haxby's modification of Bruce and Young's (1986)model of face perception, there are core bilateral brain regions that are typically involved when processing faces: the lateral portion of the fusiform gyrus (FG), the (posterior) superior temporal sulcus (STS) and the inferior occipital gyrus(Haxby, Hoffman, & Gobbini, 2000, 2002). Within the "core system" the inferior occipital gyrus is involved in early

perception of facial features. Processing of invariant features, such as identity, is accompanied by relatively greater activation in the FG. Variable aspects of faces, such as gaze and lip movement lead to increased activity in the STS. According to Haxby (2000) the "core system" for face processing performs visual analysis of face perception and is complemented by an "extended system" of brain regions that process further aspects of the face: the auditory cortex processes prelexical speech perception, spatial direction of attention is rooted in activity in the intraparietal sulcus and the anterior temporal cortex processes biographical information. In addition, the "extended system" also included processing of emotion within face perception, reflected in activations of limbic brain regions, such as the amygdala, and the insula.

Haxby's model of face perception was modified and extended by Adolphs (2002) who added a temporal dimension of faceperception and emotion recognition. Equivalent to Haxby's "core system", structural encoding, thus fast and early perceptual processing of facial stimuli, is performed by the thalamus, striate cortices, superior colliculi and the amygdala 120ms after stimulus onset. Only 50ms later, detailed perception processes are initiated by specific "recognition modules", akin to Haxby's "extended system", leading to an emotional reaction. Recruited regions include the striate cortex, the fusiform face area (FFA) of the FG, superior temporal gyrus, amygdala, orbitofrontal cortex, basal ganglia, hypothalamus and the brainstem. After 300ms of stimulus onset a "cognitive system" processes conceptual knowledge of emotional information presented in the face in the FFA, superior temporal gyrus, insula orbitofrontal and somatosensory cortices. Crucially, some regions are not exclusively part of one *system*, instead, Adolphs differentiated between "early" and "late" processing stages in some regions, such as the in amygdala, the FFA and the superior temporal gyrus(Adolphs, 2002).

Common to both Adolphs' and Haxby's model is the assumption of coordinated interaction between multiple regions, which in turn can participate in other neural system sub-serving different processing stages of face/emotion perception or other cognitive functions. For example, the inferior occipital region is thought to provide input to the FG and the STS, thus functional activations in the inferior occipital gyrus are a precursor to further processing of invariant aspects of the face.

Amongst the region recruited during processing of emotional information from faces, the amygdala has been particularly highlighted. Despite earlier perspectives on the amygdala as a "fear module", recent findings suggest that the amygdala plays a more general role in detecting and processing important and salient features in the environment (Adolphs, 1999; Adolphs, Tranel, Damasio, & Damasio, 1995; De Martino, Camerer, & Adolphs, 2010; Di Martino, et al., 2009; Whalen, 1984). With regard to facial emotion processing, neuroimaging and lesion studies suggest a specific sensitivity of the amygdala to the eye region (Kawashima, et al., 1999; Morris, deBonis, & Dolan, 2002; Whalen, et al., 2004). In line with this proposed framework of amygdala function, healthy participants showed increased amygdala activity underlying a focus on the eyes(Gamer

&Buchel, 2009; Gamer, et al., 2010). Given that the eyes carry a great portion of information about others' emotional states, these findings underline a crucial role of the amygdala in processing emotional information from faces. On a structural level, the importance of amygdala's role in facial emotion processing has been underlinedby connectivity data derived from diffusion tensor imaging (DTI). These data imply a direct pathway between the amygdala and the FG (Morris, Öhman, & Dolan, 1999; Smith, et al., 2009), emphasizing the proposed close interaction between regions of the "extended" and "core" system(Adolphs, 2002; Haxby, et al., 2000). Additional support for the contribution of the amygdala-FG connection to face processing in general and emotion processing in specific is provided by findings of a strong positive correlation between local cortical thickness in FG and amygdala volume(Dziobek, Bahnemann, Convit, & Heekeren, 2010), as well as functional connectivity between these regions during face processing(Kleinhans, et al., 2008).

In sum, social cognition in typical development recruits anetwork of brain regions during face perception and the recognition of emotions from faces. The regions of the face network are functionally and structurally connected, thus impairments in particular regions may affect later occurring processing stages and thus facial emotion recognition in general.

1.2.3. Social Cognition in ASD

As outlined in the first section of this chapter, ASD is characterized by severe impairments in social interactions and communication(Levy, et al., 2009). These social deficits result in isolation in everyday life for affected individuals, suffering for their families and caregivers and extensive societal costs. Social impairments include difficulties in recognizing others' emotions in facial expressions, gestures and tones of voice, and to use higher-level mental state reasoning to understand and predict others' behavior.

Many studies, for example, report deficits in explicit social cognition, such as decreased performance in facial emotional labeling tasks in ASD. Other studies strongly indicate that ASD involve particularly greater impairments in implicit aspects of social cognition, such as atypical reflexive gaze (see, e.g., Kirchner, Hatri, Heekeren, & Dziobek, 2011), reduced imitation and facial mimicry (see, e.g., McIntosh, Reichmann-Decker, Winkielman, & Wilbarger, 2006; Senju, et al., 2007), and implicit mental state inferences (see, e.g., Senju, Southgate, White, & Frith, 2009; Yoshida, et al., 2010). Reports of increased implicit as compared to explicit socio-cognitive impairments in ASD, however, remain inconclusive, in particular regarding facial emotion recognition. Direct evidence by an empirical comparison of performance in implicit and explicit processes is still missing.

1.2.3.1. Impaired Explicit Facial Emotion Recognition

Among the social impairments in ASD, difficulty in recognizing the emotional state of others from faces is a prominent and diagnostically relevant symptom. Impairments in facial emotion recognition severely affect this fundamental basis of human social communication and interactions.

Difficulties in using emotional information from faces are evident early in development. For example, children on the autism spectrum sort static images of emotional facial expression not based on emotions, like verbal ability matched healthy children. Instead, autistic children sort the faces according to non-emotional characteristics (for example, which type of hat the person was wearing). In addition, intensity and valence processing seems to be impaired in children with ASD as well (Celani, Battacchi, & Arcidiacono, 1999). There have been suggestions that facial emotion recognition impairments are mostly due to general confounds, such as impaired holistic face processing(Gauthier, et al., 2009; Joseph & Tanaka, 2003). However, there are other studies indicating even increased object recognition in ASD as compared to controls, while facial emotion recognition was impaired(Trepagnier, Sebrechts, & Peterson, 2002).

There is a great amount of empirical evidence that individuals on the autism spectrum showsignificantly reduced accuracy and mostly increased reaction times as compared to typically developed participants in standard explicit facial emotion recognition tasks (see, e.g., Ashwin, Chapman, Colle, & Baron-Cohen, 2006; Baron-Cohen, Wheelwright, Hill, et al., 2001; Dziobek, et al., 2010). These studies included different types of stimuli comprising pictures of faces, face parts and videos of faces, mostly however, only using the 6 basic emotions. There are also studies showing no group differences between controls and ASD (see, e.g., Baron-Cohen, Jolliffe, et al., 1997; Neumann, Spezio, Piven, & Adolphs, 2006). Several explanations may account for these contradictory findings.First and foremost, ASD is a highly heterogeneous population. Differences in symptom expression in the study-specific sample together with differences in task, stimuli and design between studies may account for the findings of preserved emotion recognition in ASD (Harms, Martin, & Wallace, 2010; Kennedy & Adolphs, 2012). In addition, given that most studies focus on high-functioning adult samples, ceiling effects might lead to a lack of group differences to healthy controls. This factor is particularly important to consider because most studies only use emotional stimuli that comprise the 6, or even less, basic emotions plus a neutral expression condition. Similar to the ceiling effect, there might be specific compensatory mechanisms at work, such as increased systemizing abilities or verbal mediation. Along the same lines, most studies to date use static stimuli, such as pictures of faces or face parts. These experimental manipulations significantly reduce the complexity of the stimulus material as compared to real life. Given that social impairments in ASD, and even in high-functioning ASD, become particularly obvious during unstructured, complex real life situations (Volkmar, Lord, Bailey, Schultz, & Klin, 2004; Volkmar, et al., 1987), more naturalistic approaches using richer and more complex stimuli (e.g. videos of facial expressions instead of pictures), might be

more suitablefor measuring subtle social impairments in ASD with greater ecological validity. Thus, it is possible and indeed very likely that reports of preserved explicit emotion recognition are due to the discussed confounding factors, and in consequence do not reflect naturalistic and diagnostically relevant facial emotion recognition abilities and respective impairments in ASD.

1.2.3.2. Atypical Implicit Gaze on Emotional Faces

Given that newborns show an innate preference for faces, implicit gaze on faces is crucial to investigations of implicit socio-cognitive impairments in ASD. Atypical social gaze, in particular on human faces, is amongst the earliest pathological signs (Dawson, 1998; Mundy, Sigman, & Kasari, 1994) and even a diagnostic criterion for ASD (American Psychiatric Association, 2000). Differences in scan paths are characterized by specific patterns on emotional faces. Affected individuals spend less time looking on faces in general(Pelphrey, et al., 2002). Most prominently, however, individuals on the autism spectrum focus less on the eye region (see, e.g., Klin, Jones, Schultz, Volkmar, & Cohen, 2002b). Within general emotional face recognition, processing information from the eyes seems to be specifically impaired (Baron-Cohen, Wheelwright, Hill, et al., 2001; Baron-Cohen, Wheelwright, & Joliffe, 1997; Leekam, Hunnisett, & Moore, 1998) due to the reported gaze patterns.

Albeit a growth in studies that investigated gaze patterns in ASD, the exact mechanisms behind the reduced eye focus remain a puzzle. Two explanations have previously been put forward. The first hypothesis suggests that ASD exhibits a generally reduced social attention, or even a complete lack thereof. According to this hypothesis, a failure to detect salient social cues in the environment would lead to a reduced reflexive, i.e. implicit, orientation of gaze toward important sources of social information, such as the eyes(Neumann, et al., 2006; Schultz, 2005).Support for this long-standing view comes from studies showing that individuals with ASD prefer to focus on the mouth region (see, e.g., Klin, et al., 2002b). The second hypothesis proposes that direct eye contact induces an aversive response in ASD. Along these lines, if direct eye contact is aversive, individuals would actively avoid to directly focus others' eyes (Dalton, et al., 2005; Hutt & Ounsted, 1966; Kylliainen, Braeutigam, Hietanen, Swithenby, & Bailey, 2006; Kylliainen & Hietanen, 2006). In addition to personal reports from affected individuals, there is psychophysiological support for this hypothesis showing increased skin conductance responses (SCR) in children with autism when confronted with direct as compared to averted gaze (for a review on gaze in ASD, see, Nation & Penny, 2008). Further, face-encoding skills of children and adolescents with ASD were mediated by emotional arousal in response to direct gaze (Joseph, Ehrman, McNally, & Keehn, 2008).

In sum, there is empirical evidence for both the reduced orientation and avoidance hypotheses regarding the mechanism leading to the markedly reduced focus on the eye region in ASD. In fact, avoidance and orientation processes do not have to be mutually exclusive, as recently suggested by

Spezio and colleagues (2007). However, no study to date compared the two possible mechanisms directly within one experimental design and with adequate measures.

1.2.3.3. Aberrant Neural Correlates of Facial Emotion Recognition

The social impairments in ASD are rooted in aberrant structural characteristics and in dysfunctional profiles of brain regions that process social information. A number of neuroimaging studies using fMRI or positron-emissions-tomography (PET) implicate dysfunctional profiles of several brain regions during face perception and facial emotion processing, including extrastriate cortices(e.g., Deeley, et al., 2007), medial frontal cortex(e.g., Loveland, Steinberg, Pearson, Mansour, & Reddoch, 2008), orbitofrontal cortices(e.g., Ogai, et al., 2003), cerebellum(e.g., Critchley, et al., 2000), STS (e.g., Redcay, 2008) and the inferior frontal gyrus (e.g., Hall, Szechtman, & Nahmias, 2003). The majority of these studies report a general BOLD signal decrease in brain regions during facial emotion processing in ASD. Other studies, however, found increases in activity as compared to controls, e.g. in the superior parietal lobe (e.g., Hubl, et al., 2003), prefrontal cortices(e.g., Wang, Dapretto, Hariri, Sigman, & Bookheimer, 2004), anterior and occipital parietal lobe(e.g., Dapretto, et al., 2006), STS and the anterior cingulate cortex(e.g., Hall, et al., 2003).

The most consistently replicated finding of aberrant brain function during emotional face perception is a decrease in activity in the FG (Bolte, et al., 2006; Dalton, et al., 2005; Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2007; Hubl, et al., 2003; Pierce, Muller, Ambrose, Allen, & Courchesne, 2001; Schultz, et al., 2003). A study by Pierce and Redcay (2008), however, suggests that aberrant BOLD responses in the FG are not due to disturbed emotion processing per se, but rather reflect impairments in the core system of face perception, such as identity processing. Given the proposed interactive nature of the face perception and facial emotion recognition network, it has been suggested that ASD use “alternative means” during emotion recognition, caused by impairments in brain regions that process pre-conscious aspects of facial emotions (Harms, et al., 2010). Regarding the proposed dissociation of implicit and explicit processing deficits in ASD, an impairment of brain systems processing implicit aspects of emotional face recognition would affect neural correlates of more explicit aspects, in turn leading to the observed behavioral deficit in explicitly labeling emotions from faces.

One key candidate region for impaired implicit emotion processing in ASD is the amygdala(Adams, Gordon, Baird, Ambady, & Kleck, 2003; Ashwin, et al., 2006; Baron-Cohen, et al., 1999; Dziobek, et al., 2010; Dziobek, Fleck, Rogers, et al., 2006; Habel, et al., 2007; Kleinhans, et al., 2008; Munson, et al., 2006; Paul, Corsello, Tranel, & Adolphs, 2010; Schulkin, 2007). There are several studies showing commonly reduced activity in the amygdala in face processing but specifically in emotion processing from faces in ASD (Ashwin, et al., 2006; Corbett, et al., 2009; Dapretto, et al.,

2006; Hadjikhani, et al., 2007). However, there are also reports of increased amygdala activity in ASD in response to emotional faces (Dalton, et al., 2005; Monk, et al., 2010). In addition to altered amygdalar function, structural neuroimaging studies and post-mortem data strongly indicate anatomical abnormalities in the autistic amygdala. For example, there are numerous findings regarding differences in overall size of the amygdala in ASD (Corbett, et al., 2009; Schultz, 2005; Sparks, et al., 2002), which have been associated with impairments in social and communicative impairments(Munson, et al., 2006; Schumann, Barnes, Lord, & Courchesne, 2009). There are also reports of altered functional or effective connectivity between the amygdala and other brain regions, such as the medial temporal gyrus(Monk, et al., 2010) or the prefrontal cortex (Wicker, et al., 2008). Albeit the recently emphasized problem of head motion artifacts(Deen & Pelphrey, 2012) in connectivity data analyses, general defects in long range connections (e.g., Booth, Wallace, & Happe, 2011) of the corpus callosum in ASD, might generally contribute to a disturbed connectivity between brain regions in ASD, in turn mediating alterations in amygdalar connectivity.

With regard to the proposed interaction of other brain regions during face perception, alterations in amygdalar function and structure have been closely linked to FG alterations. A study by Dziobek and colleagues, for example, found a negative correlation between amygdala volume and cortical thickness in the FG, whereas controls yielded strong positive correlations(2010). Along these lines, there seems to be reduced functional connectivity between the amygdala and FG in ASD (Kleinhans et al., 2008). From a developmental perspective, an early amygdalar 'implicit' dysfunction may therefore prevent infants from assigning emotional salience to faces leading to aberrant development of face processing in other cortical areas, such as the FG (Grelotti, et al., 2002).

Linking the neural correlates of facial emotion processing impairments with implicit atypical gaze in ASD; the reported hyper- as well as hypoactivations in response to emotional faces could reflect respective reduced orientation or aversion to the eye region and may account for the inconsistencies in previous findings. An increase in amygdala activity in healthy controls is associated with an immediate orientation towards the eyes (Gamer & Buchel, 2009). Together with the failure to reflexively gaze towards the eyes in patients with amygdalar lesions(Spezio, Huang, et al., 2007), these data suggest that amygdala activity triggers orientation towards salient social cues. According to this hypothesis, decreased amygdalar response to faces in ASD would rather support the reduced orientation hypothesis within atypical gaze(Schultz, 2005). In contrast, increased amygdala activation was found to positively correlate with the duration of eye contact in ASD(Dalton, et al., 2005). This effect has been interpreted as an over-arousal in response to direct eye contact indicating aversiveness of eye fixation. These results would in contrast favor the avoidance hypothesis of direct eye contact and amygdalar (dys-)function in ASD, consistent with findings indicating amygdalar involvement in general aversion processing (De Martino, et al., 2010; Haruno & Frith, 2010; Hietanen, Leppanen, Peltola, Linna-Aho, & Ruuhiala, 2008). There is, however, no study to date which directly

investigated amygdala activity with respect to the orientation and avoidance hypotheses in atypical autistic gaze.

2. Research Aims

The overall aim of this dissertation is to advance the understanding of the behavioral and neural correlates of impaired implicit and explicit aspects of facial emotion recognition within atypical social cognition in high-functioning autism spectrum disorders. The specific aims were threefold:

2.1. Identify Implicit and Explicit Facial Emotion Recognition Impairments in ASD

Human social agents are confronted with a rapidly changing environment containing overt (e.g. big smile) as well as subtle (fake smile) social information. In contrast to most facial emotion recognition tasks to date, we are rarely confronted with direct, i.e. explicit, prompts on how to process social information ("*Is the smile fake or genuine?"*). Instead, a great portion of social information has to be processed implicitly, without being triggered explicitly. Individuals on the autism spectrum show severe impairments in social cognition, comprising implicit as well as explicit aspects. It remains unclear, however, whether the social impairments, such as recognizing emotions from faces, are more pronounced for implicit as compared to explicit processing. There is a clear lack of behavioral measures assessing implicit and explicit aspects of facial emotion recognition comparably based on direct performance to allow for a comparison between respective abilities, thus informing their relation in ASD.

To address a behavioral dissociation of implicit and explicit facial emotion recognition impairments in ASD, we developed two new validated video-based tasks to sensitively measure and comparably quantify implicit as well as explicit processing deficits in ASD.

2.2. Characterize the Mechanisms behind Atypical Gaze on Emotional Faces in ASD

Within rather automatic, i.e., implicit, social information processing, effective gaze to scan the social environment is crucial for social functioning. Atypical gaze on faces represents a prominent and early observable feature of the autism social symptomatology. The exact mechanisms behind the reduced focus on the eye region in ASD remain a puzzle. Is it a failure to reflexively orient towards salient social cues, such as the eyes? Or are gaze patterns driven by active avoidance tendencies that reflect negatively arousing, or even aversive effects of direct eye contact?

To precisely identify implicit reflexive gaze tendencies during facial emotion recognition, we investigated the influence of orientation- and avoidance-related gaze on the reduced eye-focus in ASD.

2.3. Specify the Role of the Amygdala in Atypical Gaze on Emotional Faces in ASD

A number of brain imaging studies showed that social impairments in ASD are rooted in dysfunctional profiles of brain regions that process social information, including facial emotion processing. One of the key regions implicated in the autistic pathophysiology in general and specifically in impaired emotion processing is the amygdala. The amygdala seems to have a complex dysfunctional profile in ASD in response to emotional faces, including hyper-, as well as hypoactivation along with reports of reduced focus on the eye region. The exact mechanisms behind these dysfunctional activation patterns and their relation to avoidance and orientation processes on the gaze level, however, remain contradictory.

To further define aberrant brain function underlying impaired facial emotion processing in ASD in a third independent study, we assessed the specific role of the amygdala in reflexive orientation- and avoidance-related gaze.

3. Methodology

The current chapter describes and illustrates this dissertation studies' methodology and is divided into two sections. General methodology is concerned with methods that have been applied to at least two research studies. The specific methodology section includes further study–specific descriptions.

3.1. General Methodology

3.1.1. Sample Information

Each study included a sample of adult neurotypically-developed control participants (NT) with no reported history of psychiatric or neurological disorders (see Table 1 for demographic information of the three dissertation studies' samples).

	N (male)		Age (yrs (SD))			MWT-IQ (SD)			LPS-IQ (SD)		
	ASD	*NT*	*ASD*	*NT*	*p*	*ASD*	*NT*	*p*	*ASD*	*NT*	*p*
Study I	24 (15)	24 (15)	32.54 (8.51)	30.29 (8.37)	*.36*	108.04 (13.26)	106.21 (10.46)	.59	120.54 (9.87)	119.58 (9.68)	*.73*
StudyII	17 (5)	19 (5)	32.7 (8.2)	30.4 (5.9)	*.33*	104.5 (15.6)	110.4 (12.9)	.23	126.75 (11.57)	124.5 (9.61)	*.54*
Study III	16 (16)	17 (17)	30.44 (6.34)	30.47 (6.24)	*.99*	108.06 (7.38)	108.12 (14.76)	.99	128.47 10.82	126.4 (8.94)	*.55*

Table 1. Demographic variables and IQ measures of the three dissertation studies' samples.
p values reflect levels of significance from independent samples t-test. Samples size (N) values reflect total number of participants; number of males is given in parentheses for Study I and II, Study III included only male. For Age, MWT-IW and LPS-IQ, values are given in mean; standard deviation is given in parentheses. Abbreviations: N, sample size; yrs, years; SD, standard deviation; MWT-IQ, Mehrfach-Wortschatz-Test, German multiple choice vocabulary intelligence quotient test; LPS-IQ, Leistungsprüfsystem (subtest 4), German strategic thinking intelligence quotient test; ASD autism spectrum disorders; NT, neurotypically-developed.

Control participants were recruited by public notices and project databases of the Freie Universität Berlin and/or the Max Planck Institute for Human Development, Berlin, Germany. ASD participants were recruited through the autism in adulthood outpatient clinic of the Charité University Medicine, Berlin, Germany or were referred to us by specialized clinicians.

In addition to age and gender, groups were matched with respect to their intelligence level, as assessed by a German vocabulary test (Mehrfachwahl-Wortschatz-Test, MWT, Tewes, 1991) and a

strategic thinking test (Leistungsprüfsystem, LPS, Horn, 1962) to exclude potential confounds due to intellectual functioning.

All participants had normal or corrected-to-normal vision, were native German speakers, received payment for their time and provided written informed consent.Study I was approved by the ethics committee of the German Society for Psychology (DGPs). Study II and III were approved by the ethics committee Charité University Medicine, Berlin, Germany.

3.1.2. ASD Diagnoses

Diagnoses were made according to DSM-IV (4th ed., text rev., American Psychiatric Association, 2000) criteria for Asperger Syndrome and autism without mental retardation using two instruments known to be the gold standard for diagnosing autism: the ADOS (Lord, et al., 2000)and the ADI-R (Lord, et al., 1994), if parental informants were available. The diagnosis Asperger Syndrome was additionally confirmed with theASDI(Gillberg, et al., 2001).

To control for clinically significant levels of autistic traits in healthy populations, we applied the Autism Spectrum Quotient (AQ, Baron-Cohen, Wheelwright, Skinner, et al., 2001) in both groups in allstudies.

3.1.3. Statistical Analyses

Behavioral (e.g., accuracy scores, reaction times) or eye movement data were first analyzed with repeated measures analysis of variances (ANOVA) to investigate potential main and interaction effects. Respective within subject factors were determined by the study-specific experimental conditions and are specified in the results section of each manuscript. In all studies, the between-subject factor group (NT versus ASD) was included to investigate effects of group membership. Post-hoc t-tests included independent samples t-tests between groups and paired-samples t-tests within-groups, if applicable. Correlations between two measures were calculated based on Pearson's r correlation coefficients (2-tailed), whereas differences between correlations were calculated according to Fisher's r to z transformation (2-tailed). All statistical tests used a significance threshold of $p < .05$, if not specified otherwise. Data were preprocessed and analyzed with Matlab ® (version 7.10.0, The Mathworks, Inc., Natick, MA, USA), SPSS (version 17.0 for Mac, SPSS Inc., Chicago, IL, USA) or PASW (version 18.0 for Mac, SPSS Inc., an IBM Company, Chicago, IL, USA).

3.1.4. Analyses of Gaze

Gaze behavior, i.e. eye movements, was recorded at a rate of 60 data points per second (60 Hz). Resulting time series were applied to study- and eye-tracker-related specific preprocessing steps (e.g., Gaussian convolution, recursive median filtering (Nodes & Gallagher, 1982)) to suppress impulse noise. We excluded trials in which participants did not fixate the fixation cross prior to the onset of the face or contained consecutive missing data likely caused by blinks, head motion or poor calibration.

We then categorized trials as containing an eye movement after fixation, i.e.a fixation change, (downwards or upwards), or as trials not containing a fixation change to compare groups and conditions. A fixation change was thereby defined as occurring a certain number of pixels (or degree (°)) apart from the original position of the fixation cross in vertical direction. The remaining trials were then used to calculate the proportion of fixation changes downwards (when the eyes were presented at the location of the fixation cross) and upwards (when the mouth was presented at the location of the fixation cross) as reflexive gaze responses triggered by the eyes or the mouth, respectively.

To further assess potential effects between groups when combining eye movements away and towards the eyes, we calculated individual eye preference indices, as a general measure of gaze. The indices described the difference between the proportion of trials with a fixation change upwards from the mouth and the proportion of trials with a fixation change downwards from the eyes(Gamer & Buchel, 2009). Actual calculation formulas of the indices differed between the studies, and are due to study-specific different technical condition/properties and respective amounts of invalid data.

3.1.5. Explicit Emotion Recognition Task

The emotion recognition task of Study I and II comprised 120 grey-scale faces (20 male, 20 female each displaying happy, fearful, and neutral expressions) from a standardized dataset (Goeleven, De Raedt, Leyman, & Verschuere, 2008; Lundqvist, Flykt, & Öhmann, 1998). Trials started with the initial presentation of a fixation cross, followed by the presentation of a face. After a blank grey screen, participants were asked to indicate the emotional expression via button press out of three verbal label options (fearful, happy, neutral). Importantly, to investigate the effect of initially fixating the mouth or the eyes on gaze behavior, half of the faces within each emotion category were shifted either downward (Figure 1, Trial A) or upward (Figure 1, Trial B), so that the eyes or the mouth appeared at the location of the formerly presented fixation cross. The task thus allowed the investigation of reflexive gaze behavior as a response to direct eye contact as well as eye movements, when initially fixating the mouth. Please see Figure 1 for study-specific timing and task procedure.

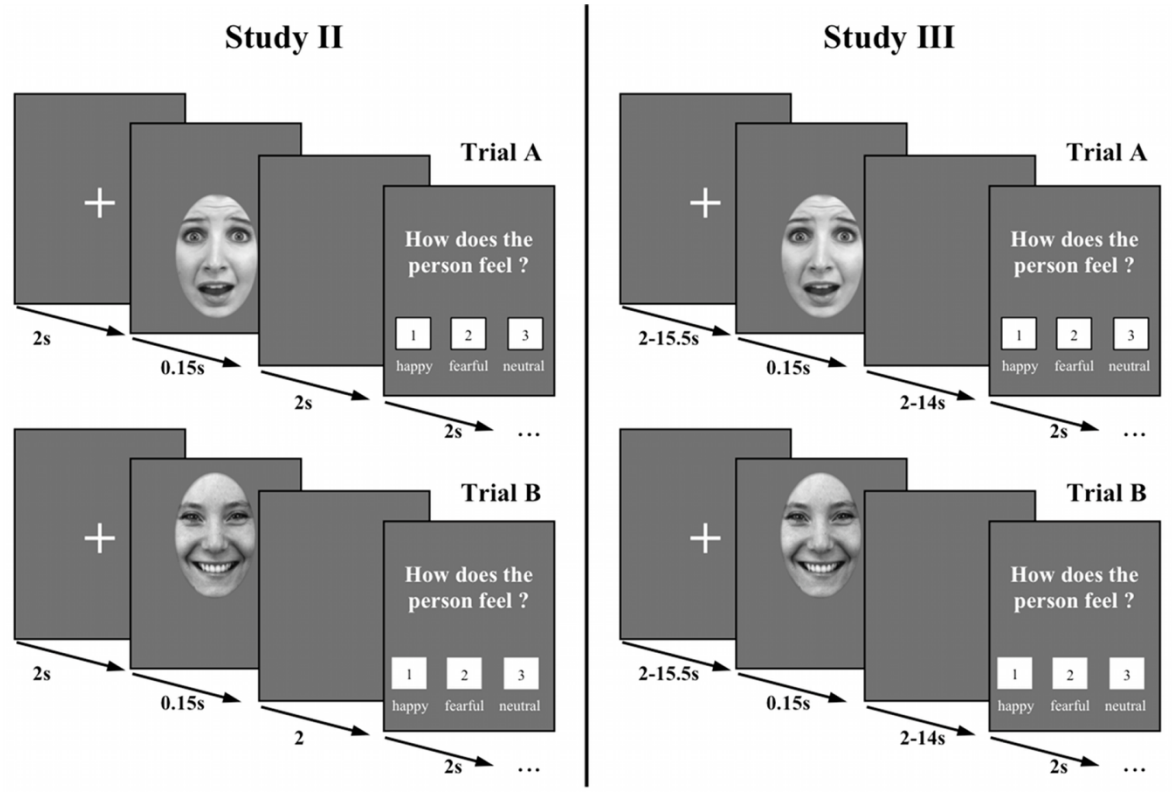

Figure 1. Explicit emotion recognition task and timing of Study II and III.

Trials started with the presentation of a fixation cross (Study II: 2s, Study III: 2-15.5s), followed by the presentation of a face for 150ms. After a blank screen (Study II: 2s, Study III: 2-14s), participants had to indicate the emotional expression (happy, fearful, neutral) via button press. Faces were shifted vertically on the screen to vary the initial fixation position on faces. In half of the trials, participants initially fixated the eyes (Trial A). In the other half of trials, participants initially fixated the mouth (Trial B). Presentation of fixation cross and blank screen were jittered for Study III, according to trial optimization with optseq2 (http://surfer.nmr.mgh.harvard.edu/fswiki/optseq2).

3.2. Specific Methodology

3.2.1. Study I

3.2.1.1. Face Puzzle Tasks

The new Face Puzzle tasks represent two independently applicable tasks for the assessment of implicit and explicit emotion recognition abilities from faces using short video clips (mean length 10.3 s) with 14 professional actors (7 male, varying age (20-50 years)) portraying 25 different emotional facial expressions (13 negative, 12 positive), covering five basic and20 complex, more social, emotions (e.g., worried, forgiving, doubtful, interested, compassionate).

Methodology

To increase ecological validity of stimuli and thus sensitivity of the tasks to real life impairments of ASD, we produced a new set of more naturalistic video stimulus material in the context of a comprehensive project to produce and evaluate new tasks and trainings for socio-cognitive functioning (Social Cognition Training Tool (SCOTT), http://www.languages-of-emotion.de/en/scott.html). Stimulus production took place at the film studio of the Humboldt University, Berlin, Germany in cooperation with its Computer and Media Service (CMS) and professional acting agencies and schools in Berlin. In sum, we produced a total set of 40 different emotional states that were depicted in facial expressions by 47 professional actors of varying age (18-65 years). To increase ecological validity, emotion selection was based on a previous study(Hepach, Kliemann, Gruneisen, Heekeren, & Dziobek, 2011)that characterized emotional words regarding their frequency and thus their relevance in everyday life in addition to the classic valence and arousal dimensions.

Stimuli and tasks were subjected to pre-validation steps to ensure item and task validity. In fact, stimulus validation yielded high average emotion recognition rates (mean = 92.6 on a scale of 0-100, SD = .07) and good believability (4.4, on a scale of 1-6, SD = .07) based on an expert validation study. Initial validation of the Face Puzzle implicit task in an independent sample of healthy participants revealed good internal consistency and item difficulty, thus no further revision was necessary. The Face Puzzle explicit task initial validation was not sufficient, resulting in revision of respective items before use in Study I.

In the Face Puzzle explicit task, a face video was presented in the upper center of the screen. For detailed inspection, participants could enlarge the video when directing the computer mouse above the video, which played automatically in loops until the task item was completed. Participants then had to choose the correct label for the presented emotional video out of four emotion labels in a multiple-choice format. To complete the task item participants had to place the chosen label into a target field below the target video through a drag and drop motion with the computer mouse. Distractor labels were constructed as follows: i) two emotions of the same valence, one with comparable valence and arousal levels, ii) one emotion that differed in arousal level but with the same valence as the target item and ii) one emotion of the opposite valence (see Figure 2, for an example). In sum, by comparing the emotional mimic with the provided labels, participants had to explicitly match the target video's emotional content.

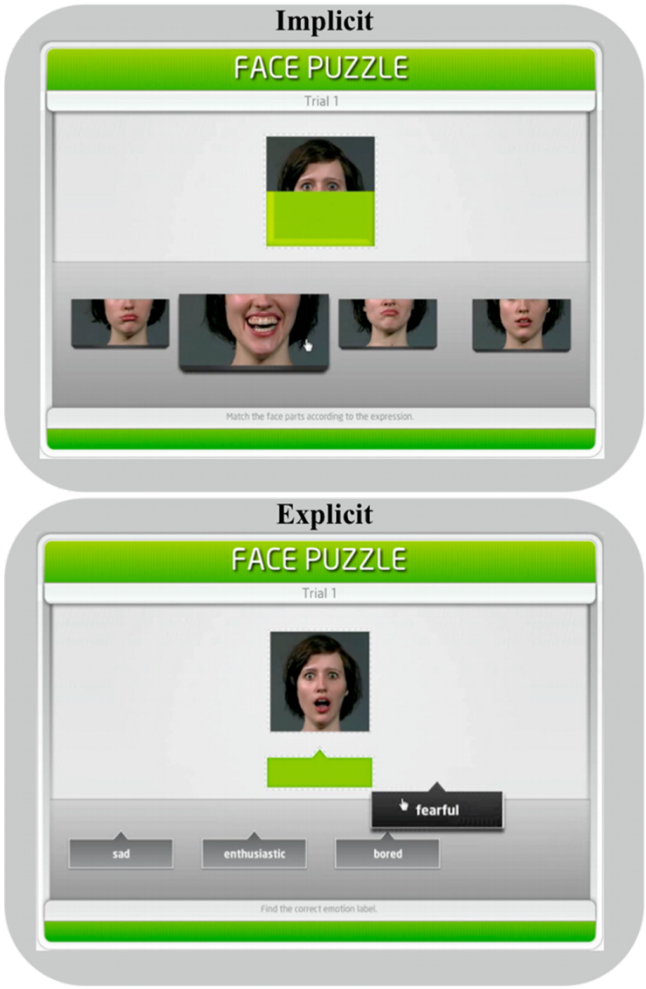

Figure 2. Face Puzzle implicit and explicit task.

(upper) Face Puzzle implicit task: Participants have to find the according mouthvideo to the target eyevideo. *(lower)* Face Puzzle explicit task: Participants have to explicitly label the target emotional expression.

In the Face Puzzle implicit task, facevideos were divided in an upper (including the eye region; eyevideo) and a lower part (including the nose and the mouth region; mouth video). An eyevideo was displayed in the upper center of the screen, representing the target item. Below the target item four mouthvideos of the same actor were displayed. As in the explicit task, the target eyevideo was started playing automatically in loops until the participant completed the item by dropping a mouthvideo into the target field. Mouthvideos remained still images until participants directed the computer mouse on a video, thereby enlarging and starting to play the video. Participants were asked to match the target eyevideo with the correct mouth video according to the emotional expression. To complete a task item, participants had to place a mouth video underneath the eyevideo through a drag and drop function. No further information about the presented emotional states was given, so that participants had to identify the depicted emotion in the face parts without explicitly being asked to

identify a specific emotion (i.e., “find the happy mouth that match the happy eyes”) or explicitly labelthe emotion.

3.2.1.2. Additional Socio-cognitive Measures

In Study I, we applied additional established socio-cognitive measures to i) investigate the new tasks’ external and constructive validity, and to ii) further differentiate between implicit and explicit emotion recognition processes.

As anexplicit measure for emotion recognition from facial stimuli, we administered a standard task for facial emotion and mental state recognition: the ‘Reading the Mind in the Eyes Test’(RMET, Baron-Cohen, Wheelwright, Hill, et al., 2001). The RMET is a performance-based test that requires participants to explicitly infer and label emotional and mental states based on photographs of eyeregions, similar to the explicit Face Puzzle task.The RMET has been used in a number of studies with healthy and psychiatric samples and has shown sensitivity to social impairments in ASD. To avoid possible ceiling effects in the control group and to increase sensitivity to subtle impairments in ASD, we additionally computed the subscale ‘difficult items’, introduced by Domes et al. (2007), in addition to the RMET total score.

As a measure of implicit socio-cognitive functioning, we additionally administered the ‘externally-oriented thinking’ (EOT) subscale of the Toronto Alexithymia Scale (TAS-26, German translation, Kupfer, Brosig, & Brähler, 2001). The EOT-TAS scale measures the tendency to focus attention internally as opposed to externally, thus representing a measure of rather implicit thinking styles without the use of external prompts.

3.2.2. Study III:

3.2.2.1. Magnetic Resonance Imaging

To inform neural correlates of facial emotional recognition in brain function, we used functional magnetic resonance imaging (fMRI) in Study III. FMRI allows the indirect investigation of neural activity, via changes inBOLD signal(Logothetis, 2008). Importantly and in contrast to other neuroimaging methods, such as positron emission tomography (PET), fMRI is noninvasive and in combination with time-efficient experimental designs suited for investigating brain function in number of psychiatric diseases, including high-functioning autism.

Participants were scanned using a Siemens Magnetom Tim Trio 3Tesla system (Siemens Medical Solutions, Erlangen, Germany) equipped with a 12-channel head coil at the Dahlem Institute for Neuroimaging of Emotion (D.I.N.E., http://www.dineberlin.de/) at the Freie Universität Berlin, Germany.

3.2.2.2. Imaging Sequences and Analyses

For each participant, we acquired 4 functional runs of 180 blood oxygen level dependent signal (BOLD) sensitive T2*weighted echo planar images (EPI) (time of repetition (TR), 2000 ms; echo time (TE), 25 ms; flip angle, 70°; field of view (FOV), 204 x 20 mm^2; matrix, 102 x 102; voxel size, 2 x 2 x 2 mm). To reduce susceptibility effects and achieve a better in-plane resolution with regard to our specific hypotheses regarding the bilateral amygdalae, we collected 33 axial slices covering a 6.6 cm block, resulting in a higher resolution of 2 x 2 x 2 mm (see Figure 3). For registration of functional images we additionally acquired high-resolution T1-weighthed structural images (TR, 1900 ms; TE 2.52 ms; flip angle, 9°; 176 sagittal slices; slice thickness 1 mm; matrix, 256 x 256; FOV, 256; voxel size, 1 x 1 x 1 mm). Data were preprocessed and analyzed using FEAT (FMRI Expert Analysis Tool) within the FSL toolbox (Version 4.1.4, FMRIB's Software Library, Oxford Centre of fMRI of the Brain, www.fmrib.ox.ac.uk/fsl, S. M. Smith, et al., 2004). Preprocessing included standard procedures (e.g., removal of non-brain tissue, slice time and motion correction, spatial smoothing (5-mm full width at half maximum (FWHM) Gaussian kernel), high-pass filtering(Gaussian-weighted straight line fitting, sigma = 50 s)), registration to the T1-weighted structural image, transformation into standard space (Montréal Neurological Institute, MNI) using 7- and 12-parameter affine transformations, respectively (using FLIRT, Jenkinson & Smith, 2001).

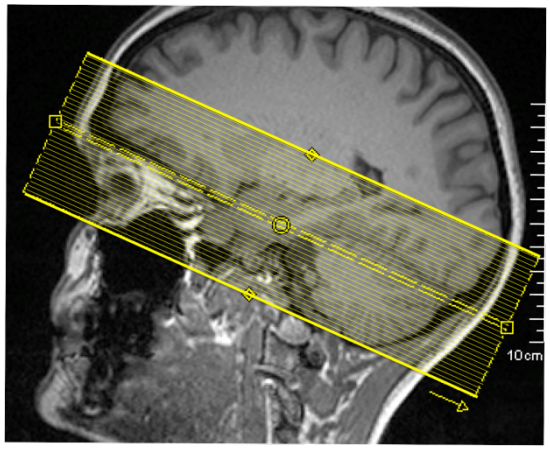

Figure 3. EPI slab location.

To achieve a better in-plane resolution and reduce susceptibility effects, we collected 33 axial slices of T2*weighted echo planar images covering 6.6 cm (including bilateral amygdalae), instead of whole-brain EPI acquisition, resulting in a higher resolution of 2 x 2 x 2 mm. Example EPI slab is displayed on one participant's T1-weighted structural image (sagittal view).

Functional analysis of imaging data included modeling of time series individually for each participant with event-related regressors according to the study conditions. Contrast images were computed for each participant, spatially normalized, transformed into standard space, then submitted to a second-order within-subject fixed-effects analysis across runs and finally applied to higher level mixed-effects analyses across participants using the FMRIB Local Analysis of Mixed Effects tool provided by FSL (FLAME, stage 1 & 2). Because of the specific hypotheses in Study III regarding amygdala functioning in ASD, we used an anatomically defined mask to restrict our analyses to the bilateral amygdalae. The mask included voxels with a 10% probability to belong to the bilateral amygdalae as specified by the Harvard-Oxford subcortical atlas (http://www.cma.mgh.harvard.edu/fsl_atlas.html) provided in the FSL atlas tool.

4. Dissertation Studies

This chapter summarizes three original research studies, which together constitute this dissertation. The studies represent empirical attempts towards advancing our understanding of implicit and explicit aspects of facial emotion recognition impairments in ASD with behavioral, eye-tracking and neuroimaging methods. In Study I we developed and evaluated two new video-based tasks for implicit and explicit facial emotion recognition to further identify and define relations between respective impairments in ASD. In Study II, we specifically assessed gaze on emotional faces in ASD as an important aspect during implicit social processing. In particular, we investigated the puzzle of reduced focus on the eye region during an explicit emotion recognition task with eye-tracking. In Study III, we investigated the underlying neural basis of atypical reflexive gaze patterns in ASD as identified in Study II, in particular the role of the amygdala.

4.1. Study I: Implicit and Explicit Emotion Recognition Impairments in ASD

In Study I (Kliemann, Rosenblau, Boelte, Heekeren, & Dziobek, under review),we sought to develop a new behavioral tool to comparably measure implicit and explicit emotion recognition processing aspects. We developed two new video-based behavioral tasks with similar answer formats to identify implicit and explicit impairments, as well as respective interrelations, in ASD. Whereas the Face Puzzle explicit task triggered emotion recognition from facial expressions explicitly by instructing participants to match videos of emotional faces with verbal labels, there were no such explicit prompts to identify a specific facial emotional expression in the Face Puzzle implicit task. Instead, the implicit task asked participants to identify emotional cues in face parts to correctly compose a complete facial expression from puzzle pieces.

Item analyses showed highly satisfactory internal consistencies: for both tasks Cronbach's alpha was > .8. In addition, correlation analyses with external implicit and explicit socio-cognitive measures were in favor of the tasks' external validity, as typically developed group's performance in the Face Puzzle explicit task correlated with accuracy in a standard explicit emotion recognition task (RMET). In contrast, there was no correlation of the RMET with performance in the Face Puzzle implicit task. Furthermore, performance in the Face Puzzle implicit task correlated marginally significantly with the implicit TAS subscale, but no such correlation was found between the TAS scale and performance in the Face Puzzle explicit task.

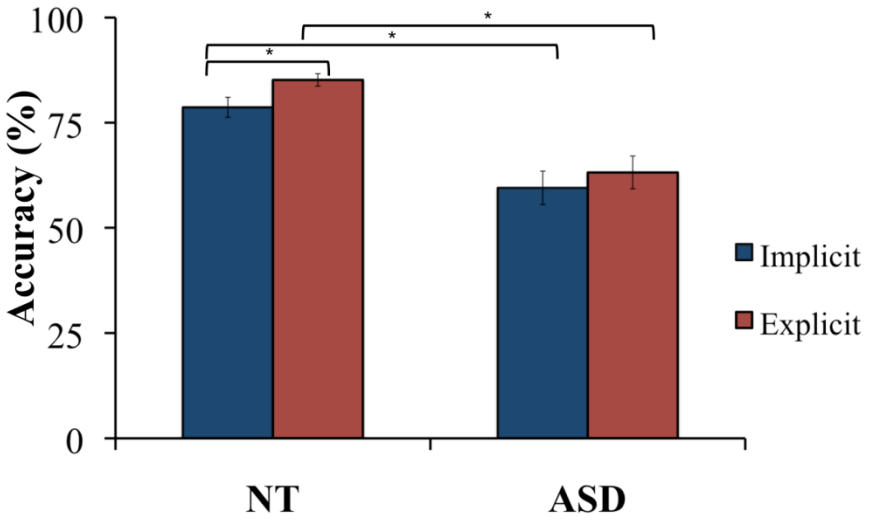

Figure 4. Accuracy (%) in Face Puzzle implicit and explicit task per group (ASD, NT).
There was no significant difference between accuracy in the Face Puzzle implicit as compared to the explicit task in the ASD group. In contrast, the NT group's accuracy was significantly greater in the explicit task as compared to the implicit task. The NT group showed greater accuracy in both tasks as compared to the ASD group.

In a second step, we investigated potential dissociations and group specific interrelations between implicit and explicit facial emotion recognition processes. The ASD group showed overall reduced accuracy scores in both tasks, as compared to the NT group (see Figure 4.). In addition, performance scores in the Face Puzzle tasks were intercorrelated and did not differ significantly in ASD. Further, accuracy in both Face Puzzle tasks correlated with diagnostic measures. The more severely affected individuals showed greater performance deficits (see Figure 5.). In contrast to ASD, the NT group's performance in the implicit and the explicit Face Puzzle task differed significantly and were not correlated (see Figure 4).

These group specific relations imply that implicit and explicit aspects of facial emotion recognition processes as measured by the Face Puzzle tasks can be dissociated behaviorally in typical development. In fact, analyses of the composite measure, combining individual accuracy and reaction time scores into a more naturalistic performance measure, suggest a greater magnitude of implicit as compared to explicit impairments in ASD (see Figure 6.).

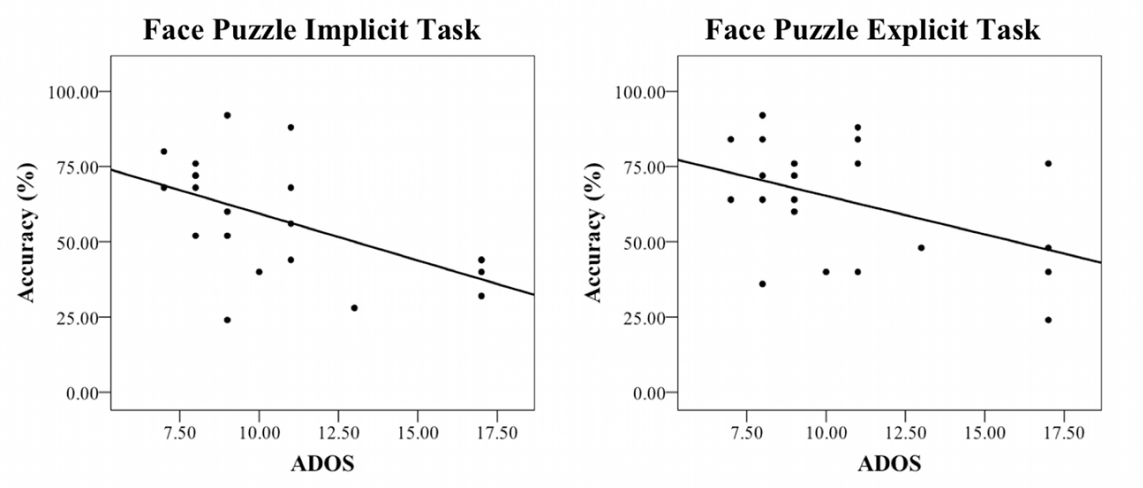

Figure 5. Correlations between ASD participants' diagnostic scores and accuracy in the Face Puzzle implicit and explicit task.

Accuracy in the Face Puzzle implicit task (left), as well as the explicit task (right) correlated significantly with individual diagnostic scores, as measured by the ADOS.

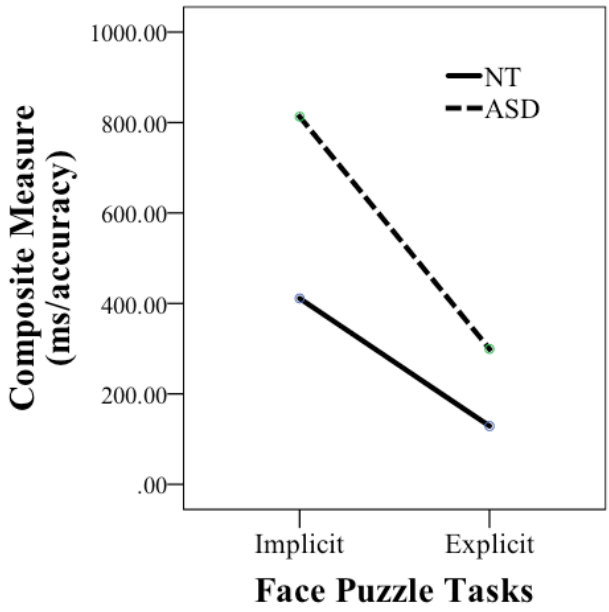

Figure 6. Task (implicit, explicit) by group (ASD, NT) interaction of the composite measure.

The group difference was greater for the implicit as compared to the explicit task, indicating increased implicit processing impairments in ASD.

4.2. Study II: Atypical Gaze on Emotional Faces in ASD

In the second study(Kliemann, Dziobek, Hatri, Steimke, & Heekeren, 2010),we sought to investigate one aspect of impaired implicit social cognitive functioning: atypical scan paths on emotional faces. Impaired social gaze is an early diagnostic sign of ASD. Probably the most prominent feature thereby is a reduced focus on the eye region. It remains a puzzle, however, whether the lack of eye focus is based on reduced general orientation towards social cues, or due to active avoidance, i.e., aversion, of direct eye contact. In Study II, we sought to identify and quantify respective portions and potential interactions of eye movements related to reduced orientation and avoidance of direct eye contact. To this end, we used a novel and classically explicit behavioral emotion recognition task, which was developed in close collaboration with colleagues in Hamburg(Gamer & Buchel, 2009). During task execution, we recorded participants' eye movements to investigate directionality of gaze and inform the avoidance and orientation hypotheses. Crucially, the experimental design varied the initial fixation position on the face.In half of the trials, participants started processing emotional faces either at the eyes *or* at the mouth while they decided whether the face depicted a fearful, happy or neutral expression. This manipulation allowed us to investigate both avoidance and orientation-guided reflexive gaze, triggered either by initially focusing the eyes or mouth region, respectively.

Analyses of eye movements replicated an overall reduced preferencefor the eyes in ASD as compared to a sample of neurotypically-developed, age-, gender- and IQ-matched healthy controls.The reduced eye-focus in ASD seemed particularly due to eye movements away from the eyes as compared to towards the eyes. In contrast, the NT group shifted gaze significantly more often towards the eyes than away from the eyes, modulated by the emotional expression (see Figure 7.).

Moreover, ASD participants tended to gaze away from the eyes faster than the NT group. These fixation changes also appeared relatively faster than gazing away from the mouth (to the eyes), most prominently for fearful faces. Finally, ASD specific gaze was associated with performance and social symptomatology, but not with impairments in repetitive behavior and communication.

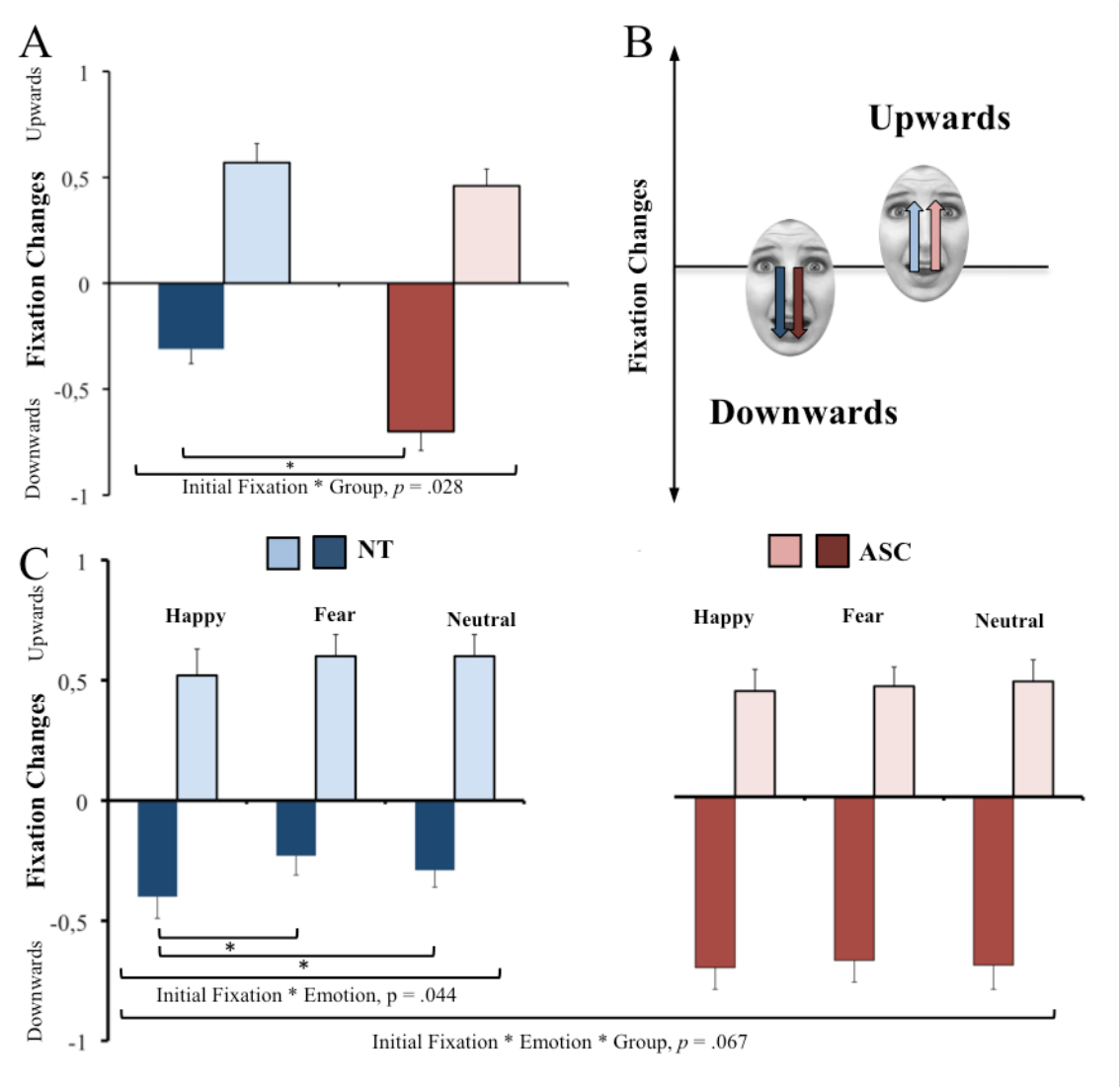

Figure 7. Fixation change patterns.

*A: Function of initial fixation (over all emotions).*ASD participants showed significantly more fixation changes away from the eyes (downwards) than the participants in the NT group. *B: Schematic demonstration of fixation changes.* Fixation changes downwards are represented in negative bars and reflect gaze away from the eyes when initially fixating the eyes. Fixation changes upwards are represented in positive bars and reflect gaze towards the eyes when initially fixating the mouth. *C: Interaction of emotion (happy, fearful, neural) and initial fixation (eyes, mouth).* Fixation changes away from the eyes (solid) were mediated by the displayed emotional expression for the NT group, whereas this effect was absent in the ASD group.

4.3. Study III: The Role of the Amygdala in Atypical Gaze on Emotional Faces in ASD

In the third study(Kliemann, Dziobek, Hatri, Baudewig, & Heekeren, 2012), we built upon the results of Study II regarding atypical gaze on emotional faces in ASD extending our study design to neuroimaging methods to assess underlying neural activity. A common neuroimaging finding in facial emotion processing in ASD is aberrant amygdala activity as compared to neurotypically developed participants. In the context of the puzzle of reduced focus on the eyes in ASD, it also remained unclear, whether amygdalar hypo- and hyperactivation in response to emotional faces in ASD support an avoidance of direct eye contact or a lack of social attention, respectively. To further specify amygdalar response patterns underlying implicit atypical gaze during explicit facial emotion recognition, we applied the explicit emotion recognition task used in Study II, whereby the timing was adapted to the scanner environment. In addition to eye movement analyses, we investigated changes in BOLD signal in the amygdala triggered by initial fixation of the eyes as compared to the mouth.

On the behavioral level, analyses of eye movements replicated the results of Study II. ASD participants showed a generally reduced focus on the eyes characterized by more gaze away than towards the eyes. Higher-level mixed effects analyses of functional MRI data in the bilateral amygdalae revealed a significant cluster of activity in the interaction contrast of group (ASD versus NT) and initial fixation position (eyes versus mouth). Extracted parameter estimates from significantly activated voxels within this cluster showed reversed group effects in response to eyes and mouth fixation (see Figure 8). ASD participants exhibited relatively greater amygdala response when initially fixating the eyes, whereas NT participants showed a relative increase when initially fixating the mouth, compared to the other facial feature and group, respectively.

In addition, emotion recognition performance appeared to be particularly impaired in ASD, when participants initially fixated the most discriminative regions of the face for a respective emotion (eyes for fearful faces, mouth for happy faces). The combination of atypical amygdala activity and gaze in ASD thus complemented group specific behavioral performance.

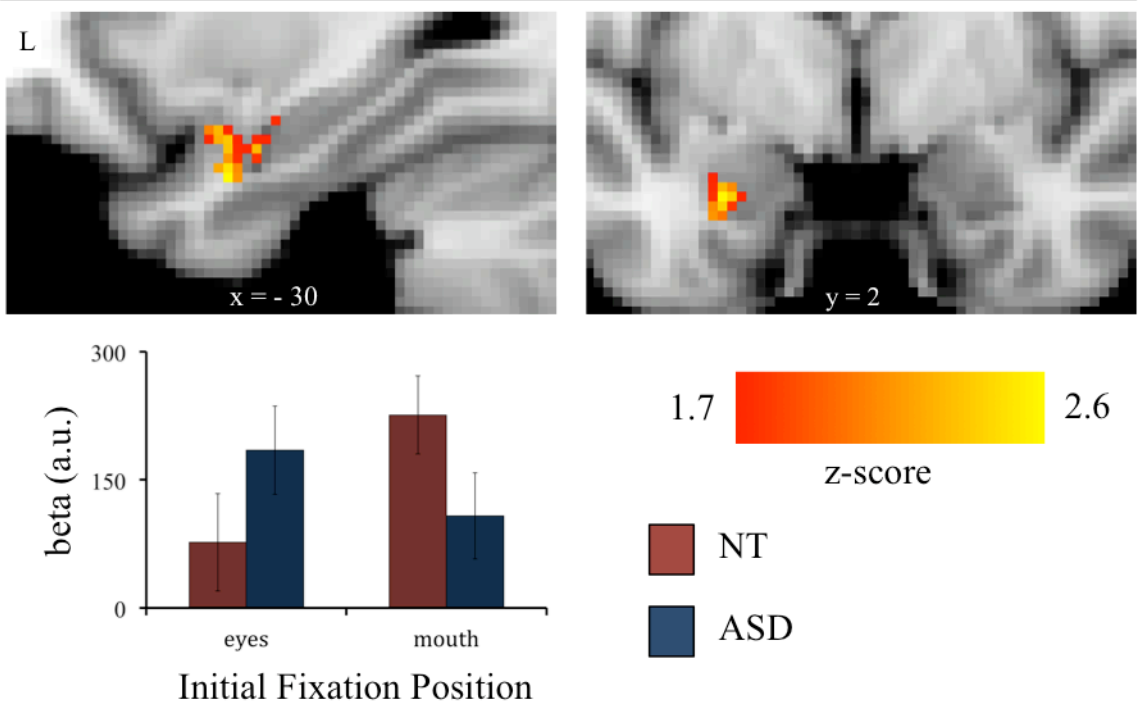

Figure 8. Significant interaction of initial fixation position (eyes, mouth) and group (ASD, NT) in the amygdala.

Upper panels show statistical maps of left (L) sagittal and coronal planes. The bar chart shows the parameter estimates (β values in arbitrary units) of the cluster reported in Table 2 (p = .005, FWE corrected). Error bars represent standard error of the mean (SE).Abbreviations: a.b., arbitrary units.

				MNI-coordinates (mm)		
Hemisphere	Multiple Comparison Correction	Voxels	Z-max	*x*	*y*	*z*
Left	FWE corrected, p = .005	148	3	-26	4	-20

Table 2. Significant interaction contrast of initial fixation (eyes, mouth) and group (ASD, NT) in the amygdala.

p values reflect levels of significance. Abbreviations: FWE, family-wise error; MNI, Montreal Neurological Institute.

5. Discussion

In the previous chapter, I have presented the results of the three studies investigating specific aspects of implicit and explicit facial emotion recognition impairments in behavior, gaze and brain function in ASD. In this chapter, I first discuss the results of the three studies, whereby Study II and III will be discussed jointly. In the second section of this chapter, I integrate the insights from all three studies within a model that describes how impairments in implicit facial emotion recognition, i.e. dysfunctional behavioral (gaze) and neural correlates (amygdala activity), affect explicit facial emotion recognition in ASD. In the last section of this chapter, I suggest how the findings and the proposed model for implicit and explicit social cognition guide future research and interventions to effectively promote the social functioning of individuals on the autism spectrum.

5.1. Specific Discussion

5.1.1. Implicit and Explicit Facial Emotion Recognition Impairments in ASD: Study I

In Study I(Kliemann, et al., under review), we assessed implicit and explicit aspects of impaired facial emotion recognition in ASD as compared to healthy controls with two new behavioral tasks: Face Puzzle implicit and explicit. The new tasks generated comparable performance-based measures of implicit and explicit aspects of facial emotion recognition, thereby offering an opportunity to empirically arrive at a dissociation of respective processes directly via individual performance scores. In sum, the results yielded excellent reliability scores (both task's Cronbach's alpha was > .8), external validity to established socio-cognitive implicit as well as explicit measures, and sensitivity to the subtle social impairments of high-functioning ASD. Results of Study I further imply that implicit and explicit aspects of facial emotion recognition can be behaviorally dissociated in typically developed individuals, while they seem to be closely associated in ASD.

The development of human social cognition is comprisedof the specification of innate spontaneous reactions into explicitly and consciously addressable representations and concepts(Adolphs, 1999; Karmiloff-Smith, 1992). A theoretical dissociation of respective implicit and explicit processes thus seeks an empirical validation to inform particular impairments in psychiatric conditions, such as ASD. First, I would like to emphasize that our approach towards measuring implicit aspects of facial emotion recognition differs from other conceptualizations of implicit social cognition in, e.g., traditional social or developmental psychology(Moors, et al., 2010). Instead of applying indirect measures based on reaction time or gaze data, we aimed at measuring direct behavioral performance as outputs of implicit and explicit processes. Our approach thus allowed quantifying performance based on accuracy, in addition to reaction times. We chose this particular approach to account for the importance of identifying and comparing implicit and explicit processing impairments in behavior (Zaki & Ochsner, 2009, 2011).

Second, both the Face Puzzle implicit and explicit task yielded excellent reliability scores, indicating sufficient internal consistency of task items. Correlation analyses with the external socio-cognitive measures additionally provided ample external validity, as performance in the Face Puzzle explicit but not the implicit task correlated with accuracy in the RMET task. The RMET classically provides explicit prompts via verbal emotion labels to trigger emotion recognition. In contrast, there was a marginally significant correlation between performance in the implicit task and the tendency to orient thinking strategies to internal as opposed to external cues, as measured by the EOT-TAS subscale. There was no such correlation with performance in the explicit task in all participants. These specific correlations for the Face Puzzle tasks indicate that our operationalization of implicit and explicit indeed dissociated respective implicit and explicit aspects of facial emotion recognition. It is important to mention, that correlations between implicit as compared to explicit measures were not significantly different and we particularly suggest caution in interpreting and generalizing this aspect of our findings. However, given the clear lack of performance based implicit measures to further proof our suggested differentiation of implicit and explicit processes, we interpret this pattern of relations in favor of the tasks' external validity.

Third, group comparisons between the ASD and NT sample provide empirical evidence that the two new Face Puzzle tasks are sensitive to impaired facial emotion recognition, and particularly to the subtle impairments of high-functioning individuals on the autism spectrum. In both tasks, the ASD group showed significantly fewer correct responses than the NT group, indicating impairments in implicit as well as explicit aspects of facial emotion recognition. Additional support for the tasks' sensitivity to atypical social cognition is reflected in positive correlations of diagnostic scores and individual performance in the Face Puzzle tasks. The greater individual symptom severity, as measured by the ADOS and the ASDI, the fewer correct responses participants made. This reflects a link between symptom severity and task performance.

Fourth, results of Study I further underline previous reports suggestinggreater implicit processing deficits in the absence of explicit cues or prompts in ASD (Senju, 2012a; Senju & Johnson, 2009; Senju, et al., 2009). We found a greater magnitude of group differences in the Face Puzzle implicit as compared to the explicit task, suggesting that ASD participants were particularly impaired in the implicit task. This is in line with findings from Volkmar and colleagues(Volkmar, et al., 2004), suggesting that the social impairments in ASD are specifically evident in unstructured, more realistic (i.e. ecologically valid) task settings and demands.

Fifth, the results of Study I provide first empirical hints for an impaired relation between implicit and explicit facial emotion recognition in ASD as compared to typical development. Because we used a performance-based approach, we were able to compare direct behavioral outputs of implicit and explicit emotion recognition intra- and interindividually and in turn assess respective relations with regard to a proposed dual-process perspective on social cognitive abilities and concepts (e.g.,

ToM, Apperly & Butterfill, 2009; Low & Perner, 2012). The NT group's performance in the explicit Face Puzzle task differed significantly from the implicit Face Puzzle task, whereby performance in both tasks was not intercorrelated. In contrast, the ASD group's performance did not differ between tasks, and correlated significantly. These results imply a dissociation of implicit and explicit aspects of emotion recognition in typical development and a lack thereof in ASD. Whereas it seems that respective processes can be independently assessed to some extent in the NT group, in turn indicating the possibility of autonomous operation, implicit and explicit processes seem to be more closely related in ASD. Different correlations of implicit and explicit performance between the groups, as measured by the composite measure, further underline the interpretation of differential relationships between implicit and explicit processes in ASD as compared to NT.

With regard to the dual-process model of implicit and explicit processes and its proposed subsequent development (Dienes & Perner, 1999; Karmiloff-Smith, 1992), our data speak towards a "two system" or "dual-process" perspective for facial emotion recognition in typical development. In ASD, however, the developmental trajectory seems to arrive at *two broken systems*. Both implicit and explicit task performance correlated with the explicit RMET scores in the ASD group, suggesting an aberrant dissociation of implicit and explicit emotion recognition in ASD. A likely outcome of abnormal implicit social processing over development, such as impaired reflexive gaze towards important social cues, would first result in generally impaired implicit processing in adulthood. If implicit processes were indeed a necessary precursor to adequate explicit social processing, explicit processing would as well be affected by aberrant implicit processing. In addition, altered development of implicit and explicit processes would ultimately lead to a lack of functionally dissociable implicit and explicit systems. This interpretation is supported by our group specific results, which show that implicit and explicit are more closely matched in ASD, but can be dissociated (or at least measured separately) in NT. As outlined in the general discussion section of this dissertation, inferences about developmental aberrations, however, are tentative and should be further tested in longitudinal designs with different age groups and based upon further operationalization of implicit and explicit emotion recognition processes.

5.1.2. Atypical Gaze and Aberrant Amygdala Activity during Facial Emotion Recognition in ASD: Study II & III

In Study II and III, we assessed the previously postulated influence of active avoidance and reduced orientation on the puzzle of reduced focus on the eye region in ASD, as well as underlying amygdala activity. In sum, the overall reduced eye preference in ASD seemed to be specifically characterized by more eye movements away from the eyes, than towards the eyes as compared to the control groups.

Group specific gaze patterns were accompanied by reversed patterns of increase and decrease in amygdala activity.

The observed eye movements replicate previous studies' findings in healthy controls. Neurotypically developed individuals show an immediate and reflexive orientation towards the eyes of human faces, indicated by increased number of fixation changes towards the eyes, than away from the eyes(Adolphs & Spezio, 2006; Argyle & Cook, 1976; Bindemann, Burton & Langton, 2008; Field, Woodson, Greenberg, & Cohen, 1982; Gamer & Buchel, 2009). The eye preference potentially reflects a developmental expertise in processing information from the eyes in typical social cognition. This aspect of implicit social information processing may thus serve as a crucial precursor to adequately develop facial emotion recognition abilities, which canthen be consciously applied and triggered by explicit prompts. On the neuronal level, the reflexive orientation towards the eyes has been reported along with increase in amygdala BOLD signal in NTs(Gamer & Buchel, 2009; Gamer, et al., 2010). We replicated this relation of gaze and amygdala activity in Study III. We found a relative increase of amygdala activity during gaze orientation towards the eyes in NT. The results thus support the view that the functional profile of the amygdala reflects social saliency mediation while triggering reflexive orientation to the eye region in healthy controls.

Study II and III add important new insights on the influence of avoidance and orientation-guided gaze within the reduced eye preference in ASD. The greater number of eye movements away from the eyes, than towards the eyes, as compared to the NT group speak for a pronounced influence of active avoidance instead of merely reduced social orientation on autistic gaze. Additional support for the active avoidance hypothesis comes from latency effects. The ASD group gazed faster away from the eyes than towards the eyes as compared to the NT group, possibly reflecting the aim to end direct eye contact as soon as possible. Could the observed scan paths simply reflect an interest in the mouth in ASD, as previously suggested(Klin, et al., 2002a, 2002b)? If so, mouth expertise due to increased exposure over development should result in greater performance when confronted with information from the mouth as compared to the eyes. However, we did not find such a beneficial effect of greatermouth fixation in ASD in both studies. In fact, the fewer fixations on the mouth, i.e., the greater the eye preference, the greater the number of correctly identified emotions in the explicit task. These relations between implicit gaze and explicit emotion recognition performance suggest that the mouth seems not to be more informative for ASD(Kirchner, et al., 2011).

On the neuronal level, fMRI data analyses aimed at investigating the role of the amygdala in avoidance and reduced orientation related gaze patterns. A previous study by Dalton and colleagues (2005) found a positive relation of amygdala activity increase and duration of direct eye contact. Together with other studies suggesting a crucial role of the amygdala in general aversion and threat processing, increased amygdala activity in response to faces and eyes in ASDas compared to controls may thus underline the avoidance hypothesis. In light of these findings, the observed increase in

amygdala activity triggered by direct eye contact, along with the current gaze patterns could be interpreted as reflecting an avoidance reaction to eye contact in ASD. In contrast to the avoidance hypotheses, a failure to reflexively orient towards the eyes in patients with amygdala lesions(Adolphs, 2007; Adolphs, et al., 2005; Spezio, Huang, et al., 2007)and the often-reported decreased BOLD response in the amygdala as compared to controls(see, e.g., Hadjikhani, et al., 2007), speak towards the reduced orientation hypothesis. Indeed, the ASD group showed decreased amygdala activity in Study III as compared to control participants when initially fixating the mouth, accompanied by reduced gaze towards the eyes. The observed increase and decrease along with gaze away and towards the eyes, respectively, thus support both the avoidance and the orientation hypotheses.

Is it possible that both processes interact, as suggested by Spezio and colleagues (2007)?Our data indeed imply an interaction of avoidance and orientation as interacting mechanisms behind reduced eye focus in ASD. In fact, wetherefore suggest that both processes coexist and interact very likely at any time, yet to intra- and interindividually varying degrees. For instance, an initial reflexive orientation towards the eyes could be attenuated by avoidance of direct eye contact, leading to fixations that are directed towards the eyes, but do not reach them. Therefore, a clear distinction between both processes cannot be performed based on the number of fixation changes alone. With respect to the heterogeneity in the expression and quality of social impairments in ASD(Abrahams & Geschwind, 2010; Dawson, et al., 2002; Jones & Lord, in press; Klin, et al., 2002a), the degree of avoiding direct eye contact may differ between individuals and potentially over development. Variations in individual symptomatology very likely lead to pronounced avoidance of eye contact *more* than reduced orientation in some individuals or vice versa. In fact, it has been suggested that the eyes trigger increased arousal even in healthy controls(Gale, Spratt, Chapman, & Smallbone, 1975; Nichols & Champne, 1971). However, this intensity of direct eye contact may reinforce the eye's saliency over development, thereby promoting effective orientation and enhancing social cognitive functioning in healthy controls. Here, we propose a framework for the effect of direct eye contact in ASD. Instead of remaining fixation on the eyes to process the presented information about the emotional expression, ASD participants showed more eye movements away from the eyes, independent of the expressed emotion. We suggest that these gaze patterns reflect maladaptive consequences of experienced arousal in response to direct eye contact. Whereas eye fixation seems to have a positive effect on social cognition in typical development, the opposite seems to be the case for ASD underlined by reversed directional group effects in overall eye preference. As outlined above, specific increase and decrease in amygdala activity triggered by the eyes or the mouth, respectively, suggest an interaction of both factors as well. The observed increase in amygdala activity when initially fixating the eyes together with the greater gaze away from the eyes supports the hypothesis of active avoidance of eye contact, modulated via amygdalar avoidance processing. The decrease in amygdala activity when initially fixating the mouth, along with the relatively reduced eye movements

towards the eyes furthermore underline amygdalar dysfunction within social saliency detection and reflexive orientation.

Further, relations of atypical gaze, explicit emotion recognition performance, underlying activity in the amygdala and diagnostic scores in ASD illustrate how the combination of avoidance and orientation tendencies reflect individual social symptomatology within the pathophysiology of ASD, instead of general illness severity.

In sum, results of Study II and III point towards i) an interaction of avoidance and orientation-guided eye movements resulting in a reduced preference for the eye region, ii) along with a specific dysfunctional profile of the amygdala in ASD.Whereas in typical social cognition the amygdala accompanies social salience mediation (i.e., orientation towards eyes), this process seems to be dysfunctional in ASD. Importantly, we do not imply that salience processing and reflexive orientation are completely absent or replaced by avoidance processes. Instead, our data suggest an interaction with maladaptive arousing or even aversive effects of direct eye contact. These insights into amygdalar dysfunctions during implicit facial emotion recognition processes further support the emerging view of the amygdala not as the cause of the autistic pathophysiology but rather as a dysfunctional node within the neuronal network underlying effective social functioning (Paul, et al., 2010).

5.2. General Discussion

5.2.1. A Model for the Relation of Implicit and Explicit Emotion Recognition Impairments in Behavior and Brain Function in ASD

Impaired facial emotion recognition in ASD comprises implicit, e.g., gaze, and explicit, e.g., emotional labeling, deficits. On the behavioral level, increased gaze away from the eyes suggests avoidance processing of direct eye contact, whereas decreased gaze towards the eyes implies reduced orientation processing within implicit social gaze in ASD. The interaction of both processes leads to the previously highlighted reduced focus on the eyes in ASD. On the brain level, increased amygdala activity and gaze away from the eyes triggered by direct eye contact suggest amygdalar avoidance processing, whereas decreased amygdala activity along with reduced gaze towards the eyes underlines reduced social orientation processing. Taken together, atypical gaze and amygdalar response profiles suggest dysfunctional aversion as well as impaired social saliency processing in ASD. The reduced focus on the eye region directly impairs explicit facial emotion recognition, e.g., by deficient information processing from the eyes. Altered BOLD response profiles in the amygdala, a region of the “core system” for facial emotion recognition, in turn affect regions of the “extended/cognitive system”, e.g., the FG(Adolphs, 2002; Haxby, et al., 2002), via aberrant structural and functional connectivity. In consequence, facial emotion recognition is impaired leading to reduced performance

in explicit emotional labeling tasks. In conclusion, impairments in facial emotion recognition in ASD derive from impaired interactions of implicit and explicit socio-cognitive processes ultimately leading to the behavioral phenotype of ASD (see Figure 9).

As outlined above, the findings of the three dissertation studies regarding facial emotion recognition in ASD suggest that greater impairments specifically in implicit social processing, as assessed by gaze and neural activity, result in deficient explicit social processing.

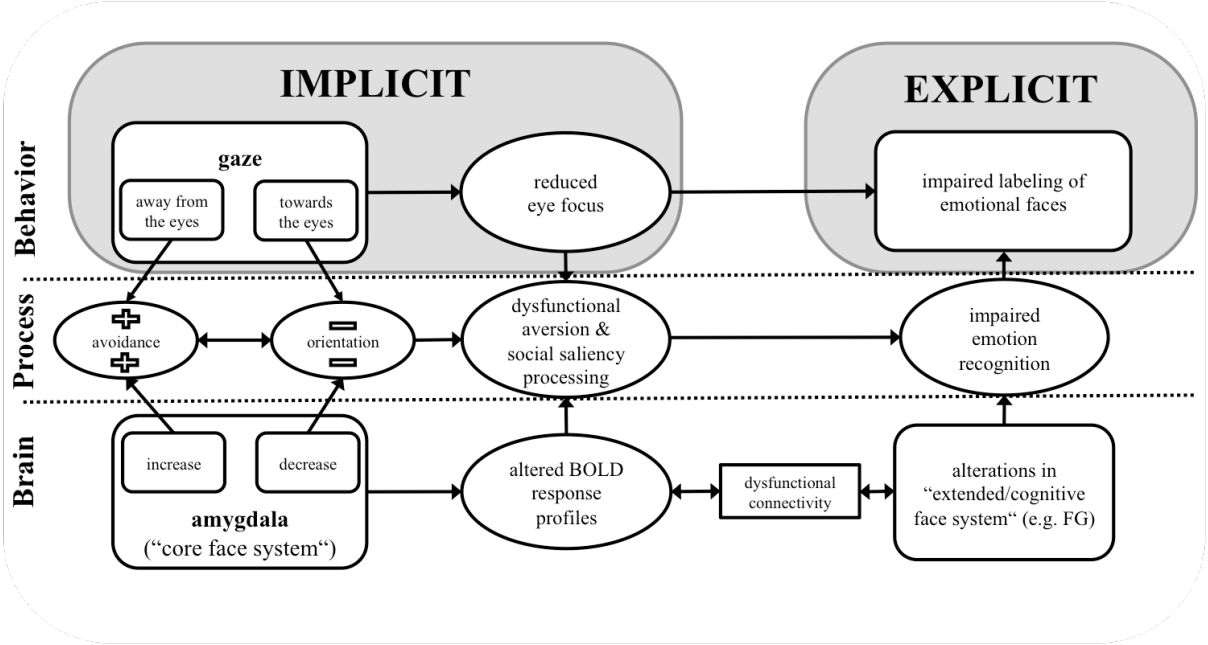

Figure 9. Model for impaired facial emotion recognition in ASD.
Implicit emotion recognition impairments in behavior (e.g., interaction of increased gaze away from the eyes and decreased gaze towards the eyes, leading to reduced eye focus) are accompanied by dysfunctional brain function (e.g., increased and decreased activity in the amygdala, a region of the 'core face system') leading towards dysfunctional aversion and social saliency processing. Aberrant gaze directly affects explicit emotion recognition performance on the behavioral level (e.g., labeling of emotional faces). Altered BOLD response profiles in the amygdala lead to alterations in the 'extended/cognitive face system' (e.g., the fusiform gyurs (FG)) via dysfunctional connectivity on the brain level, thus affecting the neural network underlying emotion recognition, in turn affecting explicit emotion recognition abilities.

5.2.2. Implications for Future Research and Interventions

Based on the findings of the dissertation studies, I suggest several implications for future research and interventions in ASD.

We are aware that our operationalization of implicit facial emotion recognition in Study I differs from the conceptualization of "implicit" in other fields, such as social or developmental

psychology. We operationalized implicit somewhat narrowly, not involving consciously controlled emotion processing. This is one possible approach towards measuring implicit processes and making them comparable to explicit processes. We encourage other and similar attempts to further test the validity, reliability and generalizability of our results.

Regarding further external validation of the new Face Puzzle tasks, potentially confounding processes should be considered, such as other cognitive functions. Motion perception and the ability to holistically process gestalt, for example, have both been shown to be impaired in autism (see, e.g., Gauthier, et al., 2009) and might contribute to the current results in ASD. Those and other potential mediating factors should be tested in future studies with adapted designs and new measures.

We found implicit social gaze, explicit emotion recognition performance and underlying brain activity in the amygdala to be modulated by initial fixation position on faces in ASD. This crucially underlines the need for using eye tracking as a standard measure when experimentally investigating social information processing in ASD and most likely all populations with affective impairments. Previously reported group differences in brain function, could simply be due to different fixation positions on social stimuli, instead of reflecting global processing differences. To exclude potential confounds in interpreting behavioral and neural data and advancing multimodal perspectives on social impairments in ASD, the results of my dissertation studies should encourage other researchers to apply gaze measures in future studies. For example, the observed interaction of avoidance and orientation in atypical gaze in ASD implicates that future studies shall use variations in task design to further specify interindividual differences in gaze characteristics. These variations may include the use of inverted faces, directing initial fixations to the nose and additional parts of faces, or showing only specific facial features instead of the whole face (see, e.g., Spezio, Adolphs, et al., 2007). With regard to further disentangling final targets of eye movements on faces beyond the methodological limitations of the eye-tracking set up of the dissertation studies, subtle latency characteristics should be further investigated with higher temporal resolution of eye movement recordings.

Despite some evidence from correlations indicating that observed gaze patterns in ASD represent social dysfunctioning, rather than global functioning and illness severity, reports of basic visual-processing differences in autism in the literature (Goldberg, et al., 2002; Minshew, Luna, & Sweeney, 1999) imply a need to further investigate current results in relation to general oculomotor functioning and potentially involved brain abnormalities (for cerebellar pathology, see, e.g., Nowinski, Minshew, Luna, Takarae, & Sweeney, 2005).

When assessing brain function, considering underlying anatomical physiology is of great importance to arrive at a consistent and comprehensive picture of the biological basis of cognitive functions. Importantly, the amygdala its not a homogenous entity, but is composed of multiple cytoarchitectonically distinct nuclei (subregions; see, e.g., (Freese & Amaral, 2009)), with distinct

connections to other brain regions (Saygin, Osher, Augustinack, Fischl, & Gabrieli, 2011). These sub-regions seem to have distinct functional roles in healthy participants (Ball, et al., 2007; Gamer, et al., 2010) and in psychiatric disorders (Etkin, Prater, Schatzberg, Menon, & Greicius, 2009). Given that neuroimaging results of Study III suggest multiple kinds of dysfunctions in the amygdala (i.e., avoidance and orientation) in ASD, it should be tested (e.g., using high-resolution fMRI at ultra-high field strengths (7Tesla)) whether disturbed avoidance and orientation processing is sub-served by different amygdalar subregions with potentially specific functional and structural connections.

In the light of the heterogeneity in individual symptom expression, the integration of other types of data, e.g., genetic information, may further promote the empirical specification of subtypes in ASD associated with different autistic phenotypes (Chakrabarti & Baron-Cohen, 2011; Yoshida, et al., 2010). In particular, individual impairments in implicit and explicit aspects of social processing may be related to distinct risk factors (e.g. epigenetic and environmental factors or genetic variants). Additionally, all three dissertation studies were based on adult samples and cross-sectional designs. Implications for the development of different implicit and explicit processes are therefore only of indirect nature. To test possible relations and interactions of genetic, environmental and developmental factors, future multimethodal approaches combining behavioral, neural, and genetic data, as well as longitudinal designs may subsequently advance a comprehensive understanding of the multifactorial nature, its individual expressions and development in ASD.

In more practical terms, interventions, trainings and therapy may benefit from considering the results of this dissertation. In general, the exact identification and quantification of interindividually differing behavioral impairments is a prerequisite for providing individual targets for interventions and thus increasing the likelihood of treatment success. In fact, trainings to date mostly emphasize explicit emotion recognition by training to compare verbal labels with other emotional information, such as emotional faces (see, e.g., FEFA, Bolte, et al., 2006; Golan, Baron-Cohen, Wheelwright, & Hill, 2006). Based on the results of Study I, which implicate a greater impairment in implicit facial emotion recognition, future trainings should focus on those implicit aspects of social cognition in everyday life, e.g., by making them more explicit via detailed descriptions or categorizations. Along the same lines, the results of Study II and III suggest a need to individually quantify the contribution of avoidance and orientation processes in atypical gaze on emotional faces in ASD. To promote implicit social orientation, for instance, intervention strategies should motivate attention toward social salient cues, such as the eyes, at best early in development. To decrease the aversive effect of direct eye contact thus reducing an avoidance reaction, the informative nature of the eye region and the behavioral benefit when focusing the eyes should be emphasized and trained.

Finally, isolated effects of pharmaceutical treatments on specific implicit and explicit aspects could be tested and applied. For instance, it could be possible that the proposed pro-social effect of the

neuropeptide oxytocin may be particularly suited to enhance social orientation or, in contrast decrease aversion of direct eye contact.

6. Conclusion

The results of this dissertation advance the understanding of implicit and explicit facial emotion recognition impairments in ASD, as well as its behavioral and biological basis.

The results suggest that:

- The new Face Puzzle implicit and Face Puzzle explicit tasks measure performance in implicit and explicit aspects of facial emotion recognition comparably, reliably, and externally validly.
- The Face Puzzle tasks are both sensitive to social impairments in ASD and imply an increased deficit in implicit as compared to explicit facial emotion recognition.
- Implicit and explicit aspects of facial emotion processing seem to be closely related in ASD, whereas in typical development they can be behaviorally dissociated.
- Within implicit social processing, the reduced focus on the eyes in ASD is characterized by an interaction of avoidance and orientation related eye movements away and towards the eyes, respectively.
- Increased amygdala activity triggered by the eyes together with increased gaze away from the eyes supports the hypothesis of active avoidance of eye contact, modulated via amygdalar avoidance processing.
- Decreased amygdala activity when starting gaze at the mouth suggests amygdalar dysfunction within social saliency detection and implicit orientation towards the eyes as social cues.
- Multiple dysfunctional amygdalar activation patterns in response to facial emotion underline the proposal that the amygdala is not the cause of the autistic pathophysiology but rather represent a dysfunctional node within the face perception and emotion recognition network leading to impaired implicit and explicit processing.
- Greater impairments in implicit social processing, as represented in atypical gaze and aberrant neural activity in the amygdala, lead to deficient explicit social processing.

7. Literature

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8. Appendices

8.1. Research Articles

Face Puzzle – Two new video-based tasks for measuring explicit and implicit aspects of facial emotion recognition

(Dorit Kliemann*[1, 2], Gabriela Rosenblau[1, 2], Sven Bölte[3], Hauke R. Heekeren[1, 2], Isabel Dziobek*[1])

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Abstract

Accurately recognizing the emotional states of others is crucial for successful social interaction. While most current facial emotion recognition tasks use explicit prompts that trigger consciously controlled processing, emotional faces in real life are almost exclusively processed implicitly. Recent attempts in social cognition suggest a dual process perspective, whereby explicit and implicit processes largely operate independently. However, due to differences in methodology, the direct comparison of implicit and explicit social cognition has remained a challenge.

Here, we sought to develop a new tool to comparably measure implicit and explicit processing aspects. We developed two new video-based tasks with similar answer formats to assess performance in respective facial emotion recognition processes: *Face Puzzle, implicit and explicit*. To assess the tasks' sensitivity to aberrant social cognition and to infer interrelationship patterns between explicit and implicit processes in typical and atypical development, we included typically developed healthy adults (NT, n= 24) as well as adults with autism spectrum disorder (ASD, n = 24).

Item analyses yielded sufficient reliability of the Face Puzzle tasks, and group-specific results indicated sensitivity to the subtle social impairments in ASD. Correlation analyses with implicit as well as explicit socio-cognitive measures were further in favor of the tasks' external validity. Furthermore, the results provide the first hints that implicit and explicit aspects of facial emotion recognition processes can be behaviorally dissociated in typical development. In contrast, reduced performance in the implicit task and intercorrelations between implicit and explicit processes were observed in the ASD group. Our results emphasize the need to separately assess explicit and implicit social cognition in order to tailor interventions aimed at promoting social cognition in developmental disorders.

1. Introduction

To effectively function as social agents, humans must process information in the social environment in order to initiate immediate behavioral responses. One important source of information concerning the internal states of others is provided in emotional facial expressions. Complex social information is not always obviously present, nor are we usually confronted with explicit prompts to interpret the information ('do you think the smile is real?'). Instead, a large fraction of subtle social information must be automatically recognized and integrated, such as the fine differences between a genuine smile ("Duchenne," involving the contraction of both the zygomatic major muscle, which raises the corner of the mouth, *and* the orbicularis oculi muscle, which raises the cheeks) and a fake smile ("non-Duchenne," only contracting the zygomatic major muscle). To successfully read the emotions of others, we therefore need to both implicitly and explicitly process aspects of the social world.

A formal theoretical dissociation of implicit and explicit processes has been proposed in the context of general knowledge within cognition. Within these frameworks, it is usually assumed that implicit knowledge is a precursor to explicit knowledge in development (Dienes and Perner, 1999; Perner and Dienes, 2003), by the re-description of implicit representations to explicit knowledge (Karmiloff-Smith, 1992). These approaches regarding more general cognition have been translated into attempts to dissociate explicit and implicit processes in the field of social cognition. Social cognition (i.e., cognitive mechanisms that underlie social behavior) in its explicit form is usually concerned with conscious and controlled processing and representations and is rather flexible, but it also demands many cognitive resources. In contrast, implicit social cognition comprises more automatic, unconscious reflexive processes that are time efficient but inflexible and limited in terms of cognitive resources (Moors et al., 2010). Reflecting this dissociation, a dual-process model of mental state inferences, i.e. Theory of Mind (ToM), has been put forward, which postulates an earlier developing implicit and a later developing explicit ToM system (Apperly and Butterfill, 2009; Low and Perner, 2012). However, the empirical comparison of individual performance in implicit and explicit processing has been difficult due to methodological incomparability in study and task designs. An additional unresolved question remains whether and to what extent respective postulated distinctions within higher-level social cognitive constructs, such as ToM, can be applied to the processing of more basic types of social stimuli, such as emotional faces.

Standard emotion recognition tasks usually present some type of visual facial stimulus (such as pictures of faces or parts of faces), and participants then have to consciously process and choose between emotional words in order to label the expression in a controlled fashion. Thus, participants have to match a target with a label by explicitly comparing the emotional aspects of the facial expression with linguistic/verbal concepts of a particular emotional expression. One advantage of these tasks is that they provide a direct performance-based measure to depict behavior or related impairments in real life. In everyday interactions, however, emotions must be recognized without the presentation of emotional labels or explicit comparisons with specific emotional concepts.

In contrast, implicit processes are usually assessed indirectly via reaction times or gaze (see, e.g., Greenwald et al., 1998; Senju et al., 2009), and they often address other implicitly processed aspects of facial information, such as race, gender, and attractiveness (see, e.g., Devine et al., 2002; Amodio et al., 2004). However, accuracy scores are of great value when investigating subtle impairments (Zaki and Ochsner, 2011). Given the methodological differences between the formats of implicit and explicit tasks, it remains a challenge to directly compare the underlying processes.

Here, we assessed the implicit and explicit processing of facial emotion recognition directly with comparable performance measures, as our newly developed explicit task triggers emotion recognition from facial expressions explicitly by instructing participants to match videos of emotional faces with verbal labels. The explicit task thus comprises controlled and conscious processing of emotional concepts. In contrast, there were no such explicit prompts to identify a specific facial emotional expression in the implicit Face Puzzle task. In the implicit task, participants must identify emotional cues in parts of the face to correctly compose a complete facial expression from puzzle pieces.

We are aware of the fact that our approach of puzzling faces without explicit prompts for controlled emotion processing reduces the concept of 'implicit' to a very specific and somewhat narrow conceptualization (see, e.g., Moors et al., 2010, for a detailed discussion on conceptualizations of implicit social cognition). Given that we demand the direct consideration of multiple answering options along with an overt response, this approach might not be shared by other researchers from fields with a long tradition of implicit emotion processing, such as developmental or social psychology. However, given the importance of comparing implicit and explicit aspects of emotion processing, we deem this approach to be warranted in order to advance our understanding of facial emotion recognition.

To inform the sensitivity of new social cognition tasks, the study of disorders involving selective socio-affective impairments, such as high-functioning autism spectrum disorder (ASD), can be valuable. ASD is a neurodevelopmental condition that comprises deficits in social communication, social interaction, and repetitive behaviors (Levy et al., 2009). While many studies report deficits in explicit emotion labeling in ASD, non-performance-based measures strongly indicate that ASD involves particularly increased impairments in implicit aspects of social cognition, such as atypical reflexive gaze (see, e.g., Kliemann et al., 2010; Kirchner et al., 2011; Kliemann et al., 2012), reduced imitation and facial mimicry (see, e.g., McIntosh et al., 2006; Senju et al., 2007), and implicit mental state inferences (see, e.g., Senju et al., 2009; Yoshida et al., 2010). Despite these reports of greater impairments in implicit as compared to explicit socio-cognitive functions in ASD, the results remain inconclusive, and direct evidence for this hypothesis via a comparison with performance in explicit processes is lacking. This gap further suggests a need to develop new tests that offer an empirical foundation to attempt a dissociation of implicit and explicit aspects of facial emotion recognition in typical and atypical samples. New performance-based measures that assess implicit processing could also inform the heterogeneity in the social phenotype of ASD and other affective disorders due to individual differences in the interactions of implicit and explicit impairments.

The aim of the current study was to dissociate implicit and explicit aspects of behavioral emotional face processing abilities with two newly developed comparable tasks that measure individual performance: Face Puzzle, implicit and explicit. In order to i) test the new tasks' sensitivity to atypical social cognition and ii) inform possible dissociations between implicit and explicit processes, we included a high-functioning ASD sample and used more naturalistic video stimuli, as opposed to pictures of still faces or face parts. With respect to previous studies' results of greater deficits in implicit as compared to explicit social processing in ASD, we expected the performance of individuals with ASD to differ to a greater extent from controls in the implicit task as compared to the explicit task.

2. Materials and Methods

2.1 Participants

Twenty-four neurotypically developed participants (15 male, mean age = 30.3 years, SD 8.37) with no history of psychiatric or neurological disorders and 24 adults on the autism spectrum (15 male, mean age = 32.5 years, SD: 8.52) participated in the current study. Control participants were recruited by public notices and from project databases of the Freie Universität Berlin, Germany. ASD participants were recruited through the autism in adulthood outpatient clinic of the Charité University Medicine, Berlin, Germany or were referred to us by specialized clinicians. Diagnoses were made according to DSM-IV criteria for Asperger syndrome and autism without mental retardation using two instruments known to be the gold standard for diagnosing autism: the Autism Diagnostic Observation Schedule (ADOS, Lord et al., 2000) and the Autism Diagnostic Interview – Revised (ADI-R, Lord et al., 1994), if parental informants were available (n = 15). For 22 individuals, the diagnosis Asperger syndrome was additionally confirmed with the Asperger Syndrome and High Functioning Autism Diagnostic Interview (ASDI, Gillberg et al., 2001).

In addition to age and gender, groups were matched with respect to their intelligence level, assessed with a German vocabulary test (Mehrfach-Wortschatz-Test (MWT), Tewes, 1991), and a strategic thinking test (LPS, subscale 4, Horn, 1962). To control for clinically significant levels of autistic traits in healthy populations, we applied the Autism Spectrum Quotient (AQ, Baron-Cohen et al., 2001b) in both groups. All participants had normal or corrected-to-normal vision and were native German speakers. Participants gave written informed consent prior to participation and received payment for their time. The study was approved by the ethics committee of the German Society for Psychology (DGPs).

2.2 Stimulus production and validation

Stimulus production was embedded in the context of a comprehensive project to produce a new set of more ecologically valid video stimulus material, comprising a total set of 40 different emotional states that were depicted in facial expressions by more than 50 professional actors of varying age (18-65 years) at the film studio of the Humboldt University, Berlin, Germany, in cooperation with its Computer and Media Service (CMS). Emotion selection was based on a previous study that characterized emotional words, not only based on valence and arousal dimensions but also regarding their frequency and relevance in everyday life (communicative frequency, see, Hepach et al., 2011).

The validity of the stimuli was tested in a separate expert validation study (psychologists working in the field of social cognition, 4 male, mean age = 29.6 years, SD = 4.3) on a representative, random selection of 100 videos (20 actors, 10 male, 5 emotions, 2 positive). The results showed high average emotion recognition rates (mean = 92.6%, SD = .07) and good believability (4.4, SD = .07; 6-point Likert scale (1 = not believable to 6 = very believable)) of the items, ensuring that the stimuli indeed depicted respective emotional expressions.

2.3 Tasks

2.3.1 Face Puzzle – implicit and explicit tasks

Face Puzzle consists of two independently applicable tasks for the assessment of implicit and explicit emotion recognition abilities from faces using more naturalistic video-based stimulus material comprising basic as well as complex emotions. After stimulus production, task development and online implementation, we conducted a separate validation study using an additional sample of healthy individuals with an initial composition of the Face Puzzle implicit and explicit tasks (N = 29, mean age = 27.21, SD = 8.5, 13 male). According to the results, items were revised if necessary.

Both tasks' target items consisted of short clips (mean length 10.3 seconds) with 14 professional actors (7 male, varying age (20-50 years)) portraying 25 different emotional facial expressions (13 negative, 12 positive; see Fig. A and supplementary video material (S1 and S2) showing example trials for the Face Puzzle implicit and explicit tasks). Five basic (angry, happy, disgusted, fearful, surprised) and 20 complex emotions (e.g., worried, forgiving, doubtful, interested, compassionate) were covered. As described above, emotions were preselected from a previous study that assessed valence, arousal and communicative relevance in order to include emotions particularly relevant for everyday human social interaction (Hepach et al., 2011).

In the implicit task, face videos were divided into an upper (including the eye region; eye video) and a lower part (including the nose and the mouth region; mouth video). The target item represented an eye video displayed in the upper center of the screen. Four mouth videos of the same actor were displayed below the target item. The target eye video started playing automatically in loops, i.e., the video played repeatedly, until the participant completed the item by dropping a mouth video into the target field. Mouth videos were displayed as still images until participants directed the computer mouse on a video, thereby enlarging and starting to play the video. Participants then had to match the eye video with the respective mouth video according to the emotion presented in the facial mimic and place it underneath the eye video through a drag and drop function (see, Fig. A, left, S1). No further information about the presented emotional states was given, so that participants had to identify the depicted emotion in the face parts without explicitly being asked to identify or label a specific emotion. Actors were instructed to minimize head movement to prevent participants from matching parts solely due to general motion.

In the explicit task, the full target video (including both, eyes and mouth) was presented in the upper center of the screen. Again, participants could enlarge the video for a more detailed inspection when directing the computer mouse above the item. As in the implicit task, the target video played automatically in loops, with the video automatically restarting until the item was completed. In the explicit task, participants were asked to choose the correct label for the presented emotion out of four emotion labels. The item was completed when the chosen label was placed into a target field below the target video through a drag and drop motion with the computer mouse. Distractor labels consisted of i) two emotions of the same valence, one with similar valence and arousal levels and one that differed more in arousal level but had the same valence as the target item, and ii) one emotion of the opposite valence (see, Fig. A, right, S2; for an example).

There was no time limit to respond to either task. Participants were asked to perform as fast and as accurately as possible. Both tasks were independent web-based applications that were accessible through a password-protected website. Completing each task took approximately 15 to 20 minutes. Tasks were designed and programmed in cooperation with a digital agency (gosub communications GmbH, www.gosub.de). The tasks could be accessed on a public webserver through any browser with a Flash Player Plugin installed. Both the implicit and explicit tasks began with a few introduction slides. Intuitive mouse interactions were used to avoid user distraction; throughout the entire application, participants navigated through introduction screens and solved the individual items only using the mouse.

2.4 Procedure

The participants completed both tasks online through the project's website in testing rooms of the Freie Universität Berlin, Germany under the supervision of trained experimenters. Task order was counterbalanced across participants to control for possible order effects.

Performance measures were comprised of accuracy (percentage of correct answers) and reaction times in choosing the correct lower face part in the implicit task and the correct label in the explicit task, respectively. The implicit task additionally counted how often mouth videos were played and enlarged ('playsum'), thereby also providing a measure for conscientious task execution.

To i) investigate the new tasks' constructive validity and ii) further differentiate between implicit and explicit emotion recognition processes, we additionally administered a standard explicit measure of emotion and mental state recognition from faces: the 'Reading the Mind in the Eyes Test' (RMET, Baron-Cohen et al., 2001a). The RMET is a performance-based measure that requires participants to label emotional and mental states based on photographs of eye regions. To avoid possible ceiling effects in the control group, we additionally computed the subscale *'difficult,'* introduced by Domes et al. (2007), in addition to the RMET total score. The RMET aims to infer and explicitly label affective states, similar to the explicit Face Puzzle task. For further external validation of the Face Puzzle tasks and the potential dissociation of implicit versus explicit socio-cognitive functioning, we additionally applied the 'externally-oriented thinking' (EOT) subscale of the Toronto Alexithymia Scale (German Version, TAS-26, Kupfer et al., 2001). The EOT-TAS scale measures the tendency to focus attention internally as opposed to externally, thus representing a measure of an implicit thinking style.

2.5 Statistical Analyses

Performance scores were first analyzed with repeated measures analysis of variances (ANOVA) to investigate potential main and interaction effects. Respective factors are specified in the results section. Post-hoc t-tests included independent samples t-tests between groups and paired-samples t-tests within groups. Correlations between two measures were calculated based on Pearson's r correlation coefficients (2-tailed), whereas differences between correlations were calculated according to Fisher's r to z transformation (2-tailed). All statistical tests used a significance threshold of $p < .05$, if not specified otherwise.

3. Results

3.1 Sample information

Groups were matched with respect to age (NT = 30.29, ASD = 30.44, $t_{(46)} = -.92$, $p > .36$), gender (NT and ASD group: 15 males, 9 females), and intelligence levels (vocabulary IQ test: NT = 106.21, ASD = 108.04, $t_{(46)} = -.53$, $p > .59$; strategic IQ test : NT = 119.58, ASD = 120.54, $t_{(46)} = -.33$, $p > .73$). We additionally applied the Autism Spectrum Quotient (AQ, Baron-Cohen et al., 2001b) in both groups to control for clinically relevant levels of autistic traits in the NT group. As expected, the groups differed significantly in AQ scores (NT = 14.38, ASD = 37.37; $t_{(46)} = 12.16$, $p < .001$). None of the controls scored above the cut-off score of 32; in fact, the highest score was 24, indicating a very low level of autistic traits in the NT group.

3.2 Reliability analyses

3.2.1 Item analyses

We assessed the tasks' internal consistency by calculating Cronbach's alpha. Both tasks demonstrated satisfactory reliability (N = 48, implicit task: Cronbach's alpha = .81, mean item difficulty = .69, range = .25 to .94; explicit task: Cronbach's alpha = .81, mean item difficulty = .74, range = .54 to .94).

The implicit task additionally offered the opportunity to control whether the participants performed the task in accordance with the instruction. We measured how often participants chose to play each of the four mouth videos for > 1 second, thereby sufficiently inspecting presented emotional information ('playsum'). Among all the participants, the mean playsum was >4 (mean= 4.7, SD = 1.1), indicating that participants sufficiently inspected the emotional information provided in the video parts. Over all 25 trials, participants in the ASD group showed a trend towards a greater number of average playsum per trial (NT = 4.38, ASD = 4.97, $t_{(46)} = -1.9$, $p = .06$). In other words, the ASD group played the mouth videos more often than the NT group.

3.3 Tasks' Sensitivity to Atypical Emotion Recognition

To test the new tasks' sensitivity to atypical emotion recognition we analyzed performance measures between and within groups.

3.3.1 Accuracy

Among all participants, the accuracy scores were considerably above chance level (i.e., 25%) and greater in the explicit than the implicit task (implicit = 69.08, explicit = 74.17). A 2x2 repeated measures ANOVA with the within-subjects factor TASK [implicit versus explicit] and the between-subjects factor GROUP [NT versus ASD] yielded a significant main effect of TASK ($F_{(1,46)} = 5.08$, $p = .029$, $\eta^2 = .1$). There was no interaction between TASK and GROUP on the mean accuracy scores ($p > .5$). Despite the absence of this interaction, we nevertheless collected group-specific data to further inform atypical and typical emotion recognition in the implicit and explicit tasks. Across all items, the groups' accuracies differed significantly for each task (implicit: NT = 78.67, ASD = 59.5, $t_{(46)} = 4.13$, $p < .001$; explicit: NT = 85.17, ASD = 63.17, $t_{(46)} = 5.26$, $p < .001$) (see, Fig. B). Within the NT group, accuracies between tasks differed significantly with increased performance in the explicit task (implicit = 78.67, explicit = 85.17, $t_{(23)} = -2.61$, $p < .016$), while there was no such effect in the ASD group (implicit = 59.5, explicit = 63.0, $t_{(23)} = -.92$, $p = .37$).

3.3.2 Reaction times

Mean reaction times for correct responses were calculated for each participant in both tasks and are referred to as RTs. Trials with incorrect responses were excluded from further analyses. We expected the RTs to differ between the implicit and explicit tasks, given that the tasks differed systematically in their answering format. In the implicit task, RTs represent the time between the start of the trial and the drop of a mouth video into the target field, including the time to inspect the target eye video and the mouth video options. In contrast, in the explicit task, participants were only presented with one face video and four emotion words. As mentioned previously, there were no time limits to respond.

As expected, the RTs differed systematically between tasks, and all participants were faster in responding correctly for the explicit as compared to the implicit task (NT: implicit = 32.12s, explicit = 10.92s, $t_{(23)} = 11.38$, $p < .001$; ASD: implicit = 44.77s, explicit = 16.27s, , $t_{(23)} = 7.46$, $p < .001$). A 2x2 ANOVA with the within-subjects factor TASK [implicit versus explicit] and the between-subjects factor group [NT versus ASD] additionally replicated the significant main effect of TASK ($F_{(1,46)} = 137.53$, $p < .001$, $\eta^2 = .75$) and also demonstrated a trend towards an interaction of both factors ($F_{(1,46)} = 2.69$, $p = .1$, $\eta^2 = .06$). Post-hoc independent samples t-tests showed that the NT group was generally faster in responding than the ASD group in both the implicit as well as the explicit tasks (implicit: NT = 32.13s, ASD = 44.77s, $t_{(46)} = -2.48$, $p = .019$; explicit: NT = 10.92s, ASD: 16.27s, $t_{(46)} = -3.24$, $p = .002$).

3.3.3 Composite measure of reaction times and accuracy

To account for the absence of a time limit to complete trials in both tasks, we further analyzed an individually calculated composite measure of reaction times and accuracy, thus accounting for compensatory strategies, i.e. speed-accuracy trade-off. The composite measure represents the division of the individual reaction times according to the respective accuracy scores. Overall, all participants yielded lower values in the implicit as compared to the explicit task (see, Fig. C). A 2x2 repeated-measures ANOVA (TASK [implicit versus explicit] * GROUP [NT versus ASD]) showed a main effect of TASK over both groups ($F_{(1,46)} = 65.17$, $p < .001$, $\eta^2 = .59$). The composite measure was additionally mediated by a significant interaction with the factor GROUP ($F_{(1,46)} = 5.5$, $p = .023$, $\eta^2 = .11$). As outlined in Figure C, the magnitude of the group difference was greater for the implicit than the explicit task. For both tasks, the NT group showed significantly lower values than the ASD group (implicit: NT = .41, ASD: .81, $t_{(46)} = -3.5$, $p = .001$; explicit: NT = .13, ASD = .29, $t_{(46)} = -3.9$, $p < .001$), indicating increased performance in general.

3.4 Relationship between implicit and explicit performance and diagnostic scores in ASD

The ASD participants' accuracy scores were significantly correlated with autism symptomatology, as measured by the ADOS ($n = 21$, implicit: $r = -.55$, $p = .009$; explicit: $r = -.45$, $p = .04$) and the ASDI ($n = 19$, implicit: $r = -.56$, $p = .02$; explicit: $r = -.58$, $p = .009$). The more severely affected individuals scored lower on both tasks, indicating a link between symptom severity and task performance and revealing the sensitivity of the new tasks to atypical emotion recognition performance.

3.5 Dissociating implicit and explicit processes

We first conducted correlation analyses on the composite measure, accounting for both accuracy and RTs within and between groups to assess potentially differential relations and to further investigate dissociations between implicit and explicit emotion recognition processes.

In the NT group, the composite scores for the implicit and explicit tasks were not correlated ($p > .25$). In contrast, performance correlated significantly between tasks in the ASD group (composite measure: $r = .51$, $p = .012$). Correlations between groups differed only by marginal significance for the composite measure ($z = -1.73$, $p = .08$), indicating a different relationship between implicit and explicit emotion recognition performance in the NT as compared to the ASD group.

3.5.1 Correlations with external measures

To assess whether accuracy in the implicit and explicit tasks differed in relation to external implicit and explicit socio-cognitive measures, we performed further correlation analyses with the externally orienting thinking scale of the TAS and the RMET, respectively. The EOT-TAS scale measures the extent to which individuals orient their thinking internally without external prompts, thus providing a measure for the individual magnitude of implicit processing when processing emotions. In contrast, the RMET asks participants to label emotional stimuli with provided labels, representing a classic explicit labeling task in emotion and mental state recognition.

Analyses for the RMET scores were performed separately for each group, given that the groups differed significantly. Because the groups did not differ in the EOT-TAS subscale, we performed the respective correlations across all participants (see Table 1).

Table1. Group means, SD, Range and group differences in accuracy and mean scores for the external socio-cognitive measures. Abbreviations: Reading the Mind in the Eyes Test

(RMET), Externally-Oriented Thinking Style scale (EOT), Toronto-Alexithymia Sclae (TAS), Mean (M), Standard Deviation (SD). p-values: two-tailed significance-value for independent samples t-tests between ASD and NT participants; *: <.05). $^{+}$: sample sizes differed for each group: RMET^{+}: NT: 24, ASD: 19.

	RMET^{+}	**RMET^{+} diff**	**EOT (TAS)**
	Accuracy		**Mean Score**
NT			
M	73.46	69.06	13.58
SD	7.68	10.5	3.2
Range	58-83	44-83	8-19
ASD			
M	63.03	56.9	15.38
SD	14.67	13.97	4.44
Range	26-83	33-78	8-24
pValue	**.004***	**.002***	.12

3.5.1.1 Explicit processes

The NT group's accuracy in the explicit Face Puzzle task correlated significantly with performance on the explicit external measure *difficult RMET* items (Pearson's r = .44, p = .033), whereas there were no significant correlations between accuracy in the implicit task and the RMET scores (all p > .15). The correlation coefficients for the implicit and explicit accuracy with the RMET scores did not differ significantly (p > .2).

In the ASD group, RMET scores correlated with accuracy in the explicit as well as the implicit task significantly or on trend level (RMET: implicit: r = .38, p = .11, explicit: r = .55, p = .015; *difficult RMET* items: implicit: r = .43, p = .068, explicit: r = .51, p = .024).

3.5.1.2 Implicit processes

Across all participants, the EOT-TAS scores' correlation with accuracy in the implicit task barely missed significance (r = -.28, p = .051). In contrast, the EOT-TAS scores did not correlate significantly with accuracy in the explicit task (p > .1). These correlations did not differ significantly between the implicit and explicit scores (z = -.024, p > .8).

Table 2. Correlational analyses between Face Puzzle Task and external socio-cognitive measures. Abbreviations: accuracy scores in the Face Puzzle implicit (FP-I) and explicit task (FP-E); Externally-Oriented Thinking Style scale (EOT); Toronto-Alexithymia Sclae (TAS); r-values: Pearson's correlation coefficient; p-values: two-tailed significance-value for bivariate correlation, $^{(*)}$:<.1, *: <.05.; $^{+}$: sample sizes differed for each group: RMET^{+}: NT: 24, ASD: 19.

	EOT (TAS)	**RMITE diff^{+}**	
	NT & ASD	*NT*	*ASD*
FP-I	r = -.28 p = .051	r = .14 p > .51	r = .43 p = .068$^{(*)}$
FP-E	r = -.23 p = .11	r = .44 p = .033*	r = .51 p = .024*

4. Discussion

The current study aimed to assess implicit and explicit aspects of facial emotion recognition with two tasks that generate performance-based measurements, thus providing an opportunity to empirically attempt a dissociation of respective processes based on individual accuracy scores. To this end, we applied the Face Puzzle implicit and Face Puzzle explicit tasks to a healthy sample as well as to age-, gender- and intelligence-matched adults with autism spectrum disorder. We tested i) the tasks' reliability and validity, ii) sensitivity to atypical social cognition and iii) dissociations of explicit and implicit processes via respective relations in typical and atypical development. In sum, our results identified the Face Puzzle tasks as reliable (both: alpha > .8), externally valid, and sensitive to atypical social cognition. Our results furthermore suggest that implicit and explicit aspects of facial emotion recognition processes can be behaviorally dissociated in typically developed individuals, while they seem to be more closely associated in ASD.

4.1 An approach towards measuring implicit and explicit aspects of facial emotion recognition

The development of human cognition, including social cognition, is comprised of the specification of basic spontaneous reactions into explicit representations and concepts, which can be externally triggered and applied within respective contexts. Given the importance of identifying implicit and explicit processing aspects and associated performance within social cognition (Zaki and Ochsner, 2009; 2011), a respective theoretical dissociation model must be empirically tested and validated to inform particular impairments in psychiatric conditions or atypical development. With the two new tasks introduced in the current study, we pursued one possible approach towards a performance-based comparison of implicit and explicit emotion recognition processes.

To further investigate the external validity of the new tasks, we correlated performance with established socio-cognitive measures. The performance of typically developed individuals in the explicit task correlated with accuracy in the RMET task, which also provides explicit prompts to identify facial emotions, while there was no correlation with implicit task performance in healthy controls. Performance in the implicit task correlated marginally significantly with the tendency to orient thinking strategies to internal as opposed to external cues, as measured by the EOT-TAS subscale, but no such correlation was found with performance in the explicit task in all participants. Despite the lack of significant differences in respective correlations with implicit/explicit measures, these results support the tasks' external validity.

4.2 Relation of implicit and explicit processes in typical and atypical development

These two new tasks allowed us to compare performance in implicit and explicit emotion processing intra- and inter-individually. The particular task relations were informative with respect to the question of whether the proposed dual-process models in other cognitive domains or social cognitive abilities (e.g., ToM, Apperly and Butterfill, 2009; Low and Perner, 2012)) can be applied to emotion recognition from faces. The current results suggest a dissociation of implicit and explicit aspects of emotion recognition in typical development and a lack thereof in atypical development, as the NT group's performance in the explicit Face Puzzle task differed significantly from that in the implicit Face Puzzle task. Furthermore, performance in both tasks was not intercorrelated, suggesting that the respective processes can be independently assessed to some extent, in turn indicating the possibility of autonomous operation. In contrast, the ASD group's performance did not differ between tasks and was correlated significantly. Because the new tasks did not restrict participants in their

decision time, investigating performance with a composite measure and combining both accuracy and reaction times provided a more comprehensive approach (Sucksmith et al., in press). Correlations between the composite scores differed between the groups on a trend level, again suggesting a differential relationship between implicit and explicit aspects of emotion recognition in typical and atypical development.

For general and social cognition, implicit processing has been suggested as a developmental precursor to explicit processing (Karmiloff-Smith, 1992; Dienes and Perner, 1999). Our data suggest that this notion also applies to facial emotion recognition abilities, as the correlation of both implicit and explicit task performance with the explicit RMET scores in the patient group suggested an aberrant dissociation of implicit and explicit emotion recognition in ASD. Abnormal implicit processing of social stimuli during development would likely result in i) impaired explicit processing and ii) the lack of functionally dissociable implicit and explicit systems, as suggested by the current study's group-specific results. Given the nature of the study's cross-sectional design in adults and our particular conceptualization of implicit processing, however, this interpretation is tentative and should be further tested in longitudinal designs with different age groups and based upon further operationalization of implicit and explicit emotion recognition processes.

4.3 Implicit processing impairments in ASD

We found that groups differed to a greater magnitude in the implicit as compared to the explicit Face Puzzle task in the composite measure, indicating a more pronounced impairment in implicit than explicit processing in ASD. This is in line with previous findings showing severe impairments in implicit processing of social information in the absence of explicit cues in high-functioning ASD (Senju, 2012). In addition, Volkmar and colleagues (see, e.g., Volkmar et al., 2004) have also proposed that social impairments in ASD are specifically evident in unstructured settings.

4.4 Implications for future research and interventions

Previous studies have mostly assessed aspects of implicit emotion processing indirectly, using such techniques as backward-masking (see, e.g., Pessoa, 2005) or age or gender differentiation tasks (see, e.g., Habel et al., 2007). The indirect assessment of implicit emotion processing does not, however, allow for a direct comparison to explicit processes. Here, we attempted to measure implicit and explicit aspects of emotion recognition processes based on performance in comparable and easily applicable tasks. In contrast to conceptualizations in other fields, such as social or developmental psychology, in our study, 'implicit' was operationalized more narrowly and did not involve consciously controlled emotion processing. We consider this as one possible approach towards measuring implicit processes to make them comparable to explicit processes.

Quantifying the accuracy of implicit processing can help provide individual targets for interventions that aim to improve socio-cognitive functioning (Zaki et al., 2010; Zaki and Ochsner, 2011). Interventions to date have mostly emphasized explicit emotion recognition, such as by training individuals to assign verbal labels to presented emotional information (see, e.g., FEFA, Bolte et al., 2006; Golan et al., 2006). Using the example of ASD, the identification of greater impairments in processing implicit aspects within social cognitive functioning in general and facial emotion recognition in particular underlines the need to place a focus on respective processes in interventions. Future trainings could, for instance, focus on those implicit aspects of social cognition in everyday life by making them more explicit via detailed descriptions or categorizations to promote compensatory effects to support effective social functioning.

4.5 Limitations

Here, we proposed one possible approach to operationalize implicit processing by asking participants to complete a puzzle of facial expressions. However, future research is needed to extend and further test this conceptualization and to explore other possible ways to allow implicit and explicit processing to be compared.

It is important to note that the external validation of new tasks is dependent on correlations with established measures that assess the psychological construct of interest as well as possibly confounding processes. While we have included tasks in our study design that reflect implicit and explicit emotion processing strategies, we have not considered other cognitive functions that might contribute to performance in our implicit Face Puzzle task. For example, the perception of motion and the ability to holistically process gestalt, both of which have been shown to be implicated in autism (see, e.g., Gauthier et al., 2009), may be present and could have contributed to the observed effects. Those and other potential confounds should be explored in future studies.

As outlined above, implications for the development of implicit and explicit processes in atypical social cognition, with the example of ASD, are only of an indirect nature given the adult samples and the cross-sectional design of the current study. We encourage future studies to directly test the proposed aberrant developmental trajectory.

4.6 Conclusion

In sum, the current study introduces Face Puzzle, which consists of two new computer-based tasks for measuring implicit and explicit aspects of facial emotion recognition performance. Whereas group-specific results suggest a behavioral dissociation of the implicit and explicit processes as measured by task performance in healthy controls, insights from ASD may suggest an aberrant developmental specification of those implicit and explicit aspects of facial emotion recognition processes, along with a particular emphasis on implicit processing deficits in ASD.

7. Acknowledgements

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8. Conflict of interest.

The authors declare no conflict of interest.

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10. Figures

Figure A. *Left: Implicit Task.* Participants have to find the according mouth-video to the target eye-video. *Right: Explicit Task*. Participants have to explicitly label the target emotional expression.

Figure B. *Accuracy scores (percentage of correct responses) by TASK (implicit vs explicit) and GROUP (ASD vs NT).* Whereas there was no significant difference between implicit and explicit task accuracy in the ASD group, typically-developed participants' accuracy was significantly increased in the explicit task. The NT group showed increased accuracy in both tasks as compared to the ASD group.

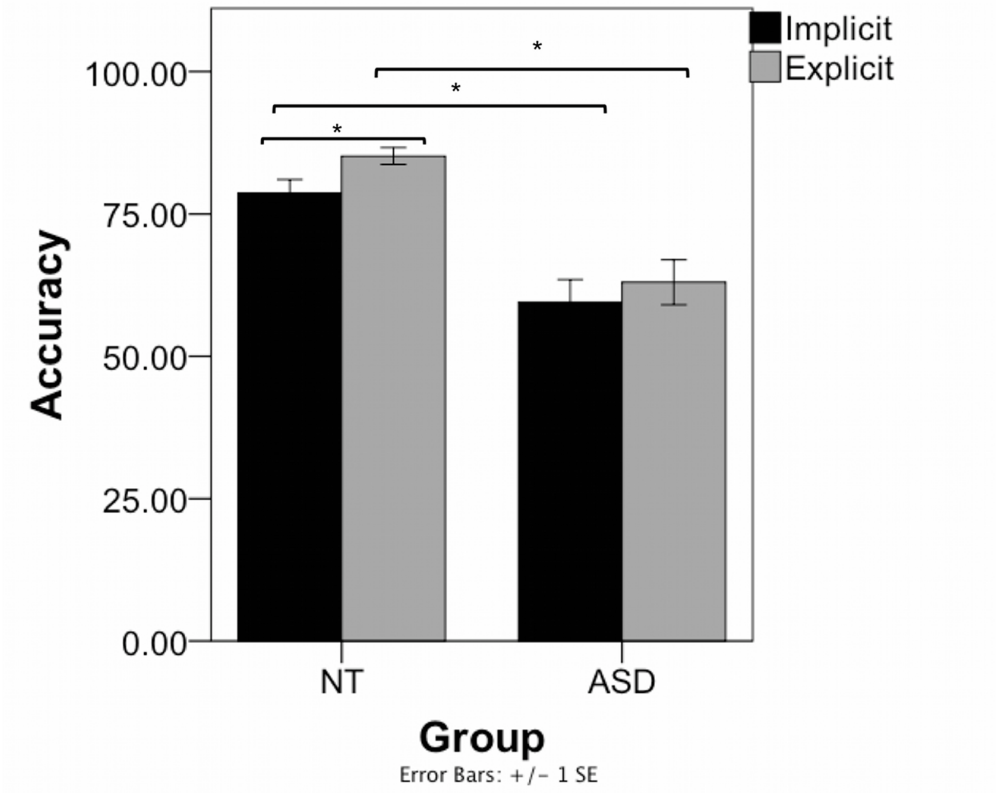
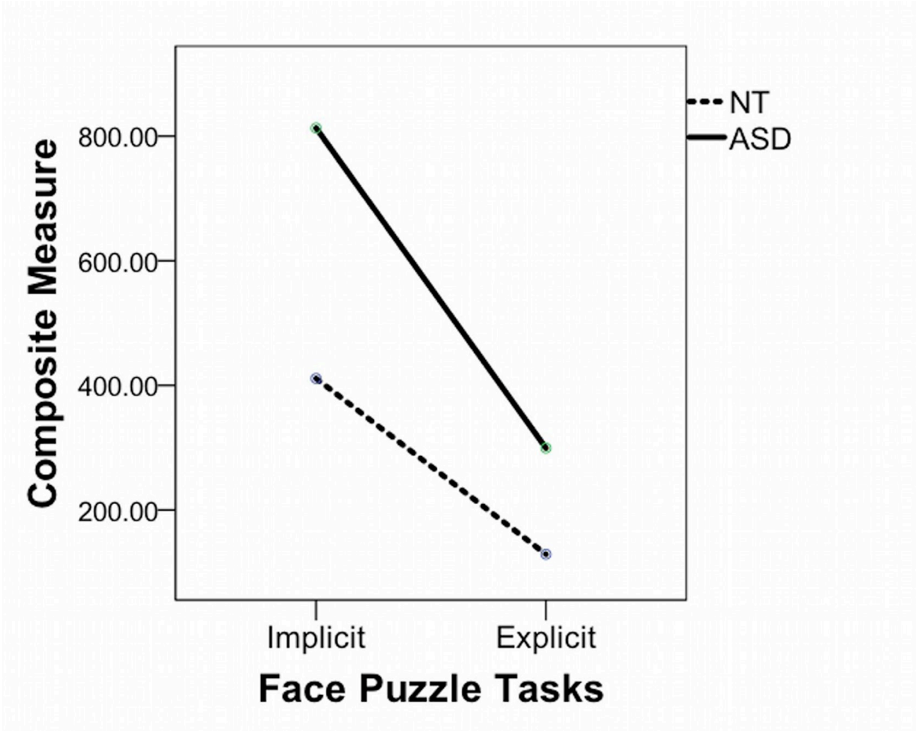


Figure C. *Significant TASK (implicit vs Explicit) by GROUP (ASD vs NT) interaction of the composite measure (RT (milliseconds) / accuracy).* The group difference was greater for the implicit as compared to the explicit task, indicating increased implicit processing impairments in ASD.



Behavioral/Systems/Cognitive

Atypical Reflexive Gaze Patterns on Emotional Faces in Autism Spectrum Disorders

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Atypical scan paths on emotional faces and reduced eye contact represent a prominent feature of autism symptomatology, yet the reason for these abnormalities remains a puzzle. Do individuals with autism spectrum disorders (ASDs) fail to orient toward the eyes or do they actively avoid direct eye contact? Here, we used a new task to investigate reflexive eye movements on fearful, happy, and neutral faces. Participants (ASDs: 12; controls: 11) initially fixated either on the eyes or on the mouth. By analyzing the frequency of participants' eye movements away from the eyes and toward the eyes, respectively, we explored both avoidance and orientation reactions. The ASD group showed a reduced preference for the eyes relative to the control group, primarily characterized by more frequent eye movements away from the eyes. Eye-tracking data revealed a pronounced influence of active avoidance of direct eye contact on atypical gaze in ASDs. The combination of avoidance and reduced orientation into an individual index predicted emotional recognition performance. Crucially, this result provides evidence for a direct link between individual gaze patterns and associated social symptomatology. These findings thereby give important insights into the social pathology of ASD, with implications for future research and interventions.

Introduction

Recent reports from the social-cognitive neurosciences have emphasized the importance of the eyes in carrying crucial information about emotional states of others. In autism spectrum disorders (ASDs), atypical eye contact is not only a diagnostic criterion (American Psychiatric Association, 1994), but also one of the earliest pathological signs, even for high-functioning individuals. Abnormalities in processing of information from the eyes (Leekam et al., 1998; Baron-Cohen et al., 2001a; Spezio et al., 2007a) are characterized by specific scan paths on emotional faces: individuals with ASD spend less time on faces in general (Pelphrey et al., 2002) and in particular focus less on the eye region (Klin et al., 2002). Despite some recent studies on gaze behavior in ASD, it still remains unclear why individuals with ASD show these abnormalities in face processing. A long-standing view suggests a general lack of social attention and specifically less attention toward the eyes as the main reason for the observed scan path on emotional faces in ASD. Some studies even found that individuals with ASD tend to focus more on the mouth region (Klin et al., 2002; Pelphrey et al., 2002). These findings may be taken as support for the missing orientation toward the eyes (Schultz, 2005). Thus, a failure to detect social saliency leads to a reduced orientation toward important sources of social information such as the eyes (Schultz, 2005; Neumann et al., 2006). Another emerging view, however, highlights the potential aversiveness of direct eye contact in ASD, resulting in an active avoidance reaction (Dalton et al., 2005; Kylliainen and Hietanen, 2006). Support for an aversion explanation of atypical gaze comes from studies showing increased skin conductance responses in children with autism when confronted with direct eye contact compared with averted gaze (for recent reviews on gaze and autism, see Nation and Penny, 2008) (Senju and Johnson, 2009). Additionally, another recent study (Joseph et al., 2008) showed that face-encoding skills of children and adolescents with ASD were mediated by emotional arousal in response to direct gaze.

Notably, those two processes do not have to be mutually exclusive, and the interplay of the two components may in fact account for the observed scan paths (Spezio et al., 2007b). Findings to date provide some evidence for each of these explanations; no study so far, however, addressed both processes specifically within the same research design. Here, we sought to further investigate the influence of (1) reduced social orientation to and (2) the active avoidance of the eyes on atypical gaze in ASD by analyzing participants' eye movements while they performed a new behavioral facial emotion classification task (Gamer and Büchel, 2009). We varied the initial fixation position on the face; thus, participants started processing a face either at the eyes or at the mouth and decided whether the face showed a happy, fearful, or neutral expression. Thereby, the task allows investigating both avoidance- and orientation-guided reflexive gaze behaviors, triggered by focusing the eyes or the mouth, respectively.

Materials and Methods

Nineteen neurotypical controls (NTs) (14 male; age range, 21–42 years; mean age, 30.37 years) with no known psychiatric or neurological disor-

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der and 17 participants with ASD (12 male; age range, 22–48 years; mean age, 32.71 years) participated in the current study. Participants in the NT group were recruited through databases of the Max Planck Institute for Human Development (Berlin, Germany). Participants with ASD were recruited through psychiatrists who specialized in autism in the Berlin area and the outpatient clinic for autism in adulthood of the Charité University Medicine, Berlin, Germany. Diagnoses were made according to *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition, criteria for Asperger syndrome and autism without mental retardation (American Psychiatric Association, 1994) using a videotaped semistructured interview, which included the assessment of childhood information. In 14 participants with available parental information, diagnoses were additionally confirmed with the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994; Bölte and Poustka, 2001) and the Asperger Syndrome (and High-Functioning Autism) Diagnostical Interview (ASDI) (Gillberg et al., 2001). Restricting the eye movement data analysis to only the subsample of ASD participants with ADI-R data revealed the same pattern of results compared with the complete sample though very minor differences in p values for some analyses (most likely because of the reduced sample size). Groups were matched with respect to gender, age, and verbal intelligence quotient (IQ) (see Results). All participants had normal or corrected-to-normal vision, were native German speakers, received payment, and gave written informed consent in accordance with the requirements of the ethics committee of the Max Planck Institute for Human Development.

Visual stimuli were shown using Presentation (Version 12.4., Neurobehavioral Systems), and gaze behavior was measured via an integrated T120 60 HZ eye tracker (Tobii Systems). The eye-tracking device was built into the screen and did not require fixing participants' heads. The device tracks both eyes separately using corneal reflection.

One hundred twenty faces from a standardized dataset (Lundqvist et al., 1998) were chosen for the emotion classification task, based on a recent validation study (Goeleven et al., 2008). Twenty women and 20 men each displayed happy, fearful, and neutral expressions in a randomized order. Each image was rotated to ensure that, when displayed, the eyes were at the same vertical height. Additionally, an elliptic mask was applied resulting in images containing just the face (Fig. 1). All images were converted to grayscale, and the cumulative brightness was normalized across images.

In each trial, a fixation cross was presented initially (2 s), followed by the presentation of a face (150 ms) (Fig. 1). After a blank gray screen (2 s), participants were asked to indicate the emotional expression via button press on a standard keyboard. The emotional classification experiment followed a 2 × 3 within-subjects design with the factors "initial fixation" (eyes, mouth) and "emotion" (happy, fearful, neutral). To investigate the effect of initial fixation on gaze behavior, half of the faces within each emotion category were shifted either downward (Fig. 1, trial A) or upward (Fig. 1, trial B), so that the eyes or the mouth appeared at the location of the formerly presented fixation cross. Thereby, the task allowed the investigation of reflexive gaze behavior as a response to direct eye contact as well as eye movements, when initially fixating the mouth.

Eye movements analysis. Eye movement behavior and pupil size were recorded at a rate of 60 data points per second (60 Hz), averaged over both eyes. Resulting time series were split into trials starting 1 s before face presentation up to 2 s thereafter. The gaze point coordinates were then convoluted with a Gaussian function (SD, 15 ms), and episodes were determined in which the first derivative of the resulting time series did not exceed 10 pixels (0.3 degree or 18°/s angular speed). Only those trials were included in which participants fixated within 50 pixels (1.5°) of the fixation cross before the onset of the face and that did not contain consecutive missing data points for >100 ms. Missing data points are typically caused by blinks, head motion, poor calibration, and, likely, by fast eye motion. To compare groups and conditions, the remaining valid trials were then categorized as trials containing a fixation change (upward or downward) or as trials not containing a fixation change within 1 s of face presentation. A fixation change was defined as occurring at least 50 pixels (1.5°) away from the original position of the fixation cross in vertical direction. We then calculated the proportion of fixation changes downward (when the eyes were presented at the location of the fixation cross) and upward (when the mouth was presented at the location of the fixation cross) as reflexive gaze responses triggered by the eyes or the mouth. Participants who had <20% valid trials for each of the six conditions were excluded from further analysis. Because of exclusion criteria (e.g., technical failure or participants' movements), the presented results are based on 12 individuals with ASD and 11 controls. Over all six conditions, included participants had, on average, 62.1% valid trials.

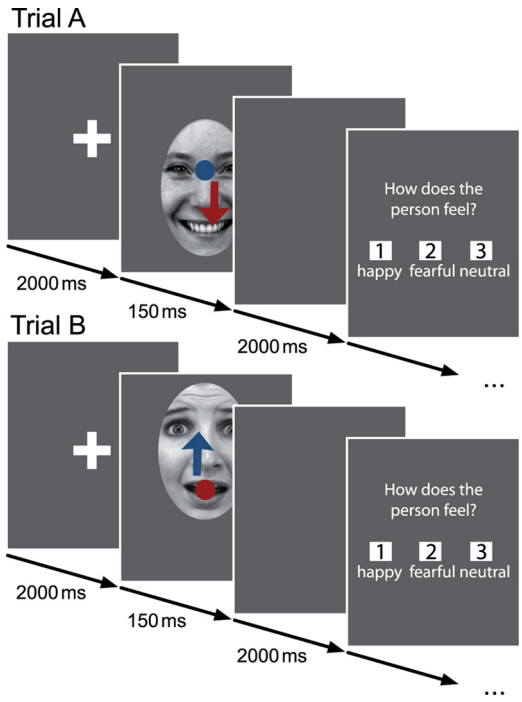

Figure 1. Emotion classification task. Each trial started with the presentation of a fixation cross (2 s), followed by an emotional face (150 ms). After a blank screen for 2 s, subjects were asked to indicate the emotion displayed. Faces were shifted vertically on the screen, so that participants started gaze either on the eyes (trial A) or on the mouth (trial B) of the presented emotional face. Hypothesized gaze behavior of ASD group in red and of NT group in blue.

Additionally, we calculated an individual eye preference index for difference between the proportion of trials with a fixation change upward from the mouth and the proportion of trials with a fixation change downward from the eyes (Gamer and Büchel, 2009), as well as an individual eye preference control index (number of trials with a fixation change upward from the mouth divided by the number of trials with a fixation change upward from the mouth plus the number of trials with a fixation change downward from the eyes) separately for each emotion and on average over all emotions. Both indices indicate how often participants gazed away from the eyes compared with the mouth. The control index, however, did not take into account the number of trials with no fixation change and therefore validates the eye preference index by controlling for the effect of missing trials. Indeed, we did not expect that trials with a fixation change downward were missed with a different probability than trials with a fixation change upward, but it was possible that trials with fixation changes were missed with a different probability than trials with no fixation changes. If this would have been the case, we expected that those indices would differ considerably. Note that we cannot make any conclusions about the final target of the observed eye movements but only about the directionality attributable to methodological limitations in recording frequency.

Statistical analysis. Since we were primarily interested in the main effects of the two factors initial fixation and emotion, as well as their interaction within and between groups, any data collected during the task were first analyzed applying a 2 × 3 [initial fixation (eyes vs mouth) × emotion (happy vs fear vs neutral)] repeated-measures ANOVA with between-subject factor group (ASD vs NT). Additionally, *post hoc* within-group ANOVAs, independent-samples t tests, as well as paired-samples t tests were conducted. All statistics used a significance level of

Table 1. Demographic variables and diagnostic scores

	ASD	NT	*p*
Complete sample			
Gender (male/female)	12/5	14/5	0.84
Age (yr)	32.7 ± 8.2	30.4 ± 5.9	0.33
MWT-IQ	104.5 ± 15.6	110.4 ± 12.9	0.23
Valid eye movement data sample			
Gender (male/female)	8/4	8/3	0.75
Age (yr)	35.4 ± 8.1	27.1 ± 2.6	0.004
MWT-IQ	102.6 ± 14.3	105.9 ± 10.6	0.53

Demographic variables and diagnostic scores for (1) the initial sample [ASD ($n = 17$) and NT participants ($n = 19$)] (upper) and (2) the subsample with valid eye-tracking data (ASD: $n = 12$; NT: $n = 11$) (lower). *p* values reflect levels of significance from independent samples *t* test and χ^2 as appropriate. Values are given as mean ± SD. MWT-IQ, Multiple choice vocabulary IQ test.

Table 2. Latency of fixation changes

	Mean (SD) (ms)	
	ASD	NT
Eye	449 (199)	508 (185)
Happy	438 (203)	437 (124)
Fearful	437 (213)	497 (204)
Neutral	423 (166)	566 (210)
Mouth	437 (114)	404 (93)
Happy	428 (179)	438 (131)
Fearful	508 (167)	402 (91)
Neutral	381 (79)	396 (142)

$p < 0.05$, unless otherwise specified. Data were analyzed using SPSS (version 17.0 for Mac; SPSS Inc.).

Results

Demographics

Groups were matched with respect to gender and age (Table 1). To assure comparable verbal IQ levels between groups, we tested participants with a vocabulary IQ test [multiple choice vocabulary test (MWT)] (Lehrl et al., 1995) (Tables 1, 2). Additionally, we used the Autism Spectrum Quotient (AQ) (Baron-Cohen et al., 2001b) in both groups to control for clinically significant levels of autistic traits in the NT group. None of the controls scored above the cutoff score of 32; in fact, the highest score was 24, indicating a very low level of autistic traits in the NT group (ASD group: mean, 38.3; SD, 8.4; NT group: mean, 13.2; SD, 5.1).

We had valid eye movement data for a subsample of 12 individuals with ASD and 11 control participants (for subsample demographic information, see Table 1). Importantly, the subsample did not differ with respect to gender and verbal IQ, but ASD participants were significantly older than NT participants (Table 1). The factor age did, however, not influence eye movements ($F_{(1,20)} = 0.37$, $p = 0.57$) and was therefore excluded from the model.

Behavioral results

Reaction times

We additionally analyzed participants' reaction times during the emotion classification task by condition with respect to the onset of the face stimuli. Individuals with ASD were generally slower in responding than NTs (ASD: 568 ms; NT: 432 ms; $t_{(34)} = 2.23$; $p = 0.03$). The above-described 2 × 3 ANOVA with between-subject factor group yielded a significant three-way interaction ($F_{(2,68)} = 5.33$; $p = 0.007$; partial $\eta^2 = 0.14$), as well as a two-way interaction between emotion and initial fixation ($F_{(2,68)} = 6.86$; $p = 0.002$; partial $\eta^2 = 0.17$). Reaction times of the ASD group were influenced by an interaction of emotion and initial fixation ($F_{(2,32)} = 7.17$; $p = 0.003$; partial $\eta^2 = 0.31$), whereas the interaction did not reach significance for the NT group ($p = 0.12$). Both NT and ASD groups were slower in responding to fearful faces when they started their gaze on the mouth compared with the eyes (ASD: fear-eyes: 561.45; fear-mouth: 645.88; $t_{(16)} = -2.19$; $p = 0.04$; NT: fear-eyes: 405.5; fear-mouth: 439.95; $t_{(18)} = -2.03$; $p = 0.057$). For neutral faces, however, individuals with ASD were significantly slower in responding to faces when the eyes were presented initially (neutral-eyes: 630.14; neutral-mouth: 503.1; $t_{(16)} = 3.05$; $p = 0.008$). Notably, individuals with ASD responded marginally faster to happy faces compared with neutral faces regardless of initial fixation (happy: 536.17; fearful: 603.67; $t_{(16)} = -2.02$; $p = 0.06$).

Emotion classification performance

For the emotion classification task, individuals in the NT group showed on average a higher correct classification percentage than individuals in the ASD group (NT: 95.46; ASD: 91.14; $t_{(34)} = -2.13$; $p = 0.04$). Performance over both groups was affected by a significant main effect of emotion ($F_{(2,68)} = 3.54$; $p = 0.035$; partial $\eta^2 = 0.09$) and a significant interaction of initial fixation and emotion ($F_{(2,68)} = 18.9$; $p = < 0.001$; partial $\eta^2 = 0.36$). The factor group marginally interacted with initial fixation ($F_{(2,68)} = 3.6$; $p = 0.07$; partial $\eta^2 = 0.09$). Especially in conditions when the mouth was fixated initially, the ASD group showed fewer correct classifications than NTs (across emotions: $p = 0.014$; happy-mouth: $p = 0.055$; fear-mouth: $p = 0.03$).

Both groups were separately influenced by an interaction of emotion and initial fixation (NT: $F_{(2,36)} = 6.89$; $p = 0.003$; partial $\eta^2 = 0.28$; ASD: $F_{(2,32)} = 12.14$; $p < 0.001$; partial $\eta^2 = 0.43$), whereas only individuals in the NT group showed a marginally significant main effect of the factor emotion ($F_{(2,36)} = 3.03$; $p = 0.061$; partial $\eta^2 = 0.14$). For happy compared with neutral faces, controls classified more emotional expressions correctly (happy: 96.58; neutral: 93.36; $t_{(18)} = 2.12$; $p = 0.048$). Larger hit rates within the NT group were also observed when the eyes were presented at the fixation cross compared with the mouth across emotions (eyes: 93.46; mouth: 96.49; $t_{(18)} = -2.53$; $p = 0.02$). Overall, individuals with ASD were able to discriminate a higher percentage of happy faces compared with fearful faces (happy: 94.37; fearful: 90.39; $t_{(17)} = 1.86$; $p = 0.08$). For fearful faces, both participants in the ASD and NT groups could discriminate a higher percentage correctly when they initially fixated the eye region (ASD: $p = 0.003$; NT: $p = 0.076$), whereas for neutral faces the hit rates were larger when participants initially fixated the mouth (ASD: $p = 0.05$; NT: $p = 0.02$).

Eye Movements results

Analysis of eye movements revealed a three-way interaction of initial fixation, emotion, and group ($F_{(2,42)} = 2.89$; $p = 0.067$; partial $\eta^2 = 0.12$), which shortly fell from significance. The factor initial fixation additionally interacted with the factors group ($F_{(1,21)} = 8.03$; $p = 0.01$; partial $\eta^2 = 0.28$) and emotion ($F_{(2,42)} = 6.44$; $p = 0.004$, partial $\eta^2 = 0.24$). Averaged over emotions (ASD: 70.09%; NT: 30.85%; $t_{(21)} = 2.37$; $p = 0.005$), as well as for all emotions separately (all $p < 0.05$), individuals with ASD gazed significantly more often downward than controls when initially fixating the eyes (Fig. 2*A*,*C*). There was no significant difference in the number of fixation changes upward from the mouth to the eyes between groups (all $p > 0.1$).

Within the NT group, eye movements were mediated by a significant main effect of initial fixation ($F_{(1,10)} = 5.06$; $p = 0.048$; partial $\eta^2 = 0.34$) and a significant two-way interaction of initial fixation and emotion ($F_{(2,20)} = 7.82$; $p = 0.003$; partial $\eta^2 = 0.44$). The ASD group showed only a trend for this interaction

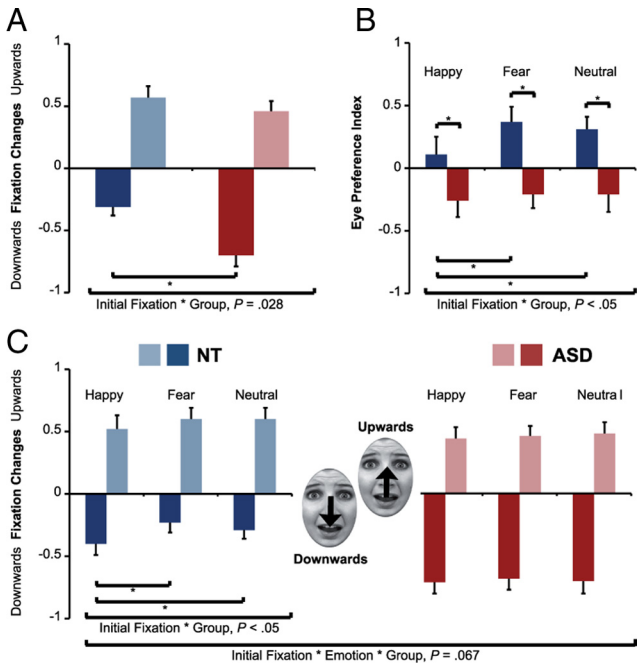

Figure 2. Fixation change patterns. ***A***, Function of initial fixation (without emotion). The ASD group showed more fixation changes downward away from the eyes than the NT group. ***B***, Eye preference index. Generally, the NT group showed an increased preference for the eyes compared with ASD. For the NT group, the preference was stronger for fearful and neutral faces compared with happy faces. There was no effect of emotion in the ASD group. ***C***, Interaction of initial fixation and emotion. Within the NT group, the proportion of fixation changes downward away from the eye region (solid) was mediated by the displayed emotional expression, whereas this effect was absent within the ASD group.

($p = 0.1$). Generally, controls oriented their gaze to a greater proportion upward toward the eyes (when initially fixating the mouth) than downward away from the eyes (when initially fixating the eyes) (upward: 57.83%; downward: 30.85%; $t_{(10)} = -2.25$; $p = 0.048$). The size of the effect was further modulated by the emotional expression, most prominently for fearful faces (upward: 60.74%; downward: 23.48%; $t_{(10)} = -2.97$; $p = 0.014$) and neutral faces (upward: 60.81%; downward: 29.05%; $t_{(10)} = -3.02$; $p = 0.013$), but not for happy faces ($p = 0.43$). In contrast to the controls, the ASD group showed a trend toward a greater proportion of fixation changes downward away from the eyes than upward from the mouth toward the eyes (upward: 46.78%; downward: 70.09%; $t_{(11)} = -1.8$; $p = 0.1$), regardless of the emotion displayed.

To further investigate eye movements with respect to a possible interaction of the two initial fixation positions, we computed an index for eye preference for each participant (the difference between the proportion of trials with a fixation change upward from the mouth and downward from the eyes). Overall, the NT group's eye movements indicated a greater preference for the eyes than the ASD group's eye movements (averaged over emotions: $p = 0.01$; fearful: $p = 0.002$; neutral: $p = 0.008$; happy: $p = 0.068$). Again, only the NT group's gaze patterns were affected by the emotional expression: the index was significantly smaller for happy than for fearful faces (happy: 11.89; fear: 37.26; $t_{(10)} = -3.17$; $p = 0.01$) and neutral faces (happy: 11.89; neutral: 31.76; $t_{(10)} = -2.74$; $p = 0.01$) (Fig. 2*B*).

The number of valid trials was influenced by interactions of group with initial fixation ($F_{(1,21)} = 10.57$; $p = 0.004$; partial $\eta^2 = 0.33$) and marginally by emotion ($F_{(2,42)} = 3.15$; $p = 0.053$; partial $\eta^2 = 0.13$). The ASD group had more valid trials when initially fixating the eyes across emotions ($p = 0.02$), especially for happy faces ($p = 0.009$). To check whether the observed differences in number of valid trials may account for the effects on gaze behavior between groups described earlier, we calculated an additional individual index (eye preference control index), which accounts for differences in number of valid trials between trials with and without fixation changes (see Eye movements analysis). The individual values of the original eye preference index and the control index were highly correlated (Pearson's $r = 0.96$; $p < 0.001$, 2-tailed), thereby reflecting the same pattern of effects. Consequently, the reported between-group differences in fixation changes were not a result of differences in the number of valid trials but represent actual differences in gaze patterns on emotional faces between the ASD and NT groups.

To further approximate the differences between avoidance and orienting response, we further analyzed the latency (time between occurrence of facial stimuli and offset of first fixation change) of participants' fixation changes. Although not reaching statistical significance, the ASD group showed a tendency toward faster eye movements away from the eyes than the NT group (averaged over emotions: ASD mean, 449 ms; NT mean, 508 ms) (Table 2), whereas the ASD group gazed more slowly upward toward the eyes (when starting fixation on the mouth) compared with the NT group (averaged over emotions: ASD mean, 437 ms; NT mean, 404 ms). For both directions, the effects were most pronounced for fearful faces, but almost absent for happy faces (away from eyes, fearful: ASD: 437 ms; NT: 497 ms; happy: ASD: 438 ms, NT: 437 ms; toward eyes, fearful: ASD: 508 ms, NT: 402 ms; happy: ASD: 428 ms, NT: 438 ms), indicating a differential response to facial emotion. In line with previous reports, fearful faces, and especially wide-open eyes, seem more arousing than happy faces, which do not indicate a potential threat in the environment (Morris et al., 1996). Importantly, individuals with autism gazed away from the eyes faster than they gazed away from the mouth for fearful faces (eyes: mean, 437 ms; mouth: mean, 508 ms), while controls showed the opposite pattern (eyes: mean, 497 ms; mouth: mean, 402 ms). In sum, those data indicate that avoidance of the eyes is indeed more pronounced in individuals with ASD because they gaze away from the eyes faster than the NT group and do so relatively faster than gazing away from the mouth toward the eyes, especially for fearful faces.

Interestingly, and in line with an interpretation of aversion, the more often ASD participants gazed away from the eye region, the faster these eye movements occurred (averaged over emotions: Pearson's $r = -0.93$, $p < 0.001$, 2-tailed). Contrarily, there was no such correlation between latency and fixation changes upward from the mouth toward the eyes in ASD (correlations differed significantly; Fisher's z to p transformation: $z = 2.89$, $p = 0.004$). The NT group, in turn, showed no modulation of number of fixation changes by latency at all.

The individual eye preference index was furthermore associated with autistic symptomatology as well as emotion recognition performance. For the ASD group, the eye preference index correlated positively with individual overall performance in the emotion classification task (Pearson's $r = 0.66$; $p = 0.019$, 2-tailed). This effect was absent for the NT group. To further assess whether general illness severity rather than socioemotional impairments account for the observed ASD-specific gaze patterns, we investigated the influence of the ADI-R total score (as a measure of illness severity) versus the influence of emotion recognition performance in a stepwise regression analysis on the eye preference index as the dependent variable. The emotion recognition performance was a significant predictor of eye movement

patterns (R^2 change = 0.74; F change$_{(1,7)}$ = 20.2; β = 0.4, p = 0.003), whereas the ADI-R total score was excluded from the model. Additionally, the eye preference index correlated negatively with the ADI-R social score (Pearson's $r = -0.7$; $p = 0.033$, 2-tailed), whereas there was no correlation for the ADI-R communication score ($p > 0.13$) and ADI-R behavior score ($p > 0.56$). Thus, the greater the preference for the eye region in the ASD group, the less pronounced the social symptomatology, indicating a link between gaze pattern and social impairments but not general illness severity in autism. In addition, there was no relationship of verbal IQ and group specific gaze patterns for both groups (ASD, $p = 0.12$; NT, $p = 0.89$), nor for the AQ scores (ASD, $p = 0.14$, NT: $p = 0.56$). Taking both the performance and diagnostic correlative relationships on the gaze patterns in ASD together, the greater the preference for the eye region in the ASD group, the higher the percentage of correct emotion classification and the less severe the social symptomatology.

Analysis of additional eye movement parameters revealed that there were no global group differences in the average y-axis offset during presentation of the fixation cross (250 ms before up to 50 ms after face presentation; ASD: 1.1 pixels; NT: 4.6 pixels; $t_{(21)} = -1.55$; $p = 0.14$), the average duration (ASD: 908 ms; NT: 1011 ms; $t_{(16)} = -1.27$; $p = 0.22$), distance (ASD: 93.9 pixel; NT: 89.44 pixel; $t_{(21)} = 3.95$; $p = 0.7$), and latency of fixation changes (ASD: 428 ms; NT: 459 ms; $t_{(21)} = -7.78$; $p = 0.44$). Additionally, the number of trials with a fixation change downward from the mouth or upward from the eyes did not differ significantly from 0. Those very few trials (<0.5% of the trials per participant on average) were excluded from any further analysis. There were no group differences in x-axis offsets of fixation changes from the mouth upward toward the left or right half of the screen (i.e., toward the left or right eye) (ASD: −3.5 pixels; NT: −0.9 pixel; $t_{(21)} = -0.32$; $p = 0.75$).

Discussion

The main goal of this study was to investigate the influence of diminished social orientation and active avoidance on the reduced focus to the eyes in ASD. The ASD group showed an overall reduced preference for the eye region compared with the NT group, which was specifically the result of an increase of fixation changes away from the eyes. Further analyses revealed group-specific directional effects when combining both components: the NT group shifted their gaze significantly more often toward the eyes than away from the eyes, replicating previous results in typically developed individuals (Gamer and Büchel, 2009). Conversely, individuals with ASD showed more fixation changes away from the eyes than toward the eyes (Fig. 2).

These results can be seen as support for the hypothesis of increased avoidance of eye contact in ASD. We are aware, however, that both avoidance and orienting responses likely coexist at any time, yet to varying degrees. For example, it is likely that an initial reflexive orientation is present in ASD (as indicated by the number of fixation changes toward the eyes), but that it is then attenuated by an avoidance of eye contact (e.g., fixations do not reach or the eyes). The combination of both forces might furthermore interact differentially in both groups, as assessed with the eye preference index. A clear disentangling of both processes can therefore not be performed based on the number of fixation changes alone. In fact, gaze away from the eyes might indicate avoidance, but it might also indicate an increased interest in the mouth, because, for example, it might be more informative.

We thus performed analyses on additional eye movement parameters, which further support the pronounced influence of active avoidance of eye contact in ASD. Though only on a descriptive level, the ASD group showed a tendency to gaze away from the eyes faster than the NT group and, importantly, did so relatively faster than gazing away from the mouth toward the eyes. This effect was most pronounced for fearful faces, indicating differential responses to facial emotion. In line with previous reports, fearful faces—and especially eyes—are more arousing than happy faces, which do not indicate a potential threat in the environment (Morris et al., 1996). Statistically reliable correlations underline these patterns, since the more often ASD participants gazed away from the eyes, the faster these eye movements occurred. Contrarily, there was no such correlation between latency and gaze away from the mouth in ASD and also no such relationship in the NT group.

Furthermore, if individuals with ASD gaze away from the eyes because the mouth is more informative, one would expect longer fixation times on the mouth to have a beneficial effect on emotion recognition performance. In contrast, we found the reverse to be true: the greater the eye preference index (i.e., the fewer fixations on the mouth), the more correct emotions were classified by the autistic individuals. Moreover, emotions differ distinctively with respect to their diagnostic features across the face (Smith et al., 2005). For fearful and neutral faces, the eye region may contain the most important information about the emotion, whereas for happy faces, the mouth might be more distinctive. Although the number of fixation changes was modulated by the emotional expression for the NT group, which replicates previous findings (Gamer and Büchel, 2009), the ASD group showed no such effect. Also, if the interest in the mouth was greater (than in the eyes), eye movements away from the mouth might occur less often and would be slower for certain emotions, where the mouth is most informative, such as for happy faces. However, the ASD group gazed away from the mouth faster for happy faces than for fearful faces, albeit only on a descriptive level. Thus, the lack of benefit from fixations toward the mouth, together with the missing modulatory effect of facial emotion on gaze can be interpreted as further support of the hypothesis of active avoidance of the eyes in ASD.

It has been suggested that avoidance of the eyes may be mediated by an increased emotional arousal (Kylliainen and Hietanen, 2006). Thus, it is important to mention that even typically developed participants seem to experience facial expressions as more intense when they focus on the eye region (Gamer and Büchel, 2009). With respect to the mechanisms of typically developed gaze behavior; however, the reported increase in intensity has been suggested to underscore the saliency of the eye region. The saliency, in turn, leads to an increased orientation toward this important region of the face (when starting to gaze on another prominent feature, such as the mouth). The increase in experienced intensity as a response to direct eye contact thus represents a rather adaptive arousal in neurotypical controls, enhancing social cognitive functioning. The results of the current study show that direct eye contact leads to differential reflexive gaze behavior within the framework of atypical gaze in ASD: instead of persisting gaze at the eyes (to collect important information about the emotional state of others), individuals with ASD gazed more frequently away from the eyes, independent of the displayed emotional expression. In line with previous findings (see above), these gaze patterns could be interpreted as maladaptive consequences of arousal, oppositional to the arousal effect on controls. Thus, direct eye contact has a rather positive effect on social cognition in controls, whereas this effect is reversed for individuals with ASD. The opposite directional group effects in overall

eye preference underline this hypothesis: in the present study, ASD specific gaze was associated with performance and social symptomatology but not with impairments in repetitive behavior and communication. Thus, these relationships further illustrate how the combination of avoidance and orientation reflect specific socioemotional dysfunctioning within the heterogeneous pathophysiology of ASD, rather than general illness severity.

In sum, we used a novel task to further disentangle avoidance and orientation-related reflexive gaze behavior. We found that atypical gaze in ASD was prominently influenced by an increased number of reflexive eye movements away compared with eye movements toward the eye region in ASD. Within the context of previous literature (Hutt and Ounsted, 1966; Richer and Coss, 1976; Dalton et al., 2005; Kylliainen and Hietanen, 2006; Joseph et al., 2008), more and at the same time faster eye movements away from the eyes compared with away from the mouth in ASD favor the assumption of a pronounced avoidance of eye contact compared with reduced orientation. We acknowledge that future studies shall apply variations in task design such as using inverted faces, directing initial fixations to the nose, or showing only specific facial features instead of the whole face. Moreover, subtle latency characteristics have to be further investigated with higher temporal resolution of eye movement recordings. Possible limitations of the current study arise from repeated reports of basic visual-processing differences in autism in the literature (Minshew et al., 1999; Goldberg et al., 2002; Luna et al., 2007; Thakkar et al., 2008). We did not find such general group differences, suggesting that the observed gaze patterns in ASD represent socioemotional dysfunctioning in ASD, rather than global differences in oculomotor functioning. There is, however, a clear need for further investigations in particular in relation to brain abnormalities (for cerebellar pathology, see Nowinski et al., 2005).

The results of the current study complement research on the role of the amygdala in autism (Baron-Cohen et al., 2000). In typically developed controls, the amygdala seems to be not only involved in orienting toward socially salient stimuli (Gamer and Büchel, 2009; Gamer et al., 2010), but also in fear and aversion processing (LeDoux et al., 1988; Adolphs et al., 1998, 2005; Phelps et al., 2004; Pessoa et al., 2005a,b; Seymour et al., 2005; Anders et al., 2008; Asghar et al., 2008; Hadjikhani et al., 2008). A behavioral dissociation of aversion- and orientation-related gaze in ASD provides an opportunity to simultaneously identify and dissociate sociocognitive functions potentially subserved by different amygdalar nuclei (Ball et al., 2007; Hurlemann et al., 2008; Gamer et al., 2010). With respect to the goal to gain a comprehensive understanding of autism pathophysiology, the present results provide crucial information for future research investigating atypical gaze in relation to brain function. Finally, interventions and behavioral training studies may benefit from considering these results: although strategies motivating attention toward social salient cues seem generally justified, special attention should be directed to the reduction of the aversive effect of direct eye contact.

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Behavioral/Systems/Cognitive

The Role of the Amygdala in Atypical Gaze on Emotional Faces in Autism Spectrum Disorders

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Reduced focus toward the eyes is a characteristic of atypical gaze on emotional faces in autism spectrum disorders (ASD). Along with the atypical gaze, aberrant amygdala activity during face processing compared with neurotypically developed (NT) participants has been repeatedly reported in ASD. It remains unclear whether the previously reported dysfunctional amygdalar response patterns in ASD support an active avoidance of direct eye contact or rather a lack of social attention. Using a recently introduced emotion classification task, we investigated eye movements and changes in blood oxygen level-dependent (BOLD) signal in the amygdala with a 3T MRI scanner in 16 autistic and 17 control adult human participants. By modulating the initial fixation position on faces, we investigated changes triggered by the eyes compared with the mouth. Between-group interaction effects revealed different patterns of gaze and amygdalar BOLD changes in ASD and NT: Individuals with ASD gazed more often away from than toward the eyes, compared with the NT group, which showed the reversed tendency. An interaction contrast of group and initial fixation position further yielded a significant cluster of amygdala activity. Extracted parameter estimates showed greater response to eyes fixation in ASD, whereas the NT group showed an increase for mouth fixation.

The differing patterns of amygdala activity in combination with differing patterns of gaze behavior between groups triggered by direct eye contact and mouth fixation, suggest a dysfunctional profile of the amygdala in ASD involving an interplay of both eye-avoidance processing and reduced orientation.

Introduction

The specific functional role of the amygdala within human social cognition remains a topic of debate. Recent findings suggest a crucial role in detecting and processing environmental features (Adolphs et al., 1995; Whalen, 2007; De Martino et al., 2010). Consistently, neuroimaging and lesion studies suggest amygdalar sensitivity to the eyes, which themselves represent cues for processing social information (Kawashima et al., 1999; Morris et al., 2002; Whalen et al., 2004). In line with this amygdala function framework, typically developed participants show increased amygdala activity underlying a focus on the eyes (Gamer and Büchel, 2009).

In contrast, autism spectrum disorders (ASD) show atypical gaze on emotional faces—marked by a reduced eye focus (Klin et al., 2002; Pelphrey et al., 2002; Kliemann et al., 2010). Within the social symptomatology in ASD, processing information from the eyes seems to be specifically impaired (Leekam et al., 1998; Baron-Cohen et al., 2001b). Previous research highlighted two potential explanations: one hypothesis suggests a general lack of social attention, resulting in a missing orientation toward social cues, such as the eyes (Dawson et al., 1998; Grelotti et al., 2002; Schultz, 2005; Neumann et al., 2006). Another hypothesis suggests an aversiveness of eye contact, leading to an avoidance of eye fixation (Richer and Coss, 1976; Kylliäinen and Hietanen, 2006). In fact, these processes do not have to be mutually exclusive (Spezio et al., 2007).

On a neural level, atypical gaze in ASD has been repeatedly reported together with aberrant amygdala activity. The exact relationship of gaze and brain findings in ASD, however, remains unclear. Previous studies reported both amygdalar hyperactivation (Dalton et al., 2005) and hypoactivation (Baron-Cohen et al., 1999; Critchley et al., 2000; Corbett et al., 2009; Kleinhans et al., 2011) triggered by faces. Within normal functioning, an increase in amygdala activation seems to be associated with immediate orientation toward the eyes (Gamer and Büchel, 2009; Gamer et al., 2010). Along the same lines, patients with bilateral amygdala lesions fail to reflexively gaze toward the eyes (Spezio et al., 2007). Thus, if the amygdala triggers orientation toward salient social cues, such as the eyes, decreased amygdalar response to faces in ASD would rather support the reduced orientation hypothesis. Increased amygdala activation, however, was found to positively correlate with duration of eye contact in a study by Dalton et al. (2005), which was interpreted as an overarousal indicating aversiveness of eye fixation. These results would rather favor the avoidance hypothesis, consistent with findings indicating amygdalar involvement in aversion processing.

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To further define the amygdala's functional role within atypical gaze in ASD we applied a facial emotion classification task, using eye tracking during fMRI, varying the initial fixation position on faces. We hypothesized that individuals with ASD would show reduced eye movements toward the eyes along with decreased amygdala activity when starting fixation on the mouth (in accordance with reduced orientation) and/or enhanced eye movements away from the eyes accompanied by increased amygdala activity when starting fixation on the eyes (in accordance with avoidance), compared with controls.

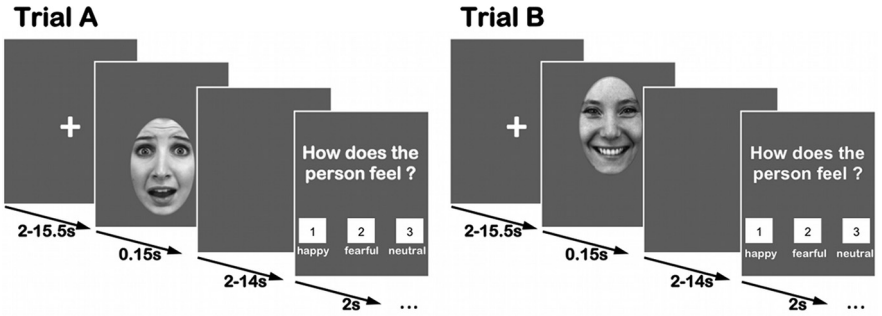

Figure 1. Emotion classification task. Trials started with a presentation cross (2–15.5 s), followed by an emotional face presented for 150 ms. After a blank screen, participants had to indicate the emotional expression displayed via button press. Faces were shifted vertically on the screen, so that participants initially fixated the eyes (trial A) or the mouth (trial B).

Materials and Methods

Participants. Seventeen neurotypically developed (NT) male controls and 16 male participants with autism spectrum disorders (ASD) participated in the current study. Controls were recruited through databases of the Max Planck Institute for Human Development, Berlin, Germany and the Freie Universität Berlin, Berlin, Germany. Participants with ASD were recruited through psychiatrists specialized in autism in the Berlin area and the outpatient clinic for autism in adulthood of the Charité University Medicine, Berlin, Germany. Controls were asked twice to indicate any psychiatric or neurological disorder during recruitment as well as before study onset and were excluded from the study in case of a report. Diagnoses were made according to DSM-IV (American Psychiatric Association, 1994) criteria for Asperger syndrome and autism without mental retardation using an in-house developed semistructured interview tapping the diagnostic criteria for autism (Dziobek et al., 2006, 2008) and the Asperger Syndrome (and High-Functioning Autism) Diagnostic Interview (ASDI; Gillberg et al., 2001), which is specifically targeted toward adults with autism spectrum disorders. In 13 participants with available parental informants, diagnoses were additionally confirmed with the Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1994). All diagnoses were made by two clinical psychologists [I.D. and J.K. (not an author on this paper)], trained and certified in making ASD diagnoses using the ADI-R based on DSM-IV (American Psychiatric Association, 1994) criteria.

All participants had normal or corrected-to-normal vision, were native German speakers, received payment for participation, and gave written informed consent, according to the requirements of the ethics committee of the Charité University Medicine, Berlin, Germany.

Experimental stimuli. We chose 120 faces (20 females, 20 males, each displaying happy, fearful, and neutral expressions) from a standardized dataset (Lundqvist et al., 1998) for the emotion classification task, based on a recent validation study (Goeleven et al., 2008). Each image was rotated to ensure that, when displayed, the eyes were at the same vertical height. Additionally, an elliptic mask was applied resulting in images containing just the face (Fig. 1). All images were converted to grayscale and the cumulative brightness was normalized across images.

Task and procedures. In each trial, a fixation cross was presented initially (jittered, 2–15.5 s), followed by the presentation of a face (150 ms) (Fig. 1) (Gamer and Büchel, 2009; Gamer et al., 2010; Kliemann et al., 2010). After showing a blank gray screen (jittered, 2–14 s), participants were asked to indicate the emotional expression via button press on a fiber optic response device (Current Designs Inc.). The emotional classification experiment followed a 2 × 3 within-subjects design with the factors Initial Fixation (eyes, mouth), Emotion (happy, fearful, neutral) and the between-subject factor Group (NT, ASD). To investigate the effect of Initial Fixation, half of the faces within each emotion category were shifted either downward (Fig. 1, Trial A) or upward (Fig. 1, Trial B), so that the eyes or the mouth appeared at the location of the formerly presented fixation cross. Thereby, the task allowed the investigation of changes in amygdalar blood oxygen level-dependent (BOLD) signal as a response to direct eye contact and when initially fixating the mouth in combination with respective eye movements.

Presentation order and timing of the six experimental conditions were optimized using the afni toolbox (3dDeconvolve, make_random_timing; http://afni.nimh.nih.gov/afni/). Visual stimuli were presented in the scanner using Presentation (Version 12.4., Neurobehavioral Systems Inc.) running on a Microsoft Windows XP operating system via MR-compatible LCD-goggles (800 × 600 pixels resolution; Resonance Technology).

Eye movement data analysis. Eye movements were recorded during scanning using a 60 Hz embedded infrared camera (ViewPoint Eye Tracker, Arrington Research). The resulting time series were split into trials starting 150 ms before and ending 1 s after stimulus presentation. To suppress impulse noise, each time series was filtered using a recursive median filter (Nodes and Gallagher, 1982; see Juhola, 1991, for median filtering of saccadic eye movement; Stork, 2003, for a discussion on recursive median filters). The filter was iterated with an increasing window size from 3, 5, 7, 9 and 11 data points respectively to eliminate spikes of varying widths due to improper pupil recognition. This technique successfully suppressed impulse noise while preserving edge information necessary for the proper recognition of saccades where previously used linear filtering techniques failed. Gaze points outside the screen (not within 5° of the fixation cross) were discarded. Trials were considered for further analysis if all gaze points within the baseline period were valid, within 2° of the fixation cross and if the total amount of invalid samples due to blinks or other data defects in the remainder of the trial did not exceed 250 ms. The baseline period started 150 ms before and ended 150 ms after stimulus presentation. The first fixation change was determined as the first deviation in the gaze position of at least 1° away from the mean gaze position during the baseline period. If the first fixation occurred during the baseline period, the trial was said to contain a saccade during the baseline and was excluded from further analysis. As a result, only trials were considered where a steady fixation close to the fixation cross with no blinks or saccades was found for the whole duration of the baseline period and with <250 ms worth of missing data during the remainder of the trial. Four participants from the ASD group had to be excluded from further analysis based on the number of invalid trials due to blinks, motion or other artifacts. Since calibration was performed separately for each run and for each participant, runs with <30% valid trials were excluded individually from further analysis. No significant differences in the percentage of valid trials could be found between the groups (NT: 45.6%; ASD: 47.2%; $p > 0.1$). For some participants the number of valid trials fell under the 30% mark for individual conditions even though the total number of valid trials exceeded 30% per run. To ensure reliable percentages while avoiding excluding whole subjects, those values were replaced by the respective group means. No significant difference in the number of valid conditions was found between the groups (NT: 91.1%; ASD: 88.9%; $p > 0.1$). For each emotion separately, the percentage of trials that contained a fixation change downward from the eyes or upward from the mouth was computed depending on whether the eyes or the mouth was presented at the fixation cross. Because of a reoccurring buffer overflow error in the eye tracker data acqui-

sition computer, a significant portion of the eye tracker trials were not recorded, randomly across the experiment. There was no significant effect for missing trials for the factors Group, Emotion or Initial Fixation, but within individual participants missing trials were unevenly distributed across conditions. To retain the trials for those conditions that had a sufficient number of valid trials a total of 17 conditions in 9 different participants (8 conditions in the ASP group and 9 in the NT group) were interpolated, which allowed inclusion of more subjects and increased power as a consequence. Overall, 9.8% of a total of $29 \times 6 = 174$ cases were interpolated. It is possible that this manipulation introduced a bias toward false positives in subsequently used mean difference-based statistics. Correlation analyses with the eye movement data were performed using non-interpolated trials only as interpolation was performed within group and per condition and the interpolated values are thus orthogonal to the remaining variables within each subject.

To further investigate potential effects between groups when combining eye movements away from and toward the eyes to a general measure, we calculated an eye preference index. The index measures the directionality of gaze shifts toward the eyes compared with the mouth while correcting for between-subject variability due to differences in the overall proportion of trials containing gaze shifts. Comparable indices have been used in two previous studies, using the same experimental paradigm (Gamer and Büchel, 2009; Kliemann et al., 2010). The proportion of gaze shifts toward the mouth or toward the eyes are corrected by dividing the respective variable by the sum of the gaze shifts toward both features in each emotion and adding one to offset the divisor away from zero:

$$S_{\text{eye}} = \frac{P_{\text{eye}}}{P_{\text{eye}} + P_{\text{mouth}} + 1}, \quad (1)$$

$$S_{\text{mouth}} = \frac{P_{\text{mouth}}}{P_{\text{eye}} + P_{\text{mouth}} + 1}. \quad (2)$$

The eye preference index (J) is defined as the difference of the corrected gaze shifts,

$$J = S_{\text{eye}} - S_{\text{mouth}}. \quad (3)$$

The eye preference index ranges in the interval $[-0.5, +0.5]$ and is first defined for each emotion separately and then additionally combined over all emotions. Positive values indicate stronger preference for the eyes (i.e., increased orientation to the eyes, as suggested in the NT group) and negative values indicate stronger preference for the mouth (i.e., avoidance of the eyes, as suggested in the ASD group). To control for possible discrepancies regarding the number of trials between groups and among conditions we performed an ANOVA on the overall proportion of trials containing fixation changes and found no significant effects or interactions for diagnosis or emotion. In addition individual independent samples t tests between the groups for each emotion separately showed no significant effects.

Statistical analysis. Since we were primarily interested in effects and interaction of the two factors Initial Fixation and Emotion within and between groups, any data collected during the task were first analyzed applying a 2×3 (Initial Fixation [Eyes vs Mouth] × Emotion [Happy vs Fear vs Neutral]) repeated-measures ANOVA with between-subject factor Group ([ASD vs NT]), unless otherwise specified. Additionally, *post hoc* within group ANOVAs, independent-samples t tests, as well as paired-samples t tests were conducted. All statistics used a significance level of $p < 0.05$, unless otherwise specified. Data were analyzed using PASW (version 18.0 for Mac, SPSS Inc., an IBM Company).

fMRI data acquisition. Participants were scanned using a Siemens Magnetom Tim Trio 3T system equipped with a 12-channel head coil at the Dahlem Institute for Neuroimaging of Emotion (D.I.N.E., http://www.dine-berlin.de/) at the Freie Universität Berlin, Germany. For each participant, we acquired 4 functional runs of 180 BOLD-sensitive T2*-weighted EPIs (TR, 2000 ms; TE, 25 ms; flip angle, 70°; FOV, 204×20 mm^2; matrix, 102×102; voxel size, $2 \times 2 \times 2$ mm). To achieve a better in-plane resolution, reduce susceptibility effects and with regard to the specific hypotheses, we collected 33 axial slices covering a 6.6 cm block, which included the bilateral amygdalae. For registration of the functional images, high-resolution T1-weighted structural images (TR, 1900 ms; TE 2.52 ms; flip angle, 9°; 176 sagittal slices; slice thickness 1 mm; matrix, 256×256; FOV, 256; voxel size, $1 \times 1 \times 1$ mm) were collected.

fMRI data analysis. The fMRI data were preprocessed and analyzed using FEAT (FMRI Expert Analysis Tool) within the FSL toolbox (FMRIB's Software Library, Oxford Centre of fMRI of the Brain, www.fmrib.ox.ac.uk/fsl; Smith et al., 2004). Preprocessing included nonbrain tissue removal, slice time and motion correction and spatial smoothing using a 5 mm FWHM Gaussian kernel. To remove low-frequency artifacts, we applied a high-pass temporal filter (Gaussian-weighted straight line fitting, $\sigma = 50$ s) to the data. Functional data were first registered to the T1-weighted structural image and then transformed into standard space (Montréal Neurological Institute, MNI) using 7- and 12-parameter affine transformations, respectively (using FLIRT; Jenkinson and Smith, 2001).

We then modeled the time series individually for each participant and run with seven event-related regressors (six regressors represented face onsets according to the six conditions of our 2×3 within-subject design, one additional regressor represented the onset of the emotion recognition question). Regressors were generated by convolving the impulse function related to the onsets of events of interest with a Gamma HRF. Contrast images were computed for each participant, spatially normalized, transformed into standard space and then submitted to a second-order within-subject fixed-effects analysis across runs. Because of our specific hypotheses regarding amygdala functioning in ASD, we used an anatomically determined mask to restrict our analyses to the amygdala. The mask included voxels with a 10% probability to belong to the bilateral amygdalae as specified by the Harvard-Oxford subcortical atlas (http://www.cma.mgh.harvard.edu/fsl_atlas.html) provided in the FSL atlas tool. Higher level mixed-effects analyses across participants were applied to the resulting contrast images using the FMRIB Local Analysis of Mixed Effects tool provided by FSL (FLAME, stage 1 and 2). We report clusters of maximally activated voxels that survived statistical thresholding with a z-value of 1.7 and FWE correction (corresponding to $p < 0.05$, corrected for multiple comparisons) (see Table 3). For visualization purposes, the resulting z-images were thresholded with a z-value from 1.7 to 2.6 and displayed on a standard brain (MNI-template) (see Fig. 5). To further characterize possible interactions, we extracted parameter estimates (PEs) of voxels for the identified cluster for relevant task conditions. In contrast to previous studies using the same design (Gamer and Büchel, 2009; Gamer et al., 2010) we did not focus our analyses on potential interactions of emotional expression and initial fixation position because (1) eye tracking data strongly suggested that gaze behavior (specifically gaze toward or away from the eyes) in autistic individuals was not modulated by the emotional expression on the face (Kliemann et al., 2010) and (2) previous studies suggest that amygdala activity is influenced by the face per se rather than by the emotional expression (Dalton et al., 2005). Thus, to gain more power in analyses of neuronal data with respect to between-group effects, we collapsed data across emotional expressions.

Results

Demographics

Groups were matched with respect to age (NT: 30,47, SD: 6.23, range 24–46; ASD: mean 30.44, SD 6.34, range: 22–42; $t_{(31)} = -0.015$, $p = 0.99$) and intelligence level (IQ) [a verbal multiple choice vocabulary test, Mehrfachwahl-Wortschatztest (MWT) (Lehrl et al., 1995), and a nonverbal strategic thinking test (Leistungsprüfsystem, subtest 4, in 16 ASD (Horn, 1962); Table 1]. We additionally used the Autism Spectrum Quotient (AQ) (Baron-Cohen et al., 2001a) in both groups to control for clinically significant levels of autistic traits in the NT group. Groups differed significantly in AQ-scores (NT: 11.82, ASD: 36.06; $t_{(31)} = 11.41$, $p < 0.001$). None of the controls scored above the cutoff score of 32, in fact, the highest score was 22, indicating a very low level of autistic traits in the NT group.

Table 1. Demographic variables, IQ measures, and diagnostic scores

	ASD	NT	*p*
n	16	7	
Age (years)	30.44 ± 6.34	30.47 ± 6.24	0.99
MWT-IQ	108.06 ± 7.38	108.12 ± 14.76	0.99
LPS-IQ	128.47 ± 10.82 (in 15 ASD)	126.4 ± 8.94	0.55
AQ	36.06 ± 1.85	11.82 ± 1.11	0.000

p values reflect levels of significance from independent samples *t* test. Values are given in mean ± SD. *n*, sample size; MWT-IQ, multiple choice vocabulary IQ test; LPS-IQ, strategic thinking IQ test; AQ, Autism Quotient.

Behavioral results

Reaction times

We analyzed participants' reaction times (RT) during the emotion classification task by condition with respect to the onset of the face stimuli. Over all conditions, individuals with ASD showed a tendency toward slower responses than control participants [ASD: 706.31 ms, NT: 623.86 ms; $t_{(31)} = 1.66$, $p = 0.11$]. The 2 × 3 ANOVA with between-subject factor Group yielded a main effect of emotion over both groups [$F_{(2,62)} = 4.9$, $p = 0.01$, partial $\eta^{2} = 0.14$], but no further main or interaction effects. Over both groups, participants were faster in responding to happy compared with fearful [happy: 643.2 ms, fearful: 676.6 ms; $t_{(32)} = -3.8$, $p = 0.001$] and neutral faces [happy: 643.2 ms, neutral: 670.1 ms; $t_{(32)} = -2.1$, $p = 0.043$].

Emotion classification performance

For the emotion classification task, the NT group showed a higher percentage of correct emotion classification than the ASD group in a *post hoc t* test [NT: 97.3; ASD: 93.8; $t_{(31)} = -2.8$; $p = 0.01$]. Performance was further modulated by a significant two-way interaction of Initial Fixation and Emotion [$F_{(2,62)} = 9.4$; $p < 0.001$, partial $\eta^{2} = 0.2$]. Over both groups, there were trends toward a main effect of Emotion [$F_{(2,62)} = 2.5$; $p = 0.09$, partial $\eta^{2} = 0.07$] and an interaction of all three factors [$F_{(2,62)} = 2.6$; $p = 0.08$, partial $\eta^{2} = 0.08$] (Initial Fixation [Eyes vs Mouth] × Emotion [Happy vs Fear vs Neutral]) × Group ([ASD vs NT]).

Both groups were separately influenced by an interaction of Emotion and Initial Fixation (NT: [$F_{(2,32)} = 46.9$; $p = 0.014$, partial $\eta^{2} = 0.24$]; ASD: [$F_{(2,30)} = 6.2$; $p = 0.006$, partial $\eta^{2} = 0.3$]). All participants classified more happy than fearful expressions correctly [NT: happy: 98.4; fear: 96.7; $t_{(16)} = 2.0$; $p = 0.06$; ASD: happy: 95.4; fear: 92.3; $t_{(15)} = 2.9$; $p = 0.01$]. Nonetheless, the NT group showed significantly increased performance for those emotional expressions compared with the ASD group (NT: happy: 98.4; ASD: happy: 95.4; $t_{(31)} = -2.2$, $p = 0.034$; NT: fearful: 96.7; ASD: fearful: 92.3; $t_{(31)} = -2.3$; $p = 0.03$). For happy faces, the effect was mainly driven by differences for the mouth conditions ($p = 0.05$), whereas for fearful faces the magnitude of effects was increased for eyes conditions ($p = 0.008$) (Fig. 2). Greater accuracy was also observed for both groups when the eyes were initially fixated for happy faces [NT: eyes: 99.4; mouth: 97.4; $t_{(16)} = 2.9$; $p = 0.01$; ASD: eyes: 97.9; mouth: 92.8; $t_{(15)} = 2.6$; $p = 0.02$]. Interestingly, both groups showed a higher rate of emotion recognition when initially fixating the mouth for neutral faces, though only on trend level for the NT group [NT: eyes: 95.6; mouth: 98.1; $t_{(16)} = -1.8$; $p = 0.09$; ASD: eyes: 92.5; mouth: 94.9; $t_{(15)} = -2.1$; $p = 0.05$].

Eye Tracking results

The repeated measure ANOVA yielded a significant three-way interaction of all three factors [$F_{(2,54)} = 3.87$; $p = 0.027$, partial $\eta^{2} = 1.25$]. Group differences for neutral faces when initially fixating the mouth region were marginally significant [NT: 22.2, ASD: 9.4; $t_{(22.4)} = -2$, $p = 0.057$]. The NT group's gaze was further modulated by a significant interaction of Emotion and Initial Fixation [$F_{(2,32)} = 6.2$; $p = 0.005$, partial $\eta^{2} = 0.28$]. For neutral faces, participants in the NT group showed a trend toward more fixation changes from the mouth to the eyes [mouth: 22.2, eyes: 9.7; $t_{(16)} = -1.86$, $p = 0.08$]. When starting fixation at the mouth, more fixation changes to the eyes were observed for neutral, compared with happy faces [neutral: 22.2, happy: 14.9; $t_{(16)} = -2.7$, $p = 0.016$], and showed a trend for neutral compared with fearful faces [neutral: 22.2, fearful: 17.6; $t_{(16)} = -1.77$, $p = 0.097$]. We further analyzed the group means on a descriptive level: though not reaching statistical significance, over each emotion and for each emotion separately, the ASD group showed more eye movements away from the eyes than toward the eyes (from the mouth). In contrast, the NT group showed the opposite pattern (Fig. 3, Table 2). The magnitudes of these descriptive differences were greatest for neutral and fearful faces, between and within groups.

A Group × Emotion ANOVA on the eye preference index revealed a significant interaction of Group * Emotion ($F_{2, 54} = 4.388$;

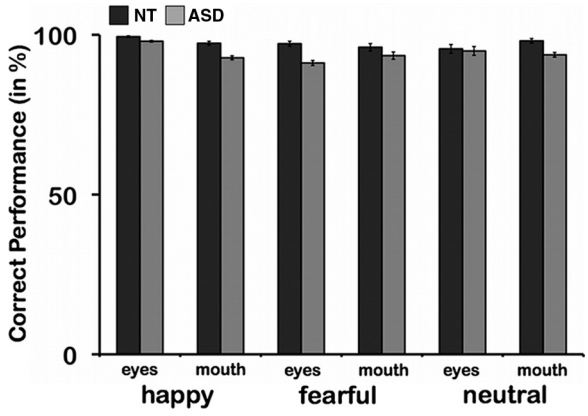

Figure 2. Emotion classification performance. The ASD group (lighter) showed on average less correct emotion classification than the NT group (darker) ($p = 0.01$). For happy faces, group differences were mainly driven by the mouth condition ($p = 0.05$), whereas for fearful faces, the difference was the largest for eyes conditions ($p = 0.008$).

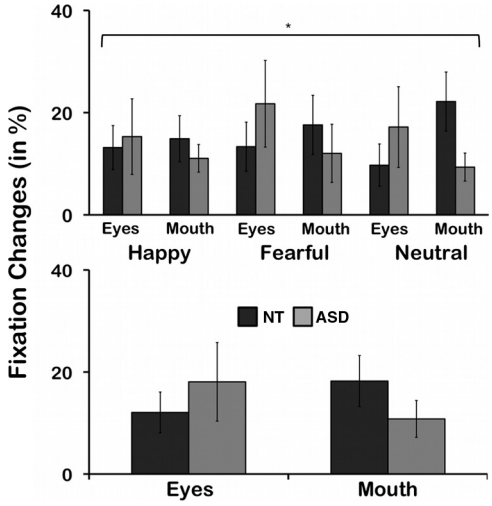

Figure 3. Eye movements as a function of Initial Fixation, Emotion, and Group (top); and Initial Fixation and Group (bottom). Eye movements were significantly modulated by all the factors (Group, Emotion, Initial Fixation, $p = 0.027$). Descriptively, the ASD groups showed more eye movements away from the eyes than toward the eyes, whereas the NT group showed more eye movements from the mouth toward the eyes, than away from the eyes (Table 2).

Table 2. Descriptives of Eye Movements per Emotion, Initial Fixation, and Group

	NT	ASD
Happy		
Eyes	13.2 (4.3)	15.3 (7.5)
Mouth	14.9 (4.5)	11.1 (2.7)
Fearful		
Eyes	13.4 (4.9)	21.7 (8.5)
Mouth	17.6 (5.9)	12.04 (5.8)
Neutral		
Eyes	9.7 (4.2)	12.8 (7.9)
Mouth	22.2 (5.8)	9.4 (2.8)

Values are given in mean and SE (in parentheses).

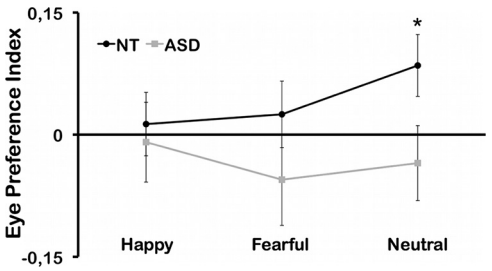

Figure 4. Eye preference index as a function of Emotion and Group. The ASD group showed consistently diminished eye preference (negative values) over all emotions, whereas controls show pronounced eye preference (positive values). The group difference was significant for neutral faces ($p < 0.05$).

$p < 0.05$; partial $\eta^2 = 0.140$) (Fig. 4). Within the NT group a one-way ANOVA revealed a significant effect for Emotion ($F_{2, 32} = 6.692$; $p < 0.01$; partial $\eta^2 = 0.295$). No effect of Emotion could be found in the ASD group again, which is replicating a lack of an emotion effect on eye movements in ASD (Kliemann et al., 2010). Within each emotion, participants in the control group showed consistently higher preference (positive index) for eye regions whereas participants in the ASD group showed consistently lower preference (and thus a negative index) for the eyes as outlined in Figure 4. The differences were again most pronounced for neutral faces for which controls showed an eye preference index of 0.085 (SD 0.160) and participants in the ASD group −0.035 (SD 0.163) ($t_{(27)} = 1.970$, $p = 0.05$). In addition, the eye preference index differed significantly from zero in the control group for neutral faces ($t_{(16)} = 2.185$, $p < 0.05$) but did not differ from zero in the ASD group. This strong effect for neutral faces is most likely due to the fact that the main effect of Emotion in the NT group is most pronounced in this condition.

Relationship between eye movements and emotion recognition performance

The number of eye movements away from the eyes was further correlated with performance on the emotion recognition task in ASD (eyes trials: $p = 0.076$, $r = -0.62$; mouth trials: $p = 0.021$, $r = -0.74$; all trials: $p = 0.026$, $r = -0.73$) but not in NT. Notably, this difference between groups (assessed with Fisher r-to-z transformation) was significant for mouth trials ($z = -2.19$, $p = 0.029$, two-tailed) and marginally significant for all trials ($z = -1.9$, $p = 0.057$, two-tailed). In other words, the more participants gazed away from the eyes, the less correctly emotions were identified, especially for mouth conditions.

fMRI results

We investigated how amygdala activity is differentially affected by the initial fixation of the eye compared with the mouth. For this purpose, we analyzed the fMRI data in predefined regions of interest (bilateral amygdalae, see Materials and Methods) with respect to the main hypotheses: (1) increased BOLD response within the amygdala for ASD compared with NT in trials where the eyes were fixated initially compared with trials where the mouth was fixated initially (ASD > NT; eyes > mouth, i.e., avoidance) and (2) increased BOLD response within the amygdala for NT compared with ASD in trials where the mouth was fixated initially compared with trials where the eyes were fixated initially (NT > ASD; mouth < eyes, i.e., orientation). The resulting 2 × 2 interaction contrast (Initial Fixation [eyes vs mouth] × Group [ASD vs NT]) revealed a significant cluster of activation within the left ($p < 0.05$, FWE corrected) amygdala (Fig. 5, Table 3). Extracted PEs averaged across emotions further illustrate the direction of the interaction effect: the ASD group showed increased amygdala activity at initial fixation of the eyes compared with initial fixation of the mouth and compared with the NT group. In contrast, the NT group showed increased amygdala activity at initial fixation of the mouth compared with initial fixation of the eyes (Fig. 5) and compared with the ASD group.

There were no significantly activated clusters showing a three-way interaction of Emotion × Initial Fixation × Group in the amygdala.

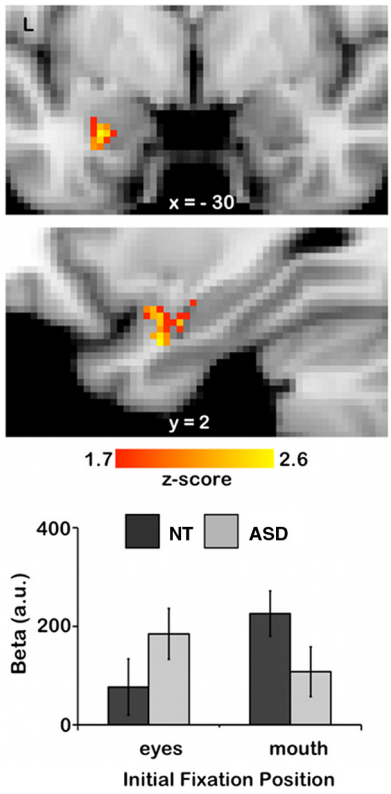

Figure 5. Amygdala region showing a significant interaction of Initial Fixation position and Group. The upper two panels show statistical maps of coronal and left (L) sagittal planes. The lower bar shows the extracted β values of the cluster reported in Table 2 ($p = 0.05$, FWE corrected). Error bars represent SE.

Table 3. Results of Interaction Contrast Initial Fixation Position and Group in the Amygdala

				MNI-coordinates (mm)		
Hemisphere	Multiple comparison correction	Voxels	*Z*-max	*X*	*Y*	*Z*
Left	FWE corrected $p = 0.05$	148	3	−26	4	−20

Discussion

To further define the role of the amygdala within atypical gaze in ASD, we investigated eye movements and amygdala activity when participants initially fixated the mouth or the eyes of faces during an emotion recognition task. Eye movements were mediated by a significant interaction of Emotion, Initial Fixation and Group. Within this interaction, ASD participants showed more eye movements away from than toward the eyes, whereas NT participants gazed more frequently toward than away from the eyes. fMRI data analyses revealed a significant interaction effect of Initial Fixation and Group in the amygdala, reflecting reversed group patterns in response to eyes and mouth fixation: ASD participants exhibited relatively greater amygdala response when initially fixating the eyes, whereas NT participants showed a relative increase when initially fixating the mouth, compared with the other facial feature and group, respectively.

Social functioning requires the recognition and orientation toward important environmental cues. During communication, the eyes carry information about the other agent's inner state as well as information about the environment. Already early in development neurotypically developed individuals focus immediately on the eyes (Nation and Penny, 2008). Our data support this, by revealing relatively more eye movements toward than away from the eyes, as additionally indicated by the positive eye preference index (Fig. 4). At the same time, NT participants classified more fearful faces correctly when fixating the eyes. Thus, the previously reported orientation toward the eyes leads to increased performance when only information from the eyes is available, potentially reflecting a developmental expertise in processing information from the eyes due to the eye preference. Recently, the orientation toward (fearful) eyes has been associated with increased amygdala activity (Gamer and Büchel, 2009; Gamer et al., 2010), suggesting that the amygdala triggers reflexive orientation in controls. In other words, the greater the orientation toward the eyes (from the mouth), the greater the increase in underlying amygdala activity. Here, we replicated these findings, showing a relative increase of amygdala activity while orienting gaze to the eyes (Fig. 5). Our data therefore support the proposed functional profile of the amygdala to represent (social) salience mediation triggering reflexive orientation toward the eyes in NTs.

In contrast, autistic individuals' gaze on faces is strongly characterized by a reduced eye focus (Klin et al., 2002; Pelphrey et al., 2002). Two—not mutually exclusive—explanations have been highlighted in the literature. First, previous research suggested that eye contact might be aversive (Hutt and Ounsted, 1966; Richer and Coss, 1976; Kylliäinen and Hietanen, 2006), grounded on behavioral and psychophysiological findings (Joseph et al., 2008). A previous study using the same paradigm, furthermore, showed a strong increase in gaze away from the eyes when starting fixation on the eyes compared with controls (Kliemann et al., 2010). The present group interaction effect replicated those findings (Fig. 3), showing relatively more eye movements away from than toward the eyes in autism compared with controls.

The correlation between gaze away from the eyes and performance could further reflect a behavioral significance of actively gazing away from the eye region. The magnitude of this effect for mouth trials does not imply a behavioral benefit for processing information from the mouth, consistent with previous studies (Kliemann et al., 2010; Kirchner et al., 2011). If current eye movements could be explained by a greater interest in the mouth in ASD, this should lead to increased mouth exposure across the lifespan, resulting in increased performance when looking at the mouth. Performance, however, was not superior when directed to fixate the mouth versus the eyes in ASD.

Dalton et al. (2005) strikingly connected the duration of eye fixation and the magnitude of amygdala activity in ASD. This amygdalar hyperresponsiveness to direct gaze has been suggested to represent a neural indicator for a heightened (and negatively valenced) emotional arousal triggered by eye contact. This is in accordance with our data and the suggested influence of avoidance: if directed to fixate the eyes versus the mouth, ASD participants showed increased amygdala activity compared with controls in the interaction contrast. The aversion then results in an avoidance of eye contact, as represented in the number of gazes away from the eyes. Of note, there are several studies showing amygdalar involvement during processing of aversive or threat related stimuli in general (Gallagher and Holland, 1994; Adolphs et al., 1995; LeDoux, 1996; Whalen, 2007). In light of these findings, increased amygdala activity in response to eye contact, as shown in the interaction contrast, together with the observed gaze patterns could be interpreted as reflecting an avoidance reaction to eye contact in ASD.

Another explanation for the reduced eye focus in ASD is diminished social attention (Grelotti et al., 2002; Schultz, 2005; Neumann et al., 2006). Thereby, ASD participants would fail to actively orient toward the eyes. Support for amygdalar involvement in the lack of attention toward the eyes comes from elegant studies in patients with amygdala lesions. These patients showed reduced reflexive orientation toward the eyes when looking at faces on photographs or videos (Adolphs et al., 2005; Adolphs, 2007; Spezio et al., 2007). Thus, if reduced orientation to the eyes would be mediated by the amygdala in autism, this should be represented in decreased activity. In fact, we found reduced amygdala activity in ASD compared with control participants when initially fixating the mouth, accompanied by reduced eye movements toward the eyes.

Amygdalar activation patterns in combination with the atypical gaze complement our behavioral findings as well: emotion recognition performance was particularly impaired in ASD participants when looking at the most discriminative regions for the respective emotion (eyes for fearful, mouth for happy faces). Atypical gaze and underlying amygdala activity may thus lead to reduced expertise in emotion recognition abilities in ASD.

In sum, the results provide new and important insights into aberrant amygdala functioning within social information processing in autism: the increase in amygdala activity triggered by the eyes along with previously reported increased gaze away from the eyes, supports the hypothesis of active avoidance of eye contact, modulated via amygdalar avoidance processing. The decrease in amygdala activity when starting gaze at the mouth further underlines amygdalar dysfunction within social saliency detection and orientation. ASD is, however, of multifactorial nature with interacting risk factors (e.g., genetic variants, epigenetic and environmental factors) producing the autistic phenotype and symptom heterogeneity (Abrahams and Geschwind, 2010; Scherer and Dawson, 2011). Variance within the autistic sample is thus very likely leading to pronounced avoidance of eye contact more than reduced orientation in some individuals or vice versa. We propose that both components may coexist, yet to varying intra- and interindividual degrees, as indicated in the eye preference index. However, the exact influence of avoidance and reduced orientation only in the ASD group cannot be disentangled here. The exact contribution should be specifically tested in fu-

ture studies, including measurements of arousal and anxiety. Along these lines, the integration of other types of data, such as genetic information, may help to better disentangle subtypes of (social) symptomatologies associated with different phenotypes (Yoshida et al., 2010; Chakrabarti and Baron-Cohen, 2011).

Both gaze and neuronal effects have been consistently reported in healthy samples. Thus the effects observed in the present study may partly be due to the greater homogeneity in individual gaze behavior and associated amygdalar response in NT, as compared with ASD samples. Although ASD participants showed increased amygdala activity for eyes > mouth trials (peak voxel: x: −16, y: −12, z: −14; z-max = 2.8), the respective cluster did not survive adequate cluster correction. Within group effects in ASD should be carefully addressed in future studies to further specify the mechanisms behind the reported group interactions.

Importantly, the amygdala is not a (cyto-)anatomically homogeneous structure, but consists of several subnuclei (Gloor, 1997; Freese and Amaral, 2009; Saygin et al., 2011) with specific connections to other brain regions (Price and Amaral, 1981; McDonald, 1992; Kleinhans et al., 2008; Dziobek et al., 2010), and presumably different functional profiles (Ball et al., 2007; Straube et al., 2008). Avoidance and orientation may therefore be modulated via distinct amygdalar subnuclei. Further, the functional specification of the subnuclei may be dysfunctional during the developmental trajectory in ASD. Current spatial resolution of functional images together with registration to standard brain templates, however, make it hard to functionally localize subnuclei activation patterns. Future research and advances in neuroimaging methods will help to further define (dys-)functions of amygdalar subnuclei.

Our data suggest a specific dysfunctional profile of the amygdala in autism: whereas the amygdala in controls accompanies social salience mediation (such as orientation toward eyes), this process seems to be dysfunctional in ASD. Orientation processing is not absent or replaced, instead, it seems to interact with aberrant aversion processing of eye contact. This interpretation further supports the emerging opinion that the amygdala is not the cause of the autistic pathophysiology but rather represents a dysfunctional node within the neuronal network underlying effective social functioning (Paul et al., 2010).

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