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Mirror images

Neural correlates of emotion processing in autism, schizophrenia, and mental health

Joanneke Bastiaansen
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Neural correlates of emotion processing in autism, schizophrenia, and mental health

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Joanneke Adriana Clasina Johanna Bastiaansen
geboren op 19 juni 1983
te Maastricht
Promotores: Prof. dr. C. Keysers
          Prof. dr. R.B. Minderaa

Beoordelingscommissie: Prof. dr. J.K. Buitelaar
                        Prof. dr. A. Klin
                        Prof. dr. D.H. Skuse

Wetenschap

Terwijl wij achter ons scherm fronzen
Een dik bekabelde walnoot met een bloemkooltje eronder aan
Componeert hij muziek op het ritmisch bonzen
Denkt zij aan het eten van vanavond (niet vergeten bij de slager langs te gaan)

In de spiegel verschijnt een jonge vrouw
Haar jukbeenspieren doen haar glimlach trillen
Hij kijkt anders naar haar, plichtsgetrouw
In onze korrelige foto's zoeken we de verschillen

Als hij zijn pyjama verwisseld heeft voor een winterse jas
En ze na een handdruk naar buiten stappen
Zet zij zich aan de boodschappen
en verstijft
In de wetenschap dat voor hem het leven blijft
zoals het al was

Wanneer ze met die gedachte afrekt
Vervolgt ze haar pad
In de goede hoop dat
Er misschien niet vandaag maar dan toch morgen
Een moeder zal zijn met minder zorgen
Voor wie ze wat hebben betekend
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General introduction
Displays of emotion are important elements of everyday social interactions. Trouble understanding the emotions of other people can lead to great difficulties in social functioning as can be seen in autism, but also in schizophrenia (Boraston, Blakemore, Chilvers, & Skuse, 2007; Chan, Li, Cheung, & Gong, 2010; Harms, Martin, & Wallace, 2010; Kee, Green, Mintz, & Brekke, 2003; Law Smith, Montagne, Perrett, Gill, & Gallagher, 2010). Emotion perception involves a complex interplay between various brain regions that is still poorly understood. In this thesis, we will examine the neural underpinnings of emotion processing with an emphasis on simulation or mirror mechanisms. We will study autism in combination with schizophrenia to gain more insight into the similarities and differences between these patient groups in terms of (social) behavior, social functioning, social cognition, and neural responses during emotion perception. Combined study of these patient groups will provide more information about the underlying neural mechanisms of social (cognitive) dysfunction and give more insight into what makes each disorder unique. These insights could provide important pointers for the development of more effective therapies. In this general introduction, we will provide the reader with the necessary background information to set the stage for the subsequent chapters.

The social arena

Human beings are intrinsically social. We are not the fastest species, nor the strongest, but we have survived because we know how to cooperate to fulfill our needs and those of our conspecifics. The demands placed on our social abilities have increased tremendously since the days of group hunting. Nowadays we are generally part of societies that are governed by complex social rules and cultural values. To navigate smoothly through this environment, we need social cognitive skills that help us manage social relationships effectively (Burns, 2006). These skills are studied in the field of social cognition, which refers to the domain of cognition that involves the perception, interpretation and processing of social information or phrased more simply: to the way we make sense of other people and ourselves (Fiske & Taylor, 1991).

A fundamental part of social cognition is the ability to understand the emotions of other people (for a scientific definition of emotion see textbook 1.1). When a person storms up at us we want to know, for instance, whether that person is angry or happy in order to respond adaptively; should we open our arms to embrace the person or use our arms to cover our face? Bodily expressions, and facial expressions in particular, provide essential information about another person’s emotional state (Ekman, Friesen, & Ancoli, 1980). Through transient changes in the configuration of facial muscles, humans both consciously and unconsciously transmit messages that can help others grasp what is going on in their minds and in their environment (Russell, Bachorowski, & Fernandez-Dols, 2003). This information is essential for maintaining good interpersonal relationships and for learning about, for instance, potential dangers in our environment (Darwin, 1998/1872; Ekman, 1992a; Ekman et al., 1980). Due to the central role of emotional signals in communicating to and influencing others, difficulties in emotion processing can lead to severe impairments in social functioning (Feldman, Philippot, & Custrini, 1991).
1.1 What is an emotion?
During the course of a normal day many of us experience a multitude of emotions, ranging from very basic ones such as happiness and disgust to complex emotions such as guilt (Ekman, 1992a). Emotions color our lives, which makes them most interesting to study. Their scientific scrutiny is, however, complicated by difficulties in defining what an emotion actually entails. In everyday conversations, emotions are often equated with feelings: we feel angry at the person cutting in line in front of us. However, emotions are not mere subjective experiences or intangible mental qualities (Damasio, 1994): when we are angry we can often feel our temperature and heartbeat rise and we might experience a tendency to approach the other person to confront him with his behavior. In addition, we probably (unconsciously) express our feeling of anger by contracting our facial muscles into a frown or by clenching our fists. This example illustrates that the domain of emotion not only comprises subjective experiences, but also expressive reactions of the face and the body, physiological reactions, and (tendencies towards) instrumental and coping behavior (Cornelius, 1996). Thus, emotions are not single mental entities, but complex psychophysiological experiences that can be studied from many different perspectives, including neurobiology.

Simulation theory of social cognition
Historically, theories of social cognition have emphasized theoretical processes involved in identifying the mental states of others. These Theory theories suggest that we attribute a mental state to another individual based on deliberate explicit inferences about that person's behavior, environment, and other mental states (Gallese & Goldman, 1998; Goldman & Sripada, 2005). Imagine there is a lady sitting in front of you, who bites into her sandwich and shows the most horrifying grimace. According to Theory theory, you would process many percepts of this scene: the sandwich with perhaps a tiny maggot peaking out, a foul smell, the woman's wrinkled face upon ingestion, and the sound of her gagging. You would then integrate these elements and use your prior knowledge about the meaning of facial expressions, the hygiene in the restaurant and so forth to infer that this woman is experiencing disgust. Cognitive elaborations on sensory representations are an important tool in social cognition, but can only provide a detached account of the experiences of others, while we often have instantaneous 'gut' feelings of what is going on in other individuals (Adolphs, Damasio, Tranel, Cooper, & Damasio, 2000; Adolphs, Tranel, & Damasio, 2003). These gut feelings are better accounted for by Simulation theory.

Simulation theories claim that humans (also) use their personal mental mechanism to come to an understanding of the mental life of another individual (Goldman & Sripada, 2005; Keysers & Gazzola, 2006). Here, simulation does not refer to mentalizing by imagining oneself in the same situation as a target. Rather, it refers to an unconscious process by which we directly link first-person (I act) and third-person (he/she acts) experiences, thereby creating a meaningful interpersonal space (Gallese, 2003). As our brain-body system is similar to that of other people, the linkage between the first and third person experiences of actions, sensations and emotions may be established by embodied simulation (Gallese, Keysers, & Rizzolatti, 2004; Keysers & Gazzola, 2006). Embodied simulation is a form of neural processing of social information that involves activating neural states during observation -in representation codes that are specific to the body- that match those that the observer would experience in a similar situation. Motor simulation, for instance, occurs when seeing other people's actions activates a pattern of motor activity in the observer that
corresponds to the pattern when the observer would perform the same action.

The entwining of action and perception is evident at the behavioral level: the execution of an action can be facilitated by simultaneously watching an action that is similar, and hindered by watching dissimilar actions (Brass, Bekkering, Wohlschlager, & Prinz, 2000; Craighero, Bello, Fadiga, & Rizzolatti, 2002). When seeing emotions, people (unconsciously) mimic the emotional expressions they see in a muscle-specific manner; a process referred to as facial mimicry (Dimberg, Thunberg, & Elmehed, 2000). Seeing a smiling face, for instance, triggers activity in the zygomaticus major muscles that make our mouth corners curl upwards. Besides these motor responses, seeing emotions in another person can also cause the emotional state to (unconsciously) spill over to the observer; a process named emotional contagion (Wild, Erb, & Bartels, 2001). A brief look at the woman’s horrified face might, for instance, make you feel nauseous yourself, which helps you realize what she is going through in an instant. The motor simulation of other people’s facial configuration seems to interact with these affective processes. Adopting emotion-specific postures can, for instance, trigger the corresponding emotion (Ekman, 1992b; Strack, Martin, & Stepper, 1988). Additionally, motor interference can modify the subjective experience of observed emotions (Effron, Niedenthal, Gil, & Droit-Volet, 2006). Several studies demonstrate that congruent mimicry of facial expressions can facilitate emotion recognition, while blocking facial movements can interfere with it (Niedenthal, 2007; Niedenthal, Brauer, Halberstadt, & Innes-Ker, 2001; Oberman, Winkielman, & Ramachandran, 2007). Thus, motor simulation may play a role in emotional contagion and emotion understanding. Facial mimicry and emotional contagion both involve an automatic coupling of information from the self and the other, which already occurs early in life (Hatfield, Cacioppo, & Rapson, 1993; Hess, Philippot, & Blairy, 1999). This direct link between self and other could facilitate attachment, provide a source of information on another person’s emotional state, and help establish empathy.

**Mirror mechanisms**

Simulation theories were greatly stimulated by the discovery of mirror neurons in premotor (F5) and inferior parietal (PF) regions of the macaque monkey’s brain. Single-cell recordings demonstrated that these motor neurons have a special property; they not only respond to the execution of hand-object interactions, but also to the sight of similar actions (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; Fogassi et al., 2005; Fujii, Hihara, & Iriki, 2008; Gallese, Fadiga, Fogassi, & Rizzolatti, 1996). A subset of ventral premotor neurons triggering mouth actions also fire to the observation of similar mouth actions, including communicative gestures (Ferrari, Gallese, Rizzolatti, & Fogassi, 2003). The discovery of this *mirroring* property provided neurobiological support for common coding theory, which challenges the distinction between action and perception, and inspired the core idea of embodied simulation theory, namely that our own motor programs may play a role in understanding the actions performed by others (Prinz, 1990; Rizzolatti, Fogassi, & Gallese, 2001). Single-cell (Mukamel, Ekstrom, Kaplan, Iacoboni, & Fried), fMRI (Buccino et al., 2001; Filimon, Nelson, Hagler, & Sereno, 2007; Gazzola, Rizzolatti, Wicker, & Keysers, 2007; Grézes, Armony, Rowe, & Passingham, 2003) and TMS (Avenanti, Bolognini, Maravita, & Aglioti, 2007) studies show that a similar system involving the premotor and posterior parietal cortex exists in humans: the **Mirror Neuron System** (MNS). The facilitation of the execution of actions by the sight of similar
actions (Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995) is, for instance, impaired when the premotor cortex is transiently impaired by magnetic stimulation (Avenanti et al., 2007). The motor simulation mechanism implemented in the human MNS may contribute to understanding the intentions behind the actions of others (Rizzolatti & Craighero, 2004).

In addition to an entwining of action perception and execution in the premotor and parietal cortex, there is evidence for a shared circuit in the somatosensory cortex that maps the perception and experience of tactile sensations (Blakemore, Bristow, Bird, Frith, & Ward, 2005; Bufalari, Aprile, Avenanti, Di Russo, & Aglioti, 2007; Keysers et al., 2004). Recently it has been proposed that beyond actions and tactile perceptions, our brain also readily simulates the emotions of others (Decety & Jackson, 2004; Keysers & Gazzola, 2006; Niedenthal, 2007). As further detailed in textbox 1.2, emotions have a complex neurobiological basis. Therefore, simulation of other’s emotions likely involves many different regions. Emotional states often become visible to others through motor acts, namely our bodily and in particular our facial expressions. Recent studies show that the MNS is indeed also activated when humans perceive these facial actions of others (Carr, Iacoboni, Dubeau, Mazziotta, & Lenzi, 2003; Hennenlotter et al., 2005; Leslie, Johnson-Frey, & Grafton, 2004; van der Gaag, Minderaa, & Keysers, 2007; Wicker et al., 2003; Wild, Erb, Eyb, Bartels, & Grodd, 2003). Interestingly, activity in the frontal part of the MNS, in pars opercularis of the inferior frontal gyrus, is associated with the participant’s tendency to empathize with other individuals (Gazzola, Aziz-Zadeh, & Keysers, 2006; Jabbi et al., 2007; Pfeifer, Iacoboni, Mazziotta, & Dapretto, 2008; Saarela et al., 2007; Schulte-Rüther, Markowitsch, Fink, & Piefke, 2007). In addition, lesions to this area are associated with deficits in emotional empathy and emotion recognition (Shamay-Tsoory, Aharon-Peretz, & Perry, 2009). This suggests that motor simulation of facial expressions may help decode the internal state of another person and provide a means to empathize with other people. In chapter 2, we further investigate the role of motor simulation and examine whether during emotion perception somatosensory and affective aspects of other’s emotions are also mirrored.

Due to the alleged role of the MNS in emotion recognition, empathy, and other social skills, such as imitation (Iacoboni et al., 1999) and joint action (Kokal, Gazzola, & Keysers, 2009), various researchers have suggested that a dysfunctional MNS is at the heart of social impairments in autism (Iacoboni & Dapretto, 2006; Oberman & Ramachandran, 2007; Rizzolatti & Fabbri-Destro, 2008; Rizzolatti, Fabbri-Destro, & Cattaneo, 2009; Williams, Whiten, Suddendorf, & Perrett, 2001). The mirror neuron hypothesis of autism is grounded in the idea that mirror neurons are involved in the formation of self-other representations, which provide the basis for low level imitation and goal understanding, from which cognitively more sophisticated processes such as the ability to understand other people’s state of mind can develop. Therefore, early developmental impairments in the MNS of children with autism may lead to a cascade of impairments ranging from impaired imitation and goal inference to poor mentalizing and other social abilities. The mirror neuron theory of autism has an intuitive appeal, because the alleged functions of the MNS seem to correspond with the social cognitive deficits seen in autism. We will investigate the role of the MNS in autism in chapters 4 and 5 of this thesis.
1.2 The neurobiology of emotion

Early attempts to identify and understand the neural systems underlying emotion searched for one specific circuit accommodating emotions, “The Emotional Brain”, which resulted in the concept of the limbic system (MacLean, 1949). Although the concept has been widely used in scientific research, it has not been supported by empirical facts (Kotter & Meyer, 1992). Classic limbic areas such as the cingulate gyrus and amygdala have indeed been proven important for emotional processes, but they are also important for non-emotional processes (Devinsky, Morrell, & Vogt, 1995; LeDoux, 2007). Additionally, non-limbic regions such as the insular and somatosensory cortices fulfil important emotional functions as well (Damasio, 1994). In effect, emotions have a multifaceted neurobiological basis. One aspect is the production of affective states (e.g. the subjective experience of feeling happy), which involves structures such as the amygdala and anterior insula (Phillips, Drevets, Rauch, & Lane, 2003a). Another aspect constitutes the bodily responses in reaction to an emotional event, which are continuously represented in the somatosensory cortices (Damasio, 1994). Furthermore, the striatum may, in concert with other areas, serve to coordinate motor responses to emotive stimuli in order to guide the organism towards a desired goal (e.g. approach or withdrawal, Phan, Wager, Taylor, & Liberzon, 2002). Expressive reactions of the face are represented in two major adjacent ventrolateral frontal areas (i.e. primary motor cortex, M1, ventral premotor cortex, vPMC), the medial Supplementary Motor Area (SMA) and the cingulate motor cortices (Morecraft, Stilwell-Morecraft, & Rossing, 2004). The precise contribution of the participating areas in emotion experience and the way these widely distributed networks interact is still largely unknown. What we know is that the experience of emotions entails a complex interplay between regions supporting different functions including affective, somatosensory and motor processes.

**Autism, Schizophrenia, and Social Dysfunction**

**Autism Spectrum Disorder**

Autism is a severe and lifelong neurodevelopmental disorder, which already manifests itself in the first three years of life by fundamental impairments in reciprocal social interaction and communication, and by stereotyped behaviors and interests (American Psychiatric Association, APA, 2000). According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR), autism is the core syndrome of the Pervasive Developmental Disorders (PDD), which also include Asperger’s syndrome, PDD-Not Otherwise Specified (PDD-NOS), Childhood Disintegrative Disorder, and Rett’s Disorder. The latter two disorders are less prevalent and presumably of different etiology. Prevalence of all PDDs is estimated to be 60 per 10,000 with an elevated incidence rate in boys compared to girls (4:1, Fombonne, 2005). Because autistic disorders are defined by a common set of behaviors, recent developments of the DSM head towards eliminating separate categories and considering autism as a single spectrum disorder (www.dsm5.org). In this thesis, we will use **Autism Spectrum Disorder (ASD)** as an umbrella term to refer to autism, Asperger’s syndrome, as well as PDD-NOS. In some cases we will abbreviate this to autism (e.g. title of this thesis). Our focus is on high-functioning individuals; individuals with average or above-average general abilities (i.e. IQ).

The clinical presentation of ASD is heterogeneous depending on clinical specifiers (e.g. severity), associated features (e.g. intellectual disability), and comorbid conditions (e.g. depression). Besides this heterogeneity, impaired social functioning is central to all forms of ASD and persists across the lifespan (Shattuck et al., 2007). Even the most high-functioning adults with
ASD experience difficulties in social interaction and participate less in various social arenas such as work and (social) recreation (Howlin, Mawhood, & Rutter, 2000). These impairments are not unique to ASD, but also exist in other psychiatric disorders such as schizophrenia.

Schizophrenia

Schizophrenia is a severe neurodevelopmental disorder with great diversity in symptoms. It typically develops in adolescence and early adulthood, with half to one percent of the population affected and a higher prevalence in men (McGrath et al., 2004). Schizophrenia, which literally means "split mind", is often confused by the general public with the presence of multiple personalities (i.e. dissociative identity disorder). In truth, split mind refers to the dissociation from reality that is typical of the disorder. During psychotic episodes, individuals may display disorganized speech or behaviors, may have unfounded and irrational beliefs (e.g. delusions of persecution), or experience sensory perceptions of things that are not present (e.g. auditory hallucinations of voices). These distortions of normal functions are commonly referred to as positive symptoms. Negative symptoms of schizophrenia entail a loss or absence of normal traits or abilities, such as affective flattening, inability to experience pleasure (anhedonia), lack of motivation (avolition), and a lack of desire to form relationships (asociality). Eugen Bleuler (1911), who coined the term schizophrenia, thought that these latter symptoms are even more fundamental to the disorder than the psychotic symptoms, but current diagnostic manuals give equal weight to both types of symptoms. Schizophrenia is a mixture of symptoms that can manifest itself in many ways, but commonly involves social and occupational dysfunction (DSM-IV-TR, APA, 2000). Disturbances in social interaction are already seen at the prepsychotic phase of the illness, suggesting that they are not simply the consequence of the social isolation and hospitalization that individuals with schizophrenia face (Abdi & Sharma, 2004; Addington, Penn, Woods, Addington, & Perkins, 2008a; Bearden et al., 2000).

Where ASD and schizophrenia meet

As previously discussed, ASD and schizophrenia both have unique features and their own developmental course, but severe difficulties in social functioning exist in both (DSM-IV-TR, 2000; Frith, 2003; Goldstein, Minshew, Allen, & Seaton, 2002; Kanner, 1943). Social behaviors of individuals with schizophrenia can resemble autistic symptoms, especially when psychotic symptoms are in remission and negative symptoms become more prominent (Frith, 2003; Sheitman, Kraus, Bodfish, & Carmel, 2004). Common to both is a withdrawal from social contact, which is illustrated by the origin of the term autism: to first describe the developmental disorder, Kanner (1943) borrowed the term autism from Eugen Bleuler (1911), who used it to describe one of the negative symptoms associated with schizophrenia: a withdrawal from contact with the outside world. Due to behavioral similarities between autistic and negative symptoms, a co-diagnosis of autism and schizophrenia can only be established when prominent hallucinations or delusions occur in the presence of a pervasive developmental disorder (DSM-IV-TR, APA, 2000). The presence of negative symptoms does not suffice.

In this thesis, we will focus on the two disorders when they are most similar. We recruit
high-functioning adult men with ASD and non-psychotic adult men with schizophrenia with (mild) negative symptomatology in a similar age and IQ range. We verify clinical diagnoses in the ASD group with the Autism Diagnostic Observation Schedule (ADOS), one of the golden standards for diagnostics in autism research (Lord et al., 2000). The ADOS is a standardized instrument that assesses social interaction, communication, and imagination during a semi-structured interaction with an examiner. To investigate whether our patient groups indeed show similar social behaviors, we apply the ADOS to the schizophrenia group as well. Of particular interest is whether this observational instrument can distinguish ASD from schizophrenia and whether there is an association of autistic symptoms as measured by the ADOS with negative symptomatology in the schizophrenia group (chapter 6). To see whether the patient groups resemble each other in terms of social functioning, we administer the Social Functioning Scale (SFS, Birchwood, Smith, Cochrane, Wetton, & Copestake, 1990, see chapters 4 and 7). The SFS taps those areas that are crucial to community maintenance (e.g. employment, pro-social activities) and is specifically designed for people with known social difficulties.

ASD and schizophrenia can resemble each other in terms of social behavior, but also in terms of the underlying social cognitive skills. In fact, in both disorders social dysfunction is related to impairments in social cognitive skills such as difficulties in recognizing other’s emotions (Couture, Penn, & Roberts, 2006; Fett et al., 2011; Hughes, Soares-Boucaud, Hochmann, & Frith, 1997; Kee et al., 2003; Klin, Jones, Schultz, Volkmar, & Cohen, 2002). Important for our study is that social cognitive deficits in schizophrenia may be more severe and resemble the deficits in ASD more when negative symptoms are prominent (Couture et al., 2010; Edwards, Jackson, & Pattison, 2002; Frith, 1994; Kohler et al., 2003; Schneider, Gur, Gur, & Shtasel, 1995; van ‘t Wout et al., 2007). To investigate whether our patient groups resemble each other in terms of social cognition (chapter 7), we administer the Ekman 60 Faces Test (FEEST), a measure of emotion recognition in which the subject has to indicate which of six named basic emotions was expressed in pictures of actors displaying prototypical emotional expressions (Young, Perrett, Calder, Sprengelmeyer, & Ekman, 2002). Additionally, we use the Interpersonal Reactivity Index (IRI, Davis, 1983) as a measure of empathy.

**Studying Neural Correlates of Emotion Processing**

In both schizophrenia and ASD abnormalities have been found in brain regions associated with a deficit in emotion processing. Structural and functional abnormalities have been found, for instance, in regions involved in face processing such as the fusiform gyrus (Abdi & Sharma, 2004) and in regions important for the coding of the emotional significance of the stimulus such as the amygdala, anterior insula, and ventral striatum (Phillips, Drevets, Rauch, & Lane, 2003b). The MNS has also been implicated in both disorders (Gallese, 2003; Salvatore, Dimaggio, & Lysaker, 2007). Although social difficulties overlap in autism and schizophrenia and similar brain regions have been implicated, the two disorders have mainly been studied separately. A combined study will provide more information about the underlying neural mechanisms of social dysfunction and give insight into what makes each disorder unique. To study in vivo how the brain processes information, we will use functional Magnetic Resonance Imaging (fMRI).
Chapter 1

Magnetic Resonance Imaging

MRI is an example of a non-invasive technique that allows researchers to study the living human brain. An MRI scanner can be found in any modern medical hospital and is used to visualize various inner parts of the human body. An MRI scanner can, for instance, create high-resolution pictures of the knee’s bones, tendons, and menisci, but is also perfectly suited to create structural images of the human brain by providing sharp contrasts between the different brain tissues. The technique cleverly takes advantage of the fact that the human body is mainly composed of water molecules. A powerful magnetic field aligns these hydrogen atoms and when this field is perturbed by radio frequencies, the cores of the atoms generate a rotating magnetic field that is detectable by the scanner. Different manipulations of the magnetic field can provide researchers and clinicians with different kinds of information. Important to the field of neuroscience, an MRI scanner not only allows the visualization of brain structures, but also provides the opportunity to study ongoing neural processes. Functional MRI measures neural activity through changes in the MR signal caused by so-called blood-oxygen-level dependent (BOLD) effects. An active brain region consumes oxygen, which leads to an increase in deoxygenated hemoglobin. This would have diminished the MR signal from that region, were it not for the vascular system that responds by sending a disproportionate amount of oxygenated hemoglobin. Because the amount of oxygenated hemoglobin outnumbers the deoxygenated hemoglobin, the MR signal increases in active regions. This information is used to create images that reflect neural activity. MRI is a wonderful technique to study the structure and the workings of the human brain, because it is harmless for the participant, has good spatial resolution, and can create an image of the brain once every 1-3 seconds. The technique, however, also poses some constraints. MRI scans, for instance, have a low signal-to-noise ratio, which means images can appear grainy. Many repetitions of a certain task and as many participants as possible are needed to increase the signal intensity relative to the noise in order to obtain enough power to detect effects. Another constraint is that a subject needs to lay as still as possible in the tube of the scanner to obtain clear images. These factors play an important role when designing fMRI experiments in general and experiments on emotion processing in particular.

Main Paradigm

In this thesis, we will study both motor mirror mechanisms and affective mirror mechanisms. The experimental study of motor mirror mechanisms in emotions requires a condition in which subjects perceive facial expressions and generate facial expressions themselves (chapter 4). The study of affective mirror mechanisms or shared circuits requires a condition in which subjects experience emotions themselves in addition to a condition in which subjects perceive emotions (chapter 3, 4, 7). Emotion perception can be relatively easily studied inside the MRI environment. The most common method is to present pictures of emotional facial expressions, which the subjects then have to label. The emotional expressions we are confronted with in daily life are, however, dynamic and usually do not come with an explicit task. As a first step in the direction of more naturalistic paradigms, we use passive observation of movies of emotional facial expressions throughout this thesis (chapters 3, 4, 7). Inducing emotions is a more daunting task, which is complicated by ethical considerations (imagine inducing fear) and the constraints imposed by the MRI environment (e.g. restricted motion, need for repetitions). In this thesis, we have chosen disgust for the emotion
experience condition, because it can be triggered in a reliable and repeatable way that is ethically sound through the administration of unpleasant flavors (chapters 3, 4, 7) and the use of scripts (chapter 3).

**Thesis outline**

This thesis examines the neural underpinnings of emotion processing with an emphasis on simulation or mirror mechanisms. In *chapter 2* we will first review studies to investigate whether the observation of emotions directly triggers activation of matching neural substrates in the observer that extend beyond the original MNS as defined for actions into regions involved in somatosensory and affective processes. In *chapter 3* we dive deeper into the emotion disgust to examine whether social perception and mental imagery of disgust share common neural substrates and what functional circuits are involved in typically developing subjects. In chapters 4 and 5 we turn to the role of motor simulation in the MNS in autism. Hypoactivation of the inferior frontal gyrus during the perception of emotional facial expressions has been interpreted as evidence for a deficit of the MNS in children with autism. *Chapter 4* examines whether this dysfunction persists in adulthood, and how brain activity in the MNS relates to social functioning outside the laboratory. In *chapter 5* we review evidence from various studies to evaluate the claim that ASD may result from an impairment of the MNS. Social abnormalities are not unique to ASD, but also occur in schizophrenia. In *chapter 6* we investigate whether adults with ASD can be distinguished from individuals with schizophrenia on the basis of behavioral observation. Similarities between ASD and schizophrenia in their social profile raise the question in what aspects neural impairments are similar and different in these two disorders. Therefore, we compare the findings on the link between the MNS and social functioning in ASD to individuals with schizophrenia that have comparable social deficits in the second part of chapter 4. In *chapter 7* we compare the groups on measures of social cognition and use a whole-brain approach to examine similarities and differences in their neural signature during emotion perception. *Chapter 8* concludes this thesis with a summary of the chapters and a general discussion.
Mirror mechanisms in emotion processing
Abstract

Why do we feel tears well up when we see a loved one cry? Why do we wince when we see other people hurt themselves? This review addresses these questions from the perspective of embodied simulation: observing the actions and tactile sensations of others activates premotor, posterior parietal and somatosensory regions in the brain of the observer which are also active when performing similar movements and feeling similar sensations. We will show that seeing the emotions of others also recruits regions involved in experiencing similar emotions, although there does not seem to be a reliable mapping of particular emotions onto particular brain regions. Instead, emotion simulation seems to involve a mosaic of affective, motor and somatosensory components. The relative contributions of these components to a particular emotion and their interrelationship are largely unknown, although recent experimental evidence suggests that motor simulation may be a trigger for the simulation of associated feeling states. This mosaic of simulations may be necessary for generating the compelling insights we have into the feelings of others. Through their integration with, and modulation by, higher cognitive functions, they could be at the core of important social functions, including empathy, mind reading and social learning.
2.1 Introduction

Humans have an astonishing capacity to intuitively grasp the mental states of other individuals. If we see someone bite into his sandwich and show a horrified grimace, we do not have to chew over what happened to sense that he is not enjoying his meal. In fact, just the sight of his disgust might cause our own stomachs to turn and prevent us from eating our own sandwich. Although people’s more subtle emotions can remain puzzling, we often have gut feelings of what is going on in other individuals. Various researchers have suggested under different designations that direct simulation (see glossary) of observed social events through mirror-like mechanisms are at the heart of this experiential understanding of others: the shared-manifold hypothesis (Gallese, 2003); unmediated resonance model (A. I. Goldman & Sripada, 2005); shared circuits (Keysers & Gazzola, 2006); direct-matching hypothesis (Rizzolatti, Fogassi, & Gallese, 2001) and hot hypothesis (Wicker et al., 2003). The basic tenet of these models is that observation of an action in another individual directly triggers activation of matching neural substrates in the observer, through which the action can be understood. While some researchers focus on the role of motor areas in social cognition (e.g. motor theory of social cognition, Jacob & Jeannerod, 2005) others see embodied simulation as a general and basic endowment of our brain that involves a linkage between the first and third person experiences of actions, sensations and emotions (Keysers & Gazzola, 2006).

2.1.1 Sharing actions in the premotor and parietal cortex

Simulation theories were greatly stimulated by the study of action execution and action observation in monkeys. Two reciprocally connected areas, namely area F5 in the ventral premotor cortex and the parietal area PF, were found to contain individual neurons that respond both to the execution of hand-object interactions and the sight of similar actions (see Keysers et al., 2004; Rizzolatti & Craighero, 2004 for reviews). Owing to their common role in first (I grasp) and third person (He grasps) perspectives, these neurons were named ‘mirror neurons’. Linking what the monkey sees people do to what it itself does might provide it with an intuitive insight into the actions of others. Given their properties, mirror neurons seem particularly well-suited to providing insights into the actions of conspecifics (Gallese, Fadiga, Fogassi, & Rizzolatti, 1996; Kohler et al., 2002; Rizzolatti, Fadiga, Gallese, & Fogassi, 1996; Rizzolatti et al., 2001; Umiltà et al., 2001). In recent years, evidence has accumulated for the existence of a mirror neuron system (MNS) for actions in humans (see Figure 1a). Arguably the most convincing evidence comes from functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation (TMS) studies. FMRI indirectly measures brain activity by estimating the level of blood oxygenation in cubes of brain tissue named voxels, whereas TMS uses magnetic stimulation to either stimulate or transiently impair a cortical region. FMRI shows that the ventral premotor cortex (BA44/6), inferior parietal lobe (IPL) as well as somatosensory areas (BA2 in particular) involved in executing the actions become reactivated while subjects view or hear similar actions performed by others (Buccino et al., 2004; Gazzola et al., 2006; Gazzola, Rizzolatti, Wicker, & Keysers, 2007; Grèzes, Armony, Rowe, & Passingham, 2003). Finding the same voxel involved in execution and perception, however, cannot ensure that the same neurons within the voxel (which is usually around 3 x 3 x 3 mm in size) are involved in both cases (Dinstein, Thomas, Behrmann, & Heeger, 2008). Various researchers are now taking on the challenge.
to create experimental fMRI designs that can better address the neural response selectivity in the MNS than the usual movement observation and imitation protocols (Dinstein, Hasson, Rubin, & Heeger, 2007). In contrast, TMS experiments show that observing the actions of others specifically facilitates the execution of similar actions (Fadiga et al., 1995) and that applying repetitive TMS on the premotor or somatosensory cortex impairs this motor facilitation (Avenanti et al., 2007; Catmur, Walsh, & Heyes, 2009). This demonstrates both that the vision of an action directly activates motor programmes for executing similar actions and that the link between vision and action occurs in the somatosensory and premotor areas identified by the fMRI experiments. This suggests that the MNS is indeed where perception meets action in the brain.

The possible roles of the MNS are still a matter of debate. Many would agree that mirror neurons are well-positioned to support understanding what action another individual is performing and how it is being performed (Thioux, Gazzola, & Keysers, 2008). Anyone witnessing a person’s hand reach for an orange on a table will instantaneously the goal of the action: reaching to grasp. This mirroring seems to occur primarily at the level of motor goals. For example, aphasic subjects born without hands and arms activate foot and mouth representations when they observe hand actions they would perform with these effectors (Gazzola et al., 2007). Indeed, the disruption of putative mirror neuron areas in humans either through lesions (Pazzaglia, Pizzamiglio, Pes, & Aglioti, 2008; Pazzaglia, Smania, Corato, & Aglioti, 2008) or repetitive TMS (Pobric & Hamilton, 2006) impairs the capacity of subjects to process the actions of others (see, however, Bell, 1994; Halsband et al., 2001; Moro et al., 2008). A second important function attributed to the MNS in humans is imitation. This idea is supported by observations that a portion of the mirror neuron system (in pars opercularis of the inferior frontal gyrus) is more active during imitation than the sum of activity during execution and observation (see Catmur et al., 2009 for a discussion; Iacoboni et al., 1999). A third function attributed to the MNS is that of empathizing with others, based on the finding that subjects that score higher on a questionnaire measuring their tendency to place themselves in the other person’s shoes activate their MNS more strongly while hearing the actions of others (Gazzola et al., 2006).

\[\text{Figure 1} \text{ Anatomical location of the motor and somatosensory components of simulation.}\]

(a) Lateral view of the human brain with the location of the ventral premotor cortex (BA6/BA44) and the inferior parietal lobule (IPL). (b) Lateral view showing the location of the primary and secondary somatosensory cortex (SI/SII).
2.1.2 Sharing sensations in the somatosensory cortex

In addition to a shared circuit for actions in the premotor and parietal cortex, there is evidence for a shared circuit in the somatosensory cortex that maps the perception and experience of tactile sensations. Keysers et al. (2004) showed their subjects movies of someone else’s legs being touched with a stick. The same subjects were later touched on their own legs to localize their primary and secondary somatosensory cortices (SI/SII, see Figure 1b). Part of SII that was active while the subjects felt touch on their own body became reactivated while viewing someone else being touched in similar ways. Activations in SI during touch observation were present, but were substantially weaker. Blakemore et al. (2005) found that the observation of touch was associated with an activity in SI that was somatotopically organized: different regions of SI reacted to the observation of someone being touched on the neck and the face. In the same experiment, the authors found that a subject with vision-touch synaesthesia, who reported vivid tactile QUALIA while seeing the tactile sensations of others, had increased activity in SI during the vision of touch. Electroencephalography (EEG) studies allow for a temporally precise measurement of components of electrical activity in the brain. Recently, an elegant EEG study by Bufalari and co-workers (2007a) showed that in non-synaesthetes electrical activity in response to stimulation of the hand can be modulated by the sight of someone else being touched. The latency of this component (45 ms) suggests that within the mosaic of brain areas composing SI, those receiving direct thalamic input (BA3) are only active while experiencing touch on one’s own body. The second stage of processing (BA1/2) is modulated by perceiving other people’s experiences: stronger activity in these regions predicts how intense observers judge the sensations of others. Together, these studies suggest that under normal circumstances, the earliest stages of cortical somatosensory processing (BA3) remain private, that is reserved for our own tactile sensations, while later stages (BA1/2 and SII) can serve to vicariously share the tactile sensations of others. Individuals with synaesthesia show that if the earliest stages are activated more strongly, the observer will experience touching of others literally as if being touched herself. Similarly, while the MNS codes for both the execution and the perception of actions, the primary motor cortex (MI) is usually not active while viewing the actions of others (Gazzola & Keysers, 2009). The absence of vicarious MI activity is quite natural as observers do not normally move while viewing the actions of others. The existence of patients that cannot refrain from overtly imitating behaviors they observe (Lhermitte, Pillon, & Serdaru, 1986) suggests that active inhibition is responsible for blocking the outflow of activity from premotor regions to MI (Gazzola & Keysers, 2009; Preston & de Waal, 2002). These mechanisms could help us distinguish our own actions and sensations from those of others which are shared in our (pre)motor and somatosensory regions.

In summary, primates readily activate premotor and parietal cortical areas involved in action execution when they see someone perform a goal-directed action. This simulation might be useful for understanding the action and its goal, and enable a more or less automatic imitation of someone’s actions. There is mounting evidence to suggest that a similar neural mechanism involved in action imitation may also apply to the domain of sensations. Recently it has been proposed that beyond actions and tactile perceptions, our brain also readily simulates the emotions of others (Decety & Jackson, 2004; Keysers & Gazzola, 2006; Niedenthal, 2007). Although this phenomenon seems superficially different from motor imitation, similarity in the neural processes suggests they are deeply related. This review examines the evidence for the presence of mirror mechanisms in
sharing the emotions of other individuals.

2.2 Affective sharing of emotions

One of the key challenges in studying the sharing of emotions using neuroscientific methods is being able to trigger the emotion. Although this is generally difficult, there are some exceptions such as disgust and pain. Therefore we will focus on the affective sharing of these particular emotions in the following section. In the case of pain, we will illustrate that emotion simulation not only involves an affective component (i.e. concerning sensations of pleasure and displeasure), but also a motor and sensory component, which will be addressed in the subsequent section. The interaction between these components is largely unknown, but as we will see in a subsequent section, recent experimental evidence suggests one potential route of communication from motor to affective mirror systems. The term mirror system implies that there is a certain degree of specificity: we map what we see on our own neural substrates for that specific action, sensation or emotion. We will use the emotion fear to show that there is little evidence for a consistent mapping of particular emotions on particular brain regions. Instead, different networks seem to be involved dependent on the process by which the emotion is accessed. In addition, the activation strengths of the different components are likely to be related to the quality of the emotion and its associated output. In the concluding comments, we will discuss the various functions that emotion simulation could subserve through its integration with, and modulation by, higher cognitive functions.

2.2.1 Sharing of disgust

Disgust is closely related to the phylogenetically primitive sensation of distaste. In its most basic form, from which more developed forms such as moral disgust may have evolved, it involves an oral defence to potentially harmful foods and body products (Haidt, Rozin, McCauley, & Imada, 1997; Rozin, Haidt, & McCauley, 2000). This makes disgust relatively easy to trigger repeatedly using aversive tastes and odours. The primary experience of taste and distaste can be located in the transition zone between the anterior part of the insular cortex together with the frontal opercular taste cortex (Small et al., 1999; Yaxley, Rolls, & Sienkiewicz, 1990), a region we refer to as the IFO. The experience of unpleasant odours triggers activity in a similar region (Royer, Plailly, Delon-Martin, Kareken, & Segebarth, 2003). Through its numerous connections to structures such as the orbitofrontal cortex (OFC), frontal operculum, anterior cingulate cortex (ACC), lateral premotor cortex, basal ganglia, temporal lobe and amygdala, the insula (see Figure 2b) can anatomically fulfil the requirements for associating offensive tastes and smells with other people’s expressions of disgust (Augustine, 1996). This is supported by the finding of distinct electrophysiological responses in the anterior insula to facial expressions of disgust in the observer (Krolak-Salmon et al., 2003).

A seminal study in 2003 established a functional co-dependence of disgust experience and perception on the IFO. To reliably induce disgust, Wicker and colleagues (2003) puffed unpleasant odours in a mask placed over the subject’s nose and mouth. When brain activations were compared between this condition and one in which subjects only viewed movies of an actor expressing disgust after sniffing the content of a glass, they demonstrated an overlap in the left IFO between the perception and experience of disgust. This result was later confirmed by showing that the
experience of unpleasant tastes also overlaps in the IFO with the observation of others’ facial expressions of disgust (Jabbi et al., 2007).

Interestingly, the IFO not only seems to be recruited while viewing and experiencing disgust, but it also seems essential both for the first and third person perspective of disgust. Two patients with lesions encompassing the anterior insular activations found above are unable to feel disgust and are impaired in recognizing this emotion in other individuals (Adolphs et al., 2003; Calder, Keane, Manes, Antoun, & Young, 2000). This is consistent with a large lesion study showing that the somatosensory cortex / anterior supramarginal gyrus and surrounding insular region are essential for recognizing emotions from visually presented facial expressions (Adolphs et al., 2000). In addition, the anterior insula is implicated in disorders associated with an impaired ability to recognize disgust such as obsessive-compulsive disorder (Breiter et al., 1996; Calder, Lawrence, & Young, 2001; Sprengelmeyer et al., 1997), Wilson’s disease (Wang, Hoosain, Yang, Meng, & Wang, 2003), and Huntington’s disease (Gray, Young, Barker, Curtis, & Gibson, 1997; Hennenlotter et al., 2004; Sprengelmeyer et al., 1996; Wang et al., 2003).

The role of the IFO goes beyond the perception and experience of disgust. The fact that electrical stimulation of the anterior sector of the insula evokes nausea and visceromotor activity (Calder, Keane, Manes et al., 2000; Penfield & Faulk, 1955) demonstrates its role in controlling visceral sensations and related autonomic responses. The IFO not only instantiates the representation of bodily states, but it also makes these representations consciously available as subjective feeling states. More activity and grey matter in the insula for instance predicts that people are better at judging their visceral bodily states (Critchley, Wiens, Rotshstein, Ohman, & Dolan, 2004). In addition, the strength of activation in the IFO when witnessing expressions of disgust is stronger in individuals that report experiencing more distress while witnessing the distress of others (Jabbi et al., 2007). This suggests that the IFO is involved in the involuntary sharing of emotional states, often referred to as emotional contagion (Hatfield et al., 1993). In sum, this indicates that the IFO might have a dual function: it can translate the observation of other people’s facial expressions into similar visceral states of the self (Critchley et al., 2005), and make these states consciously available for sensing the emotional state of other people (Keysers & Gazzola, 2007).

Figure 2 Anatomical locations of affective components of simulation

(a) Sagittal view of a human brain with the location of the anterior cingulate cortex (ACC).
(b) Coronal view of a human brain showing the location of the insula and the amygdala.
2.2.2 Sharing of pain

Although pain is not traditionally considered a basic emotion, it is a strong feeling state that can, akin to disgust, be triggered repeatedly and reliably in a research environment. Neuroimaging studies show involvement of the dorsal anterior cingulate cortex and anterior insula (AI) in processing the unpleasantness of physical pain (for a review see Peyron, Laurent, & Garcia-Larrea, 2000). In the ACC (see Figure 2a) nociceptive-specific neurons are found which respond to contralateral noxious thermal and/or mechanical stimulation, but not to their unpleasant equivalent (Hutchison, Davis, Lozano, Tasker, & Dostrovsky, 1999; Vogt, 2005), which is consistent with the ACC’s role in pain experience. Importantly, Hutchison et al. (1999) also demonstrated the existence of single neurons in the ACC which are active both during the sensation and perception of pain. This suggests that, similar to touch and disgust, pain is a feeling that we simulate.

In a seminal study, Tania Singer and co-workers (2004) tested romantic couples in a situation where one was lying in the scanner and was informed by a symbol on the computer screen when her lover was receiving a painful stimulation. Knowing that her lover was in pain activated parts of the pain matrix that were also active when a noxious stimulus was applied to the subject herself: the AI and ACC. Numerous other studies (reviewed in Singer & Lamm, 2009) found activation of the insula and the anterior cingulate cortex associated with the observation of stimuli depicting pain-inducing events. For instance, static images of a knife cutting a hand, or a foot stuck in the door (Jackson, Meltzoff, & Decety, 2005), and videos of needles being inserted into human body parts (Cheng et al., 2007) all activate these areas. Even in the absence of a direct pain-inducing event, the observation of a facial expression of pain activates the ACC and AI. This was shown for the observation of dynamic facial expressions of moderate pain compared to neutral expressions (Botvinick et al., 2005), and when comparing painful expressions with angry ones (Simon, Craig, Millner, & Rainville, 2006). In addition, these regions respond more strongly to intense than mild facial expressions of pain (Saarela et al., 2007). Akin to disgust, pain simulation in affective centers (ACC, AI) is correlated with inter-individual differences in empathy (Singer et al., 2004). This led some researchers to emphasize the representations of the other person’s subjective unpleasantness in understanding someone else’s pain (de Vignemont & Singer, 2006; Singer et al., 2004; Singer et al., 2006). Recent studies suggest, however, that sensory and motor components may also play a role.

2.3 Sensory and motor components of emotion simulation

Pain is often characterized by a motor response (e.g. facial expression of pain) and frequently has a clear sensory component (e.g. a needle entering the skin), which resembles other emotions. In fact, the pain matrix, which designates the collection of areas involved in the experience of pain, consists of a somatic/sensory as well as an affective/motivational component (Jackson, Rainville, & Decety, 2006). Brain areas that are involved in representing the sensory aspect of physiological pain are the thalamus, SI/SII, and the (posterior) insular cortex (Peyron, Laurent, & Garcia-Larrea, 2000; Price, 2000). Activation of the motor cortex and the cerebellum is also reported in studies of pain experience (Peyron et al., 2000). The next section reviews evidence of motor and somatosensory simulations during pain perception in particular and emotion perception in general, suggesting there
is more than merely affective simulation.

2.3.1 The case of pain

In some cases, people might share not only affective but also motor and somatosensory representations with other people in pain. In the EEG study cited earlier, Bufalari and co-workers (2007a) show that the degree to which the sight of other people's tactile and nociceptive sensations modulates neural activity from the crown of SI (BA1 and 2) depends on the rated stimulus intensity. This suggests that sensory components of the pain matrix can be activated by the vision of other people's pain. At first glance, fMRI studies seem more ambiguous about the role of SI and SII in pain perception. As far as the sensory cortex is concerned, about half of the studies found activity in SI/SII (e.g. Decety, Michalska, & Akitsuki, 2008; Lamm, Nusbaum, Meltzoff, & Decety, 2007; Moriguchi et al., 2007) and half did not (e.g. Jackson et al., 2005; Singer et al., 2004; Singer et al., 2006). At least three factors could explain these differences. First, vicarious SI/SII activity might be of modest intensity and the sensitivity of the method and sample size of the experiment then determine whether it is significant. For example, Jackson, Meltzoff and Decety (2005) failed to find SI/SII activation in their fMRI study; Cheng and colleagues (2008), using the same stimuli with hands and feet in painful situations, demonstrate suppression of the mu rhythm at the postcentral gyrus using magnetoencephalography (MEG), a technique that measures electrical activity in the brain by use of magnetic fields. The mu rhythm is suppressed during both the execution and performance of actions and is for that reason seen as an indicator of mirror neuron activity (Pineda, 2005). Also Jackson et al. (2005) fail in their sample of 15 subjects to find the SI/SII activity that Moriguchi and collaborators (2007) did find in their group of 30 subjects using the same stimuli. Second, the type of comparison may also play a role. Singer et al. (2004, 2006) subtracted conditions in which subjects receive an electric shock above the tactile threshold from one above the pain threshold. Likewise Saarela et al. (2007) subtract mild facial expressions of pain from more intense ones. By subtracting one tactile condition from another, somatosensory activation present in both conditions may be lost. In addition, Cheng et al. (2007) find somatosensory activation both when subjects view needles (pain) being inserted into different body parts and when these body parts are touched by a q-tip (no pain). Moreover, activation of the somatosensory regions disappears when pain scenarios are contrasted with the neutral ones. Although somatosensory regions do not survive in a whole-brain analysis, Cheng et al. (2007) show that S1 activation correlates with pain intensity. In addition, region of interest (ROI) analysis on the left postcentral region (functionally defined by the somatosensory signal change during the pain condition) shows that watching painful situations results in stronger activation than watching the neutral equivalents. Again, when using more sensitive methods, somatosensory involvement during pain perception is demonstrated. A third factor explaining the contradictory findings concerning the involvement of the sensory cortex in pain perception could be the experimental design used: some neuroimaging studies draw more attention to general unpleasantness instead of focusing on a specific body part. For example, Singer et al. (2004) did not find an overlap in somatosensory cortices for perception of pain in self and a loved one. However, pain in the other was indicated by a cue and no pain-related behavior was visible. Similarly, studies using facial expressions of pain do not localize the source of the pain on the body. This could be the reason they often fail to find somatosensory activations in response to
pain (Botvinick et al., 2005). While putting more emphasis on the affective side of pain reduces somatosensory engagement, evaluating the sensory consequences of pain conversely leads to increased activity in somatosensory areas (Lamm, Nusbaum et al., 2007).

Evidence that simulation of pain can involve the motor system as well comes from Avenanti and co-workers (2005). They found that during the observation of pain applied to hands, motor excitability (as measured using TMS-induced motor evoked potentials, MEP) in the corresponding hand muscles of the observer is decreased. In addition, the amplitude of MEP inhibition correlates with sensory aspects such as pain intensity. Along the same lines, an fMRI study contrasting pictures of faces displaying pain varying from high to low intensity found that various nodes of the motor system (BA45, SMA, BA6 and left IPL) were sensitive to intensity differences in displayed pain (Saarela et al., 2007). The role of motor activation could be two-fold, with the MNS registering the actions of the face and body, while the supplementary motor area (SMA) could be involved in programming defensive movements during pain perception (Decety et al., 2008). These studies indicate that the pain of others is represented in a mosaic of brain regions involving affective, somatosensory and motor representations, but the precise factors determining the relative importance of these various nodes remain to be elucidated.

2.3.2 Other emotions

The study of pain demonstrates that sharing the emotions of others may not be limited to sharing their affective states: motor and somatosensory aspects of emotions may also be shared. In most cases we deduce the emotional states of others from their motor behavior: we know people are happy because they smile when they are happy, and we know when people are disgusted because they turn up their noses. Could a system similar to the mirror system for goal-directed actions allow an observer to share the facial and bodily emotional expressions of others?

The repertoire of the motor mirror system indeed extends from hand actions to a wide range of body actions including facial actions (e.g. Buccino et al., 2001). In monkeys, mirror neurons were documented that react to the observation of specific mouth actions; some ingestive mouth actions such as sucking, but also, and most interestingly, some communicative ones such as lip smacking (Ferrari et al., 2003). Several brain-imaging experiments in humans also suggest that we activate our premotor cortex upon viewing an emotional facial expression. Activity in the pars opercularis of the inferior frontal gyrus (IFG) and the ventral premotor cortex (vPMC) was reported in several brain-imaging studies in which subjects observed emotional facial expressions. Interestingly, premotor activity is found for the observation of both dynamic (Hennenlotter et al., 2005; van der Gaag et al., 2007; Wicker et al., 2003) and static stimuli (Carr et al., 2003; Leslie et al., 2004; Wild et al., 2003). Additional evidence for the role of the motor cortex in emotion perception comes from two studies showing that viewing an emotional facial expression interferes with a simple facial motor task, which translates into an increase of activity in the vPMC/IFG that is correlated with the intensity of the emotion (Lee, Dolan, & Critchley, 2008; Wild et al., 2003). Additionally, the amount of facial movement during the imitation of emotional expressions correlates with activity in the MNS (Lee, Josephs, Dolan, & Critchley, 2006).

The somatosensory cortex together with the ventral premotor cortex and IFG seem to be recruited when perceiving mouth actions (Gazzola et al., 2006) and natural emotional facial
expressions (Hennenlotter et al., 2005; Wicker et al., 2003; Winston, O’Doherty, & Dolan, 2003). Expressions causing the most somatosensory activity during execution also caused the most activity during observation (van der Gaag, Minderaa, & Keysers, 2007). In line with pain studies, activity of the somatosensory cortex is not reported consistently across studies. This might be explained by sensitivity of the measurement, the type of stimulus (i.e. static vs. dynamic), a reporting bias (e.g. report of only the peak coordinates of an active cluster), or the type of comparison (i.e. against baseline or a neutral condition). Importantly, a large lesion study has shown that lesions to the right somatosensory cortices (centred on the most ventral part of the somatosensory cortex, where the face is represented) impair the ability to recognize emotions from visually presented faces (Adolphs et al., 2000). Apparently, activation of somatosensory representations of the face when viewing emotions is crucial for emotion recognition.

In summary, we may get access to the facial state of another person (i.e. the configuration of facial muscle groups) by reproducing in our premotor cortices the contractions of the muscles we observe, and by feeling the effect of these (simulated) contractions in our own somatosensory cortices. This idea is strongly supported by observations that even subliminal exposure to emotional facial expressions triggers measurable movements of the observer’s facial musculature that resemble those observed. This phenomenon is called facial mimicry (Dimberg et al., 2000). Whether such sensorimotor simulation could be important for generating a model of the affective state of others will be discussed in the following section.

2.4 From motor to affective simulation

Psychological theories have linked overt facial mimicry (as measured by an electromyograph or through observation) to emotional contagion and emotion understanding (James, 1884; Lipps, 1907; Niedenthal, 2007). Given that our brain has a lifelong experience with the correlation between our own facial configuration and our personal internal affective states, the simulation of other people’s facial configuration could trigger matching affective states. Intriguingly, there is only limited evidence that the amount of facial mimicry correlates with the amount of emotional contagion and/or understanding. While Niedenthal and collaborators (2001) show that blocking facial mimicry leads to a slower detection of facial expression changes, Hess and Blairy (2001) could not demonstrate a direct link between degree of facial mimicry and emotional recognition accuracy. Additionally, studies in disorders affecting facial expressivity such as Möbius syndrome and facial paralysis show no striking emotion recognition impairment (Calder, Keane, Cole, Campbell, & Young, 2000; Keilhor, Barrett, Crucian, Kortenkamp, & Heilman, 2002). While it is difficult to directly translate the concepts of facial mimicry and emotional contagion into testable neural hypotheses, it seems likely that if facial mimicry were to trigger emotional contagion, areas such as the primary somatosensory or motor cortex, known to directly sense or cause facial movements, would be most strongly connected to the insula, which is thought to represent a neural correlate of emotional contagion. To explore this possibility, Jabbi and Keysers (2008) performed a functional connectivity study using Granger causality. A functional connectivity study can identify brain regions whose connectivity (i.e. correlation in activity) is modulated by the task. A correlation between two brain regions implies a connection, but does not necessarily indicate causation. Granger causality can, however, determine whether a time series A is useful in forecasting another time series B more than
B is able to predict A. Jabbi and Keysers (2008) used the region of the IFO that is common to the experience and observation of disgust as a seed or reference region in their study. They found activity in the IFO is Granger-caused by activity in the region of the inferior frontal gyrus that is active both while observing and generating facial expressions. In contrast, there was no enhanced effective connectivity with the somatosensory cortex or the primary motor cortex. This may explain why the inferior frontal gyrus is not just responsive to facial movement, but is more active when attention is drawn to emotional or socially relevant properties (Gur et al., 2002; Lawrence et al., 2006; Scheuerecker et al., 2007; Schilbach, Eickhoff, Mozejisch, & Vogeley, 2008). It also suggests that the link between motor simulation and emotional contagion may not be through overt facial mimicry, as suggested by early psychological theories (Lipps, 1907), but instead through a covert simulation in high-level motor regions (Carr, Iacoboni, Dubaue, Mazziotta, & Lenzi, 2003). This may help clarify why motor simulation can be important in emotion understanding even in the absence of a tight correlation between overt facial mimicry and emotional contagion. Future studies are needed to further investigate this provocative hypothesis.

In summary, regions involved in simulating facial expressions indeed seem to trigger an affective simulation of the hidden inner states of others. In this process, the link between our own (visible) facial expression and (invisible) internal states could serve as a Rosetta stone to derive hidden internal states from the observable actions of others. It is likely that bodily expressions of emotions (such as body postures) could be processed in similar ways (de Gelder, Snyder, Greve, Gerard, & Hadjikhani, 2004). There are, however, likely to be many routes to equally many types of emotions which are processed in various regions of the brain.

2.5 The specificity of sharing emotions

An important theme in the neural study of emotion has been the search for brain areas that are selectively involved in particular emotions. Many for instance associate the ACC with the emotion of pain (Hutchison et al., 1999), the IFO with the emotion of disgust (Adolphs et al., 2003; Calder et al., 2001) and the amygdala with the emotion of fear (Adolphs, Tranel, Damasio, & Damasio, 1994). Such an organization would be a powerful instrument to examine the degree to which the observation of a particular emotion in others is translated into representations of a similar emotion in the self. Unfortunately, most studies do not lend themselves well to answering this question: some lump together the observation of various facial expressions in the design of the experiment (e.g. Carr et al., 2003; Leslie, Johnson-Frey, & Grafton, 2004), while others focus on only one emotion (e.g. Botvinick et al., 2005; Grosbras & Paus, 2006; Hennenlotter et al., 2005). Additionally, many other studies do not use a condition in which the subjects experience the emotion themselves (e.g. no studies have been performed yet combining perception and experience of fear). Overall, the available data shed increasing doubts on the existence of a reliable mapping of particular emotions on particular brain regions.

2.5.1 The case of fear

Fear is probably the most widely studied basic emotion, and much interest in the literature on fear has focused on the amygdala (see Figure 2b). This structure is often thought to be involved in
processing facial, vocal and bodily signals of fear as well as in experiencing fear and fear conditioning (e.g. de Gelder et al., 2004; Halgren, Walter, Cherlow, & Crandall, 1978; LeDoux, 2003; Phan et al., 2002). The fact that certain patients with lesions in the amygdala seem to show deficits in the recognition and experience of fear (e.g. patient SM: Adolphs et al., 1994; patient YW: Broks et al., 1998; patient NM: Sprengelmeyer et al., 1999; Tranel, Gulllickson, Koch, & Adolphs, 2006) but not of other basic emotions has led some to propose that the brain simulates the fear of others by activating states of fear in a fear-selective amygdala (Goldman & Sripada, 2005). However, recent neuroimaging and patient studies challenge this view. First, it is unclear whether patients with lesions in the amygdala are unable to experience fear: two-week old primates with amygdala lesions display more fear (more grimaces and screams) during social interactions than non-lesioned conspecifics (Amaral et al., 2003); Anderson and Phelps (2002) report an amygdala-lesioned patient who expresses a normal range of emotion; and even the most widely tested lesion patient SM displays a normal range of affect and emotion during social interaction (Tranel et al., 2006). Second, it is unclear whether lesions in the amygdala directly impair the recognition of fear: half the reported patients with amygdala lesions display normal fear recognition (see Keysers & Gazzola, 2006); SM is unimpaired in recognizing fear from vocal and bodily expressions of fear (Adolphs & Tranel, 1999; Atkinson, Heberlein, & Adolphs, 2007) and if instructed to look at the eyes of people (which she does not spontaneously do), even her recognition of fearful facial expressions is normal (Adolphs et al., 2005). The role of the amygdala in recognizing fear may have less to do with the actual recognition, but more with directing attention to the salient parts of the environment (e.g. eyes) through its connections with high-level visual areas (Vuilleumier, Richardson, Armony, Driver, & Dolan, 2004). Indeed, an increasing number of fMRI studies find that the amygdala is similarly recruited by movies of positive and negative facial emotions (Kilts, Egan, Gideon, Ely, & Hoffman, 2003; van der Gaag et al., 2007), except in fearful individuals (Ewbank et al., 2009) or after administration of norepinephrine and cortisol to simulate stress (Kukolja et al., 2008). When all these results are taken together, they suggest that the role of the amygdala in experiencing and recognizing fear is more indirect than previously suggested, which sheds doubt on the proposal that this structure embodies a selective simulation of fear.

2.5.2 Other emotions

Similar problems apply to other brain regions that have been considered relatively selective for particular emotions. Van der Gaag et al. (2007) systematically compared the observation of movies of happy, fearful, disgusted and neutral facial expressions. They could not find fear selectivity in the amygdala. Furthermore, disgust and the other emotions activated the IFO without significant differences between any of the emotions. The fact that the IFO seems similarly important for the simulation of pain and disgust (see above), also makes the lack of specificity of this structure apparent. Moreover, this structure is not only recruited by both pain and disgust, but it is activated more strongly in more empathic individuals for both of the emotions. Furthermore, inter-individual differences in empathy also explain activity in the very same voxels during the observation of positive facial expressions (Jabbi et al., 2007) with no difference between emotions. Therefore the insula does not seem to be a center for disgust. One alternative hypothesis is that the insula may play a broader role in emotion processing by translating what we perceive into visceral responses.
that colour our subjective feelings (Craig, 2002). Since disgust is related to visceral responses in particular (retching, nausea), we may in some cases rely more strongly on the insula for recognizing that particular emotion in ourselves and others. Finally, even the link between the cingulate cortex and a particular emotion such as pain may be an oversimplification. Although neurons responding to painful stimuli exist in the ACC, Vogt (2005) suggests that many regions of the cingulate cortex are not specific for a particular emotion but for a particular output. For instance, viewing sad faces is associated with increased activity in the subgenual ACC because of the role of this region in autonomic integration, while the perception of pain and fear causes overlapping activations in the anterior midcingulate cortex (aMCC) because of its strong motor connections, which prepare the body to react to these challenges (Vogt, 2005).

In summary, although there is vast evidence for a role of the amygdala in fear, a role of the anterior insula in disgust and a role of the ACC in pain, these activations are not emotion specific. Signals of fear could enhance amygdala activity (in particular in stressful situations) because they indicate a potential threat and as a result visual attention to the outside world is increased. Similarly, visceral responses mediated by the anterior insula are probably more important for disgust and the aMCC with its strong motor connections might be particularly relevant for pain. This might explain why activity in the amygdala is often found for fear, activity in the anterior insula is often found for disgust, and activity in the ACC is often found for pain. However, activity in these regions is unlikely to be directly linked to a particular emotion and for this reason simulation of a particular emotion is also unlikely to be related to a particular brain region.

A key challenge for the field of emotion in general, and the simulation of emotions in particular, will be to examine whether individual neurons - within brain regions that are not emotion specific as a whole - may represent some emotions more than others both during self-perception and other-perception. In the case of pain, for instance, there seems to be a rostro-caudal functional organization of the ACC and insular cortex with self-perception involving more caudal areas than other-perception (Jackson et al., 2006; Morrison & Downing, 2007). Applying methods such as (cross-modal) adaptation (Dinstein et al., 2007), which are used in the study of motor actions, might help address the question of neural specificity in the emotional domain.

We should however be wary of treating brain regions as separate entities. Simulation is a highly integrated process which is likely to depend on the networks connecting various regions. Indeed, much of the distinction between self and other during social interactions may depend on differences in the networks in which shared circuits are engaged. For example, although the IFO is active when observing, feeling and even imagining disgust, effective connectivity analysis shows that the involved networks are quite different (Jabbi, Bastiaansen, & Keysers, 2008): during experience, the IFO is embedded in a network composed of somatosensory, gustatory/motivational and motor output regions; during mental imagery (triggered by written scripts) into a network of language processing, semantic memory (temporal pole) and mental imagery (SMA) areas; finally, during observation, the IFO receives its strongest emotional input from the right BA45, which is involved in execution, observation and imitation of facial expressions. The same is true for pain: the ACC and AI are involved in both the experience and the observation of pain, but the functional network during self-perception is different from the network that is activated during the perception of others in pain (Zaki, Ochsner, Hanelin, Wager, & Mackey, 2007).
2.6 Concluding comments

2.6.1 Role of simulation

Neuroimaging experiments show that we activate common circuits when observing sensations or emotions felt by others, and when experiencing these sensations and emotions ourselves. This clearly suggests that seeing someone else experiencing touch, disgust, or pain triggers much more in us than a purely theoretical, disembodied interpretation of other people’s mental states. Witnessing someone experiencing an emotion or a sensation is associated with a pattern of activity in our brain embodying their actions, sensations and affective states. What could be the role of this automatic cortical simulation?

The motor component of simulating other people’s facial expressions can have two purposes. One is directly social, and arises when the observer of a facial expression not only simulates the facial expressions of others, but allows this simulation to show on his/her face. Such facial mimicry facilitates social contacts, and could increase the survival of individuals by increasing their social success (Chartrand & Bargh, 1999; van Baaren, Janssen, Chartrand, & Dijksterhuis, 2009). The overt outflow of simulated facial expressions, however, depends on the social context: people refrain from imitating people’s smiles if they are in competitive contexts or deal with an outgroup member (Lanzetta & Englis, 1989; van Baaren et al., 2009); motor simulation of goal-directed actions can be overt during imitation but remains covert in most situations. The second function of motor simulation seems to be a way of bridging the observable behavior of others with hidden internal states that correspond to these behaviors. It could do this by triggering a simulation of affective states through the connections of premotor regions with the IFO (Jabbi & Keysers, 2008). This circuitry does not require the primary motor cortex and therefore does not require the motor simulation to become overt. As was previously discussed, this could explain why the amount of overt facial mimicry does not directly predict how accurate observers are at judging the emotions of others, or how much they are affected emotionally by the emotions of others (Blairy, Herrara, & Hess, 1999; Gump & Kulik, 1997; Hess et al., 1999; Niedenthal et al., 2001; however see Sonnby-Borgstrom, 2002). The importance of such motor simulation for feeling what goes on in others derives from lesion studies that show that lesions in these regions impair the recognition of affect in others (Adolphs et al., 2000). The affective simulation that can be triggered by the motor simulation of others’ behavior and/or by mental imagery of their states derived from other sources of information (Jabbi et al., 2008) is likely to have a dual function as well. On the one hand, it probably allows us to feel what goes on in others: lesions in regions involved in sensory (SI/SII+posterior insula) and/or emotional (IFO) simulation indeed impair an individual’s capacity to judge the emotions of others (Adolphs et al., 2000; Adolphs et al., 2003; Calder, Keane, Manes et al., 2000). On the other hand, beyond providing a direct understanding of the emotions felt by others and allowing the selection of appropriate behavioral responses, affective simulation may help ‘synchronize’ the emotional states of members of a group.

The study of the neural basis of simulation makes a further functional prediction. Given that emotions are shared through a mosaic of motor, somatosensory and affective simulations, people’s reactions to other’s emotions may be expected to differ in fine-grained ways. For instance, certain people could engage in more motor and less affective simulation. Others may have the reverse relationship. The psychological literature indeed supports the idea that empathy has multiple
Chapter 2

separable subcomponents, which is in contrast to the layman’s vision of empathy as a unitary system. Many separate cognitive empathy from affective empathy (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001; Davis, 1983; Mehrabian & Epstein, 1972), while others additionally distinguish motor empathy (Blair, 2005). Finally, even affective empathy can be further divided into personal distress (the contagious sharing of others’ distress) and emotional concern (the wish to help that is triggered by the distress of others), with these forms developing at different ages (Preston & de Waal, 2002). Although the distinctions made by neuroscientists and psychologists differ - the former being driven by neuroanatomical and the latter by functional considerations - recent evidence suggests that these distinctions may be linked. Affective forms of empathy correlate with brain activity in affective brain regions (IFO while witnessing the disgust or pleasure of others (Jabbi et al., 2007) and while sharing the pain with a loved one (Singer et al., 2004), respectively). The less affective forms (cognitive perspective taking), however, correlate with the activity in non-affective brain regions (premotor and somatosensory areas during action observation, Avenanti, Minio-Paluello, Bufalari, & Aglioti, 2009; Gazzola et al., 2006). Some psychologists would rather not label personal distress as a form of affective empathy because it involves a self-oriented rather than an other-oriented affective response to the emotions of others (Batson, Fultz, & Schoenrade, 1987; Eisenberg, 2006). In fact, there are studies showing that the more a person attributes their own traits to another person and the higher the person’s own distress to discomfort in others, the less strong the empathic responses are in motor and somatosensory regions (Avenanti et al., 2009; Lawrence et al., 2006). Notwithstanding the debate about whether personal distress should be labeled as a form of empathy or not, it is still largely unclear why individuals differ in the composition of their empathy to begin with and how such differences could be influenced by training.

Finally, the automatic sharing of both affect and action with others may have a very fundamental role for learning. While it remains unclear whether the MNS involved in actions may be partially inborn, it certainly is plastic. For instance, the training involved in becoming a dancer or pianist increases the MNS response to perceiving others perform that particular dance (Calvo-Merino, Glaser, Grezes, Passingham, & Haggard, 2005; Lahav, Saltzman, & Schlaug, 2007), and practice can virtually reverse the behavior of the mirror system (Catmur, Walsh, & Heyes, 2007). It has been suggested that the association between performing an action and perceiving oneself perform the action may form the basis for this plasticity (Catmur, Walsh, & Heyes, 2009; Heyes, 2001; Keysers & Perrett, 2004). For actions that we do not see ourselves perform (e.g. facial expressions), experience of early imitation by parents may be the key to learning (Del Giudice, Manera, & Keysers, 2009). While motor simulation alone has often been taken as the neural basis of learning by observation, this explanation falls short of explaining how observers can learn which of the actions of others are worth learning. This problem might be naturally solved by the brain using a combination of affective and motor simulation. If viewing another individual perform action A resulted in a positive outcome, and action B resulted in a negative outcome, the brain of the observer would vicariously co-activate affective reward areas and motor representations of A, but co-activate pain areas together with representations of B. This would lead to assimilating behavior A but not behavior B through the mechanisms of individual trial and error learning and operant conditioning.
2.6.2 Beyond simulation

A variety of authors have criticized simulation theory based on the fact that it cannot explain all facets of social cognition (e.g. Gallagher, 2007; Jacob & Jeannerod, 2005; Saxe, 2005) and that we still fail to have conclusive evidence in humans that the exact same neurons are involved in action perception and execution (Dinstein et al., 2008). As previously discussed, this criticism also applies to the case of emotions and sensations. We view the first critique as an experimental challenge that should inspire researchers in the next decade. As for the second, we believe that it is fruitless to create a competition between simulation views versus more cognitively inspired ‘mentalizing’ approaches in current social neuroscience research. There is no doubt that in many instances we rely on our knowledge of the person or the situation to make inferences about the state of mind of the other. If a salesman of second-hand cars smiled broadly while bragging about the quality of a rusty old car, our simulation circuitry could make us share his enthusiasm, but semantic knowledge about second-hand car salesmen could lead to a different conclusion. There is ample evidence that what we know about someone else can influence the simulation mechanism. For instance, the perceived fairness of the observed individual in pain influences how much pain will be shared (Singer et al., 2006). Similarly, the gender of the observed individual can influence our neural response (Simon et al., 2006). In addition, the sensory part of the pain matrix is engaged more when the perceived pain reaction of another person matches how we would respond ourselves (Lamm, Meltzoff, & Decety, 2010). Therefore, we believe the interesting question is how these two processes are integrated in the brain (Keysers & Gazzola, 2007). Brass and colleagues (2009), for instance, suggest that it is the control of shared representations by the temporo-parietal junction (TPJ) and medial prefrontal regions (e.g. by virtue of assigning agency and suppressing externally triggered response tendencies) and not the shared representations per se that pave the way to understanding others. Various authors have implicated these regions in mentalizing and determining agency (Mitchell, Macrae, & Banaji, 2006; Saxe, 2006). Brass (2009) suggests these regions not only control automatic imitative response tendencies but also shared representations involved in higher order social cognition. In fact, Cheng and colleagues (2007) show that when acupuncture practitioners watch needles being inserted in someone’s body parts they do not activate their own pain matrix (ACC, AI, PAG) as naïve subjects do, but they activate medial and superior prefrontal cortices and the TPJ instead. Possibly due to their knowledge of acupuncture, experts cognitively inhibit affective simulation and reduce their vicarious experience of pain intensity and unpleasantness. Understanding the influence of higher level cognitive representations on simulation will be one of the key challenges in the coming years, and will be essential to understanding how our species has adapted to a world in which simulation can sometimes be adaptive and sometimes not (e.g. when having to fight an enemy).

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## Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tr>
<td><strong>Simulation</strong></td>
<td>A form of neural processing of social information that involves activating neural states during observation that match those that the observer experiences in a similar situation. An example of motor simulation would be activating a pattern of motor activity while watching other people's actions that corresponds to the pattern found when the observer performs the same actions (Etzel, Gazzola, &amp; Keysers, 2008).</td>
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<tr>
<td><strong>Embodied cognition</strong></td>
<td>By embodied cognition we mean any form of cognitive processing that is performed in representation codes that are specific to the body (Goldman &amp; de Vignemont, 2009). Such codes include the motor codes implemented in primary motor, premotor and supplementary motor cortices; the somatosensory codes implemented in primary and secondary somatosensory cortices as well as in the insula; the visceromotor representations implemented in the insula.</td>
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<tr>
<td><strong>Qualia</strong></td>
<td>The phenomenological aspects of feelings and sensations making up our conscious experience.</td>
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<tr>
<td><strong>Emotional contagion</strong></td>
<td>Simulation as we use it refers to a process, namely how the brain attaches meaning to other people's states by recruiting representations of similar states of the self. Emotional contagion refers to the effect that this can have on the observer's mood or emotions: the emotional state of an observer comes to resemble that of the observed individual, for instance an infant starting to cry upon hearing another baby crying (Hess &amp; Blairy, 2001).</td>
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<tr>
<td><strong>Empathy</strong></td>
<td>We use empathy in its broadest sense unless otherwise specified: the process through which we are sensitive to other people's inner states (affective states in particular) by placing ourselves into either a similar state (feeling sad while seeing a friend cry) or a compassionate state (having tender feelings for a person in pain). This broad definition includes emotional contagion as well as what has been termed empathy in the narrow sense, that is an other-focused congruent emotion (Batson et al., 1987; de Vignemont &amp; Singer, 2006).</td>
</tr>
<tr>
<td><strong>Imitation</strong></td>
<td>We use imitation in its broadest sense here: the generation of a behavior that follows and matches an observed behavior. This broad definition includes what has been termed emulation (generating a behavior that achieves the same goal as the observed behavior) and ‘true imitation’ (generating an otherwise unlikely movement that matches the one observed in its details). This term also includes both automatic imitation, as measured using interference paradigms (Brass et al., 2009), and voluntary imitation, as measured using more explicit instructions to imitate.</td>
</tr>
<tr>
<td><strong>Motor goals</strong></td>
<td>The proximal purpose of an action. We speak of motor to specify the pragmatic nature of the goals as can be implemented in the motor system, as opposed to more abstract goals or intentions that would be implemented in non-motor brain regions. The motor goal of grasping a glass of water would for instance be to hold the glass. This particular goal can be achieved in a variety of ways (e.g. you could grasp it with your left or right hand or with your feet), which places it above detailed motor programs in the motor hierarchy. It is however different from a more distal goal or intention of grasping 'to drink' or 'to throw the content into my enemy's face'. It refers to the 'what' level of an action (Thioux et al., 2008).</td>
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Seeing, feeling, and imaging disgust

A version of this manuscript is published as:
A common anterior insula representation of disgust observation, experience and imagination shows divergent functional connectivity pathways (2008)
Jabbi, M., Bastiaansen, J., & Keysers, C.
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Chapter 3

Abstract

Similar brain regions are involved when we imagine, observe and execute an action. Is the same true for emotions? In this study, the same subjects were scanned while they (a) experience, (b) view someone else experiencing, and (c) imagine experiencing gustatory emotions (through script-driven imagery). Capitalizing on the fact that disgust can be repeatedly induced within the scanner environment, we scanned the same subjects while they (a) view actors taste the content of a cup and look disgusted, (b) taste unpleasant bitter liquids to induce disgust, and (c) read and imagine scenarios involving disgust. To minimize habituation, we added neutral and pleasant trials in all three fMRI experiments. We found that voxels in the anterior insula and adjacent frontal operculum (IFO) are involved in all three modalities of disgust, which suggests that simulation in the context of social perception and mental imagery of disgust entails common neural substrates. Effective connectivity analyses, however, showed that during the three modalities the IFO is embedded in distinct functional circuits, which could explain why observing, imagining and experiencing an emotion feel so different.
Imagine disgust in your insula

“Disgust refers to something revolting, primarily in relation to the sense of taste, as actually perceived or vividly imagined; and secondarily to anything which causes a similar feeling, through the sense of smell, touch and even eyesight”

Charles Darwin (1872/1965)

3.1 Introduction

The concept of ‘simulation’ is important for our understanding of imagination and social perception. For actions, simulation accounts of imagination propose that we can accurately imagine what it feels like to perform actions, because common brain areas are involved in the execution and imagination of these actions. Empirical studies showing parietal, premotor, and supplementary motor (SMA) cortex activations during both action imagination and action execution support this account (Fadiga & Craighero, 2004; Gerardin et al., 2000; Jeannerod & Frak, 1999; Porro et al., 1996; Solodkin, Hlustik, Chen, & Small, 2004). Simulation accounts of action perception posit that we intuitively feel what others do and can anticipate their future actions, because our perceptual apparatus links their actions with neural structures planning our own actions. Empirical support for this comes from the discovery of mirror neurons that respond to the perception and execution of similar actions in the premotor and inferior parietal cortex of the macaque monkey (Fogassi et al., 2005; Keysers et al., 2003; Kohler et al., 2002; Umiltà et al., 2001) in conjunction with the observation of human premotor and inferior parietal responses to the observation and execution of actions (Gallese & Goldman, 1998; Gallese et al., 2004; Gazzola et al., 2007; Gazzola et al., 2007; Grafton, Arbib, Fadiga, & Rizzolatti, 1996; Iacoboni et al., 1999; Keysers & Gazzola, 2006, 2007).

Together, these studies suggest that brain areas such as the premotor and posterior parietal cortex form a neural substrate underlying three functions: motor execution, observation and imagination. Apparently, the human brain does not need to duplicate the motor expertise stored in motor areas in order to permit imagination and social perception. Instead, it seems to (at least partly) employ the very hardware of our own actions. Our question here is whether this notion can be extended to the realm of emotions.

Neuroimaging studies have shown that while individuals view or become aware of the delight (Jabbi et al., 2007), pain (Jackson et al., 2005; Lamm, Batson, & Decety, 2007; Saarelä et al., 2007; Singer et al., 2004) or disgust of others (Carr et al., 2003; Jabbi et al., 2007; Wicker et al., 2003), they activate the anterior insula and adjacent frontal operculum (IFO), which is known to be recruited during the experience of similar emotions and is modulated by empathic tendencies. The important role of the IFO in emotion simulation and emotion understanding is further supported by lesion studies showing that damage to the IFO disrupts both the experience and recognition of disgust (Adolphs et al., 2003; Calder, Keane, Manes, Antoun, & Young, 2000). Interestingly, similar IFO regions are recruited during affective autobiographical recall (Damasio et al., 2000; Preston et al., 2007) and taste imagination of pictured food items (Kikuchi, Kubota, Nisijima, Washiya, & Kato, 2005). Moreover, lesions to the IFO have not only been shown to result in deficits in disgust recognition and experience, but also in a marked reduction of feelings of craving for cigarettes in long-term smokers (Gray, Harrison, Wiens, & Critchley, 2007; Naqvi, Rudrauf, Damasio, & Bechara, 2007), and in an apparent unawareness of one’s own functional impairment in individuals with anosognosia (Karnath, Baier, & Nägele, 2005). These and other findings point to the functional
significance of the IFO in facilitating interoceptive awareness (i.e. sensing the inner state of the body) per se (Craig, 2008).

Given the well-documented role of the IFO in coding the experience and social observation of feeling states such as disgust, two interesting questions arise: does the part of the IFO that is commonly involved in the experience and observation of disgust (Jabbi et al., 2007; Wicker et al., 2003) also respond to individuals' vivid imagination of disgusting experiences? And how does the IFO's functional circuitry differ during the imagination, observation and experience of disgust? In line with the emerging evidence for a role of the IFO in coding awareness of feeling states, we expect that this region will also be involved in disgust imagination. Furthermore, we expect that the functional connectivity between this region and the rest of the brain will differ across the experience, observation and imagination of disgust.

3.2 Methods and Materials

3.2.1 Participants
Twelve healthy right-handed volunteers (6 females) that participated in a previous study on the observation and experience of gustatory emotions (Jabbi et al., 2007), were invited to participate in this fMRI experiment. All 12 individuals were free of psychiatric, neurological and other physical conditions, and had normal or corrected-to-normal vision. All subjects completed the consent forms approved by the Institutional Review Board of the University Medical Center Groningen and were paid 50 Euros in total for their participation in the observation, experience and imagination experiments.

3.2.2 Experimental Procedures

Observation and Experience
The procedures for the observation and experience experiments described in this study have been reported earlier (Jabbi et al., 2007) and are briefly illustrated in Figures S1 and S2.

Imagination
Similar to the observation and experience experiments, the imagination runs consisted of three different conditions: disgust, neutral and pleasure. Written scenarios (i.e. scripts) with an approximate reading time of 35 seconds were developed to induce disgusted (9 scripts), neutral (7 scripts) and pleasant (8 scripts) emotional feeling states (see Supplementary Materials for sample scripts). Initially, the scripts were evaluated by 11 subjects that did not take part in the fMRI study. These subjects rated the amount of disgust and pleasure they experienced while reading the scripts, and while imagining themselves going through the scenarios (on scales ranging from 0 to 6). Additionally, they rated how challenging the scripts were to imagine. For two additional subjects, we measured the reading time with a chronometer to examine what script was quickest to read. We used the shortest reading time (20s) as the upper limit for the surface under the curve analysis.
described below.

For the final experiment, each of the subjects rated all 25 scripts on how disgusting, how pleasant and how hard to imagine they were. These ratings served two purposes. First, they allowed us to choose the six most disgusting, the six most pleasant and the six most neutral (i.e. least disgusting or pleasant) scripts for the fMRI experiment on an individual basis. Second, these personal ratings of the scripts informed us how disgusted or pleased the subjects were during the fMRI experiment (Figure S3).

During scanning, each trial began with a red fixation cross lasting 6 seconds, followed by a script presented as a single screen of text lasting 35 seconds, followed by a fixation cross lasting 6 seconds. The subjects then viewed a screen with a simple arithmetic task for 6 seconds and had to indicate their choice by pressing with their right index finger the right or left button of a response box (Figure S4). Then the next trial started again with the fixation cross. We included the arithmetic task between two scripts to maintain attention and wash out the emotional state induced by the scripts. We administered two runs each comprising 9 trials (3 disgust, 3 neutral and 3 pleasant scripts in random order) and lasting 9.35 minutes.

3.2.3 Image Acquisition and Analysis

Images were acquired using a Philips 3T whole-body scanner (Best, The Netherlands) using a circular sense head coil. T2*-weighted echo-planar sequencing was performed with 39 interleaved 3.5mm thick axial slices with 0 mm gap (TR=2000 ms, TE = 30 ms, flip angle = 80°, FOV = 224 mm, 64 x 64 matrix of 3.5 x 3.5 x 3.5 mm voxels). At the end of each functional scan, a T1-weighted anatomical image (1 x 1 x 1 mm) that covered the whole brain was acquired parallel to the bicommissural plane.

Statistical Parametric Mapping (SPM2; Wellcome Department of Cognitive Neurology, London UK; http://www.fil.ion.ucl.ac.uk) was used for preprocessing and data analysis. All functional volumes were realigned to the first acquired volume; coregistered to the subject’s anatomical space; spatially normalized to obtain images with a voxel size of 2 x 2 x 2 mm (Friston et al., 1994); and smoothed with an 8 mm full-with half-maximum isotropic Gaussian kernel. For the time series, we used the same high-pass filters for all subjects with cut-off points at 106s, 310s and 380s for the observation, tasting and imagination conditions, respectively, in order to remove low-frequency noise and slow-drifts from the signal. Condition-specific effects were estimated for each voxel using the General Linear Model. Contrast images were then tested at the group level using a one tailed t-test against zero. We additionally extracted the time course from the IFO region of interest (ROI) that was found to be commonly active during the observation and experience (Jabbi et al., 2007) for all three experimental conditions using Marsbar (http://marsbar.sourceforge.net; M.Brett, J.-L. Anton, R. Valabregue, and J.-B. Poline, ROI SPM toolbox).

3.2.4 Connectivity Analysis

To explore the functional integration between the IFO and other related regions during the three disgust modalities, we employed psychophysiological interaction (PPI) analysis implemented in SPM. Through PPI procedures advised by Friston and colleagues (1997), we identified voxels whose time
course correlated more strongly with the time course of activity in the IFO during the disgust compared to the neutral condition for the imagination, observation, and experience modalities separately. The seed region for this analysis was determined for each modality and subject separately by opening the relevant contrast in SPM (e.g. vision of disgust - vision of neutral), placing the cursor at the center of the IFO ROI (x=42, y=18, z=-6) derived from the previous paper (Jabbi et al., 2007), and defining a 5mm radius sphere using the function ‘VOI’. This function automatically moves to the closest voxel with a significant contrast (uncorrected p<.005). Given our voxel size, the actual center of the sphere deviated on average 4 mm from the center of the ROI (see Table S1). There was no significant difference between the spatial distribution of centers in the three modalities (two-tailed matched-pair t-test performed separately on the x, y and z coordinates for imagination vs. observation, imagination vs. taste and observation vs. taste, all p>0.25). First, the PPI analysis multiplied point by point the time course of activity in the sphere seed region with a psychological variable containing the value 1 for the condition disgust, -1 for the condition neutral and zero elsewhere. Second, this interaction vector, the time course of the seed region, and the psychological variable were entered as regressors in a whole-brain GLM analysis. Comparing the parameter estimate of the interaction term with zero in the population of 12 subjects at the second level of analysis (one-tailed t-test comparing n=12 parameter estimates against zero) then revealed voxels that are on average functionally more strongly connected to the seed region in the disgust condition compared to the neutral condition (Friston et al., 1997). We thresholded the PPI maps at uncorrected p<.001 with an extent threshold (k) of 10 voxels.

3.3. Results

3.3.1 Script ratings

Before scanning, the 12 subjects included in the fMRI experiment rated (Figure S3) the 6 disgust scripts as more disgusting than the neutral ones (two-tailed matched-pair t-test, uncorrected p<.001), but there was no significant difference between the disgusting and neutral scripts in terms of how pleasant (p>0.07) or how hard they were to imagine (p>0.87). The pleasant scripts served to balance the experimental design and were not further analyzed in terms of MRI data. Their ratings differed from the other scripts in that they were less disgusting than the disgust script (p<.001), and more pleasant than both the other types of scripts (p<.001). Finally, the disgusting scripts were slightly more disgusting than the pleasant scripts were pleasant (p<.04).

3.3.2 Time courses

In Jabbi, Swart & Keysers (2007), a region of the IFO was significantly more active during the vision and the experience of disgust compared to their neutral counterparts (p<.005 vision of disgust - vision of neutral, and p<.005 taste of quinine - taste of neutral solution, Figure 1a). To examine whether this region is also recruited during the imagination of scenarios involving disgust (compared to those without emotional valence), we extracted the signal from this ROI in the imagination condition for all 12 subjects (Figure 1b; traditional GLM results for all three modalities are specified in Table S2). It is difficult to assess how the emotional states of the subjects fluctuate while reading
the scenarios. Therefore, we did not use a standard GLM approach. Instead, we calculated the surface under the average difference curve between the disgust and neutral scenarios. The first 4s were excluded because of the hemodynamic response delay, while time points after 20s were excluded because some of the subjects could have finished reading some of the scripts (see Experimental Procedures). To examine whether the IFO is commonly involved in the representation of the imagination, experience, and social observation of emotional feeling states, we employed a one-sample t-test (one tailed) to compare the surfaces of the 12 subjects against zero. We found that the disgust scenarios recruited the IFO ROI significantly more (p<.004) than the neutral scenarios during imagination. Time courses of the IFO ROI during the observation and experience of disgust derived from Jabbi, Swart & Keysers (2007) are also shown in Figure 1c-d for illustrative purposes.

**Figure 1** Time courses in the IFO during disgust imagination, observation, and experience

![Coronal slice](a) showing the location of the ROI (white), which has previously been shown to be involved in the experience and observation of disgust (p < 0.005, k > 10 voxels, Jabbi et al., 2007). b-d) Time courses of the average disgust-neutral difference relative to (b) the to be imagined scripts, (c) the onset of the movies of facial expressions, and (d) the administration of the tastes. Error bars represent the standard error of the mean.

### 3.3.3 Functional Connectivity

Next, we wanted to examine the functional circuitry within which the IFO is embedded in the three disgust modalities (observation, imagination and experience). To this end, we used as a seed the time course of the IFO ROI, which was based on a 5 mm sphere centred on the voxel with a significant omnibus test closest to x=42, y=18, z=-6 (see Methods and Materials). Effective connectivity was mapped using three separate PPI analyses (Friston et al., 1997), one for each modality. These analyses were performed separately for each subject. Then, the parameter estimates of the interaction term were tested against zero at the population level using a one-tailed
t-test to determine which voxels consistently increased their functional connectivity with the IFO during the disgust condition compared to the neutral condition. Results are shown in Figure 2 and Table 1.

During observation, we found that only the ipsilateral right inferior frontal gyrus (IFG, pars triangularis or BA45, Amunts et al., 1999) was more effectively connected with the right IFO for the observation of disgusted facial expressions compared to neutral faces (Table 1). During experience and imagination, much wider networks involving in particular somatosensory, motor, gustatory and ‘limbic’ regions were shown to be more effectively connected to the IFO during the disgust compared to the neutral conditions (Table 1). Overlap in the functional connectivity networks observed in the three modalities was rare; only one single cluster in the left temporal pole showed overlap between the imagination and experience conditions.

**Figure 2** PPI maps of the whole brain connectivity with the IFO (as seed)

Images are thresholded at $t=4.64$, which corresponds to $p<.05$ false discovery rate corrected for the imagination of disgust relative to neutral. The numbers in the figures show the corresponding $Z$-coordinates in MNI space. Left is left and right is right.
Table 1 Regions of effective connectivity with the IFO during observation, experience and imagination of disgust relative to neutral at p<.001, k>10 voxels, t>4.64

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Imagine disgust in your insula
3.4 Discussion

The IFO involvement in both the observation and experience of disgust and pleasure (Jabbi et al., 2007; Wicker et al., 2003) has been speculated to provide a simulation mechanism of the inner state of disgust during disgust observation in others. Findings showing IFO involvement in the experience and observation/awareness of other people’s pain (Jackson et al., 2005; Lamm, Batson, & Decety, 2007; Saarela et al., 2007; Singer et al., 2004), suggested that this purported simulatory IFO response may not be specific for emotions linked to gustatory or olfactory stimulation, but may be more generally linked to the simulation of bodily feeling states during social cognition (Damasio, 2003; James, 1884). The IFO may thus play an important role in general interoceptive awareness (Craig, 2002; Critchley, 2005; Critchley et al., 2004; Gray et al., 2007; Naqvi et al., 2007). In fact, independent evidence suggests that some sectors of IFO are also involved in the imagination of basic emotions and sensations such as taste (Damasio et al., 2000; Kikuchi et al., 2005; Preston et al., 2007).

Our findings show that the part of the IFO that is active during the experience and observation of other people’s disgust (Jabbi et al., 2007), is indeed also active during the imagination of one’s own disgust. To our knowledge, our findings of a common IFO activation while the same subjects experience, observe, and imagine being disgusted, provide the first direct evidence that two apparently distinct forms of simulation (social perception and imagination) may actually rely on a common neural substrate. These findings have two implications. First, they support the idea that imagination and social perception of emotions may share neuroanatomical underpinnings. This is in line with similar findings in the mirror neuron literature showing common neural representations for perceived, executed and imagined motor actions (Fogassi et al., 2005; Keysers et al., 2003; Kohler et al., 2002; Umiltà et al., 2001). Second, they provide insights into the neural basis of the captivating experience of reading a book. While previous studies on social perception used movies of other people’s experiences or arbitrarily colored symbolic cues, we combined movies and written material. Thanks to this combination we were able to demonstrate that reading (mental imagery) and watching other people can both recruit brain regions involved in experiencing an emotion.

The IFO ROI selected in this study appears to be a key location in the phenomenon of simulation that somehow makes feeling an emotion, seeing that emotion on someone else’s face, and imagining that emotion share a similar feeling component. Notwithstanding this partial overlap, the three disgust modalities do feel clearly different, emerge through distinct processes, and are triggered by different events. The trigger during experience was an unpleasant taste; during observation it was the sight of a disgusted facial expression; and during imagination the trigger was formed by mental imagery through written scripts. Interestingly, these differences seem to be reflected in our connectivity findings.
Our IFO ROI involved in all three modalities includes anterior aspects of the insula and the adjacent frontal operculum. In this location, postmortem cytoarchitectonic analysis of five human brains found a dysgranular cytoarchitecture (Bonthius, Solodkin, & Van Hoesen, 2005), which probably corresponds to the dysgranular zone of the frontal operculum/insula (Mesulam & Mufson, 1982a). Tracer injections in the monkey show that the insula is highly interconnected with most of the brain (Augustine, 1996; Chikama, McFarland, Amaral, & Haber, 1997; Mesulam & Mufson, 1982a, 1982b), and in particular the motor cortices (premotor, (pre)SMA, primary motor and cingulated motor cortex), regions involved in gustation (basal ganglia, amygdala, ACC, orbitofrontal cortex), somatosensation (SI, SII and posterior Insula), high-level vision (STS), and memory and semantics (temporal pole, hippocampus). The monkey insula, however, does not have a homologue of the IFO (Fudge, Breitbart, Danish, & Pannoni, 2005), which seems to underscore the probably prominent role for this phylogenetically new region in the higher-order physiological awareness “that might be absent in monkeys”. Our effective connectivity findings showed changes in the temporal correlation between the BOLD signal in the IFO and a variety of putative human homologues of the structures that are connected to the monkey insula (Augustine, 1996; Chikama, McFarland, Amaral, & Haber, 1997; Mesulam & Mufson, 1982a, 1982b).

During experience, changes in effective connectivity occurred primarily with somatosensory (left SI/SII and posterior insula, Dijkerman & de Haan, 2007), gustatory/motivational (basal ganglia, orbitofrontal cortex), and motor output regions (cingulated and primary motor cortex). What do these changes in effective connectivity mean? The SI/SII and the posterior insula are involved in somatosensation (Small & Prescott, 2005) and could represent the tactile experience of the tasted fluids, which is relevant for both neutral and unpleasant gustatory stimuli. The IFO, however, increases activity as the intensity of the taste of a solution increases, and it integrates the taste and texture of food (Small & Prescott, 2005). Therefore, the observed IFO effective connectivity with somatosensory areas during disgust experience relative to tasteless artificial saliva may reflect the integration of texture and taste in the IFO. The orbitofrontal cortex, the basal ganglia and motor regions (M1 and cingulate motor cortex) are involved in evaluating the valence of a taste (Small & Prescott, 2005) and regulating behavior accordingly (Fudge et al., 2005). Therefore, the increase in effective connectivity between these regions and the IFO may underlie the valence-related relevance of taste processing.

During imagination, subjects need to transform the written material involved in the scripts into a mental simulation of the actions, sensations and feeling states of the protagonists. All scripts, be they disgust-inducing or neutral, involved actions and sensations. However, unlike the neutral scripts, imagining the disgusting scripts triggered strong feeling states of disgust. Therefore, increased effective connectivity for the disgust scripts between the IFO and regions such as Broca’s area (left BA44/45) and the left temporal pole, which are known to be important for understanding stories (Vigneau et al., 2006), may reflect a cognitive-affective integration mechanism. The (pre)SMA plays a key role in the mental imagery of actions (Fadiga & Craighero, 2004; Gerardin et al., 2000; Jeannerod & Frak, 1999; Porro et al., 1996; Sacco et al., 2006; Solodkin et al., 2004), while somatosensory regions (right SI/SII/posterior insula) play an important role in the mental imagery of tactile and proprioceptive sensation (Dijkerman & de Haan, 2007; Sacco et al., 2006). Therefore, these regions may play an important role in the imagery of actions and sensations in general. The change of effective connectivity with the IFO, however, reflects that motor and
somatosensory imagery seems to be linked to activity in the IFO and feeling states more strongly when these actions and sensations are disgusting. Finally, increases of connectivity with the hippocampus could reflect autobiographic memories triggered by the scripts (Rekkas & Constable, 2005).

During social observation, the most prominent region with stronger connectivity during disgust compared to neutral faces was the ipsilateral right BA45. This region has been shown to be involved in the execution, observation and imitation of facial expressions (Carr et al., 2003; Pfeifer et al., 2008; van der Gaag et al., 2007). Possibly, the vision of any facial movement can trigger a motor simulation of facial expressions in BA45, which might be related to the phenomenon of facial mimicry (Dimberg, 1990; Hess et al., 1999). Here, however, we show that effective connectivity between this region and the IFO is specifically increased during disgust, which links simulation of the bodily feeling state of disgust with the simulation of the disgusted facial expression. Indeed, whereas lesions to the IFG resulted in widespread deficits in the perception of facial expressions (Adolphs et al., 2000), lesions to the IFO lead to more focused deficits in disgust recognition (Adolphs et al., 2003; Calder, Keane, Manes et al., 2000).

3.4.1 Conclusions

Humans can experience vivid emotional feeling states in the absence of actual emotional encounters in a myriad of ways, including the recall of past experiences, the imagination of hypothetical experiences, reading a good book, watching a good movie or witnessing a friend’s experience. By making subjects view disgusted facial expression of others, read disgust provoking scenarios and taste an unpleasantly bitter solution, we found a modality a-specific involvement of a region of the IFO during disgust. However, the functional connectivity between this region and the rest of the brain was orchestrated in a modality specific way. This suggests that the IFO is a convergence zone, where bodily feeling states relevant for the emotion of disgust are represented according to a common code (Pessoa, 2008; Prinz, 1997), regardless of stimulus modality. Our findings of IFO involvement in all three modalities supports the idea that simulation through both pre-reflective (viewing someone else’s disgust) as well as reflective (deliberate mental imagery and language) routes may therefore be complementary rather than independent of each other (Keysers & Gazzola, 2007). The functional relationship between the IFO and interconnected regions during social perception, as opposed to imaginary and actual emotional experience remains an important question for future research. However, the relative lack of overlap between the results of our effective connectivity analysis between the three modalities confirms the idea that these modalities feel different, because they are embedded in distinct and modality specific neural circuitries (Keysers & Gazzola, 2006; Pessoa, 2008). Our findings of IFO involvement in the actual imagination of gustatory disgust are in support of the important role of this region in regulating awareness and the embodiment of feeling states.
3.5 Supplementary Figures

**Figure S1** Observation stimuli

Frames represent different time points of the 3s movies depicting facial expressions during disgusting, neutral, and pleasant gustatory experiences. See Jabbi, Swart & Keysers (2007) for detailed methods.

**Figure S2** Sequence of events within a single taste trial

The person with the headphone represents the experimenter, while the individual lying supine represents a subject in the scanner. Three tubes protrude into a pacifier in the mouth through which various tastes are delivered. See Jabbi, Swart & Keysers (2007) for detailed methods.
Figure S3 Script ratings

The 12 participants of the fMRI experiment rated all 25 available scripts on a scale ranging from 0-6 according to how disgusting, how pleasant and how hard to imagine they found them. The six most disgusting, the six most pleasant, and the six most neutral (i.e. least disgusting and least pleasant) scripts were then chosen on an individual basis for inclusion in the fMRI experiment. The average rating of the chosen scripts are shown in this figure with error bars representing the standard error of the mean over the 12 subjects. * denotes significant matched-pair t-tests (two-tailed, uncorrected p<.01).

Figure S4 Sequence of events within an imagination trial

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3.6 Supplementary Tables

Table S1 Center of the 5mm radius sphere used for the PPI analysis in MNI coordinates

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Imagine disgust in your insula
Table S2 Regions of activation during the observation, experience and imagination of disgust relative to neutral

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Random effects analysis of the GLM at p<.001 with an extent threshold (k) of 10 voxels
3.7 Sample Scripts

The original scripts are written in Dutch. Below you will find an example script for each category, translated in English.

Disgust
You are sleeping for a night in a decrepit inn in a remote area. When you park yourself on the bed, you see the decomposing head of a rat rocking with your movement on the mattress. In a reflex you smack the cadaver off the sheets with your bare hand. To prevent yourself from gagging your hand reaches for your mouth. But when your hand makes contact with your face, you become even more nauseated. Your fingers are covered with a red gunk, covered with swarming maggots! You feel the tacky substance stick to your cheeks and lips. Finally, the horrid taste of tainted blood enters your mouth....

Pleasure
It is a rainy day and you have spent the whole day inside wearing a warm sweater. In front of you there is now a big, steaming pan on the table. The delicious, spicy smell of traditional soup spreads across the room. You are handed a full bowl of soup and snuggle yourself into the couch. The soup is very clear and generously filled with vegetables, vermicelli and crispy croutons. Once you have brought the spoon to your mouth, you taste the delightful combination of slightly salted soup with soft vermicelli and fresh vegetables. You feel how a warm glow runs through your body, while the slightly salty taste caresses your palate...

Neutral
You just came back from grocery shopping and have your hands full with all kinds of things. While you try to hold all the bags with one hand, the other one searches for the keys of your front door. You come across a smooth, plastic pass in your coat pocket en feel the relief of the letters on it. When you take it out of your pocket it turns out to be a pass for the supermarket. You clasp the blue object in between your lips. Your tongue pushes against the blunt brim of the tasteless, plastic pass. When you put your bags down, you take the pass out of your mouth again...
Motor simulation of emotional facial expressions in autism

Published as:
Age-related increase in inferior frontal gyrus activity and social functioning in autism (2011)
Biological Psychiatry
Chapter 4

Abstract

Background Hypoactivation of the inferior frontal gyrus during the perception of facial expressions has been interpreted as evidence for a deficit of the mirror neuron system in children with autism. We examined whether this dysfunction persists in adulthood, and how brain activity in the mirror neuron system relates to social functioning outside the laboratory.

Methods Twenty-one adult males with Autism Spectrum Disorder and 21 typically developing subjects matched for age, gender, and IQ were scanned in three conditions: observing short movies showing facial expressions, performing a facial movement, and experiencing a disgusting taste. Symptom severity and level of social adjustment were measured with the Autism Diagnostic Observation Schedule and the Social Functioning Scale.

Results Inferior frontal gyrus activity during the observation of facial expressions increased with age in autism, but not in controls. The age-related increase in activity was associated with changes in gaze behavior, and improvements in social functioning. These age-related neurocognitive improvements were not found in a group of individuals with schizophrenia, who had comparable levels of social functioning.

Conclusions The results of this cross-sectional study suggest that mirror neuron system activity augments with age in autism and that this is accompanied by changes in gaze behavior and improved social functioning. It is the first demonstration of an age-related neurocognitive improvement in autism. Increased motor simulation may contribute to the amelioration in social functioning documented in adolescence and adulthood. This finding should encourage the development of new therapeutic interventions directed at emotion simulation.
4.1 Introduction

Autism is a lifelong disorder defined by impairments in social and communicative functioning and by pronounced behavioral rigidities (Lord, Cook, Leventhal, & Amaral, 2000; F. R. Volkmar, Lord, Bailey, Schultz, & Klin, 2004). Autism Spectrum Disorder (ASD) have a strong genetic component, but no biological marker is available to date. An influential (Iacoboni & Dapretto, 2006; Rizzolatti & Fabbri-Destro, 2008; Rizzolatti et al., 2009; Williams et al., 2001) but controversial (Dinstein et al., 2008; Southgate & Hamilton, 2008) theory holds that the core social difficulties in ASD originate from a dysfunction of the putative mirror neuron system (MNS). Mirror neurons are found in macaques, in ventral premotor and inferior parietal regions involved in action execution. Single-cell recordings demonstrate that these neurons fire when the monkeys perform an action, and when they observe a similar action (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; Fogassi et al., 2005; Fujii, Hihara, & Iriki, 2008; Gallese et al., 1996). The discovery of this mirroring property challenges the distinction between action and perception and suggests motor programs may play a role in action understanding (Rizzolatti et al., 2001). A subset of ventral premotor neurons triggering mouth actions also fire to the observation of similar mouth actions, including communicative gestures (Ferrari et al., 2003). Single-cell (Mukamel et al., fMRI (Buccino et al., 2001; Filimon et al., 2007; Gazzola et al., 2007; Grèzes et al., 2003) and TMS (Fadiga et al., 1995; Urgesi, Moro, Candidi, & Aglioti, 2006) studies show that a similar system exists in humans. The motor simulation mechanism implemented in the human MNS may contribute to understanding the intentions behind the actions of others (Rizzolatti & Craighero, 2004). This also seems to be true for emotional facial expressions (Bastiaansen, Thioux, & Keysers, 2009), which trigger an increase of activity in the precentral motor face area of the observer (Carr et al., 2003; Fox, laria, & Barton, 2009; Leslie et al., 2004; Schilbach et al., 2008; van der Gaag et al., 2007; Wicker et al., 2003) that is associated with facial mimicry (Schilbach, Eickhoff, Mojtisch, & Vogele, 2008). The observer (unconsciously) mimics the emotion in a muscle-specific manner (Dimberg, 1982, 1990; Dimberg et al., 2000), which can facilitate emotion recognition (Niedenthal, 2007; Niedenthal et al., 2001; Oberman et al., 2007). Adopting emotion-specific postures triggers the corresponding emotion (Strack et al., 1988), while motor interference modifies the subjective experience of observed emotions (Effron et al., 2006). The interaction between emotion perception and motor simulation might be instantiated by the inferior frontal gyrus (IFG: Brodmann’s area [BA] 44/45) and the anterior insular cortex (Carr et al., 2003; Jabbi et al., 2007), which are anatomically connected (Nanetti, Cerlani, Gazzola, Renken, & Keysers, 2009). The anterior insular cortex, thought to represent bodily sensations (Craig, 2002), may serve as a relay between the premotor cortex and the limbic system (Carr et al., 2003; Dapretto et al., 2006; Jabbi et al., 2007). Activity in the IFG during the perception of a disgusted expression indeed seems to cause increased activity in the anterior insular cortex (Jabbi & Keysers, 2008). High empathizers activate these regions more strongly (Jabbi et al., 2007; Pfeifer et al., 2008) and mimic more (Sonnby-Borgström, 2002), which underlines the importance of motor simulation for emotion recognition and empathy (Bastiaansen et al., 2009).

In this context, the finding that children and adolescents with ASD fail to activate the IFG normally during the perception and the imitation of emotional facial expressions (Bookheimer, Wang, Scott, Sigman, & Dapretto, 2008; Dapretto et al., 2006; Greimel et al., 2010; Uddin et al., 2008) suggests a MNS dysfunction that can potentially impact social comprehension. The first
experiment tested children of 12 ± 2 years of age, and found a significant (negative) correlation between IFG activity and symptom severity (Dapretto et al., 2006). In fact, at the group level children with ASD did not show any significant IFG activity during the observation of emotional facial expressions. Three subsequent investigations with children and adolescents produced similar findings in tasks where the subjects had to match upright and inverted faces (Bookheimer et al., 2008), had to recognize themselves on a set of morphed pictures (Uddin et al., 2008), or had to judge their own emotional response while empathizing with a face on the screen (Greimel et al., 2010). Previous investigations with adults have provided mixed results, with two out of three studies failing to show significant group differences in the IFG for face perception (Ashwin, Baron-Cohen, Wheelwright, O’Riordan, & Bullmore, 2007; Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2007; Pierce, Haist, Sedaghat, & Courchesne, 2004). However, the sample size in these studies was small (approximately 10 subjects per group), and groups were not matched on critical variables. Here, using fMRI and dynamic facial expressions, we examine the relationship between IFG activity, autistic symptoms, and social behavior outside the laboratory in an adult population of 21 males with ASD that are pair-matched on age and IQ with 21 typically developing males.

4.2 Methods and Materials

4.2.1 Participants
Twenty-one adult males with ASD (age M=30.6, SD=10.09, range=18-54 years) were recruited via local mental health institutions and through mailing lists. All subjects were diagnosed with autism, Asperger Syndrome, or PDD-NOS by a clinical psychologist or psychiatrist according to DSM-IV-TR criteria (American Psychiatric Association, APA, 2000). Clinical diagnoses were verified with the Autism Diagnostic Observation Schedule (ADOS, Lord et al., 2000). One of the subjects scored below the communication domain cut-off; his diagnosis was confirmed by the Autism Diagnostic Interview Revised (ADI-R, Rutter, Le Couteur, & Lord, 2003). The subjects were considered to be high-functioning by their clinicians and none had an IQ score below 70 (IQ M=102.5, SD=14.81) on the Groninger Intelligence Test 2 (GIT2, Luteijn & Barelds, 2004). The control group consisted of 21 typically developing males (age M=30.5, SD=9.85, range=18-53 years) that were pair-matched on age and IQ (IQ M=101.5, SD=17.40) with the subjects in the ASD group (Supplementary Table 1). The presence of major psychiatric disorders was ruled out by the administration of the Dutch version of the Schedules of Clinical Assessment in Neuropsychiatry (SCAN 2.1, Giel & Nienhuis, 1996). In addition, they were interviewed to verify that first-degree relatives did not have a pervasive developmental disorder or a history of psychosis. All subjects had normal or corrected-to-normal hearing and vision, were eligible for MRI research, and gave written informed consent to participate in the study, which was approved by the Institutional Review Board of the University Medical Center Groningen (METc).

4.2.2 Behavioral Measures
We assessed each subject’s current level of social adjustment through the Social Functioning Scale (SFS, Birchwood, Smith, Cochrane, Wetton, & Copestake, 1990), which has originally been
developed for schizophrenia. The SFS, which is filled out by both the subject (SFS-client) and an informant (e.g. a parent, SFS-other), is preeminently a measure of current social adjustment in people with known social difficulties, because it is a continuous measure that taps those areas that are crucial to community maintenance (e.g. prosocial activities, independent living skills, employment). For the ASD group, we additionally used the social domain of the ADOS as a measure of symptom severity.

4.2.3 FMRI Tasks
The study of mirror mechanisms not only requires measuring brain activity when subjects perceive, for instance, the emotion of another individual, but also when they themselves feel or express an emotion. Therefore, subjects first performed an observation task, followed by two control tasks: facial movement execution, and emotion experience through a disgusting taste (see Supplementary Methods).

Observation of dynamic facial expressions
The observation task comprised two visual runs, during which subjects were asked to carefully watch short movies of facial expressions (3 s, 14° x 18°). Each run consisted of the same 60 movies presented in random order, which showed (a) actors making a disgusted, pleased or neutral facial expression (i.e. blowing up the cheeks) or (b) actors responding as naturally as possible to one of three tastes: water (neutral condition), lemon juice (disgust condition) or a sweet juice (pleasure condition). In these cases, the actors responded with a clear emotional facial expression after tasting the liquid through a straw (Figure 1a). For each stimulus type there were eight different actors (male/female), who were recruited from a local professional theatre company and a youth theatre school. The movies were validated and used in two previous experiments (Jabbi et al., 2007; van der Gaag et al., 2007). Movies were separated by a red fixation cross (1° x 1°) with an intertrial interval that varied randomly between 5 and 12 s (baseline). A still image of the background against which the actors were filmed was presented at the beginning of each run and served as a background for the fixation crosses to improve stability of the eye tracking signal (see Supplementary Methods) by keeping the pupil size constant. Stimuli were presented using Presentation software (Neurobehavioral Systems Inc., Albany, CA, USA).

4.2.3 Magnetic Resonance Images acquisition and preprocessing
See Supplementary Methods.

4.2.4 fMRI Analysis

Subject level analysis
Statistical analyses were implemented using the Statistical Parametric Mapping software package (SPM2, Wellcome Department of Cognitive Neurology, London, UK: http://www.fil.ion.ucl.ac.uk) and region of interest (ROI) toolbox MarsBaR (http://marsbar.sourceforge.net/). Time series were
high-pass filtered at 385s for the visual runs to remove low-frequency noise and slow drifts in the signal. At the subject-level, separate predictors were used as boxcar functions convolved with the hemodynamic response function for the six movie types (disgust, pleasure, neutral either with or without a cup).

**Group analyses**

Dapretto and colleagues (Dapretto et al., 2006) found that the strongest difference between children with autism and typically developing (TD) children during the imitation of facial expressions was located in the pars opercularis of the right IFG (BA44) around peak coordinates (57, 10, 16). To examine activity in this region (Figure 1b) in adults, we first created a spherical ROI centred on the corresponding MNI coordinates (Figure 1c). In the absence of information on the cluster size of the activated region, we used a 5 mm radius sphere for our analyses. We checked whether our ROI had mirror properties (Figure 1c) by examining its activity in the control group during facial expression execution and during emotion experience (see Supplementary Methods). Next, contrast estimates for all six movie types were extracted from the ROI at the subject level and subjected to a Mixed Model ANCOVA with factors Emotion x Context x Group, including IQ and Age as covariates. Because the effect of group did not interact with factors Context or Emotion, we then averaged the contrast estimates per subject over emotion and context to compute the general effect of watching facial expressions. Subsequently, we set up a multiple regression analysis in MarsBaR with six columns in the design matrix: one constant for each group, and separate IQ and age covariates for each group to account for the broad age and IQ range in both groups (18-54 yrs, 73-133 IQ pts). This analysis was repeated after the removal of two outliers, whose BA44 activity was more than two standard deviations apart from the group mean. To explore whether the effects found in the ROI were spatially limited, we repeated this analysis for all voxels in the brain using SPM (without removing the outliers).

To examine whether there was hypoactivity in the ROI for the younger subjects with ASD, we selected the eight youngest and eight oldest subjects of each group and ran two independent sample t tests. A sample size of eight is enough to enable parametric statistics, while preserving sufficient difference in age between the subgroups.

Large variability in brain responses to a stimulus reduces the information that a region can provide about that stimulus. It has recently been proposed that in ASD premotor regions show more variable responses to the vision of action (Dinstein et al.), which challenges their contribution to social perception. To examine whether premotor responses were less consistent in the ASD group during facial expression observation, we calculated in our ROI each subject’s correlation between the modeled and measured time courses across the two perception runs. The correlations were analyzed across participants using a multiple regression analysis in MarsBaR with a single entry per participant, separate constants for the ASD and TD groups, and covariates for IQ and age.

Finally, since in children with ASD BA44 activity predicts symptom severity (Dapretto et al., 2006) and social competence (Pfeifer et al., 2008), we examined the link between social symptoms, social adjustment, and brain activity in adults with ASD. To this end, we calculated the linear pairwise regressions of BA44 activity, age, ADOS (social domain) and SFS scores, and compared the regression slopes with those of the control group if applicable and with those of a group of
participants with a diagnosis of schizophrenia (see Supplementary Methods). The variability in SFS scores was too low in the TD group to perform regression analyses.

4.3 Results

4.3.1 Behavioral Measures

**Social Functioning Scale**

SFS scores were significantly lower in the ASD group compared to the TD group (SFS-client: T(22.8)= -6.234, p=.000, SFS-other: T(23.4)= -7.205, p=.000). The variability in SFS scores was very low in the TD group, reflecting a ceiling effect (TD: $\sigma^2$=9, ASD: $\sigma^2$=107; p<.005).

**Movie ratings**

The ratings collected after scanning for the different emotions are summarized in Figure S1.

4.3.2 fMRI analysis

**fMRI group comparison**

During the observation of dynamic emotional facial expressions, high-functioning adults with ASD activated a similar neural network as TD subjects, including BA44 (Supplementary Tables 2 and 3). Compared to the TD group, the ASD group did not show reduced activity in any region of the brain using a standard threshold (T=3.33, uncorrected p=.001, k=20, Figure S2), nor was there any group difference in the BA44 ROI (one-tailed T(40)=1.16, p=.13). To further examine if there is an effect of emotion (Disgust, Neutral vs. Pleasure) and/or the presence of a context (Cup vs. No Cup) on the group difference, we analyzed the signal measured in these six conditions in the ROI using an Emotion (3) x Context (2) x Group (2) Mixed Model ANCOVA including IQ and age as nuisance variables. This analysis confirmed the absence of a main effect of Group (F(1,38)=1.07, p=.31) and found no evidence for interactions of Group x Emotion (F(2,37)=1.66, p=.20), Group x Context (F(1,38)=.78, p=.38) or Group x Emotion x Context (F(2,37)=1.41, p=.25). Accordingly, we examined the average activity across all six stimulus types in all further analyses.

**Age effect on brain activity**

The regression analysis in the predefined BA44 ROI suggests that age may be a critical factor in determining BA44 activation: while there was no main effect of Age (F=3.33, p=.57), nor IQ (F=.03, p=.87), there was a significant interaction for IQ x Group (F=4.73, p=.04), and a highly significant interaction for Age x Group (F=10.55, p=.003). After the removal of two outliers (see methods), the Age x Group interaction became even more significant (F=15.93, p=.000), while the interaction of IQ x Group disappeared (F=2.51, p=.13). As shown in Figure 1d and Figure 2a-b, activity in BA44 during emotion perception increased with age for the ASD group (n=19, slope=3.1, T=2.89, p=.003), but not for the TD group (n=21, slope=-2.9, T=-2.75, p=.99) with the slopes being significantly different (p=.003). The whole-brain analysis did not reveal any regions showing a significant IQ x Group
interaction. In contrast, the Age x Group was significant in one single region of the brain: right BA44 (Talairach coordinates: 58, 12, 12), which matches the area of hypoactivation in children with ASD perfectly (Fig. 1e). Selection of the eight youngest subjects in each group showed that young adults with ASD (BA44 M=.01, age M=21.9, IQ M=93.6) activated the BA44 ROI significantly less than their TD peers (Figure 1d, BA44 M=.53, age M=21.3, IQ M=89.9, F(1,13)=6.16, p=.03). For the oldest subjects there was no significant difference between the groups, F(1,13)=.47, p=.51.

If a group has higher variability in brain response, the predicted brain response (i.e. time course of the task convolved with the hemodynamic response) should correlate less with the measured brain response. We found no significant difference in this correlation in our BA44 ROI between the ASD and TD group (one-tailed T=-.37, p=.64). However, there was a differential effect of age in the two groups (F=6.99, p<.01): the correlation increased (i.e. unexplained variance decreased) with age in the ASD group (slope=.3, T=2.41, p=.01), but not in the TD group (slope=-.2, T=1.34, p=.91).

Social functioning and BA44 activity
To examine the behavioral significance of our findings, we investigated the relationship between age, BA44 activity, a measure of autistic symptoms (ADOS social domain), and a measure of social adjustment (SFS) that assesses the subject’s engagement in activities that are crucial to community maintenance. In the ASD group, age, BA44 activity, and SFS scores were significantly and positively associated (Figure 2): older subjects not only activated BA44 more, they were also more socially adjusted than younger individuals. In contrast, the social domain of the ADOS was not significantly correlated with age, BA44 activity, nor SFS scores (all p>.24), suggesting that age-related changes in BA44 activity were associated with social adjustment as measured using the SFS, but not with the remission of autistic symptoms as measured using the ADOS. Because the TD group is characterized by high social functioning and little variation in SFS scores, we cannot assess whether the link between age and SFS and between IFG activity and SFS is specific to autism or whether it would be observed in any population with social functioning deficits. To disentangle these possibilities, we tested a group of individuals with schizophrenia having predominantly negative symptoms, which are frequently associated with social deficits (Uta Frith & Happé, 2005) and autistic-like symptoms (Bastaanssen et al., 2011b; Sheitman et al., 2004) in schizophrenia. These analyses demonstrate that age-related increases in BA44 activity and social functioning seen in ASD, do not occur in schizophrenia (see Supplementary Methods and Supplementary Figure 3).
Figure 1 ROI definition, stimuli and fMRI results

(a) Still frames at maximum intensity of the disgusted, neutral, and happy facial expressions with and without the presence of a gustatory stimulus. Neutral movies involved movement of the face to make them more comparable to the emotional facial expressions (third from left: blowing up of the cheeks, fourth from the left: tasting and lip movements). (b) Hypoactivation found in children with ASD (Dapretto et al., 2006). c) MNS ROI (5 mm sphere) based on peak coordinates in right BA44 where a group difference was reported in children (MNI: 56, 10, 14). In the TD group the ROI was not only significantly active during the observation of emotional facial expressions, but also during the execution of a facial expression, and during emotional experience (*** = uncorrected p<.001). This suggests that the ROI in our sample of TDs has mirror properties. (d) Scatter-plot of the Age x Group interaction in the BA44 ROI: the older the subjects with ASD (pink), the stronger the activity and vice versa for the TDs (blue). The bar graph in the top left shows the activity in BA44 for the youngest adults with ASD (pink, n=8, age M=21.9) compared to the youngest TDs (blue, n=8, age M=21.3), p<.05. (e) The whole-brain analysis showed that the interaction between age and group is maximal in BA44 (k=94).
Figure 2 Regression diagram

The diagrams show linear pairwise regressions between BA44 activity, age and social functioning (if applicable) in the (a) TD group, and (b) ASD group. Regression slopes b are expressed in arbitrary units (x 100) per year/ SFS point and are reported in combination with their respective significance levels (* p<.05, ** p<.01, *** p<.001). Regression slopes that are significantly different from the ASD group are marked by stars in panel (a). SFS-client refers to the questionnaire that was filled out by the subject, SFS-other refers to the version that was filled out by an informant (e.g. parent or caretaker).

4.4 Discussion

In this cross-sectional study, we measured brain activity during the observation of dynamic facial expressions in a group of adults with Autism Spectrum Disorder compared to pair-matched controls. While three previous investigations with children around 12 years of age had consistently found significant hypoactivity of the IFG (Bookheimer et al., 2008; Dapretto et al., 2006; Uddin et al., 2008), in our relatively large sample of adults both groups activated this location to the same extent, even when the analysis was restricted to the region of hypoactivity in children. This confirms the results of two other studies reporting whole-brain analyses for an adult population, in which no group difference was found involving the IFG (Ashwin et al., 2007; but see Hadjikhani et al., 2007; Pierce et al., 2004). The discrepancy between findings in children and adults is intriguing. Our study demonstrates that age might be a critical factor determining IFG activity in ASD: activity increased with age in the autism group but not in the control group, so that by age 30, individuals with ASD no longer differed from typically developing individuals. In addition, the within-subject variance decreased with age in the ASD group. This suggests that neural ‘noise’ in the IFG (Dinstein et al., 2010) decreases with age in ASD, which may be indicative of improved functioning of the MNS. Our results suggest prima facie that motor simulation of facial expressions follows a developmental trajectory with a deficit affecting individuals with ASD during their first years of life, and vanishing somewhere in late adolescence or early adulthood. Importantly, we found that the age-related increase of activity in pars opercularis of the IFG (BA44) in ASD was associated with improvements in social functioning. Increased simulation of facial expressions in the IFG is likely to affect emotion recognition, and enhance the ability of some adults with ASD to share the feelings of others (see introduction). This probably has a positive impact on social affiliation, and plays a
positive role in the construction of a tissue of social relationships (Lakin, Chartrand, & Arkin, 2008; Lakin, Jefferis, Cheng, & Chartrand, 2003). It is well-established that there is a certain degree of abatement in the behavioral difficulties experienced by individuals with ASD throughout adolescence and adulthood (Farley et al., 2009; McGovern & Sigman, 2005; Piven, Harper, Palmer, & Arndt, 1996; Seltzer et al., 2003; Shattuck et al., 2007). Improvements are mostly seen in high-functioning individuals (Howlin, Goode, Hutton, & Rutter, 2004; Shattuck et al., 2007) and concern social behaviors, as well as language, repetitive/stereotyped behaviors, and emotional responsiveness to other’s distress. Here, we found an improvement of social functioning (as measured using the SFS) with age, while autistic symptoms (as measured using the ADOS) did not change significantly. This could be the consequence of a selection bias, since we selected only participants who scored on the ADOS. Alternatively, it could indicate that while autistic symptoms predominantly persist, the way individuals with ASD cope with their social difficulties improves with age. Although speculative, this would be consistent with a longitudinal study showing that age significantly predicts a decline of maladaptive behaviors such as withdrawal and inattentiveness, but not of autistic symptoms (Shattuck et al., 2007). The relationship between IFG activity and social functioning could not be investigated in typically developing individuals, because they showed little variation in scores on the social functioning measure. To examine whether the association between IFG activity, age, and social functioning was specific to autism, we included a group of individuals with schizophrenia (see Supplementary Methods). Although the scores on the social functioning scale were comparable to those in the ASD group, IFG activity was not associated with age or social functioning in schizophrenia, suggesting that the developmental pattern might be unique to ASD. Here, it is important to note that the SFS has not been age-normed in an older adult population (>30 yrs). However, the fact that we found no evidence of increased SFS scores with age in individuals with schizophrenia argues against the idea that the age-related increase of SFS scores in the ASD group reflects an inherent property of the SFS measure. Instead, our findings are compatible with the idea that increased motor simulation could lead to the documented age-related improvements in social functioning in autism and the improved responsiveness to other’s distress evidenced throughout adolescence (McGovern & Sigman, 2005).

Further research is needed to determine the origin of the increased activity in the IFG during face perception, but the analyses conducted on the available eye tracking data (see Supplementary Methods) suggest that eye gaze behavior might be determinant (Corden, Chilvers, & Skuse, 2008; Dalton, Nacewicz, Alexander, & Davidson, 2007; Dalton et al., 2005; Senju et al., 2009; Spezio et al., 2007a; Vivanti, Nadig, Ozonoff, & Rogers, 2008). On a group level, eye gaze behaviors in our study did not differ between the individuals with ASD and TDs. This could mean that motor simulation is normal in adults with ASD -as long as they pay attention to the same aspects of the face as controls (the same conclusion has also been reached for the fusiform gyrus during face processing: Dalton et al., 2005). Again, age seems to play a critical role. In normal ageing, time spent looking at the eyes decreases, while fixations to the lower part of the face increase (Sullivan, Ruffman, & Hutton, 2007; Wong, Cronin-Golomb, & Neargarden, 2005). Here, we found that in adults with ASD the amount of time spent on the lower half of the face also increases with age. The associated increase in BA44 activity suggests that this could be a beneficial strategy for individuals with ASD. Individuals with ASD reach higher levels of accuracy on emotion recognition (Spezio et al., 2007a) and familiar face recognition tasks (Langdell, 1978) when presented with information from
the lower regions of the face, particularly the mouth region, compared to the eye region. Perhaps high-functioning individuals with ASD, while growing older, learn to look more at the parts of the face that are most relevant for them to decode emotional facial expressions. Further research is needed to investigate this hypothesis and its implications. For instance, increased fixations to the lower part of the face might lead to better recognition of some (e.g. disgust), but not other (e.g. fear) emotions not tested in this experiment (Wong et al., 2005).

If there is an actual improvement of facial simulation abilities in ASD during adolescence and early adulthood (Beall, Moody, McIntosh, Hepburn, & Reed, 2008; Magnée, De Gelder, van Engelend, & Kemner, 2007), and if it contributes to social adjustment as suggested by our study, therapeutic interventions targeting the same mechanism should be experimentally tested in children. Some recently developed training methods produce significant improvements for face recognition (Tanaka et al.) as well as emotion recognition (Golan et al., 2009; Golan & Baron-Cohen, 2006), but the generalization from training material to real-life is not guaranteed (Golan et al., 2009; Golan & Baron-Cohen, 2006). We are not aware of any study reporting the effect of training motor simulation of facial expressions. The MNS is flexible, and learning is possible even in adulthood (Catmur et al., 2007; Lahav, Saltzman, & Schlaug, 2007). Furthermore, expertise in a motor domain is associated with increased activity in the MNS during the observation of similar movements (Calvo-Merino et al., 2005; Cross, Hamilton, & Grafton, 2006; Haslinger et al., 2005). Therefore, children with ASD might particularly benefit from imitation training.

In conclusion, activity in the IFG during the perception of dynamic facial expressions increases with age in autism, and this is associated with improved social functioning. It is the first published evidence of an age-related neurocognitive improvement in autism, and suggests that individuals with ASD may learn to improve their social skills over the course of life. There was no significant age-related change in a group of individuals with schizophrenia with comparable levels of social functioning, suggesting that our findings might be specific to autism. Because autism is a developmental pathology with changes occurring over the lifetime, researchers should examine how individuals with ASD develop to deal with their initial dysfunctions, and how therapeutic interventions aimed at promoting motor simulation of emotional expressions can support this process.

**Acknowledgements**

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4.5 Supplementary Methods

4.5.1 fMRI Control Tasks

The study of mirror mechanisms not only requires measuring brain activity when subjects perceive, for instance, the emotion of another individual, but also when they themselves feel or express an emotion. Therefore, the fMRI study consisted of three tasks: observation of short movies showing emotional facial expressions, execution of a facial movement, and experience of a disgusting taste. The three tasks were performed in the same specific order for all subjects to prevent influences from the facial movement execution (task 2) or the emotional experience (task 3) on the activity during the observation of emotional faces (task 1). Subjects were initially informed about the first task (see main text). After its completion, they were instructed about the other two tasks. Two subjects came back within two weeks for an extra visual or taste run due to technical problems.

Task 2: Movement of facial muscles

After task 1 (observation of dynamic facial expressions), subjects were trained on a motor task, which required them to pull up their noses as soon as a red cross on the screen changed to green. In the scanner, subjects performed 15 trials of the task. They contracted their facial muscles for the entire duration of the green cross (4 s) and relaxed them when the cross returned to red (16 s, baseline). The task instructions were given in pure motor terms (‘pull up your nose’) to avoid any explicit emotional connotations. However, the movement was selected to resemble the facial movements observed during a disgust response. The experience of disgust was induced by task three.

Task 3: Emotion experience

To repeatedly and reliably induce emotions in the MR environment, we delivered unpleasant and neutral liquids to the subjects. The unpleasant liquid consisted of a concentration of quinine diluted with sterile water as used in previous studies (Jabbi et al., 2007; Small et al., 2003). The neutral liquids consisted of artificial saliva diluted with sterile water in three different concentrations of 2.1%, 3.3%, and 5% (Farmachemie BV Haarlem, The Netherlands, art. nr. 39.701.130). During two taste runs, quinine and the concentration of saliva that the subject rated as the most neutral were delivered as a 0.5 cm³ bolus over a 5 s interval. Two CC of sterile water were delivered as a rinse at the end of each trial. The experimenter, who was standing next to the scanner, delivered the three types of liquids via three tubes that led to a pacifier which was placed in the mouth of the subject. Due to the length of the tubes (200 cm), the experimenter could keep distance from the subjects, which ensured that they were not distracted by her presence. Each taste run consisted of six quinine and six saliva trials presented in pseudorandom order to avoid the same condition from repeating itself more than twice in a row. In addition, the first condition of the first run was always different from the first condition of the second run. Two practice trials preceded the functional runs to ensure that subjects were comfortable with the task and able to swallow the liquids without moving their heads.
4.5.2 fMRI Analysis Control Tasks

Time series were high-pass filtered at 25 s for the motor task and 242 s for the taste task to remove low-frequency noise and slow drifts in the signal (the high-pass filter is based on the maximum time interval between two events of the same condition, which is very different between the tasks). For the experience task, tasting, rinsing and swallowing were modeled as separate conditions. Motion parameters were used as covariates in the motor and taste tasks, during which motion could be expected due to task demands. However, for the majority of subjects head motion never exceeded the acquired voxel size (3.5 mm). For one control subject we removed part of a run because of excessive motion. We checked whether our region of interest (ROI) had mirror properties (Figure 1c) by examining its activity in the control group (n=21) during facial expression execution (movement vs. baseline) and during emotion experience (disgust vs. baseline). The ROI was significantly activated in both cases (p<.001).

Magnetic resonance images acquisition

Scans were acquired using a 3T Phillips Intera Quasar (Best, The Netherlands) equipped with a synergy SENSE eight-channel head coil. Functional images were acquired using a T2*-weighted echo-planar sequence with 32 interleaved axial slices aligned with ac-pc, a thickness of 3.5 mm and no slice gap to cover the entire cortex (TR=1.5 s, TE = 28 ms, TA = 1.45 s, flip angle = 70 degrees). In addition, two T1-weighted anatomical images (1 x 1 x 1 mm) containing 160 slices were acquired parallel to the bicommissural plane.

Magnetic resonance images preprocessing

Data were preprocessed using the Statistical Parametric Mapping software package (SPM2, Wellcome Department of Cognitive Neurology, London, UK: http://www.fil.ion.ucl.ac.uk). Dummy scans, during which magnetization steady state was reached, were excluded from analysis. In addition, the first and last scans, where nothing was presented to the subjects, were excluded from analysis. Functional images from all sessions were corrected for slice timing (reference slice = 12, time bin=22) and subsequently realigned to the first volume of the first run to correct for shifts in head position. Because the experiment was done in two sessions (subjects went out of the scanner between Task 1 and the other two tasks to perform the motor and gustatory training), coregistration was performed in two steps. First, the subject’s T1-weighted structural scan of the first experimental session was coregistered to the mean functional volume. Second, the anatomy of the second experimental session was coregistered to the first anatomy. This latter high-quality anatomy was segmented into gray matter, white matter and cerebrospinal fluid (CSF). The gray matter segment was normalized to a Montreal Neurological Institute (MNI) gray matter template and resulting parameters were used to normalize all functional images. Functional images were spatially smoothed with a 10 mm full-width half-maximum (FWHM) isotropic Gaussian kernel to improve the signal-to-noise ratio and to accommodate residual variations in functional neuroanatomy between subjects.
4.5.3 Eye Tracking

An infrared video camera (SMI, iView) was mounted onto the scanner bed to track subjects’ gazes during the observation runs. The same video camera was also used to record facial movements during the motor task in order to confirm that all subjects pulled up their nose according to the instructions.

Eye tracking analysis

Because we used movies of facial expressions as stimuli, we defined dynamic ROIs in order to determine the time subjects spent looking at the face, the eyes only, and the rest of the face (face minus eyes). To this end, we first manually tracked for each movie frame the position of the pupils and mouth corners using a slowed-down version of the movies. We then used these moving coordinates to dynamically define an elliptical ROI around the face of the actors and a rectangular ROI covering both eyes (Figure S4). The face ROI not only takes horizontal and vertical displacement into account, but also follows the head tilts of the actor. The amount of time spent on the face was calculated by counting the number of samples falling within the face ROI during all movies. In addition, we counted separately the number of samples that fell within the eye region, and within the rest of the face (i.e. within the face ROI but outside of the eye ROI). We then correlated these two measures with age and the amount of activity in BA44 in the two groups separately.

Eye tracking results

Calibration errors or excessive noise prevented the analysis of the data of roughly half of the subjects. Stable eye tracking data were obtained and analyzed in 15 individuals with Autism Spectrum Disorder (ASD) and 10 typically developing (TD) subjects. There was no group difference in the time spent looking at various parts of our stimuli (face region, eye region, and face minus eyes region, all p>.38). This indicates that individuals with ASD and controls looked at the same parts of the tightly cropped dynamic facial expressions. We then examined whether changes in the way subjects look at the stimuli may account for part of the age-related changes in brain activity. We found that for both groups older subjects looked less at the eyes (r=-.577, p=.003), and more at the rest of the faces (r=.633, p=.001). This could be a beneficial strategy for our stimuli, because the lower part of the face often contained much information and movement (Figure 1a). Interestingly, we found that for the ASD group looking less at the eyes was accompanied by increased BA44 ROI activity (the correlation between the time spent looking at the eye region and BA44 activity was r=-.54, p=.04).

4.5.4 Control Experiment

Participants with schizophrenia

In addition to the 21 adult males with an autism spectrum disorder (age M=30.6, SD=10.09, IQ M=102.5, SD=14.81), and 21 typically developing males (age M=30.5, SD=9.85, IQ M=101.5, SD=17.40), 20 adult males with schizophrenia were recruited (age M=37.1, SD=10.53, IQ M=91.0,
SD=17.25) as part of a larger research project on the neurobiological basis of empathy. Individuals with schizophrenia and predominantly negative symptomatology were selected by experienced clinicians from a local mental health organization (Psychosencluster, GGZ Drenthe, Assen, The Netherlands). Diagnoses were confirmed by the administration of the Dutch version of the Schedules of Clinical Assessment in Neuropsychiatry (SCAN 2.1, Giel & Nienhuis, 1996). Current symptomatology was assessed by the Positive and Negative Syndrome Scale (PANSS, Kay, Fiszbein, & Opfer, 1987). The Social Functioning Scale (SFS, Birchwood et al., 1990) was used to assess the current level of social adjustment. All subjects with schizophrenia had normal or corrected-to-normal hearing and vision, were eligible for MRI research, and gave written informed consent to participate in the study, which was approved by the Institutional Review Board of the University Medical Center Groningen (METc).

**Behavioral and fMRI analyses**

Participants with schizophrenia performed the same fMRI tasks and were given the same behavioral tests as the typically developing individuals and the individuals with ASD. For the SFS, 19 questionnaires were available for the ‘client’ version, and 15 for the ‘other’ version. Independent samples t-tests were performed to compare scores on the SFS in the group with schizophrenia and the group with ASD. MRI data preprocessing and subject-level analyses were performed in the exact same manner as described in the main paper for the other two groups. To compare activity in the BA44 ROI in the patient groups, we used a similar multiple regression analysis in MarsBaR as we did for the comparison between the ASD and TD group (two constants, two separate covariates to account for each group’s IQ, and two for each group’s age). To further examine the link between age, social adjustment, and brain activity that was found in ASD, we calculated the linear pairwise regressions within the group with schizophrenia and directly contrasted the regression slopes with those in the ASD group using a regression model including a constant for each group.

**Results**

The group with schizophrenia had SFS scores comparable to the ASD group (SFS-client: T(36.0)=-.55, p=.59, SFS-other: T(32.3)=-1.21, p=.23). In addition, mean activity in the BA44 ROI was not significantly different between the groups (F=.04, p=.83). In the group with schizophrenia, age and SFS scores were not positively related to each other nor to BA44 activity (Figure S3). A direct comparison between the slopes showed that the relations between age and BA44 activity, and age and SFS scores were significantly stronger in the ASD group (Figure S3).
4.6 Supplementary Figures

**Figure S1** Perceived intensity of disgust (a) and pleasure (b) as a function of movie and group.

Error bars represent the standard error of the mean over subjects. After scanning, the subjects rated all movies on the perceived intensity of disgust and pleasure (0 to 6). A 3 Emotions (Disgust, Neutral, Pleasure) x 2 Contexts (Cup vs. No Cup) x 2 Groups (ASD, TD) mixed MANOVA on these ratings confirmed that the different emotions were perceived differently (F(4,37)= 314.8, p=.000 for emotion). There was also a difference between groups in the MANOVA (F(2,39)=3.9, p=.03). Separate ANOVAs showed that subjects with ASD rated the movies slightly higher on disgust and lower on pleasure than the TDs. Apart from this slight negative bias for the ASD group, there was no significant interaction between Group and either Emotion or Context for the disgust and pleasure ratings (all p>.29). This means the relative differences between the movies were perceived similarly in the groups. ASD, Autism Spectrum Disorder group; D, disgust; N, neutral; P, pleasure; TD, typically developing group.
Figure S2: Renders of the activity during the observation of emotional facial expressions.

Faces (~ baseline): TD and ASD group analysis, T=3.55, uncorrected p<.001, k>20, and between-group comparison TD > ASD (blue), T=3.33, uncorrected p<.001, k>20. All results for the TD and ASD group survive FDR<.05. Results are presented on a standard MNI brain. ASD, Autism Spectrum Disorder group; FDR, false discovery rate; MNI, Montreal Neurological Institute; RH, right hemisphere; TD, typically developing group.

Figure S3: Regression diagram

The diagrams show linear pairwise regressions between BA44 activity, age and social functioning (if applicable) in the (a) TD group, (b) ASD group, and (c) schizophrenia group. Regression slopes b are expressed in arbitrary units (x 100) per year/ SFS point and are reported in combination with their respective significance levels (* p<.05, ** p<.01, *** p<.001). Regression slopes that are significantly different from the ASD group are marked by stars in panels (a) and (c). SFS-client refers to the questionnaire that was filled out by the subject, SFS-other refers to the version that was filled out by an informant (e.g. parent or caretaker). ASD, Autism Spectrum Disorder group; SFS, Social Functioning Scale; TD, typically developing group.
**Figure S4** Movie regions of interest (ROIs).

Frame of a disgusted facial expression with in green the tilted elliptical face ROI and the rectangular eye ROI.
4.7 Supplementary Tables

Table S1 Subject demographics

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ADOS, Autism Diagnostic Observation Schedule; ASD, Autism Spectrum Disorder group; SFS, Social Functioning Scale; TD, typically developing group.
**Table S2** Peaks of activity during the observation of emotional facial expressions for the TD group

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Faces - baseline: TD group. Cluster size (k) and MNI coordinates (mm). Uncorrected p<.001, T=3.55, k>20. All results survive FDR<.05. BA, Brodmann area; FDR, false discovery rate; H, hemisphere; L, left; MNI, Montreal Neurological Institute; R, right; TD, typically developing.
Table S3 Peaks of activity during the observation of emotional facial expressions for the ASD group

<table>
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<th>(k)</th>
<th>T</th>
<th>H</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
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Faces - baseline: ASD group. Cluster size (k) and MNI coordinates (mm). Uncorrected p<.001, T=3.55, k>20. All results survive FDR<.05. ASD, Autism Spectrum Disorder; BA, Brodmann area; FDR, false discovery rate; H, hemisphere; L, left; MNI, Montreal Neurological Institute; R, right.
Autism and the mirror neuron system

A version of this manuscript is under review as:
Autism spectrum disorders and the putative mirror neuron system
Thioux, M., Bastiaansen, J. A., & Keysers, C.
Abstract

The idea that the core symptoms of autism could have originated from a dysfunctional mirror neuron system has received much attention from the media in the past five years. In this review, we evaluate this controversial claim by examining a wide array of behavioral, neurophysiological, and neuroimaging studies on imitation, action perception, and face perception in Autism Spectrum Disorder. We observe that the available evidence for a disruption of the mirror neuron system in autism is quite inconsistent. Several factors that could contribute to an explanation of these inconsistencies are: the nature of the stimuli, the degree of identification with the actor, and the attention paid to the actor’s movements. Furthermore, the age of the participants may play an important role, since there are indications that in autism functioning of the mirror neuron system might improve with age. The available evidence does not support the claim that a dysfunctional mirror neuron system can explain the whole constellation of autistic symptoms, but does instigate an interesting modified hypothesis. Autism might be characterized by a developmental delay that predominantly affects the functioning of strictly congruent mirror neurons, which are rarer than broadly congruent ones and are necessary to represent the precise body kinematics of observed actions. Such a specific and transient difficulty might be the consequence of differences in synaptic plasticity and/or early deficits in orienting attention towards biological motion and faces, and could account for some of the imitation difficulties found in autism.
5.1 Introduction

Mirror neurons were discovered in the ventral premotor cortex of the monkey (area F5), which was studied for its involvement in action preparation. Mirror neurons have the astonishing property of firing not only when the monkey performs an action, but also when the monkey observes or hears another individual perform a similar action (di Pellegrino et al., 1992; Fujii et al., 2008; Gallese et al., 1996; Keysers et al., 2003; Kohler et al., 2002; Umiltà et al., 2001). With the firing of these neurons, the monkey can be said to ‘simulate’ the actions of others: it activates premotor neurons as if it were performing a similar action. More recently, neurons with the same property were also found in the inferior parietal cortex (area PF/PFG) of the monkey (Fogassi et al., 2005; Fujii et al., 2008; Rozzi, Ferrari, Bonini, Rizzolatti, & Fogassi, 2008). A continuum of response properties has been uncovered, which ranges between the features of two types of prototypical mirror neurons. On one end of the continuum there are strictly congruent mirror neurons, which only fire when the observed action is performed with the same effector and in the same manner as the action they serve to execute. On the other end there are broadly congruent mirror neurons, which fire during the observation of a range of actions that share the same goal as the one they serve to execute, irrespective of the effector used by the other individual (Gallese et al., 1996; Keysers, Thioux, & Gazzola, 2011; Rizzolatti & Craighero, 2004). For example, a mirror neuron that responds selectively to the observation and the execution of a precision grip is classified as strictly congruent. A mirror neuron that is responsible for the observation and execution of a precision grip, but that also fires to the observation of another individual grasping with the whole hand, the foot, or the mouth, is classified as broadly congruent. Strictly congruent mirror neurons may encode the specific details of how an observed action is performed, while broadly congruent mirror neurons are well-positioned to encode the goal of the action, that is what it tries to achieve, irrespective of how it is achieved - with the hand, with the mouth, or even with the foot (Keysers et al., 2011; Thioux et al., 2008).

5.1.1 The human mirror neuron system

Humans also ‘simulate’ the actions of others in their action execution system: activity in the parietal and premotor regions increases when a subject observes someone else performing an action (Buccino et al., 2001; Gazzola & Keysers, 2009; Gazzola et al., 2007; Grèzes et al., 2003; Iacoboni et al., 1999; Shmuelof & Zohary, 2005; Tai, Scherfler, Brooks, Sawamoto, & Castiello, 2004), or hears the sound of an action (Gazzola et al., 2006). Furthermore, action perception facilitates congruent motor output as measured using motor evoked potentials (Aziz-Zadeh, Iacoboni, Zaidel, Wilson, & Mazziotta, 2004; Urgesi et al., 2006) and reaction times (Brass, Bekkering, Wohlschlager, & Prinz, 2000). Recently, several research teams have used functional magnetic resonance imaging (fMRI) to examine more closely whether the same neuronal populations in the parietal and premotor cortices are recruited during both action execution and observation. Two experiments have confirmed this by showing cross-modal adaptation: the blood-oxygen-level-dependent (BOLD) signal in the parietal and premotor cortices is diminished during action observation when it is preceded by the execution of the same action, relative to a different action (Chong, Cunnington, Williams, Kanwisher, & Mattingley, 2008; Kilner, Neal, Weiskopf, Friston, & Frith, 2009). Two other studies
have demonstrated that a pattern classification algorithm, which has learned to discriminate brain activity corresponding to the participants listening or watching actions A and B, can also discriminate above chance whether on different trials the participants executed action A or B (Etzel et al., 2008; Oosterhof, Wiggett, Diedrichsen, Tipper, & Downing, 2010). The cross-modal adaptation of the BOLD signal and the successful pattern classification across execution and perception suggest that the activity across the premotor and parietal regions during action observation reflects the firing of neuronal populations that encode information about the actions (what and how) in the same manner as when executing similar actions.

In humans, mirror neurons are thought to play an important role in imitation and in understanding the goal behind the actions of others (Iacoboni, 2009; Keysers, 2009; Rizzolatti & Craighero, 2004; Rizzolatti & Luppino, 2001). In addition, a subset of mirror neurons encoding mouth and face movements may contribute to the perception of facial expressions (Ferrari et al., 2003; Mukamel et al., 2010). The observation of facial expressions is associated with increased activity in regions of the inferior precentral and inferior frontal gyri that are also involved in producing similar expressions (Carr et al., 2003; Leslie et al., 2004; van der Gaag et al., 2007). This activity is thought to trigger congruent activity in emotional brain regions such as the insula and the amygdala (Carr et al., 2003; Jabbi & Keysers, 2008), which are involved in experiencing similar emotions (Jabbi et al., 2007; Wicker et al., 2003). According to this hypothesis motor simulation could also contribute to decoding the emotional value of facial expressions (Bastiaansen et al., 2009; Iacoboni & Dapretto, 2006; Jabbi & Keysers, 2008).

The same network, comprising the inferior frontal gyrus (IFG), the insula, and the amygdala, is also likely to be active during ‘facial mimicry’ (Carr et al., 2003; Lee et al., 2008). Electromyography (EMG) recordings during the observation of facial expressions demonstrate the occurrence of spontaneous reactions in those muscles of the face that are also involved in the production of that facial expression -for instance in the zygomaticus major muscle when viewing a series of happy faces (Dimberg et al., 2000). These spontaneous reactions of the facial muscles occur approximately 500 ms after stimulus onset. The fact that witnessing an angry expression is often associated with activity in the frontalis muscle, which is involved when producing a fearful expression (Beall et al., 2008), and the fact that viewing body postures can also trigger an emotionally congruent reaction in the muscles of the face (Magnée, Stekelenburg, Kemner, & De Gelder, 2007; Tamietto et al., 2009), suggest that these facial reactions may occur following an emotional response. The reader is, however, also seems to be true: sometimes facial reactions influence the way emotions are perceived (Niedenthal, 2007). In fact, blocking facial mimicry affects the perception of the boundary between different emotional expressions (Niedenthal et al., 2001), as well as the perceived duration of an emotional expression (Effron et al., 2006). Taken together, these observations indicate that motor simulation and emotional processing interact reciprocally during the perception of facial expressions.

5.1.2 Development of the human mirror neuron system

Little is known about the early development of the mirror neuron system (MNS). Two studies have collected indirect measurements of mirror neuron activity in infants using electroencephalography (EEG, Lepage & Théoret, 2006) and Near-Infrared Spectroscopy (Shimada & Hiraki, 2006). The
results suggest that activation of the (pre)motor cortex during action observation is present by the age of 6 months. At this age, infants show a propensity to encode the goal of the actions they observe (Woodward, 1998), which might reflect the work of the MNS. The tendency to encode the goal of observed actions is not present earlier in life, but seems to develop together with the infant's ability to execute the same actions (Sommerville & Woodward, 2005; Sommerville, Woodward, & Needham, 2005, but see Csibra, 2008; Kamewari, Kato, Kanda, & Ishiguro, 2005; Southgate, Johnson, & Csibra, 2008). Therefore, the MNS might gradually develop between 0 and 6 months, hand in hand with the child's ability to execute goal-directed actions.

According to Keysers and Perrett (2004), Hebbian learning could explain the development of the MNS. Since during grasping the child is also spectator of her own hand actions, parietal and premotor neurons fire at the same time as some neurons in the posterior superior temporal sulcus (pSTS), which respond to the observation of hand actions irrespective of the viewpoint. Those neurons that fire at the same moment strengthen their connections through Hebbian synaptic potentiation. This increases the specificity of the connections between the pSTS neurons, which are involved in the perception of body movements, and the grasping circuits of the parietal and premotor cortex, where mirror neurons will acquire their property. The same pairing between execution and observation also occurs in situations where one is being imitated (Brass & Heyes, 2005; Del Giudice et al., 2009; Heyes, 2001). For instance, a child cannot observe its own facial expressions, but the adult who imitates the child's expression can serve as a mirror. This could trigger an activation in the child's pSTS that becomes associated with the premotor cortex activity corresponding to the expressed emotion (for a different perspective see Meltzoff & Decety, 2003). Recently, Del Giudice et al. (2009) proposed that the early development of the MNS could be canalized by several genetic factors such as the spontaneous and cyclical movements present in childhood between 0 and 6 months, and a hard-wired preference for biological motion and for perfect contingencies between perception and sensation. Spontaneous movements occurring cyclically could help develop stable neuronal circuits in the (pre)motor cortex. At the same time, a child's preference for biological motion and perfect contingencies could guide their attention towards their own movements and optimize Hebbian learning.

5.1.3 The mirror neuron theory of autism

Some researchers have proposed that the core symptoms of autism could result from an impairment of the MNS. The central idea is that if mirror neurons support the ability to imitate others and to understand the goal behind their actions, the disruption of this system might also impair the later development of the ability to understand the state of mind of other people (Iacoboni & Dapretto, 2006; Meltzoff & Decety, 2003; Oberman & Ramachandran, 2007; Rizzolatti & Fabbri-Destro, 2008; Rizzolatti et al., 2009). To examine this hypothesis in detail, we review studies that have investigated the cerebral substrate of imitation and action perception in Autism Spectrum Disorder (ASD) in the following sections. We begin with studies where participants were explicitly requested to imitate the observed actions and then turn to studies investigating the observation of hand action without imitation, which can be regarded as automatic imitation or action simulation from a MNS perspective. Before concluding, the penultimate section will examine in detail automatic facial reactions and the involvement of simulation mechanisms during the perception of emotional
expressions, which seems to constitute an area of particular weakness in autism.

5.2 On Imitation

5.2.1 Neuroimaging findings on imitation

Three studies have compared brain activity in individuals with ASD and controls during tasks that explicitly required the imitation of facial expressions or hand/finger movements. One study used magnetoencephalography (MEG) to test imitation in eight adults with ASD and 10 control participants (Nishitani, Avikainen, & Hari, 2004). Subjects had to reproduce three different mouth configurations that corresponded to the ones seen on pictures of a face. Source analysis revealed that the active brain circuit during imitation, comprising occipital cortex, STS, inferior parietal lobule, IFG, and primary motor cortex (MI), was similar in the two groups. However, in the ASD group activation of the IFG was delayed approximately 50 ms. In addition, the activation of the IFG and MI was weaker compared to controls. In both groups, MI activation was observed approximately 50 ms after activation of the IFG. The two other experiments used fMRI to investigate the cerebral network involved during imitation in autism. In the first study, Dapretto et al. (2006) scanned children with autism and matched controls (12 ± 2 y.-o.) while they were imitating and observing emotional facial expressions. In contrast to the typically developing children, the children with ASD did not activate the pars opercularis of the inferior frontal gyrus (BA44) during imitation. This difference was found both in the contrast between imitation and rest within the ASD group, and in the direct comparison between the groups. In addition, BA44 was also less active in the ASD group compared to controls during the passive observation of emotional expressions. Finally, activity in the pars opercularis was negatively correlated with symptom severity in the ASD group during imitation. These results are unlikely to be attributable to a failure in attending to the faces: the children in the ASD group showed reliable activity in the fusiform gyrus and amygdala, and their imitation performance did not differ from that of control participants. The authors conclude that the failure to activate BA44 denotes a deficit of the mirror neuron system in autism. In the second fMRI study, Williams et al. (2006) tested the imitation of finger movements in 16 adolescents with ASD and 15 matched controls (15 ± 2 y.-o.). In this experiment (adapted from Iacoboni et al., 1999), the subjects had to move their right index or middle finger in response to a stimulus, which was either a movie of the finger movement, a still picture of a hand with a cross over the finger that had to be moved, or a grey background with only the cross that indicated which finger to move. The direct comparison between groups showed that several areas were less active in the participants with autism during the imitation of finger movements (- rest), including the inferior parietal cortex. The authors interpreted this as a deficit of the MNS. This conclusion is weakened, however, by the fact that for the same contrast several areas were hyper-active in participants with autism, including the left precentral gyrus, which is a major component of the human MNS. Furthermore, there was no significant group difference in the network of brain areas that was more active during the imitation condition compared to the simple execution of the finger movement (in front of a still hand or a grey background). Moreover, in contrast with previous research (Iacoboni et al., 1999), differences in activity were not seen in the inferior frontal gyrus (BA44) for this contrast, not even in the control group.
Although the three aforementioned studies suggest that the cerebral network involved in the imitation of hand actions and facial expressions might be abnormal in autism, they do not reveal consistent differences in MNS activity. One study found a delayed and weaker activation of the IFG, one reported a complete absence of activity in BA44, and one study found a hypo-activation of the inferior parietal lobe, along with many other areas of hypo- and hyper-activation. These inconsistencies may largely result from the different stimuli used in the three experiments. Participants had to imitate non-meaningful mouth movements in the first study (Nishitani et al., 2004), meaningful emotional expressions in the second study (Dapretto et al., 2006), and non-meaningful finger movements in the third study (Williams et al., 2006). These findings suggest that imitation of facial expressions may be particularly challenging for children with ASD. Before drawing any conclusions about the role of mirror neurons in imitation difficulties and in the etiology of ASD, the nature and severity of the imitation difficulties in autism should be carefully considered.

5.2.2 Nature of imitation difficulties in autism

The most carefully designed studies on imitation performance have included a control group of children that is matched to the ASD group on the basis of mental age and verbal IQ. These studies have shown that the overall level of development explains most of the interindividual variability in imitation performance, and that the overlap of performance between groups is large (e.g. Beadle-Brown & Whiten, 2004; Charman & Baron-Cohen, 1994; Charman et al., 1997; Perra et al., 2008; Rogers, Hepburn, Stackhouse, & Wehner, 2003). Part of the imitation difficulties may even be accounted for by factors that are not specific to imitation or action perception-execution matching. For instance, Vivanti and collaborators (2008) recently showed that the low performance of their ASD group in imitating non-meaningful gestures could be explained by the amount of time these individuals spent fixating the demonstrated action. This important finding suggests that the performance of subjects with ASD on imitation tests may suffer from their difficulties in the perception of biological motion more generally (Blake, Turner, Smoski, Pozdol, & Stone, 2003; Klin, Lin, Gorrindo, Ramsay, & Jones, 2009). This ability involves the pSTS, a cortical area that is considered to be the main source of input to the MNS, in which decreased grey matter volume has been reported in autism (e.g. Boddaert et al., 2004; Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2006). The contention that imitation can be impaired in autism for reasons other than a dysfunction of the observation-execution matching mechanism was tested by Leighton et al. (2008). The authors studied a group of 16 adults with ASD, who displayed a deficit for both mirror and non-mirror imitation compared to well-matched controls. They gave the participants two new variations of the imitation task the ASD group had performed poorly on. In one version, the to-be-performed action was indicated by the movements of geometrical shapes on the screen, instead of the human hand movement. In another condition, the execution aspect of the task was removed, and the participants had to describe what the human agent was doing. Participants with autism were impaired on the two alternative versions of the test as well. Therefore, the authors concluded that the imitative nature of the main task was not responsible for the low performance in this group. It should be noted, however, that the origin of the difficulties observed in the two alternative versions of the imitation task remains unexplained. Following the instructions indicated by the movements of the shapes might, for instance, be accomplished in controls by using the same neuronal ensembles
as those used for action perception-execution matching (Schubotz, 2007). Furthermore, a deficit in the processing of biological motion could contribute to the imitation deficit in the other test (i.e. verbal description of hand actions). The merit of this study is that it stresses the importance of testing more precisely what aspects of the imitation task are fundamental to the difficulties experienced by individuals with ASD, as the problem might not be restricted to matching action perception with execution, but might occur ‘upstream’ (Tessari, Canessa, Ukmar, & Rumiati, 2007).

When examining the role of the MNS in the imitation difficulties evidenced in ASD, it is also important to realize that not all aspects of imitation performance are equally affected. The difficulties experienced by individuals with autism seem much greater when they have to understand the beliefs of someone else than when they have to imitate hand actions (Hamilton, Brindley, & Frith, 2007). Critical for the mirror neuron hypothesis, children with autism do not seem to have much difficulty reproducing the goal of an action that was demonstrated by an experimenter (Aldridge, Stone, Sweeney, & Bower, 2000; Carpenter, Pennington, & Rogers, 2001). Carpenter et al. (2001) tested infants between two and five years old on a task in which children were shown an action performed on an object, and were then given the opportunity to manipulate the object themselves (Meltzoff, 1995). Both children with autism and mentally retarded children accomplished the target actions more often after being shown a failed attempt to perform the action than after witnessing a non-purposeful action or during prior spontaneous interaction with the objects. Furthermore, in both groups children performed the expected target action after viewing a failed attempt as often as after the experimenter showed the correct end state. In this experiment, children with autism were perfectly able to interpret actions in the context of their goals. According to Hobson and collaborators (2008; 1999), one aspect that seems more problematic for these children is the reproduction of the style of an action—for instance, throwing a ball into a trashcan gently (underhand toss) or more forcefully (overhand throw). The authors showed that the style in which an action is performed is unlikely to be reproduced by children with autism, especially when the style is not necessary for the successful accomplishment of the action. Children with autism did perform similarly as controls in the imitation of goal-directed actions demonstrated on unfamiliar objects. According to Hobson and Hobson (2008), the specific difficulty imitating the style of an action might indicate a difference in the quality of the intersubjective engagement during the test (Hobson & Meyer, 2006; see also Carpenter, 2006). From our own perspective, the dissociation between goal and style is reminiscent of the distinction between strictly and broadly congruent mirror neurons. While both strictly and broadly congruent mirror neurons respond to the sight of the action they serve to execute, broadly, but not strictly, congruent mirror neurons also discharge during the observation of other actions that achieve the same goal using different means (or styles, following the denomination of Hobson and collaborators). Given that strictly congruent mirror neurons make up only one third of the MNS (Gallese et al., 1996), the accurate encoding of the style that depends on this subpopulation should be three times more vulnerable to neural dysfunctions than the encoding of the action goal, which might explain the dissociation between goal and style replication found in autism.

5.2.3 Comments

In conclusion, evidence for an impairment of imitation skills in autism as a result of a dysfunction of
the MNS is still scarce and inconsistent. Recent investigations that try to characterize more precisely the nature of the imitation difficulties help clarify the possible role of the MNS. The dissociation between the imitation of goal and style (Hobson & Hobson, 2008) suggests that the MNS might function sufficiently well to successfully match the goal of observed actions, but not well enough for the more vulnerable reproduction of style. At the same time, the finding that the time spent looking at the action can explain the performance on the imitation of meaningless gestures (Vivanti et al., 2008) suggests that an impairment of the pSTS, upstream of the MNS proper, may also play a critical role in imitation difficulties. The pSTS is implicated in the perception of biological motion and is the main source of input of the MNS. Any deficit at this level can be expected to have serious effects on the functioning and development of this system. Finally, the participation of the MNS in the processing of emotional facial expressions might be critically impaired in children with autism, which may contribute to some of the social difficulties evident in ASD as we shall discuss later.

5.3 Observation of hand actions

Other researchers have looked for evidence for a dysfunction of the MNS in autism in the simulation of observed actions without an explicit action imitation requirement. Here again, studies that have looked at the neural response during action perception have produced relatively inconsistent results.

5.3.1 MEG, EEG, and fMRI investigations

One early study used MEG (2x61 channels) to record the response of the motor cortex during action execution and action observation in five adults with autism and eight controls (Avikainen, Kulomaki, & Hari, 1999). The left and right median nerves (running the length of the arm) of the subjects were stimulated alternatively, while the rolandic oscillations in the Beta frequency range (~20 Hz) were used as an indicator of MI activity. During rest, stimulation of the median nerve is followed by a 20Hz-rolandic rebound. This induced rebound is abolished when the participant executes an action, and is also greatly reduced during action observation. The same action-observation-induced suppression of the rolandic rebound was found in controls and participants with autism. This indicates that in this group of young adults with autism, the motor cortex activity was modulated during action observation in a similar way as in controls. The groups were, however, small and the variability between individuals with autism was large.

In addition to this MEG study, several research groups have used EEG to measure the suppression of mu rhythms over the central electrodes during action execution and action observation in individuals with ASD. Mu rhythms are EEG oscillations recorded over the sensorimotor cortex in (or slightly above) the alpha frequency range (8.5-10.5 Hz), which are suppressed during movement execution and observation in typically developing subjects; a phenomenon that is thought to indirectly reflect the firing of neurons in the (pre)motor cortex (Pineda, 2005). In one experiment, Oberman et al. (2005) found a complete absence of mu rhythm suppression during the observation of hand actions in autism. In this experiment, participants opened and closed their hand, watched a movie of the same action, watched a movie with two bouncing balls, and watched
a display consisting of background white noise. In controls, significant mu suppression was observed for both the action execution and action observation conditions relative to baseline, but not during the observation of bouncing balls. In ASD, mu suppression was present during execution, but it was completely absent for the observation of hand movements. In a complementary experiment with a group of children between eight and 12 years of age, Oberman et al. (2008) found that the mu wave suppression was significant in the autism group if the hand performing the action in the movie was familiar, but not when it was unfamiliar. The absence of mu wave suppression in ASD during the observation of hand actions has, however, not been replicated by three other research teams. In one study, Bernier and collaborators (2007) asked young adults with and without ASD to watch a grasping action, execute the same action, and imitate the action. Significant mu suppression was found in all three conditions in participants with ASD, though the effect was less pronounced compared to controls. In another study, Fan and collaborators (2010) had their participants (n=20 ASD, 11-26 y.-o., n=20 matched controls) observe videos of a hand manipulating a small object, and execute the same action. Significant mu suppression was found in both groups during imitation as well as passive observation of hand actions. Furthermore, in both groups mu suppression was larger for the observation of hand actions than for the observation of a moving dot. Finally, Raymaekers and collaborators (2009) reported significant mu wave suppression in two groups of children aged between eight and 13 years, who observed and executed a meaningless hand movement (i.e. closing and opening of the hand). Here also, mu suppression was larger in both groups during the observation of hand movements than during the observation of a bouncing ball (non-biological motion). Interestingly, in this study involving younger children the authors found a significant correlation between the index of mu suppression and the age of the participants in the ASD group only. Similar to the original studies by Oberman and collaborators (2005; 2008), this study tested meaningless hand movements that are usually less accurately imitated than meaningful ones in autism (Rogers, Cook, & Meryl, 2005; Vivanti et al., 2008; Williams, Whiten, & Singh, 2004). Therefore, normal mu suppression in this experiment demonstrates that the use of meaningless movements may not explain the absence of effect found in the original study. The more recent study by Oberman et al. (2008) suggests that instead the engagement of ASD individuals in the task may be a major determinant of the amount of simulation. In the familiar hand condition the children were shown a picture of the actor (family member or self) before the movie depicting the hand action, which might have helped those with ASD to identify with the actor.

Further research is needed to reveal the effects of different experimental variables on mu wave suppression in ASD. Since autistic symptoms appear early in life and may change over the course of development, the presence of mu wave suppression should ideally be tested in very young children. Mu rhythms are, however, not always easy to find using EEG. For instance, testing children between five and seven years of age, Martineau and collaborators (2008) failed to find a modulation of the mu rhythms that they had previously found in adults (Cochin, Barthelemy, Roux, & Martineau, 1999). In this experiment, children watched complex dance movements or control stimuli representing landscapes. Interestingly, a modulation of theta rhythms (1 - 5.5 Hz) was found in typically developing children that was absent in children with ASD (Martineau et al., 2008). As there was no execution condition in the experiment, it is impossible to know whether this difference in theta rhythms modulation should be attributed to a dysfunction of the MNS. This finding indicates, however, that exploring other frequency bands might be necessary to capture mirror neuron activity
in younger children.

Surprisingly, only a couple of studies have used fMRI to investigate the passive observation of hand actions in ASD. The results of these two studies are rather straightforward: there seems to be no major deficit in the cerebral network involved in simulating hand actions. Martineau and her colleagues (2010) compared the cerebral activity of seven adults with ASD and eight control participants while they observed and executed flexion-extension hand movements. No cerebral area was hypo-active in the ASD group. On the contrary, relative to controls, the ASD participants showed hyperactivation of the inferior frontal gyrus pars opercularis (BA44) in both hemispheres during the observation of meaningless hand movements compared to the observation of a hand at rest. In a sophisticated experiment, Dinstein et al. (2010) used a repetition suppression technique to investigate the response of the MNS during action execution and observation. Thirteen adults with ASD and 10 controls observed meaningful hand postures and performed the same gestures. For action execution, the same reduction in BOLD signal was observed in both groups in the premotor cortex and the intraparietal region for repeated relative to novel finger movements. During observation, the reduction of the signal with stimulus repetition was also observed in both groups, but only in the intraparietal region. The direct comparison between groups also did not reveal major differences in mirror neuron areas. In both groups, the intraparietal region and the ventral premotor cortex were active during both action observation and execution. Interestingly, a supplementary analysis of the within-subject variability (across blocks) showed that the variability of the BOLD response was higher in individuals with autism, despite similar average levels of whole-brain hemodynamic responses.

5.3.2 Measuring interference from observed actions

A behavioral interference paradigm makes it possible to obtain an indirect measurement of the motor cortex activity elicited by action observation. In a simple but powerful experiment, Bird et al. (2007) asked adult participants with and without ASD (n=15 in each group) to watch short movies of a human or a robotic hand changing from a neutral intermediary position to an open or closed position. The participant’s task was to open or close their dominant hand as soon as the stimulus started to move. The onset of the visual stimulus served as a go signal, but whether the stimulus was of the open or closed type was irrelevant to the action of the participant. Participants performed blocks of the same movement in which the observed action could be either congruent or incongruent with the executed movement. Similar to controls, participants with autism were slower when the action was performed while viewing an incongruent stimulus. This is suggestive of normal automatic activation of the (pre)motor cortex during action observation. Furthermore, in both groups the interference effect was more pronounced when the observed action was performed by a human than by a robot, which is in agreement with the idea that mirror neurons are preferentially tuned to react to biological motion (Dayan et al., 2007). In the same vein, Spengler et al. (2010) recently reported significant interference of observed actions on the executed actions of adult participants, who were requested to lift a finger in response to a number that appeared superimposed on a movie of a hand performing a congruent or incongruent finger movement. Although these results suggest normal interference from observed actions in autism, an interesting observation from another research group provides some support to the idea that individuals with
autism sometimes fail to simulate other people’s action in their (pre)motor cortex. Welsh and collaborators (2009) asked the participants in their experiment to reach for a target position on the left or on the right side of a table, alternating turns with another subject, who was sitting in front of them. In the course of each turn, the subjects performed two consecutive reaching actions. When reaching twice for the same location, both controls and participants with ASD showed an inhibition of return, which means that they were slower reaching for a location when they had reached for the same location just before (within-subject IOR). Interestingly, an inhibition of return was also found when the subjects had to reach for a location just after the other participant had reached for the same location (between-subjects IOR). Here, an interesting difference emerged between the groups. Participants with ASD showed a between-subjects IOR if, and only if, the LED that triggered the other’s response was visible. When the LED could not be seen, and the subjects could only see the end of the other participant’s movements, the inhibition of return disappeared in the group with autism, but remained in the control group. This suggests that in contrast to controls, participants with ASD were not influenced by a representation of the action of the other individual in their motor cortex. At first glance, these results seem to contradict the two other studies’ reports of normal interference (Bird et al., 2007; Spengler et al., 2010). One explanation could be that in the context of this game, participants with ASD looked at the LED and paid little attention to the hand of the other player, since it was not required by the task. If this interpretation is correct, it would support the idea that individuals with ASD are somehow less likely to automatically orient their attention towards the movements of other humans (Klin et al., 2009).

5.3.3 Monitoring hand muscles activity

Two additional studies must be described before closing this section on the simulation of hand actions. Both studies found significant group differences when looking at muscle-specific reactions triggered by action observation. In an experiment involving 10 adults with ASD and 10 controls, Théoret et al. (2005) used transcranial magnetic stimulation (TMS) over the motor cortex to measure the modulation of motor evoked potential (MEP) by the observation of finger movements. The hand on the video was presented either in egocentric or allocentric view (i.e. fingers facing away or towards the observer, respectively). During action observation, MEPs of control participants increased in the muscle involved in executing the observed movement (relative to the other muscles). In autism, MEP facilitation was found for hands in an allocentric orientation, but not for hands in an egocentric orientation. This may indicate a specific difficulty in identifying with the actor when the hand is seen from an egocentric perspective. A more drastic difference was described in another study. Cattaneo et al. (2007) used EMG to record the activity of the mouth-opening mylohyoid muscle while participants were grasping or were observing someone grasping an object with the purpose of either eating it or placing it in a container. The participants were seven children with ASD and eight controls between five and nine years old. In anticipation of the subsequent action, children in the control group already activated the mylohyoid muscle during the reaching phase of the “grasping to eat” action. The same was true when they observed the same action being performed by the experimenter. During execution, children with ASD failed to show preparatory activity of the mylohyoid muscle before the bringing-to-the-mouth phase of the action. Moreover, they showed no activation of this muscle during observation, not even during the last
phase, when the experimenter was moving the food to his or her mouth. In a complementary experiment, the execution of a chain of hand-foot actions was tested. Participants were asked to reach and grasp a piece of food and throw it in a garbage can, which could be opened using a foot pedal. Activity was recorded from the ankle dorsiflexor muscle. Control participants anticipated the pedal opening during the grasping phase, whereas the children with ASD did not. These results suggest that children with ASD fail to anticipate the next action they are going to perform in their motor cortex, while typically developing children do (see also Fabbri-Destro, Cattaneo, Boria, & Rizzolatti, 2009). In addition, while typically developing children showed a modulation of the ankle muscle during and shortly before observing that of the demonstrator, children with ASD failed to show any evidence of simulating the observed actions in their motor cortices. Naturally, simulation need not occur if the subject is unable to execute the observed action. Therefore, if the children with ASD did not anticipate the next action they were going to perform themselves, they may not have anticipated the action of the experimenter in their motor cortex. The results indicate, however, a complete absence of motor activity during observation in the group with ASD, while they were capable of performing the action themselves - albeit without the typical sequential anticipation (Fabbri-Destro et al., 2009).

5.3.4 Comments

In summary, concerning the automatic simulation of hand actions/finger movements, three studies reported abnormal motor simulation (Cattaneo et al., 2007; Oberman et al., 2005; Welsh et al., 2009), three studies found partial support for a simulation deficit (Bernier et al., 2007; Oberman et al., 2008; Théoret et al., 2005), and seven studies reported normal (or enhanced) motor simulation (Avikainen et al., 1999; Bird et al., 2007; Dinstein et al., 2010; Fan et al., 2010; Martineau et al., 2010; Raymaekers et al., 2009; Spengler et al., 2010). It is often argued that negative findings should be considered with caution, because the study may have simply lacked the statistical power to detect the hypothesized effect. However, in the case of the studies failing to find a group difference between controls and participants with ASD, this argument is much more difficult to apply. Given that those studies that failed to find group differences are those that did demonstrate significant evidence for action simulation within the ASD group, and given that those that did find group differences are those that failed to find evidence for simulation in ASD, it is unclear which of the two groups of studies should be discarded as possible ‘false negatives’. Instead, it seems more promising and important to examine the factors that could explain why some studies failed to find significant motor simulation in their cohort of participants with autism. The results of the three experiments that reported an interference effect of observed actions suggest that, while the automatic activation of motor simulation circuits may be preserved (Bird et al., 2007; Spengler et al., 2010), orientation towards biological motion is probably an important variable (Welsh et al., 2009). Furthermore, mu wave suppression and MEP experiments show that the familiarity or level of identification with the actor is also likely to influence the amount of motor simulation in autism (Oberman et al., 2008; Théoret et al., 2005). These factors (attention to biological motion and familiarity/identification with the actor) may contribute to the high variability of BOLD response described in individuals with ASD (Dinstein et al., 2010). Finally, most experiments investigating the simulation of hand actions have tested adult participants. Those that tested children between seven
and 12 years of age have reported either a significant difference between groups (Cattaneo et al., 2007; Oberman et al., 2008) or a positive effect of age on action simulation in the ASD group (Raymaekers et al., 2009).

5.4 Facial and bodily expressions

Facial expressions constitute a very particular class of stimuli for individuals with autism, who tend to look less at the eye region or even at the face as a whole (e.g. Corden, Chilvers, & Skuse, 2008; Warren Jones, Carr, & Klin, 2008; Klin & Jones, 2008; Klin, Jones, Schultz, Volkmar, & Cohen, 2002; Pelphrey et al., 2002; Spezio, Adolphs, Hurley, & Piven, 2007a, 2007b), and who as a group tend to demonstrate difficulties in discriminating emotions from facial expressions (e.g. Ashwin, Chapman, Colle, & Baron-Cohen, 2006; Corden et al., 2008).

5.4.1 Inferior frontal gyrus pars opercularis and face perception

Although early fMRI studies of face perception in autism often concentrated on the functioning of the fusiform face area, several teams of investigators have now reported whole-brain group comparisons during passive observation of emotional or neutral faces (Ashwin et al., 2007; Dapretto et al., 2006; Hadjikhani et al., 2007), and also during perceptual tasks requiring the participants to detect female faces (Pierce et al., 2004), detect their own face in morphed pictures (Uddin et al., 2008), or match upright and inverted faces (Bookheimer et al., 2008). As far as potential differences in motor simulation are concerned, it is difficult to draw any straightforward conclusion from these experiments. Two out of three studies involving adult participants failed to find any group differences in mirror neuron areas (Ashwin et al., 2007; Pierce et al., 2004). The third study found a hypo-activity of the pre- and post-central regions, and the inferior frontal gyrus during passive viewing of neutral faces (Hadjikhani et al., 2007). However, in this case, the difference could also be attributed to the greater proportion of female participants in the control group, as women are thought to be more empathic and simulate more than males (Baron-Cohen, Knickmeyer, & Belmonte, 2005). Three studies, which tested younger participants between eight and 18 years of age, revealed significant hypo-activation of the inferior frontal cortex (Bookheimer et al., 2008; Dapretto et al., 2006; Uddin et al., 2008). The areas of hypo-activity are, however, scattered over the inferior frontal region; some areas with group differences are more than 25 mm apart from each other (Marc Thieux & Keysers, 2010). Unquestionably, these studies used very different tasks and stimuli. It is still unclear whether all these tasks and stimuli are equally well-suited for investigating the simulation of facial expressions.

We recently obtained fMRI data from a relatively large group of 21 adult participants with ASD and 21 controls while they watched short movie clips of actors displaying neutral, pleased, and disgusted facial expressions (Baas, et al., 2011a). We did not find any group difference between participants with and without ASD, even when focusing the analysis on a region of interest in the inferior frontal gyrus (BA44), where Dapretto et al. (2006) found a significant reduction of activity in a group of (only 12) children with ASD. However, in our study involving adults between 18 and 55 years of age, activity in pars opercularis (BA44) increased significantly with age in participants with ASD, but not in controls. While the youngest adults with autism showed significant
hypo-activation of BA44, by age 30 activity in this region was indistinguishable between the two
groups. Importantly, this age-related increase of activity in BA44 in autism was associated with
improvements in social functioning. The same relationships between BA44, age, and social
functioning were not found in the control group of participants, who were pairwise-matched with
the participants with autism on age, gender and full-scale IQ. Furthermore, these correlations were
not found in another control group that was scanned using the same protocol. This group consisted
of individuals with a diagnosis of schizophrenia, who had scores that were comparable to the ASD
group on a social functioning questionnaire. Therefore, the increase of activity in BA44 with age
might be specific to the developmental trajectory of ASD, and may have a significant impact on the
social functioning of these individuals. One possible explanation for the observed age-related
increase in BA44 activity could be a change in the way older individuals look at the face. Analysis of
the participants “points of regard” during the experiment showed that older participants, ASD and
controls alike, had a tendency to look longer at the mouth region, which contained most of the
relevant information about the displayed emotion in the movie clips. In a seminal study involving
adolescents, Klin et al. (2002) also found that social competencies in ASD were significantly
correlated with the time spent looking at the mouth region. These observations suggest that the
pattern of gaze behaviors in children with ASD might be responsible for a delay in the development
of the neural circuits supporting the motor simulation of facial expressions, which in turn would
have direct consequences on the ability to share others’ emotions and on the development of social
competences (Bastiaansen et al., 2011a).

5.4.2 Monitoring facial muscles activity

Recording facial muscle reactions with EMG is another approach for investigating (pre)motor cortex
activity that is triggered by the perception of facial expressions. The results of three studies that
compared individuals with and without ASD seem to confirm that age matters. In one study,
McIntosh and collaborators (2006) recorded the EMG response of the cheek and brow muscles in 11
individuals with ASD and 14 controls between 13 and 64 years of age, who watched displays of happy
and angry facial expressions. The authors looked at the rate of automatic EMG responses, and found
no difference between the groups. However, while in the control group the muscle responses were
most often congruent with the observed facial expression (e.g. activity in the zygomaticus for happy
faces), in participants with ASD there were as many incongruent as congruent responses. In a
supplementary task, the authors also asked the participants to voluntarily imitate the expressions
they observed. In this condition, more activity was recorded in the congruent than in the
incongruent muscle in both groups. In a subsequent study, Beall et al. (2008) recorded the EMG
activity of three different facial muscles while children between seven and 12 years of age watched
displays of happy, fearful, and angry facial expressions. In the typically developing children, the
experiment revealed muscle-specific reactions to happy and angry facial expressions, but not to
fearful faces. In children with autism, the pattern was very different; there was no response for
happiness and anger, and an undifferentiated response to fearful faces. Interestingly, there was a
relationship between age and muscle activity in autism, with older children showing a higher rate of
congruent responses to happy faces. Finally, in a study involving only adults, Magnée et al. (2007)
found that the EMG response was actually larger in participants with ASD, and was perfectly
congruent with the displayed happy or fearful emotion.

Taken together, the results of these three EMG investigations are consistent with the hypothesis that motor simulation of facial expressions improves with age in ASD. Importantly, these studies also highlight the fact that facial motor reactions can be of normal overall intensity in autism, but at the same time lack the congruence observed in typically developing individuals (McIntosh et al., 2006). Translated to the limited resolution of fMRI, which still struggles to discriminate motor activity patterns across emotions (van der Gaag et al., 2007), such an abnormal pattern of activity in motor cortices could masquerade as BOLD activity of normal overall intensity. One possibility could be that the pattern of normal overall intensity coupled with a lack of specificity in facial reactions is an intermediate state between a total absence of simulation in children (Beall et al., 2008; Dapretto et al., 2006), and a perfectly normal or even enhanced simulation of facial expressions in adults (Bastiaansen et al., 2011; Magnée, De Gelder, van Engeland, & Kemner, 2007). Further studies will be necessary to validate this hypothesis, and to investigate the relationship between empathy (i.e. sharing the feelings and the emotions of others) and motor simulation of facial expressions throughout the lifespan, both in ASD and typically developing individuals.

5.4.3 Empathy and motor simulation

Two recent publications have offered a first glimpse on the fragile relationship between empathy and motor simulation in individuals with autism. Avoiding the use of faces as stimuli, Grèzes et al. (2009) scanned 10 adults with ASD and 10 controls during the observation of neutral and fearful body postures. The authors concluded that there were no major group differences in the pattern of parietal and premotor activity related to the observation of dynamic body postures (neutral and fearful together). Group differences were found, however, for the processing of fearful compared to neutral stimuli, with the ASD group showing hypo-activation of the dorsal premotor cortex and the pars triangularis of the inferior frontal gyrus in the right hemisphere, hypo-activation of the amygdala, and an absence of modulation of the connectivity between the amygdala and the other regions. Therefore, this experiment suggests the presence of a problem (much likely pervasive) in the relationships between mirror neuron functioning and activity in emotional/affective centers of the brain. The second study conducted by Minio-Paluello and collaborators (2009) supports this conclusion. In this experiment, 16 adults with ASD and 20 well-matched controls underwent single-pulse TMS while they observed pictures of a hand that was affected in a painful or non-painful manner. The painful stimuli depicted a static image of the hand being pricked by a needle. Three types of control stimuli were used that showed the hand being touched with a cotton stick, the hand alone, or a tomato being pricked with the needle. The TMS-induced MEPs were recorded in two hand muscles: the pain-affected muscle and an unaffected control muscle. In line with the results of a previous study (Avenanti et al., 2005), MEPs in controls decreased in the muscle receiving pain when the participants watched the painful movie condition (relative to the three other conditions). The same modulation was not found in the group of participants with ASD. Moreover, the amount of MEP modulation for the painful condition correlated with the imagined sensory qualities of pain in control participants, but with reports of self-arousal experience in individuals with ASD. These interesting results suggest that individuals with ASD do not share the
sensory and motor components of the pain experienced by others to the same extent as typically developing individuals do.

5.4.4 Comments

In summary, the available evidence indicates that motor simulation of facial expressions may improve with age in ASD (Bastaanssen et al., 2011a; Beall et al., 2008; Dapretto et al., 2006). This phenomenon may be related to changes in gaze behaviors and may have important consequences for social functioning (Bastaanssen et al., 2011a; Klin et al., 2002). Interestingly, the investigation of facial muscle reactions suggests that the activation of the (pre)motor cortex, even if present, may not always match the observed emotion faithfully (McIntosh et al., 2006). This pattern of facial reactions with normal intensity, but lack of normal muscle-specificity, may represent an intermediate stage between a complete absence of facial simulation in children (<12 y.-o.), and a normal or enhanced simulation in adults (>30 y.-o). Such a developmental delay, if verified, could originate from two complementary sources. First, the autistic brain may be characterized by abnormal activity-dependent synaptic plasticity due to abnormalities in synaptic proteins such as neurexins, neuroligins, and Shank3 (Bourgeron, 2009; Pardo & Eberhart, 2007). In a Hebbian learning framework (Del Giudice et al., 2009; Keysers & Perrett, 2004), this reduced plasticity would imply that infants diagnosed with ASD need more frequent pairings of their own facial expressions with those of others in order to develop strictly congruent mirror neurons and muscle-specific facial mimicry. Infants with autism, however, seem to preferentially orient towards non-social contingencies and to lack the normal propensity to look at others (Warren Jones et al., 2008; Klin & Jones, 2008; Klin et al., 2009; Osterling, Dawson, & Munson, 2002; Volkmar, Chawarska, & Klin, 2005). This second factor could lead to a deprivation of the congruent visual signals that are necessary in ASD to form Hebbian associations between their own facial expressions and those of others, which could lead to a retarded development of congruent mirror neurons for facial expressions.

There is empirical evidence that links the abnormal pattern of gaze behaviors in ASD to a hyper-reactivity of the amygdala (Dalton et al., 2005). According to some researchers, individuals with ASD actively avoid looking at the face and the eyes of other people to prevent aversive over-arousal (Corden et al., 2008; Dalton et al., 2005; Nacewicz et al., 2006). Therefore, a dysfunction of the amygdala could, through its influence on gaze behaviors, contribute to an explanation of the delay in the maturation of strictly congruent mirror neurons and the late appearance of congruent facial muscle reactions. In contrast, the communication between emotional centers of the brain and motor simulation mechanisms may remain quite problematic in ASD throughout adulthood (Grèzes, Wicker, Berthoz, & De Gelder, 2009; Minio-Paluelo et al., 2009). The relationships between motor reactions, emotion recognition, and emotional empathy, and the changes that may occur during development in autism, should be further investigated.

5.5 Conclusion

In this review, we critically examined the claim that Autism Spectrum Disorder could result from a dysfunction of the mirror neuron system. The available evidence indicates that action perception
can trigger a motor response in individuals with ASD, albeit not as consistent as in typically developing individuals. In this concluding section, we will summarize the factors that likely influence motor simulation in ASD and discuss their possible impact on the development of the mirror neuron system. We will finish this section with a brief comment on the possible relationship between understanding action goals and understanding other people’s state of mind.

5.5.1 Factors that influence motor simulation in autism

The reason why some researchers have failed to find evidence of motor simulation whilst others have might be partly due to the heterogeneity within and across studies of the recruited ASD groups. Simulation mechanisms may, for instance, not be affected to the same extent in every individual, and measurement errors may be larger in groups of participants with ASD (Dinstein et al., 2010; Fan et al., 2010). Multiple differences between studies may also account for the discrepancies in the reported results. The present review of the literature identified several potential candidates that seem to influence the amount and quality of motor simulation in autism: the age of the participant, the nature of the stimuli, the degree of identification (or familiarity) with the actor, and the participant’s tendency to orient towards biological motion.

Automatic orientation towards biological motion is thought to be disrupted in toddlers with ASD, who tend to orient towards non-social contingencies instead (Klin et al., 2009). Such a deficit early on is likely to have negative consequences on the maturation of the pSTS circuits supporting the processing of biological motion (Blake et al., 2003; Boddaert et al., 2004), and consecutively on the development of the mirror neuron system. Furthermore, it could explain why the amount of time children with ASD spend looking at the demonstrator is a good predictor of imitation accuracy for meaningless gestures (Vivanti et al., 2008). In addition, it could explain the occasional lack of action simulation that is observed in adults with ASD when explicitly attending to the movement of another individual is not required by the task (Welsh et al., 2009).

The influence that the degree of identification with an actor has on motor simulation in ASD is suggested by the results of two experiments. The first showed typical patterns of mu rhythms suppression only when a picture of the child (or a family member) was presented prior to the hand stimulus (Oberman et al., 2008). The second found simulation for hands shown in an allocentric, but not egocentric viewpoint (Théoret et al., 2005), -as if individuals with ASD were prevented from simulating in the condition where the hand looks like their own.

The nature of the stimulus is also likely to have an influence on motor simulation in ASD. Emotional faces might, for instance, be more challenging (Beall et al., 2008; Dapretto et al., 2006; McIntosh et al., 2006), than goal-directed meaningful hand actions (Avikainen et al., 1999; Bernier et al., 2007; Bird et al., 2007; Dinstein et al., 2010; Fan et al., 2010; Raymaekers et al., 2009).

Finally, age seems to be an important factor. Three studies have reported a significant relationship between age and the amount of motor simulation in ASD (Bastiaansen et al., 2011a; Beall et al., 2008; Raymaekers et al., 2009). In addition, those studies that have found a complete absence of (pre)motor cortex activity during the observation of hand actions (Cattaneo et al., 2007) or facial expressions (Dapretto et al., 2006) have all tested ASD groups comprising young participants (under 14 y.-o). The recruitment of the simulation network may normalize with age in ASD, which might positively affect social adjustment in adulthood (Bastiaansen et al., 2011a). This
finding is in agreement with the results of clinical studies demonstrating social improvements throughout adolescence and adulthood (e.g. McGovern & Sigman, 2005; Shattuck et al., 2007; for a review of earlier studies see Seltzer, Shattuck, Abbeduto, & Greenberg, 2004). If the associations between age, social functioning, and increased motor simulation are confirmed by new studies, it could have a very positive consequence for interventions in ASD, as it suggests that promoting the simulation of facial and bodily expressions at an early age might improve social functioning in ASD. The MNS is actually plastic, and a good deal of experiments have shown that expertise in a motor domain is associated with increased activity in the simulation network during the observation of similar movements (e.g. Calvo-Merino, Glaser, Grezes, Passingham, & Haggard, 2005; Cross, Hamilton, & Grafton, 2006; Haslinger et al., 2005). In the same vein, training one specific finger movement in reaction to a stimulus representing a different movement, can create a muscle-specific response in the trained finger that occurs when the stimulus is presented again for passive observation (Catmur et al., 2007). This indicates that it should be possible to promote muscle-specific facial reactions while viewing various emotional stimuli. The hope is that this will help improve decoding emotions in ambiguous situations and ultimately enable sharing others’ emotions “on the fly”. We believe this sort of intervention may have positive effects even if the primary deficit lies outside the mirror neuron system, as for instance in the tendency to orient towards biological motion, in the reactivity of the amygdala to emotional stimuli, or in the communication between emotional centers of the brain and the (pre)motor cortex.

5.5.2 Broadly versus strictly congruent mirror neurons

Regarding the possible involvement of the MNS proper in Autism Spectrum Disorder, the report of a dissociation between imitation of goal and style (Hobson & Hobson, 2008) seems to be critical. This finding, namely, suggests that the MNS may work well enough to match the goal, but not the style of an observed action. This is consistent with the literature on autism and facial mimicry, which suggests that even when there is a response at the muscle level, this response is not always congruent with the observed emotion (McIntosh et al., 2006). Furthermore, this idea is consistent with two studies that have found an impairment of hand action simulation in autism based on the measurement of muscle activity (Cattaneo et al., 2007; Théoret et al., 2005). Muscle activity in response to hand action observation means that the result of the simulation in the premotor cortex is sent all the way down to the body muscles that are specifically involved in executing the same action with the same effector. This process requires more than the simulation of the goal, which can be accomplished by broadly congruent mirror neurons. It probably also requires the participation of the rarer strictly congruent mirror neurons and output from this premotor system to the spine (probably through MI). In sum, a new hypothesis may be tentatively advanced stating that simulation difficulties in autism may result from a partial dysfunction or delay in the maturation of MNS that specifically affects the less numerous, and therefore more vulnerable, strictly congruent mirror neurons.
5.5.3 Mirror neurons and theory of mind

Several researchers (e.g. Iacoboni & Dapretto, 2006; Oberman & Ramachandran, 2007; Rizzolatti & Fabbri-Destro, 2008; Rizzolatti et al., 2009; Williams et al., 2001) have argued that a dysfunction of the MNS could impair both the comprehension of the immediate goal of observed actions (e.g. she is going to grasp the pencil), and the later development of the ability to read the mind of others (e.g. she wants to draw a sketch of the new lab facilities for us to see). The causal link between the integrity of the MNS and the development of a capacity to reflect upon the state of mind of others has, however, not been established (de Lange, Spronk, Willems, Toni, & Bekkering, 2008; Spunt, Satpute, & Lieberman, 2011). Reflecting upon the state of mind of someone else is known to engage structures outside the MNS, including the medial prefrontal cortex, the superior temporal sulcus, the temporal poles, and the temporo-parietal junction (Amodio & Frith, 2006; Gallagher & Frith, 2003; Saxe, 2006). There is evidence suggesting that these brain regions might be hypo-active when individuals with ASD are engaged in theory of mind tasks (Castelli, Frith, Happé, & Frith, 2002; Happe et al., 1996). Investigating the relationships between the MNS and those regions that support mind reading will be necessary for a thorough assessment of the mirror neuron theory of autism. The present review demonstrates that individuals with Autism Spectrum Disorder do not suffer from major difficulties in understanding the immediate intentions of others, and are capable of re-enacting others’ actions in their (pre)motor cortices. Given the comparatively greater difficulties they seem to experience in theory of mind tasks (Hamilton et al., 2007), it seems very unlikely that a properly functioning mirror neuron system would be sufficient to independently enable mind reading.

Acknowledgments

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Behavioral similarities in autism and schizophrenia

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Abstract
Autism Diagnostic Observation Schedule (ADOS) module 4 was investigated in an independent sample of high-functioning adult males with an autism spectrum disorder (ASD) compared to three specific diagnostic groups: schizophrenia, psychopathy, and typical development. ADOS module 4 proves to be a reliable instrument with good predictive value. It can adequately discriminate ASD from psychopathy and typical development, but is less specific with respect to schizophrenia due to behavioral overlap between autistic and negative symptoms. However, these groups differ on some core items and explorative analyses indicate that a revision of the algorithm in line with Gotham et al. (2007) could be beneficial for discriminating ASD from schizophrenia.
6.1 Introduction

Although for a diagnosis of an autism spectrum disorder (ASD) symptoms should be present from infancy or early childhood, the disorder may not be detected until later because of several reasons: a well-structured support system, compensation for limitations through high intelligence, the presence of more subtle autistic symptoms, and confusion with or overshadowing by another psychiatric disorder (Kan, Buitelaar, & van der Gaag, 2008; Wing & Potter, 2002, see also www.dsm5.org). Partly due to increasing knowledge of milder forms of autism and more awareness that autistic conditions can be found in individuals of high ability, ASDs are starting to become more widely recognized in adults (Brugha et al., 2009; Fombonne, 2005; Kan et al., 2008; Wing & Potter, 2002). In clinical practice, we notice a growing demand for diagnostic procedures concerning ASD in adults. However, there is no established diagnostic tradition for ASD in older individuals. It is very challenging to disentangle social and communicative problems associated with ASD from the often complicated clinical picture in adulthood, especially when developmental information is unavailable. Standardized instruments are needed that can facilitate the diagnostic process. Poor self-referential cognition present in many individuals with ASD may hamper self-report measures of autistic symptoms (Johnson, Filliter, & Murphy, 2009; Lombardo, Barnes, Wheelwright, & Baron-Cohen, 2007). Therefore, observation of the individual during social interaction is important in addition to information about difficulties experienced in daily life.

The Autism Diagnostic Observation Schedule (ADOS, Lord et al., 2000) is a standardized instrument that assesses social interaction, communication, and imagination during a semi-structured interaction with an examiner. The ADOS includes four modules suited for individuals with different developmental and language levels, ranging from children with no expressive language to older and verbally more capable individuals. The psychometric properties of modules 1-3 are well-studied and present the ADOS as a reliable and valid instrument to assess the presence of ASD in children (de Bildt et al., 2004; Gray, Tonge, & Sweeney, 2008; Lord et al., 2000; Noterdaeme, Sitter, Mildenberger, & Amorosa, 2000; Papanikolaou et al., 2009). Module 4 was developed for adolescents and adults with fluent speech. In the original paper on the ADOS, Lord and colleagues (2000) included module 4 administrations for adolescents and young adults with autism (AD, n=16), PDD-NOS (n=16) and with various other diagnoses (n=15). Their results indicate that, after training as described in the manual (Lord, Rutter, DiLavore, & Risi, 1999), ADOS module 4 can be used effectively to distinguish between autism spectrum and non-spectrum, and to a lesser degree between AD and PDD-NOS. Thus far, no further specific studies into the value of module 4 have been reported. When establishing a diagnosis, clinicians need to rule out specific conditions that can cause similar symptoms. Because the control group in Lord’s study (2000) was relatively small and very diverse with respect to diagnosis, it is still unclear to what extent ADOS module 4 can support such differential diagnostics.

One disorder that shares symptoms with autism is schizophrenia. Kanner (1943) even borrowed the term autism from Eugen Bleuler, who used it to describe withdrawal from contact with the outside world in adults with schizophrenia (1911). Although autism and schizophrenia have different developmental trajectories, cross-sectionally their clinical presentations overlap (Frith, 2003; Goldstein, Minshew, Allen, & Seaton, 2002; Volkmar & Cohen, 1991). Especially individuals with schizophrenia and negative symptoms show many of the same social deficits as adults with
autism (Frith, 2003; Sheitman et al., 2004). Autism also shares features with psychopathy, a personality disorder which partly overlaps with antisocial personality disorder (APD). Besides poor behavioral control and a disregard for the rights of other people, individuals with psychopathy have deficits in the emotional and interpersonal domain, such as insensitivity or lack of empathy towards other people. Impairments in empathy are also central to ASD, characterized by a cognitive impairment to take the perspective of other people (Baron-Cohen & Wheelwright, 2004; Gillberg, 1992). Rogers and colleagues (2006) indicate that there could be a subgroup of people with ASD that have additional callous-unemotional traits reminiscent of psychopathy. Others report that some individuals with ASD may seem cold and heartless, because they are unaware of how their behavior affects other people, which could lead to a diagnosis of APD or psychopathy by mistake (Bartels & Bruinsma, 2008; Howlin, 2000; Kohn, Fahum, Ratzoni, & Apter, 1998). Especially in forensic settings, it is important to differentiate ASD from psychopathy, because they require different approaches. It should be noted, however, that unlike in psychopathy there is little evidence of any excess of crimes among people with autism (Howlin, 2000).

The current study will examine the psychometric properties of ADOS module 4 by including relatively homogeneous non-autistic groups: a group of adult males with schizophrenia and marked negative symptoms, males with psychopathy, and typically developing males. Analyses will center on the original ADOS algorithm (Lord et al., 2000), based on the operationalization of the DSM-IV and ICD-10 criteria for autistic disorder (American Psychiatric Association, APA, 1994; World Health Organization, WHO, 1993), but will also include some preliminary analyses based on revised algorithms for the ADOS. In line with proposals for the revision of the DSM (www.dsm5.org), the revised algorithms of the ADOS for modules 1-3 synthesize the items from the original social interaction and communication domains into the new domain Social Affect (SA, Gotham et al., 2007). This new notion of communicative and social behaviors as a single set of symptoms is supported by recent studies showing that non-verbal communication and social items load onto the same factor (Constantino et al., 2004; Lord et al., 1999; Robertson, Tanguay, L’Euyer, Sims, & Waltrip, 1999; van Lang et al., 2006). In addition, the revised algorithms include restricted and repetitive behaviors (RRB) as opposed to the original algorithm. Although the narrow time frame of the ADOS might not provide adequate opportunity to measure these behaviors (Lord et al., 2000), they seem to make an independent contribution to diagnostic stability (de Bildt et al., 2009; Lord et al., 2006). While adults with ASD may have a slightly different behavioral phenotype compared to children (Gotham et al., 2007), the core difficulties persist in adulthood (Seltzer et al., 2004; Shattuck et al., 2007). Therefore, it is of interest to explore the utility of this promising new metric in our adult population.

6.2 Methods

6.2.1 Participants

Thirty-two adult males with an ASD were recruited via local mental health organizations (mainly through the specialized Autism Team North Netherlands of Lents, Groningen, the Netherlands), and through mailing lists for high-functioning individuals with ASD. Six individuals with ASD were recruited from a local forensic clinic (FPC Dr. S. van Mesdag, Groningen, the Netherlands). The
participants were considered to be high-functioning by their clinicians and none had an IQ score below 70. All participants were diagnosed with an ASD by a clinical psychologist or psychiatrist according to DSM-IV-TR criteria (n=8 AD, n=17 AS, n=13 PDD-NOS), based on review of developmental history, current daily functioning, and observation. For this study, the ASD group will be investigated as one diagnostic entity along a continuous dimension of severity for two reasons. First, it is proposed for the near future that distinctions will no longer be made among different types of autism in clinical practice, because they have proven to be “inconsistent over time and place, and to be associated more with severity, language level, and intelligence than specific features” (www.dsm5.org). Individuals with autism and PDD-NOS have also shown qualitatively similar behavioral patterns on the ADOS with varying degrees of severity (Lord et al., 2000). Second, investigating the subtypes would lead to overly small subgroups.

Eighteen adult males with schizophrenia and predominantly negative symptomatology, mainly outpatients, were selected by a specialized local mental health organization (Psychosenceluster, GGZ Drenthe, Assen, the Netherlands). Diagnosis was confirmed by a structured clinical interview, the Dutch version of the Schedules of Clinical Assessment in Neuropsychiatry developed by the WHO (SCAN 2.1, Giel & Nienhuis, 1996). Current symptomatology was assessed by the Positive and Negative Syndrome Scale (PANNS, Kay et al., 1987).

The psychopathy group consisted of 16 males recruited from two forensic psychiatric clinics (FPC Dr. S. van Mersedag and FPC Veldzicht). As part of the standard clinical procedure, these individuals were assessed with the Psychopathy Checklist Revised (PCL-R), an instrument widely used for the diagnosis of psychopathy (e.g. Hare, 1991). Two diagnosticians obtained consensus on this instrument after separately scoring the items using file information extended with, if necessary, a semi-structured interview.

The typically developing group consisted of 21 typically developing males, who were interviewed to verify that first-degree relatives did not have an ASD or a history of psychosis. Age and IQ was matched with the participants with ASD who also took part in the neuroimaging part of the study (n=21). There are no significant differences between the groups in terms of age and IQ. For an overview of the group characteristics see Table 1.

Table 1 Group Characteristics

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>31.82</td>
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<tr>
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<td>29</td>
<td>101.14</td>
<td>14.67</td>
<td>73-133</td>
</tr>
<tr>
<td>Schizophrenia</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>18</td>
<td>37.00</td>
<td>10.73</td>
<td>19-61</td>
</tr>
<tr>
<td>IQ</td>
<td>18</td>
<td>89.17</td>
<td>13.89</td>
<td>68-112</td>
</tr>
<tr>
<td>Psychopathy</td>
<td></td>
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<td></td>
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</tr>
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<td>Age</td>
<td>16</td>
<td>39.00</td>
<td>10.67</td>
<td>23-60</td>
</tr>
<tr>
<td>IQ</td>
<td>15</td>
<td>92.73</td>
<td>16.10</td>
<td>63-117</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>21</td>
<td>34.24</td>
<td>9.14</td>
<td>21-53</td>
</tr>
<tr>
<td>IQ</td>
<td>21</td>
<td>97.19</td>
<td>16.37</td>
<td>73-128</td>
</tr>
</tbody>
</table>

Note. IQ scores were based on the Groninger Intelligence Test 2 (GIT 2, Luteijn & Barelnds, 2004), except for four individuals with ASD who were administered the Wechsler Adult Intelligence Scale (WAIS, Wechsler, 1997) and nine individuals with ASD for whom IQ scores were not obtained (they only took part in the ADOS part of the research project). For these cases, IQ was estimated to be in the normal range based on former IQ tests and clinical impression / daily functioning. GIT 2 scores for one individual with psychopathy were deemed unreliable and discarded.
6.2.2 Measures and procedure

Administration of the ADOS was part of the standard procedure of two large neuroimaging studies into the neural basis of empathy conducted in the Social Brain Laboratory (www.bcn-nic.nl/socialbrain.html). All participants gave written informed consent. The studies were approved by the Institutional Review Board of the University Medical Center Groningen (METc). The administration of ADOS module 4 included all standard activities and the optional daily living items to obtain relevant background information. The interviews were administered and scored by trained and certified psychologists. In total, five raters participated in the project including two certified ADOS trainers (AdB, SH). To ensure that agreement between raters remained at the high level requested by the ADOS, we discussed (fragments of) videotapes in two-monthly group meetings. The interviews were scored for consensus from videotape in changing pairs of raters, but included the examiner in the far majority of cases. In contrast to the second rater, the examiner was not blind to clinical diagnosis. The consensus scores were determined on the basis of the video-recording through a discussion in which the judgment of each rater was weighted equally. We only made an exception to this procedure when there was major disagreement (0 vs. 2) for the items B1 (Eye Contact) and B2 (Facial Expressions). Then, we gave priority to the examiner’s opinion, because we anticipated that these items might be difficult to judge from videotape alone. Fortunately, due to the high quality of the video-recordings, there was major disagreement in only two out of 93 administrations for eye contact, while for facial expressions such disagreement never occurred. Therefore, it is unlikely that the examiner’s previous knowledge influenced the consensus scores.

6.2.3 Design and analysis

Algorithms

In this paper, we will use the terms “original algorithm” when referring to the standard algorithm (Lord et al., 2000) and “revised algorithm” when referring to the application of the revised algorithm based on Gotham et al. (2007). To reach a classification of AD or ASD on the original algorithm of the ADOS, an individual needs to meet thresholds for the communication domain (COM), the social interaction domain (SOC), and for the summation of these two domains (COMSOC), but not for the restricted and repetitive behaviors domain (RRB, Lord et al., 1999). For the revised algorithm, classification is based on solely thresholding the SARRB domain, which combines social, communication, and restricted behavior items. Since algorithm items across modules 3 and 4 are comparable and our sample size does not permit independent factor analyses in order to establish specific algorithm items, we applied the revised algorithm for module 3 to our group of high-functioning adults to calculate domain scores and a total score. In line with the explanation on the original algorithm, scores of 3 were converted to 2, and all scores other than 0-3 were treated as 0.

Interrater agreement

Interrater agreement was assessed on the original algorithm at the level of ADOS classification, domains, and items. Agreement between raters at the level of diagnostic classification (AD, ASD, nonspectrum) was calculated through Cohen’s weighted kappa in addition to the percentage of
agreement. Cohen’s kappa takes into account the agreement that can occur by chance between two raters and is therefore more stringent than the mere calculation of the percentage of times the raters’ scores lead to the same ADOS classification. Interrater agreement on the domains and the total score was calculated by means of intraclass correlations (ICC). ICC scores represent correlations across pairs of raters and are higher the more consistent the scores across two different raters are. ICC scores, internal consistency and correlations could not be reliably calculated for the RRB domain, because variance was too limited: for four out of the five items less than five subjects scored different from zero. To assess interrater agreement for separate items, we used mean linearly weighted Cohen's kappa's in line with Lord et al.(2000). Cohen’s linearly weighted kappa takes into account the agreement between two raters occurring by chance and considers the difference between a score of zero and one to be smaller than a difference between zero and two. Item B3 was ignored because its score depends on items A9, B1 and B2. In addition, only items were included for which more than five subjects scored different from zero (excluding nine items: A1, A3, A5, D1, D2, D3, D5, E1, E2).

Internal consistency
To measure the internal consistency of the original and the revised domains, we applied Cronbach’s alpha. This statistic increases as the intercorrelations among test items within a domain increase.

Comparison of domain means
We used an ANOVA for each scale of both algorithms with fixed factor group, followed up by Tukey’s HSD post-hoc comparisons. We performed one-tailed Mann-Whitney tests to examine whether the forensic ASD group scored higher than the psychopathy group. To compare group differences at item level, we performed a MANOVA with fixed factor group on all items except the previously mentioned nine items that had limited variance and item B3. Post-hoc tests were performed for those items that showed a significant group effect.

Criterion-related validity
Here, criterion-related validity refers to the degree to which the outcome on the ADOS instrument is in agreement with the clinical diagnosis of having ASD or not. We used logistic regression to measure the success of both algorithms in predicting whether a participant received a diagnosis of ASD in clinical practice. Because ADOS classification is based on COM and SOC for the original algorithm and on the combined SARRB domain for the revised algorithm, we used these domains as predictors in two separate analyses. Logistic regression provides information on the sensitivity and specificity for the fixed cut-off point used in clinical practice. Receiver Operating Characteristic (ROC) curves provide information on the sensitivity and specificity of all other possible scores. In addition, it provides an Area under the Curve statistic (AuC), which represents the overall level of agreement between criterion (i.e. clinical diagnosis of ASD) and instrument (i.e. ADOS). The higher the AuC, the higher the probability that a randomly chosen participant with ASD will have a higher score on the instrument than a randomly chosen participant without ASD.
Correlations with participant characteristics

To investigate the relationship of domain scores with participant characteristics, we calculated bivariate correlations for the patient groups between domain scores, and age, IQ, and scores on the negative scale of the PANNS (schizophrenia only).

6.3 Results

6.3.1 Interrater agreement

Interrater agreement at the level of ADOS classification was 81.7% with Cohen’s adjusted weighted kappa 0.66, which corresponds to good or substantial agreement (Landis & Koch, 1977). When merging the ADOS-classifications AD and ASD (based on the proposed criteria for DSM V) the agreement increased to 89.2% with kappa 0.73. Intraclass correlations (ICC, Table 2) show high interrater agreement on SOC and COMSOC, and good agreement on COM. Mean agreement across the items was 81.7% with mean weighted kappa 0.66. Weighted kappa’s exceeded 0.60 for 14 out of the 21 items with the remainder exceeding 0.50.

Table 2 Intraclass Correlations for Interrater Agreement

<table>
<thead>
<tr>
<th>N</th>
<th>Social Interaction</th>
<th>Communication</th>
<th>Social-Communication</th>
<th>Social-Communication</th>
</tr>
</thead>
<tbody>
<tr>
<td>93</td>
<td>0.92</td>
<td>0.79</td>
<td>0.92</td>
<td>(Lord: 0.88-.97)</td>
</tr>
<tr>
<td></td>
<td>(Lord: 0.74-.90)</td>
<td></td>
<td>(Lord: 0.84-.98)</td>
<td></td>
</tr>
</tbody>
</table>

Note. Interrater agreement as mentioned for Lord et al. (2000) represents the range for all four modules.

6.3.2 Internal consistency

For the original algorithm, the internal consistency is high for SOC (Cronbach’s α=.84), but rather low for COM (α=.52). This indicates that the items of that domain do not intercorrelate well in our population. Item A4 (Stereotyped Language) performed the worst and its deletion from COM increased alpha to an acceptable level (α=.60). The reorganization of communication and social interaction items in the SA domain of the revised algorithm creates a consistent domain (α=.87).

6.3.3 Comparison of domain means

Original algorithm

All three domains and the total score showed a significant difference between the groups (Table 3). Tukey post-hoc comparisons show that for COM, SOC, and COMSOC, the ASD group scores significantly higher compared to the psychopathy group and the control group, but not compared to the schizophrenia group. The schizophrenia group scored significantly higher than the control group on COM, and higher than both the psychopathy and the control group on SOC and COMSOC. For RRB, the ASD group scored significantly higher than the control group, while there was a trend compared to the psychopathy group (p=.06). The forensic subgroup with ASD (n=6) scored higher than the
group with psychopathy on all domains (data not shown).

Revised algorithm
Both domains and the total score showed a significant difference between the groups (Table 3). Tukey post-hoc comparisons indicated that the ASD group scored significantly higher compared to the psychopathy group and the control group on SA, and there was a trend in comparison to the schizophrenia group ($p=.06$). The schizophrenia group scored significantly higher than the control group. For RRB, the ASD group again scored significantly higher than the psychopathy and control groups, but there was no significant difference with the schizophrenia group. For the total SARRB score, the ASD group scored significantly higher than the psychopathy, the control group, and the schizophrenia group, making it the only score for which the ASD group significantly differs from the schizophrenia group. The forensic subgroup with ASD (n=6) scored higher than the group with psychopathy on all domains (data not shown).
Table 3 Summary Statistics Based on the Original and Revised Algorithms

<table>
<thead>
<tr>
<th></th>
<th>ASD (n = 38)</th>
<th>Schizophrenia (n = 18)</th>
<th>Psychopathy (n = 16)</th>
<th>Control (n = 21)</th>
<th>F(3,89)*</th>
<th>Post-hoc Tests**</th>
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</thead>
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<td>Original algorithm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Communication domain (COM)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mean</td>
<td>2.55</td>
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<td>1.00</td>
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<td>8.82***</td>
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<td>SE</td>
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<td>0.22</td>
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<td>0.3</td>
<td></td>
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<td>1.50</td>
<td>0.95</td>
<td>18.69***</td>
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<tr>
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<td>Mean</td>
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<td>0.33</td>
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<td>0.00</td>
<td>4.07**</td>
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</tr>
<tr>
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<td>1.48</td>
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<td>0.38</td>
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<td>0.5</td>
<td>0.5</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.97</td>
<td>0.56</td>
<td>0.25</td>
<td>0.10</td>
<td>7.73***</td>
<td>ASD &gt;P **</td>
</tr>
<tr>
<td>SE</td>
<td>0.16</td>
<td>0.15</td>
<td>0.11</td>
<td>0.07</td>
<td></td>
<td>ASD &gt;TD ***</td>
</tr>
<tr>
<td>Range</td>
<td>0.4</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SARRB total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.37</td>
<td>5.22</td>
<td>2.19</td>
<td>1.57</td>
<td>17.50***</td>
<td>ASD &gt;S *</td>
</tr>
<tr>
<td>SE</td>
<td>0.84</td>
<td>0.95</td>
<td>0.40</td>
<td>1.81</td>
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<td>ASD &gt;P ***</td>
</tr>
<tr>
<td>Range</td>
<td>0.17</td>
<td>0.17</td>
<td>0.6</td>
<td>0.5</td>
<td></td>
<td>ASD &gt;TD ***</td>
</tr>
</tbody>
</table>

* For the univariate comparisons (main effects of group), F scores and their respective significance levels are reported: * = p<.05, ** = p<.01, *** = p<.001. ** Using the same symbols, Tukey’s HSD post-hoc comparisons are reported in the outer right column to indicate what diagnostic groups are significantly different from each other. ASD refers to autism spectrum disorder, P to psychopathy, TD to typically development, and S to schizophrenia.
6.3.4 Group comparison at item level

The multivariate test showed that there was a significant main effect of group, F(66,210)=1.688, p<.005. Results for the univariate tests are visually presented in Figure 1. Only four out of 22 items did not differ significantly between the groups. The majority of the remaining items showed a (almost) significant difference between the ASD group compared to the psychopathy and control groups, but not compared to the schizophrenia group. On some of these items the schizophrenia group also scored significantly higher than the psychopathy and/or control group: B2 (Facial Expressions), B6 (Empathy/ Comments on Others' Emotions), and B7 (Insight). Only three items distinguished the ASD from the schizophrenia group: A4 (Stereotyped Language), B10 (Quality of Social Response), and B12 (Overall Quality of Rapport). In addition, there was a trend for the ASD group to score higher than the schizophrenia group on item B11 (Amount of Reciprocal Social Communication, p=.07). Individuals with psychopathy scored comparable to the control group.

Figure 1 Between-group Comparisons at Item Level

Post-hoc comparisons of the ASD group versus the other three groups at item level (S = schizophrenia, P = psychopathy, TD = typical development). Dark grey boxes filled with *** represent a statistically significant difference at p<.001. Middle grey boxes filled with ** represent a statistically significant difference at p<.01. Light grey boxes filled with * represent a statistically significant difference at p<.05. Unfilled light grey boxes represent a statistical trend (p<.1). In all these cases, the mean of the ASD group was higher compared to the respective group.

6.3.5 Criterion-related validity

The ADOS was able to correctly classify 74.2% of the cases in our sample as having ASD or not (based on the clinical diagnosis assigned). Logistic regression analysis showed that SOC (p<.005) but not COM (p=.27) made a significant contribution in predicting whether a participant in our sample had a clinical diagnosis in the autism spectrum or not (Table 4). The SARRB domain significantly contributed to prediction (Table 4, p<.005). The odds ratios presented in Table 4 indicate that
augmenting scores of one point on SOC or SARRB, increase the probability that the individual has received a clinical diagnosis of ASD by 38% and 33%, respectively.

Table 4 Logistic Regression Analyses for Criterion-related Validity

<table>
<thead>
<tr>
<th>ADOS</th>
<th>Clinical classification</th>
<th>B</th>
<th>S.E.</th>
<th>Wald</th>
<th>df</th>
<th>Sig.</th>
<th>Odds ratio</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>Communication</td>
<td>0.21</td>
<td>0.20</td>
<td>1.21</td>
<td>1</td>
<td>0.271</td>
<td>1.23</td>
<td>0.85 - 1.79</td>
</tr>
<tr>
<td></td>
<td>Social Interaction</td>
<td>0.32</td>
<td>0.09</td>
<td>11.77</td>
<td>1</td>
<td>0.001</td>
<td>1.38</td>
<td>1.15 - 1.66</td>
</tr>
<tr>
<td>Revised</td>
<td>SARRB</td>
<td>-.29</td>
<td>0.07</td>
<td>18.52</td>
<td>1</td>
<td>0.000</td>
<td>1.33</td>
<td>1.17 - 1.52</td>
</tr>
</tbody>
</table>

Note. Odds ratios express how much the probability that an individual has received a clinical diagnosis of ASD increases with augmenting scores on the ADOS.

ROC curves for the original and revised algorithms resulted in AuC values of 0.812 and 0.796, respectively (1 = perfect agreement). This indicates that in general the ADOS scores quite adequately predicted whether someone had a clinical diagnosis of ASD or not. Application of the standard cut-off for autism spectrum on the original algorithm (i.e. 7) gives only moderate sensitivity (0.61) but good specificity (0.82) in our sample. Lowering the threshold to 6 increases the sensitivity (0.68) and keeps the specificity at the same level (0.82). Lowering the threshold to 5 increases the sensitivity further (0.79), but it decreases the specificity (0.73). For the revised algorithm, a cut-off of 5 seems optimal in the current population with adequate sensitivity (0.71) and specificity (0.82).

6.3.6 Correlations with participant characteristics

There were no significant correlations between the domain scores, and IQ or age for the groups with ASD, schizophrenia, nor psychopathy (data not shown). In the group with schizophrenia, the presence of negative symptoms as measured by the PANNS correlated positively with SOC (r=.59, p<.05) but not COM (r=.12). The PANNS also correlated positively with SA (r=.66, p<.005). Thus, the more negative symptomatology an individual with schizophrenia had, the higher his scores on the ADOS. PANNS scores correlated in particular with items that are similar to negative symptoms, such as (flat) facial expressions (B2, r=.59, p<.05), (lack of) shared enjoyment (B4, r=.81, p<.01), (lack of) asking the examiner for information (A6, r=.66, p<.01), and (difficulty with) communication of own emotions (B5, r=.53, p<.05).

6.4 Discussion

Systematic instruments are needed that can facilitate the complicated diagnostic process concerning ASD in adults. The current study is the first that examined the psychometric properties of ADOS module 4 in an independent sample of high-functioning adult males with an established clinical ASD diagnosis compared to meaningful and relatively homogeneous clinical and non-clinical groups. Our findings show that ADOS module 4 is a reliable instrument. At all levels (i.e. classification, domains and items) raters obtained substantial agreement. In addition, ADOS module 4 has good general criterion-related validity. It is able to correctly classify the majority of individuals and higher scores on the ADOS predict a higher probability of having a clinical ASD.
diagnosis. The high Areas under the Curve are further indications that ADOS scores can predict whether an individual actually has an ASD. Furthermore, group comparisons between the ASD and other groups show that the ADOS is valuable in differentiating between ASD, and psychopathy and typical development. The distinction between psychopathy and ASD even holds when only taking into account forensic individuals with ASD (although the group size was rather small to perform such an analysis). The finding that ASD and psychopathy are so well-discriminated by means of ADOS scores is promising for forensic psychiatric settings.

Another finding is the similarity between ASD and schizophrenia with respect to ADOS scores. Clearly, individuals with schizophrenia and marked negative symptoms show behavior that is very similar to ASD (Frith, 2003). Some patients with schizophrenia even have autistic-like symptoms that covary with negative symptoms (Sheitman et al., 2004). In line with these data, we show that the degree of negative symptomatology correlates significantly with ADOS scores, in particular with items resembling negative symptoms, such as (lack of) directed facial expressions and shared enjoyment. This resemblance makes it difficult for an observational instrument such as the ADOS to differentiate these groups on that behavior (see Reaven, Hepburn, & Ross, 2008 for a similar finding in children with childhood-onset schizophrenia). The findings underscore previous recommendations of using a comprehensive assessment that incorporates information on daily functioning and early development with direct observation to reach a clinical diagnosis (Lord et al., 1999). Nevertheless, four items did show a difference between these groups: individuals with ASD use more stereotyped language, less reciprocal social communication, and display qualitatively poorer social responses and overall rapport. This suggests that core social items and stereotyped language discriminate individuals with ASD from those with schizophrenia.

Although findings are preliminary, the revised SARRB domain, which combines social, communication and repetitive behavior items, seems promising in this and other respects. It not only discriminates ASD from all other groups including schizophrenia, but also has high internal consistency, and does well in identifying ASDs: a higher score on this domain predicts a higher probability of a clinical ASD diagnosis with 33% per additional point. Another positive indication for the revised algorithm is the confirmation that stereotyped language fits better with the RRB factor than with the original communication domain. Notwithstanding the caution of interpreting ASDs in adults in exactly the same way as in children, the revised algorithm as developed for modules 1-3 seems promising for module 4 as well. More research is needed in a larger sample containing individuals with more severe autistic symptoms and lower levels of daily functioning to further investigate the revised algorithm.

A marked finding is the limited role of the original communication domain in the identification of ASDs in this sample. Despite group differences between ASD and psychopathy/typical development, the communication domain does not predict a clinical ASD diagnosis. Combined with its low internal consistency, the communication domain as such does not seem to add to the validity of ADOS module 4 in the current sample. However, when communication items are incorporated in the revised algorithm, a consistent scale (SA) emerges that is valuable in the diagnostic procedure for ASD. Similarly, although restricted and repetitive behaviors were rare in our ASD sample, their contribution to SARRB supports the distinction of ASD from schizophrenia. The relatively short duration of the ADOS interview naturally could have played a role in the paucity of RRBs (Lord et al., 1999). However, combining these two findings also fits the general clinical
picture: in adolescents and adults with ASD there is a greater prevalence of impairment in nonverbal communication and social reciprocity than in verbal communication or repetitive behaviors and stereotyped interests (Shattuck et al., 2007). In fact, repetitive behaviors decline most strongly with age (Seltzer et al., 2003). Apart from ageing, individuals in our sample might have had relatively more intact verbal skills from the outset as they were all considered to be highfunctioning. Stereotyped language, however, does differentiate the ASD group from all other groups in our sample. This may be typical of our high-functioning group, because idiosyncratic language and language complexity are positively associated (Volden & Lord, 1991). Cultural differences in the use of gestures might also have played a role. Typically developing adults in our sample, for instance, used fewer emotional and only occasional descriptive gestures themselves.

The sensitivity in our sample was rather low (0.61), which means that not every individual with a clinical diagnosis of ASD obtained a concurrent classification on the ADOS. It is probable that the characteristics of our group played a role in this. Our sample consisted of high-functioning individuals that signed up for an extensive research project. They are probably situated at the milder end of the spectrum and some might have been able to (partly) compensate some behavior due to their high intelligence. Resulting relatively low scores make it difficult for the ADOS to identify these individuals. Our findings resemble the outcomes in ADOS modules 1-3, in which lower sensitivity (SE) was found for distinctions involving children with milder ASDs (module 3 by Lord, Cook, Leventhal, & Amaral, 2000 SE = 0.80, versus later studies: de Bildt et al., 2009, SE = 0.64; Gotham et al., 2008, SE = 0.49; Gotham et al., 2007, SE = 0.68). The high specificity (0.82), on the other hand, means that a positive ASD classification on the ADOS is a very strong indication for a clinician to consider diagnosing ASD. Sensitivity and specificity are tightly linked and the aim of the assessment determines which one is most important. High specificity is essential when one needs to be certain that the individuals selected actually have an ASD, for instance in autism research. High specificity can, however, lead to underinclusiveness. When the aim of the assessment is to screen for ASD, high sensitivity is crucial in order not to miss any potential case. For this purpose, lower thresholds could be considered at the expense of specificity. To prevent overinclusiveness, developmental history and current daily functioning should then be carefully reviewed. As this study included only a specific ASD group and specific control groups, further research is needed to establish the optimal cut-off points on the ADOS module 4.

This study has a number of limitations that should be taken into account when interpreting the results. First, compared to studies on the psychometric properties of modules 1-3 (de Bildt et al., 2009; Gotham et al., 2008; Gotham et al., 2007; Oosterling et al., 2010), our study has a small sample size (n=93). However, it is the first study examining module 4 in an adult population with ASD compared to specific and meaningful groups. Second, we are focused on high-functioning adult males with ASD, which means results cannot be generalized to the entire ASD population. Future studies on module 4 should comprise a larger sample, including individuals with lower levels of daily functioning, since the high-functioning character of our sample may have influenced the results. On the other hand, exactly these individuals are not always recognized during childhood. Therefore, increasing knowledge on module 4 seems most important for individuals showing milder autistic symptoms. In this light, it will also be important to include a group of high-functioning adult females, who run the risk of being undiagnosed because they might be especially good at compensating their behavior (Attwood, 1999; In ’t Velt-Simon Thomas & Mol, 2008). Third, no
standardized measures were available for the clinical diagnosis of ASD, which characterizes current practice in adult psychiatry. However, the normal clinical procedure included review of developmental history and current functioning and observation. In addition, most participants with ASD were recruited through a specialized center. Fourth, we did use standardized measures to diagnose schizophrenia, but not to review early developmental history in this group. Therefore, we cannot eliminate the possibility that ASD was present before the onset of schizophrenia. However, this possibility is minimized by the fact that these individuals were extensively tested in a specialized psychosis center and selected for this study by experienced clinicians. The control groups in the current sample were comparatively homogeneous and aimed to challenge the ADOS by comparing ASDs with other psychiatric groups with social deficits. For the investigation of ADOS’ value in differential diagnostics, examining different subtypes of schizophrenia and other diagnostic groups will be of great relevance as well (e.g. anxiety disorder, depression, ADHD, and OCD).

In summary, the ADOS module 4 is a reliable instrument that has good predictive value for ASD. It can adequately discriminate ASD from psychopath and typical development in an adult population. With respect to schizophrenia, discrimination is more difficult due to behavioral overlap. These groups are, however, different on some core items. Although ADOS module 4 fails to classify ASD in a significant proportion of our higher functioning and more mildly affected ASD group, its ASD classification is a strong lead for a clinician to at least consider an ASD diagnosis. Explorative analyses of the revised algorithm indicate that a revision -in line with modules 1-3 and developments in criteria for ASD- could be beneficial for discriminating ASD from schizophrenia.

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Neural similarities and differences in autism and schizophrenia during emotional facial expression processing

A version of this manuscript is under review as:
Shared social deficits in schizophrenia and autism spectrum disorders are partly reflected in similar neural profiles during emotional facial expression processing
Bastiaansen, J. A., Pijnenborg, M., van der Gaag, C., & Keysers, C.
Chapter 7

Abstract

Background The social behavior of individuals with autism spectrum disorders and those with schizophrenia can resemble each other, especially when negative symptoms are present in the latter. Common to both is a withdrawal from social contact and a lack of spontaneous prosocial behavior.

Objective To examine whether similar neural mechanisms are involved in autism and schizophrenia by comparing neural responses when subjects view short movies of positive, neutral and negative social stimuli (emotional facial expressions) using functional magnetic resonance imaging.

Participants Twenty-one adult males with DSM-IV diagnosed autism spectrum disorders, 20 adult males with DSM-IV diagnosed schizophrenia selected on the basis of negative symptomatology, and 21 typically developing subjects.

Results Both patient groups perceive faces more negatively and show relatively stronger neural responses to disgust than typically developing individuals in regions representing their own body and emotional salience. Additionally, the schizophrenia group shows a reduced sensitivity for pleased faces in regions involved in approach behavior and reward processing. The overall pattern of activity, irrespective of emotion, is very different in the two patient groups with hyper-activity in autism and a tendency towards hypo-activity in schizophrenia.

Conclusions Shared social difficulties in autism spectrum disorder and schizophrenia are partly reflected in similar neural responses during emotion processing. A common mechanism explaining social withdrawal in both groups may be an increased sensitivity for negative social stimuli. A reduced sensitivity for positive social stimuli may further contribute to reduced social approach in individuals with schizophrenia and negative symptoms. Approaches to re-establish this tipped balance in sensitivity to social stimuli should take into account the patient groups’ opposing patterns of overall brain activity.
7.1 Introduction

Autism spectrum disorder (ASD) and schizophrenia are neurodevelopmental disorders that have unique features and their own developmental course (American Psychiatric Association, APA, 2000; Kanner, 1943; Weinberger, 1995). The clinical presentation of both syndromes is heterogeneous, but social dysfunction is present and persistent across the entire autism spectrum and common in individuals with schizophrenia, even in presympathetic phases of the illness (Addington et al., 2008a; APA, 2000; Bearden et al., 2000; Shattuck et al., 2007). Social behaviors of individuals with schizophrenia can resemble autistic symptoms, especially when psychotic symptoms are in remission and negative symptoms become more prominent (Frith, 2003; Sheitman et al., 2004). Common to both are a withdrawal from social contact and a lack of spontaneous prosocial behavior. Recently, we reported that the phenotypic overlap can complicate distinction of the two disorders on the Autism Diagnostic Observation Schedule (ADOS), a widely used diagnostic instrument that assesses social and communicative behaviors during a semi-structured interview (Bastiaansen et al., 2011b; Lord et al., 2000). Deficits in the ability to recognize other’s emotions and other social cognitive skills have also been widely documented in both ASD and schizophrenia and may underlie the social impairments (Abdi & Sharma, 2004; Burns, 2006; Couture, Penn, & Roberts, 2006; Frith, 2003; Pijnenborg et al., 2009). Similar to social impairments, social cognitive deficits in schizophrenia may be more severe and resemble the deficits in ASD more when negative symptoms are prominent (Couture et al., 2010; Edwards, Jackson, & Pattison, 2002; Frith, 1994; Kohler et al., 2003; Schneider, Gur, Gur, & Shtasel, 1995; van’t Wout et al., 2007).

Neuroimaging studies have shown widespread structural and functional disturbances in both disorders in regions underlying social and emotional processing (Abdi & Sharma, 2004; Burns, 2006). However, to date no study has examined the neural underpinnings of emotional processing in ASD and schizophrenia using the same paradigm. Taking a closer look at where the phenotypes of ASD and schizophrenia converge, could increase our understanding of the pathogenesis of these disorders and may provide new insights for diagnostics and treatment (King & Lord, 2010). In this study, we examine neural signatures of adults with ASD and schizophrenia, who showed behavioral overlap in our ADOS study (Bastiaansen et al., 2011b), compared to typically developing (TD) adults during the spontaneous processing of dynamic negative, neutral, and positive facial emotions. In addition, we use measures of social functioning and social cognition to investigate to what extent social (cognitive) deficits overlap in these patient groups.

Based on learning theory, we hypothesize that two processes may play a role in the social withdrawal seen in both disorders. First, increased social avoidance may occur because of increased sensitivity to negative social stimuli. Second, reduced social engagement or approach may occur due to reduced sensitivity to positive social stimuli, such as a smiling face. Neurobiologically, these biases in sensitivity to visual information would first manifest themselves as reduced activity to positive and increased activity to negative facial expressions in occipital and temporal regions (Lane, Chua, & Dolan, 1999; Lang et al., 1998). These visual processing biases would then trigger similar effects down-stream in regions involved in the embodied simulation of other people’s emotions: premotor and posterior parietal regions involved in the perception and the execution of facial expressions (van der Gaag et al., 2007), somatosensory regions involved in seeing and feeling body motion and pain (Keysers, Kaas, & Gazzola, 2010), and the insula and anterior cingulate cortex.
(ACC) involved in associating positive and negative affect to interoceptive and visual stimuli (Bastiaansen et al., 2009; Singer et al., 2004). Damage to these premotor, somatosensory, and insular regions, which are also involved in our own emotion experience, impair the ability to understand and feel with the emotions of others (Adolphs et al., 2000; Adolphs et al., 2003; Calder et al., 2000; Shamay-Tsoory, Aharon-Peretz, & Perry, 2009). Finally, we expect differences in the amygdala and striatum, because of their roles in avoidance (amygdala) and approach (striatum) behaviors (Ernst & Fudge, 2009). Additionally, we hypothesize that differences between the psychiatric groups and the TD group will be most pronounced when facial expressions without an obvious external origin are directed at the subjects, because this is closest to what happens during social encounters. To explore this, we included two types of emotional facial expressions: negative, neutral, and positive expressions that are clearly triggered by the content of a cup (i.e. a sweet, sour or neutral drink) and hence seem to have nothing to do with the subject, and equivalent expressions that are directed at the camera without being triggered by taste, which have been used more often and seem more social.

### 7.2 Methods

#### 7.2.1 Subjects

The ASD group comprises 21 adult males diagnosed with autism (n=7), Asperger Syndrome (n=9), or PDD-NOS (n=5) by a clinical psychologist or psychiatrist according to DSM-IV-TR criteria (APA, 2000). Clinical diagnoses were verified by the administration of the ADOS (Lord et al., 2000) by a trained and certified psychologist. One of the subjects scored below the cut-off of the communication subscale, but his diagnosis was confirmed by the administration of the Autism Diagnostic Interview Revised (ADI-R, Lord, Rutter, & Le Couteur, 1994). The subjects were considered to be high-functioning by their clinicians and none had an IQ score below 70 on the Groninger Intelligence Test 2 (GIT2, Luteijn & Barelds, 2004).

Twenty adult males with schizophrenia were selected by experienced clinicians on the basis of negative symptomatology. The level of targeted negative symptomatology was moderate in order to resemble the social deficits encountered in high-functioning ASD. Diagnoses were confirmed by the administration of the Dutch version of the Schedules of Clinical Assessment in Neuropsychiatry (SCAN 2.1, Giel & Nienhuis, 1996). Current symptomatology was assessed by the Positive and Negative Syndrome Scale (PANNS, Kay et al., 1987). A paired samples t-test showed that negative symptoms were indeed more prominent than positive symptoms in the schizophrenia group (t=-3.019, p=.007), but overall symptomatology as measured by the PANNS was mild: positive M(SD)=11.9(3.34), negative M(SD)=14.7(4.14), composite M(SD)=2.8(4.37), general M(SD)=27.1(5.93).

The two patient groups were included in a study on the use of the ADOS as a diagnostic instrument to establish autism spectrum disorder (ASD) in adults and were mainly recruited via specialized mental health organizations (Bastiaansen et al., 2011b). Medication for both patient groups is reported in Tables S1a-b. Three individuals with ASD and eight with schizophrenia were taking antidepressants at the time of the study. According to the SCAN 2.1, five individuals with ASD and four individuals with schizophrenia were experiencing a mild depressive episode.
The control group consisted of 25 typically developing (TD) adult males. The presence of major psychiatric disorders was ruled out by the administration of the SCAN 2.1. In addition, they were interviewed to verify that first-degree relatives did not have a pervasive developmental disorder or a history of psychosis. All subjects had normal or corrected-to-normal hearing and vision, were eligible for MRI research, and gave written informed consent to participate in the study, which was approved by the Institutional Review Board of the University Medical Center Groningen (METc).

7.2.2 Experimental Tasks
The fMRI task comprised two visual runs, during which subjects were asked to carefully watch short movie clips of facial expressions (3 s, 14" x 18", see Jabbi et al., 2007; van der Gaag et al., 2007). Each run consisted of the same 60 randomly presented movie clips, which showed a) actors making a disgusted, pleased or neutral facial expression (i.e. blowing up the cheeks) or b) actors responding as naturally as possible to one of three tastes: lemon juice (disgust condition), sweet juice (pleasure condition) or water (neutral condition). In these cases, the actors responded with an intense emotional facial expression after tasting the liquid through a straw. These (“Cup”) movie clips placed the facial expressions into a gustatory context, while the others (“No Cup”) were directed at the subjects without any hints about their origin. Movie clips were separated by red fixation crosses (1" x 1") with an inter-trial interval that varied randomly between 5 and 12 s (baseline). After scanning, subjects rated each movie on the intensity of displayed disgust and pleasure (0 none to 6 very intense). They also rated the “Cup” movies on the valence of the drink (-6 very bad to 6 very good).

As a measure of each subject’s current level of social adjustment we used the Social Functioning Scale (SFS, Birchwood et al., 1990), which was filled out by the subject and by an informant (e.g. a parent or caretaker). The SFS taps those areas that are crucial to community maintenance (e.g. employment, pro-social activities) and is specifically designed for people with known social difficulties. Of particular interest is the social engagement/withdrawal subscale, which is formed by items measuring the degree of social avoidance, the amount of time spent alone, and how often individuals initiate conversation. As a measure of emotion recognition we used the Ekman 60 Faces Test (FEEST), in which the subject has to indicate which of six named basic emotions was expressed in pictures of actors displaying prototypical emotional expressions (Young et al., 2002). Additionally, we used the Interpersonal Reactivity Index (IRI, Davis, 1983) as a measure of empathy. Statistical methods for the behavioral measures are discussed in the Supplementary Methods.

7.2.3 Magnetic Resonance Image Acquisition and Preprocessing
Scans were acquired using a 3T Phillips Intera Quasar (Best, The Netherlands) equipped with a synergy SENSE eight-channel head coil. Functional images were acquired using a T2*-weighted echo-planar sequence with 32 interleaved axial slices aligned with ac-pc, a thickness of 3.5 mm and no slice gap to cover the entire cortex (TR = 1.5s, TE = 28ms, TA = 1.45s, flip angle = 70 degrees). In addition, two T1-weighted anatomical images (1x1x1 mm) containing 160 slices were acquired parallel to the bicommissural plane. Data were preprocessed using the Statistical Parametric
Mapping software package (SPM2, Wellcome Department of Cognitive Neurology, London, UK: http://www.fil.ion.ucl.ac.uk) in the following order: functional images were corrected for slice timing, realigned to the first volume of the first run to correct for shifts in head position, coregistered to the anatomy, normalized based on parameters derived from the gray matter segment of the anatomy, and then spatially smoothed with a 10 mm full-width half-maximum (FWHM) isotropic Gaussian Kernel.

7.2.4 Magnetic Resonance Image Analysis

At the first (subject) level, time series were high-pass filtered at 385s and separate predictors were used as boxcar functions convolved with the hemodynamic response function for the six movie types (disgust, pleasure or neutral, all three with or without a cup). Parameter estimates for each movie type were averaged across runs for each subject, and analyzed at the second (population) level using analyses of variance. Only gray matter was considered in the analyses: a mean gray matter mask was constructed by averaging the normalized gray matter segments of all subjects and thresholding this mean image at 0.3. Conducting the full factorial design (3 Emotions x 2 Contexts x 2 Groups x Age x IQ) is currently not possible in SPM, as some factors are within and some between subjects. Because the main interest of this work regards differences between the groups, we restricted our analyses to the main effect of Group, the two-way interactions of Group x Emotion and Group x Context, and the three way-interaction of Group x Emotion x Context, for which we set up separate design matrices including age and IQ as nuisance variables. Because it can be misleading to interpret effects of a certain order if interactions of higher order exist, we performed our analyzes in a hierarchical order, such that if a voxel shows a significant three-way interaction, we exclude it from the analysis of two-way interactions and the main effect, and if it shows a two-way interaction, we exclude it from the analysis of the main effect. Unless reported otherwise, clusters are deemed significant when they (i) contain at least 20 voxels (with the exception of the amygdala, because of its small overall size), (ii) survive voxelwise thresholds of \( p_{\text{unc}} < .001 \) and (iii) False Discovery Rate (FDR) correction for multiple comparisons \( p_{\text{FDR}} < .05 \). This was achieved by examining the critical t-value obtained using \( p_{\text{unc}} < .001 \) and \( p_{\text{FDR}} < .05 \), and using the larger of these two t-values as the final voxelwise threshold for that analysis. For analyses with clusters not surviving a voxelwise threshold of \( p_{\text{FDR}} < .05 \), we deemed clusters significant when voxels survive a threshold of \( p_{\text{unc}} < .001 \) in combination with an overall clusterwise false-positive rate of \( p < .05 \). This was done by using AlphaSim (Ward, 2000) to calculate the threshold of the necessary cluster size (k) to maintain the chance of a false positive \( p < .05 \) at the cluster level within our gray matter mask. For significant clusters we examined the parameter estimates of the most significant (peak) voxel to shed light onto the nature of the effect detected by the whole-brain analysis in that cluster. Plots of all these peaks are presented in Figures S1-3.
7.3 Results

7.3.1 Behavioral analysis

One-way ANOVAs show there are no significant differences between the groups in terms of age and IQ, but there are differences on measures of social functioning and social cognition (see Table 1). Both patient groups score significantly lower compared to TDs on social functioning, and social engagement (i.e. more withdrawal). There is a trend towards lower scores for the patient groups in emotion recognition (FEEST), but groups are not significantly different on the happiness or disgust scales. On the happiness scale, all three groups score close to the maximum (i.e. 10). The IRI shows that both patient groups are more prone to personal distress caused by other people’s emotions and are less prone to adopt the perspective of another person. The ASD group additionally experiences less empathic concern compared to the schizophrenia and TD groups.

Table 1 Group Characteristics

<table>
<thead>
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<th>Measures</th>
<th>ASD (n=21)</th>
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<td>Age</td>
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<td>32.56 (10.18)</td>
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<td></td>
</tr>
<tr>
<td>IQ</td>
<td>102.57 (14.83)</td>
<td>90.95 (17.25)</td>
<td>100.48 (16.83)</td>
<td>2.96\textsuperscript{T}</td>
<td>ASD &gt; S\textsuperscript{T}</td>
</tr>
<tr>
<td>SFS client</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>112.02 (9.73)</td>
<td>113.47 (6.70)</td>
<td>125.89 (3.19)</td>
<td>28.57\textsuperscript{***}</td>
<td>ASD/S &lt; TD\textsuperscript{***}</td>
</tr>
<tr>
<td>Engagement</td>
<td>106.5 (13.2)</td>
<td>112.6 (15.3)</td>
<td>126.0 (10.4)</td>
<td>13.8\textsuperscript{***}</td>
<td>ASD/S &lt; TD\textsuperscript{***}</td>
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<tr>
<td>SFS other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>109.77 (10.36)</td>
<td>113.04 (4.91)</td>
<td>126.30 (3.00)</td>
<td>39.93\textsuperscript{***}</td>
<td>ASD/S &lt; TD\textsuperscript{***}</td>
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<tr>
<td>Engagement</td>
<td>107.0 (15.1)</td>
<td>113.0 (12.7)</td>
<td>129.4 (7.7)</td>
<td>21.0\textsuperscript{***}</td>
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<td>FEEST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>46.19 (6.79)</td>
<td>46.15 (5.70)</td>
<td>49.76 (4.82)</td>
<td>3.00\textsuperscript{T}</td>
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</tr>
<tr>
<td>Disgust</td>
<td>6.52 (2.46)</td>
<td>8.0 (1.97)</td>
<td>7.52 (2.16)</td>
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<tr>
<td>Happy</td>
<td>9.71 (0.72)</td>
<td>9.5 (0.83)</td>
<td>9.92 (0.28)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Perspective Taking</td>
<td>13.48 (4.89)</td>
<td>16.03 (4.55)</td>
<td>18.92 (3.50)</td>
<td>9.22\textsuperscript{***}</td>
<td>ASD\textsuperscript{***}/S &lt; TD</td>
</tr>
<tr>
<td>Empathetic Concern</td>
<td>14.48 (5.35)</td>
<td>18.95 (3.50)</td>
<td>17.32 (3.61)</td>
<td>5.80\textsuperscript{**}</td>
<td>ASD &lt; TD\textsuperscript{**}/ S\textsuperscript{T}</td>
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<td>Fantasizing</td>
<td>13.48 (6.06)</td>
<td>14.74 (4.79)</td>
<td>14.24 (6.31)</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Personal Distress</td>
<td>15.50 (5.56)</td>
<td>14.13 (5.32)</td>
<td>10.24 (4.19)</td>
<td>7.79\textsuperscript{**}</td>
<td>ASD*/S &gt; TD</td>
</tr>
</tbody>
</table>

Note. Scores’ means with standard deviations in parentheses. Significant group differences are indicated by asterisks (** p<.01, * p<.05) and trend levels are indicated by \textsuperscript{T} (0.05 <p<.08). Significant F tests are followed up by Tukey post-hoc comparisons. ASD = Autism Spectrum Disorder, S = Schizophrenia, TD = typically developing group. SFS = Social Functioning Scale, FEEST=Ekman 60 Faces Test, IRI = Interpersonal Reactivity Index.

7.3.2 Magnetic Resonance Image Analysis

Main effect of group

Various regions including anterior cingulate cortex (ACC), middle cingulate cortex (MCC), primary (SI) and secondary (SII) somatosensory cortex, and precuneus show a main effect of group at a voxelwise threshold of $p_{unc}<.001$ (Table S2). Three of these clusters survive a cluster-threshold of $p<.05$ (Alphasim: $k >71$, see Methods): right ACC, right SII, and left MCC. Examination of parameter
estimates in the peak voxels shows that the ASD group does not deactivate ACC, MCC and SII as strongly as the schizophrenia and TD groups (Figure S1). Additionally, the TD group activates the MCC more strongly compared to the schizophrenia group. Lowering the threshold, the main pattern seems to be that the ASD group hyper-activates, while there is a trend for the schizophrenia group to hypo-activate compared to the TD group. Independent voxelwise sample t-tests between the groups show that the largest differences indeed exist between the ASD and schizophrenia group (results even survive FDR-correction at the voxel level, Table S3). Regions that the ASD group activates more than the schizophrenia group include ACC, MCC, precuneus, SI, SII, and insula (Figure 1a). Nothing survives for the reverse contrast (p_{uncert}=.001).

**Figure 1** Main and Interaction Effects

A) Difference in mean activity between Autism Spectrum Disorder (ASD) and schizophrenia
In red regions the ASD group activated more than the schizophrenia group (T=3.23, p_{uncert}<.001, critical F for FDR<.05 would have been 2.69) on sagittal slices of the average anatomy across all subjects.

B) Render of Interaction Effects
In red the three-way interaction of Emotion x Context x Group (F=3.93, p_{uncert}<.001, F_{FDR<.05}=3.76). In blue the two-way interaction of Emotion x Group (F=3.93, p_{uncert}<.001, F_{FDR<.05}=3.26). Results rendered on the average gray matter mask across all subjects, depth=15mm.

C-D) Plots of parameter estimates in the peaks of two regions with Emotion x Group interactions. The plots illustrate the two themes: reduced pleasure preference in schizophrenia (C) and a disgust bias in ASD (D). In the right fusiform gyrus depicted in panel C, the differences between pleasure versus neutral and disgust are larger in the typically developing (TD) group compared to the schizophrenia group. In the right hippocampus depicted in panel D, the ASD group shows a stronger response to disgust than neutral and pleasure, which is not the case in the TD group.
**Context x Group**

The groups do not respond differently across the two different contexts (Cup vs. No Cup). Only one cluster larger than 20 voxels in the left fusiform gyrus (k=26; -30, -48, -10) survives a voxelwise threshold of \( p_{unc} < .001 \), but not a voxelwise threshold of \( p_{FDR} < .05 \), nor a cluster-threshold of \( p < .05 \) (k=75, see Methods).

**Emotion x Group**

Various regions show an Emotion x Group interaction (see blue clusters in Figure 1b, Table S4). Examination of parameter estimates in these regions revealed two themes.

*Reduced pleasure representation in schizophrenia:* in high-level visual areas (e.g. right posterior superior temporal gyrus, fusiform gyrus, left lingual gyrus), right precentral gyrus, right SI, and left middle frontal gyrus, where TDs show the strongest response to pleasure, individuals with schizophrenia show a relatively reduced representation of pleasure (versus disgust and/or neutral). A small cluster in the putamen (k=23) does not show this pattern. Additionally, the schizophrenia group shows less emotion differentiation in the hippocampus (bordering the amygdala) and ACC compared to the other groups.

*Disgust bias in the patient groups:* In comparison to the TD group, the ASD and to a lesser extent the schizophrenia group have a relative overrepresentation of disgust (compared to pleasure and/or neutral) in middle occipital and temporal regions, left middle frontal cortex and left SI. In left inferior parietal lobule, and hippocampus/amygdala this was only true for the ASD group. Two exemplary plots are show in Figure 1c; other plots can be found in Figure S2.

**Emotion x Context x Group interaction effect**

Various regions show a three-way interaction of Emotion x Context x Group (see red clusters in Figure 1b and Table S5). Examination of parameter estimates in peak voxels (see Figure S3) revealed a number of regions in which the presence or absence of a cup modulated the effects observed in the Emotion x Group interaction:

*Reduced pleasure representation in schizophrenia stronger in Cup context:* A number of regions showed a preferential response to pleasure in the No Cup context for all groups, but a reduced pleasure preference in the Cup context for individuals with schizophrenia compared to the other two groups. These regions included high-level visual areas (e.g. bilateral middle temporal gyrus, right superior temporal gyrus, left superior occipital gyrus), and SI. In addition, compared with the No Cup context and the other groups, individuals with schizophrenia distinguish between pleasure and the other emotions less in the Cup context in subcortical clusters (i.e. right hippocampus, right brainstem/hippocampus, left brainstem/amygdala, right cerebellum). Many of the regions showing a reduced pleasure preference in the Cup context are adjacent to clusters that show a general reduction in pleasure preference in schizophrenia for both contexts (see Figure 1b).

*Disgust bias patient groups strongest in No Cup context:* The Emotion x Group interaction showed regions with a relatively stronger representation of disgust in the patient groups. Here, the three-way interaction shows that this ‘disgust bias’ is strongest in the No Cup context for both patient groups compared to TDs in the left middle temporal gyrus, right superior occipital gyrus,
and right pallidum. For the schizophrenia group this was also true in left primary visual cortex and left middle temporal gyrus. In the ASD group this was also true in the right brainstem/hippocampus and right hippocampus.

A number of regions showed a pattern that did not fall within these two themes. For instance, while the schizophrenia and TD groups show different emotional response patterns depending on the context in the right and left supramarginal gyrus, the emotional response pattern of the group with ASD is unmodulated by context in these regions. Finally, in the medial temporal pole emotional patterns look similar across groups in the No Cup context, but in the Cup context patients do not process emotions more than the neutral stimulus, which TDs do.

**Conditions versus baseline**

The complex three-way interaction effects suggest that group differences depend on both emotion and context. To simplify the inspection of these results, we compared the groups for each of the six stimulus categories separately (disgust, pleasure, neutral with and without a cup) by setting up multiple regression analyzes with one constant for each group, and Age and IQ as nuisance variables ($p_{unc} < .001$). The results confirm the two themes from the factorial analysis and sketch a clear picture (Figure S4, Table S6). ASD is mainly characterized by increased activity compared to TDs when viewing disgusted faces without an external trigger (No Cup) in midline structures (ACC, MCC, Precuneus), somatosensory regions (S1, SII, insula) and superior temporal gyrus. Schizophrenia is characterized by specific hypo-activity compared to TDs when viewing pleased faces, particularly when the cup is present, in frontal regions (BA45), putamen, and insula. The most striking differences are illustrated in Figure 2.

**Figure 2** Group Differences for the Separate Movie Types

The most striking mean differences in brain activity during the observation of the movie types (Disgust No Cup, Pleasure Cup) versus baseline using independent sample t-tests at $p_{unc} < .001$ ($T=3.23$) and a cluster extent threshold (k) of 20 voxels. In orange areas that are more activated by either the ASD or schizophrenia group compared to the TD group. In blue the reverse contrast (TD > ASD or Schiz).
7.3.3 Movie ratings

Concerning the movie ratings, we approached the analysis along the lines of the themes in the MRI data (see Supplementary Methods). First, we investigated whether there was a disgust bias in the movie ratings in the patient groups compared to the TD group. Neither the ASD group, t(44)=.97, p=.34, nor the schizophrenia group, t(41)=1.16, p=.25, rated the disgusted faces as more intense compared to the TD group. Comparison of the movies’ intensity difference scores (happy - disgust) shows that the ASD group does see disproportionally more disgust than happiness across the different emotion types compared to TDs, t(44)=−2.40, p<.05, and there is a non-significant trend in the same direction in schizophrenia, t(41)=−1.61, one-tailed p=.06. Similarly, both the ASD, t(43)=−2.43, p<.05, and schizophrenia group, t(41)=−2.29, p<.05, generally rated the drinks in the cup movies as worse than the TDs. These group differences were not modulated by Context, Emotion, or Emotion x Context (all p>.44). Second, we examined whether the group with schizophrenia was impaired in the perception of happiness. The group with schizophrenia did not rate the happy movies as less happy than the TDs, t(41)=.47, p=.64, nor did they differ from the TDs in the amount of happiness they saw in the happy movies relative to the neutral, t(41)=−1.06, p=.30, and to the disgust movies, t(41)=−.42, p=.68.

7.4 Comment

ASD and schizophrenia are two different diagnostic entities, but their social behaviors can resemble each other when the psychotic symptoms of schizophrenia are in remission and negative symptoms are more on the foreground (Bastiaansen et al., 2011b). Here, we investigate neural responses during emotion perception in typically developing individuals compared to these patient groups with overlapping social phenotypes (according to the ADOS), similar impairments in social cognition (FEEST), and comparable levels of social dysfunction characterized by a withdrawal from social participation (SFS). From a learning theory perspective, this shared social withdrawal may be caused by an increased sensitivity to negative social stimuli, which would increase social avoidance, and/or by a reduced sensitivity towards positive social stimuli, which would reduce social approach. Although the patient groups differ in their overall level of activation - with hyper-activity in the ASD group and a tendency towards hypo-activity in the schizophrenia group - the balance between sensitivity to negative, neutral, and positive social stimuli seems to be tipped in both patient groups compared to controls.

When watching dynamic emotional facial expressions, both patient groups show a relatively increased representation of disgust in high-level visual and somatosensory regions, even though there were no differences in the amount of time the groups spent looking at the face, the mouth, or the eyes in the stimuli (Supplementary Methods). These findings suggest that negative facial expressions may be more salient to both patient groups (Lane et al., 1999; Lang et al., 1998) and are ‘embodied’ more, that is, trigger more representations of the observer’s own body in somatosensory and insular cortices (Adolphs et al., 2000; Bastiaansen et al., 2009; Keysers et al., 2010). Increased responses in the insula while viewing disgusted facial expressions have been associated with higher levels of personal distress (Jabbi et al., 2007). This finding helps bridge two observations in the present study: our patients demonstrated relatively elevated responses in the
insula and elevated personal distress in response to other people’s negative emotions (as measured in the PD subscale of the IRI). Due to the differences in overall brain activity between the two patient groups, the relative overrepresentation of disgust only lead to activity that exceeded that of the TDs in the ASD group (Figure 2). Possibly, excessive attention to and vicarious sharing of negative emotions of other people leads to higher levels of personal distress and subsequently social withdrawal in both disorders. In our ASD group, increased activity in midline structures that are normally deactivated during task performance dovetails with the recent suggestion that ASD might be characterized by abnormal functioning in the default network (Iacoboni, 2006; Kennedy, Redcay, & Courchesne, 2006) and might reflect further abnormal self-referential processing of the stimuli during our task. Ratings of the stimuli used in our paradigm show that both patient groups tend to perceive faces as more negative. Further research is needed to investigate whether this negative bias generalizes to other negative emotions or indicates a specifically enhanced sensitivity for disgust (Ille, Schony, Kapfhammer, & Schienle, 2010; Kohler et al., 2003; Peer, Rothmann, Penrod, Penn, & Spaulding, 2004).

Besides the increased sensitivity to the negative emotion, the schizophrenia group shows a relatively reduced sensitivity for the positive emotion (Figure 1C). The reduced pleasure representation in visual areas and amygdala suggests that positive social stimuli are less salient to them (Lane et al., 1999; Lang et al., 1998; Phan et al., 2002; Vuilleumier, Richardson, Armony, Driver, & Dolan, 2004). Reduced activity in somatosensory regions suggests that they also embody positive signals less (Adolphs et al., 2000; Keysers et al., 2010). Moreover, activity for pleased faces was below that of TDs in the insula, striatum, and frontal regions involved in reward processing and approach-related behavior (Figure 2, Hietanen, Leppanen, Peltola, Linna-Aho, & Ruohiala, 2008; Phillips et al., 2003a). These findings suggest that an additional reason for reduced social engagement in schizophrenia might be a reduced sensitivity to the rewarding aspects of social contact. Individuals with (chronic) schizophrenia have indeed been shown to be more apathetic towards social praise (Layne & Wallace, 1982), experience less joy and interest in daily life (Suslow, Roestel, Ohrmann, & Arolt, 2003), and show reduced attention and responsivity to positively valenced stimuli (Schlenker, Cohen, & Hopmann, 1995; Strauss, Allen, Duke, Ross, & Schwartz, 2008). Reduced neural responses to pleasure in our study were, however, not reflected in lower intensity or valence ratings of the pleased faces. This disconnection between normal subjective ratings and abnormal brain responses in regions involved in reward learning fits with similar findings on primary rewards (Waltz et al., 2009). Although positive emotional face cues seem to be rated normally, they may be less rewarding and less related to approach behavior in schizophrenia.

Contrary to our expectations, differences between the patient groups and the TD group were not generally more pronounced when the facial expressions were directed at the subjects (No Cup condition) compared to the facial expressions that were visibly triggered by an object. Instead, the effect of this manipulation depended on the observed emotion: brain activity of ASD subjects differed most from that of TDs for disgusted faces without a cup, while that of individuals with schizophrenia differed most for pleased faces with a cup. These findings have intuitive credibility: the absence of a cup could have made disgusted facial expressions more punishing for the ASD group, because the observer is more likely to be the trigger of the expressed emotion; the presence of a cup could have made the pleased facial expressions less rewarding for the group with schizophrenia, because the observer is less likely to have been the trigger of that emotion. These
preliminary findings suggest that it might be revealing to probe more specifically how the degree to which patients feel the emotion says something about them (Fenigstein & Vanable, 1992; Hooker & Park, 2005) influences neural responses. Other factors that deserve further investigation are the roles of medication and more subtle differences in gaze behavior between the patient groups (e.g. in attention orienting, Sasson et al., 2007) on neural activity. Antipsychotic medication might play a role in the (tendency towards) general hypo-activity seen in schizophrenia, although similar results have been found in unmedicated individuals with schizophrenia during a valence evaluation task (Paradiso et al., 2003), and our attempts to correlate chlorpromazine (CPZ) equivalents of medication to brain activity did not reveal an association between level of medication and brain activity. To further investigate the specificity of our findings, future studies should also comprise individuals with depression and (social) anxiety, because these disorders have also been linked emotion recognition impairments and an attentional bias for negative information (Bourke, Douglas, & Porter, 2010; Demenescu, Kortekaas, den Boer, & Aleman, 2010; Winton, Clark, & Edelmann, 1995).

In short, we found that the neural signature of emotion processing in patients with ASD and schizophrenia is biased in a way that might provide a learning theoretical explanation for their social isolation: increased sensitivity to negative social stimuli could be involved in social withdrawal in both ASD and schizophrenia, while reduced sensitivity to positive social stimuli might further reduce social engagement in schizophrenia. This might be informative for cognitive behavioral therapies by suggesting that normalizing the sensitivity to social stimuli may help re-establish an adaptive equilibrium between social approach and withdrawal. Due to differences in overall sensitivity, individuals with ASD might benefit most from desensitization to social stimuli in general and negative stimuli in particular (e.g. by reappraisal strategies). Individuals with schizophrenia might benefit more from sensitization to positive social stimuli or from finding other reinforcers of prosocial behavior to compensate for the lack of reinforcement induced by interpersonal interactions.

Acknowledgments

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7.5 Supplementary Methods

7.5.1 Statistical Analyses Behavioral Measures

Social functioning scale (SFS)
We used one-way ANOVAs followed-up by Tukey’s post-hoc comparisons to examine whether there are significant group differences in social functioning, and social withdrawal in particular, on the client version and the other version of the Social Functioning Scale (SFS). SFS client and other scales were available for all 25 typically developing individuals (TDs), and all 21 individuals with autism spectrum disorders (ASD). For the group with schizophrenia, 19/20 client scales and 15/20 other scales were available.

Ekman 60 Faces Test (FEEST)
As a parallel with the stimuli we used in the MRI task, we ran one-way ANOVAs for the disgust and pleasure subscales in addition to the total score of this emotion recognition test. Significant results were followed up by Tukey post-hoc comparisons.

Interpersonal Reactivity Index (IRI)
We used one-way ANOVAs followed-up by Tukey’s post-hoc comparisons to compare group differences on the four domains of the IRI: perspective taking, empathic concern, fantasizing, and personal distress. Data are unavailable for one participant with schizophrenia.

Movie ratings
After scanning, participants rated each movie on the intensity of displayed disgust and pleasure (0 none to 6 very intense). In addition, they rated the movies with a cup on the valence of the drink (-6 very bad to 6 very good). Due to fatigue, movie ratings are missing for two individuals with schizophrenia, and valence ratings are missing for one individual with ASD. Analysis of movie ratings was approached along the lines of the two major themes in the MRI data. First, we investigated whether there was a disgust bias in the movie ratings in the patient groups (ASD in particular). To this end, we performed independent sample t-tests on the disgust ratings for the disgust movies between the ASD or schizophrenia group, and the TD group. In addition, pleasure and disgust intensity ratings were subtracted to obtain a difference score, which represents the relative amount of perceived pleasure and disgust for each movie. Independent sample t-tests between the ASD and schizophrenia group on the one hand, and the TD group on the other hand were performed on the mean of these difference scores and on the mean valence rating of the cup movies to see whether there was a general negative bias in the ratings of the patient groups. To investigate whether the negative bias in the patient groups depended on Emotion, Context, or Emotion x Context, we performed a Group (3) x Emotion (3) x Context (2) mixed ANOVA on the difference ratings and a Group (3) x Emotion (3) mixed ANOVA on the valence ratings (only available for cup movies).
Second, we examined whether the group with schizophrenia showed reduced perception of pleasure. To this end, we performed an independent sample t-test between the schizophrenia and TD group on the pleasure ratings for the pleasure movies. To investigate whether the amount of pleasure individuals with schizophrenia saw in the pleasure movies relative to the neutral and disgust movies was different from the TDs, we performed two additional t-tests on the difference between pleasure scores on the pleasure versus neutral movies, and on the pleasure versus disgust movies.

7.5.2 Eye Tracking

Eye Tracking Analysis
An infrared video camera (SMI, iView) was mounted onto the scanner bed to track subjects’ gazes during the observation runs. Because we used movies of facial expressions as stimuli, we defined dynamic ROIs in order to determine the time subjects spent looking at the face, the eyes only, and the rest of the face (face minus eyes). To this end, we first manually tracked for each movie frame the position of the pupils and mouth corners using a slowed-down version of the movies. We then used these moving coordinates to dynamically define an elliptical ROI around the face of the actors, and two rectangular ROIs covering both eyes and the mouth region. The face ROI not only takes horizontal and vertical displacement into account, but also follows the head tilts of the actor. The amount of time spent on the face was calculated by counting the number of samples falling within the face ROI during all movies. In addition, we counted separately the number of samples that fell within the eye region and the mouth region. We performed three mixed ANOVAs with between-subject factor Group, within-subject factors Emotion (3 levels) and Context (2 levels) for the time spent looking at the faces, eyes, and mouth.

Eye tracking results
Analyses are preliminary as calibration errors or excessive noise prevented data analysis for approximately half of the subjects. Stable eye tracking data were obtained and analyzed in 15 individuals with autism spectrum disorders (ASD), 14 individuals with schizophrenia, and 10 typically developing (TD) subjects. For the amount of time spent on the eye region, there was no significant main effect of Group (p=.78), Emotion x Group interaction (p=.99), Context x Emotion x Group interaction (p=.34), but a trend towards significance for Context x Group (p=.06). Post-hoc analysis shows that the schizophrenia group spends more time in the eye region for the No Cup movies compared to the Cup movies in comparison to the ASD group. For the amount of time spent on the face (FA) in general and on the mouth (MO) in particular, there were no significant effects involving Group: main effect of Group (FA p=.35, MO p=.39), Emotion x Group (FA p=.69, MO p=.99), Context x Group (FA p=.50, MO p=.25), Emotion x Context x Group (FA p=.81, MO p=.61).
### 7.6 Supplementary Tables

#### Table S1a Psychoactive medication of the ASD group

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<tr>
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#### Table S1b Psychoactive medication of the Schizophrenia group

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</tr>
<tr>
<td>10</td>
<td>Aripiprazole (Abiliy)</td>
<td>15</td>
<td>Mirtazapine (Remeron)</td>
<td>15</td>
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<tr>
<td>11</td>
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<td>7.5</td>
<td>Paroxetine (Seroxat)</td>
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<td></td>
<td>Quetiapine (Seroquel)</td>
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</tr>
<tr>
<td>12</td>
<td>Risperidone (Risperdal)</td>
<td>25*</td>
<td></td>
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<tr>
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<td>Aripiprazole (Abiliy)</td>
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<tr>
<td>14</td>
<td>Clozapine (Leponex)</td>
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<tr>
<td>15</td>
<td>Clozapine (Leponex)</td>
<td>300</td>
<td>Venlafaxine (Efexor)</td>
<td>150</td>
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<tr>
<td>16</td>
<td>Pimozide (Orap)</td>
<td>2</td>
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<tr>
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<td>75*</td>
<td>Venlafaxine (Efexor)</td>
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<td>18</td>
<td>Olanzapine (Zyprexa)</td>
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<tr>
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<td>15</td>
<td>Citalopram</td>
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<td>200</td>
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<tr>
<td>20</td>
<td>Aripiprazole (Abiliy)</td>
<td>30</td>
<td></td>
<td>400</td>
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</tr>
</tbody>
</table>

Note. Subject columns contain arbitrary numbers. Daily dosages in mg.
CPZ = Chlorpromazine equivalent. * = dosage per 2 weeks

130
Table S2 Differences in brain activity between the three groups during the observation of emotional facial expressions

<table>
<thead>
<tr>
<th>Cluster size</th>
<th>F</th>
<th>H</th>
<th>MNI coordinates</th>
<th>Location peak voxel</th>
<th>Cluster description</th>
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<tbody>
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<td>12.18</td>
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<tr>
<td>104</td>
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<td>-62</td>
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<td>51</td>
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<td>L</td>
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<td>-56</td>
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<td>R</td>
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</table>

Faces-rest at uncorrected p=.001 (F=7.75) and a cluster extent threshold of 20 voxels. Cluster-correction for multiple comparisons at p=.05 corresponds to 71 voxels, which is represented by the black line in the table. F-values and MNI coordinates correspond to the maximum of the activated cluster. Column ^a reports the functional name or location that belongs to the peak voxel, while column ^b lists brain areas that are part of the cluster according to Anatomy Toolbox.
Table S3 Post-hoc tests for differences in average brain activity during the observation of emotional facial expressions

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<tr>
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<td>-8</td>
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<td>R</td>
<td>18</td>
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**TD > ASD**

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<th>Location peak voxel</th>
<th>Cluster description</th>
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<td>R</td>
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<td>R</td>
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<td>R</td>
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<td>4</td>
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<td>24</td>
<td>3.50</td>
<td>R</td>
<td>52</td>
<td>-8</td>
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</table>

**Schizophrenia > ASD**

**TD > Schizophrenia**

<table>
<thead>
<tr>
<th>Cluster</th>
<th>T</th>
<th>H</th>
<th>MNI coordinates</th>
<th>Location peak voxel</th>
<th>Cluster description</th>
</tr>
</thead>
<tbody>
<tr>
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<td>X</td>
<td>Y</td>
<td>Z</td>
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<tr>
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<td>56</td>
<td>4.10</td>
<td>L</td>
<td>-36</td>
<td>-78</td>
<td>26</td>
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</table>

**Schizophrenia > TD**

Mean differences in brain activity during the observation of emotional facial expressions (Faces - rest) using independent sample t-tests at uncorrected p=.001 (T=3.23) and cluster extent threshold of 20 voxels. Only results for the comparison ASD > Schizophrenia survive FDR-correction (FDR-corrected p=.05=2.69). Column a reports the functional name or location that belongs to the peak voxel, while column b lists brain areas that are part of the cluster according to Anatomy Toolbox.
Table S4 Differences in brain activity between the three groups that depend on emotion type

<table>
<thead>
<tr>
<th>Cluster size</th>
<th>F</th>
<th>H</th>
<th>MNI coordinates</th>
<th>Location peak voxel</th>
<th>Cluster description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2719</td>
<td>21.70</td>
<td>L</td>
<td>-44 -80 -2</td>
<td>Middle occipital gyrus (MOG)</td>
<td>MOG, MTG, FG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-40 -58 -18</td>
<td>Fusiform gyrus (FG)</td>
<td></td>
</tr>
<tr>
<td>2187</td>
<td>35.90</td>
<td>R</td>
<td>-42 -60 4</td>
<td>Middle temporal gyrus (MTG)</td>
<td>MTG, MOG, FG, STG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-42 -40 -18</td>
<td>Fusiform gyrus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td></td>
<td>52 -40 14</td>
<td>Superior Temporal Gyrus (STG)</td>
<td></td>
</tr>
<tr>
<td>697</td>
<td>12.59</td>
<td>R</td>
<td>28 -46 64</td>
<td>Primary somatosensory cortex (BA2)</td>
<td>BA2, BA3, BA1</td>
</tr>
<tr>
<td>357</td>
<td>8.79</td>
<td>L</td>
<td>-30 -44 52</td>
<td>Primary somatosensory cortex (BA2)</td>
<td>BA2, BA1 (BA3), IPL, SPL</td>
</tr>
<tr>
<td>126</td>
<td>5.65</td>
<td>L</td>
<td>-20 -74 -8</td>
<td>Lingual gyrus</td>
<td>Lingual/fusiform gyrus</td>
</tr>
<tr>
<td>111</td>
<td>5.76</td>
<td>L</td>
<td>-26 32 38</td>
<td>Middle frontal gyrus</td>
<td>Middle/superior frontal gyrus</td>
</tr>
<tr>
<td>41</td>
<td>4.73</td>
<td>L</td>
<td>-46 -50 44</td>
<td>Inferior parietal lobule (IPL)</td>
<td>IPL</td>
</tr>
<tr>
<td>28</td>
<td>5.13</td>
<td>R</td>
<td>46 2 46</td>
<td>Precentral gyrus</td>
<td>Precentral gyrus</td>
</tr>
<tr>
<td>23</td>
<td>5.34</td>
<td>R</td>
<td>24 -4 6</td>
<td>Putamen</td>
<td>Pallidum, putamen</td>
</tr>
<tr>
<td>22</td>
<td>4.58</td>
<td>L</td>
<td>-20 -78 6</td>
<td>Primary visual cortex (V1)</td>
<td>V1 (BA17)</td>
</tr>
<tr>
<td>20</td>
<td>5.01</td>
<td>R</td>
<td>14 46 10</td>
<td>Anterior cingulate cortex (ACC)</td>
<td>ACC</td>
</tr>
<tr>
<td>16</td>
<td>5.39</td>
<td>R</td>
<td>36 -8 -20</td>
<td>Hippocampus</td>
<td>Hippocampus (CA), Amygdala (LB/SF)</td>
</tr>
</tbody>
</table>

At uncorrected p=.001 (F=3.93) and a cluster extent threshold of 20 voxels (all voxels survive FDR-correction, \( F_{FDR,.05}=3.26 \)), F-values and MNI coordinates correspond to the first maximum of the activated cluster. For the two largest clusters we report additional peaks. Column 4 reports the functional name or location that belongs to the peak voxel, while column 5 lists brain areas that are part of the cluster according to Anatomy Toolbox.
### Table S5 Clusters with peaks that show a three-way interaction during the observation of emotional facial expressions

<table>
<thead>
<tr>
<th>Cluster size</th>
<th>F</th>
<th>H</th>
<th>MNI coordinates</th>
<th>Location peak voxel</th>
<th>Cluster description</th>
</tr>
</thead>
<tbody>
<tr>
<td>833</td>
<td>14.34</td>
<td>L</td>
<td>X = -50, Y = -58, Z = 4</td>
<td>Middle temporal gyrus (MTG)</td>
<td>MTG, MOG</td>
</tr>
<tr>
<td>656</td>
<td>12.11</td>
<td>R</td>
<td>X = 48, Y = -66, Z = 0</td>
<td>Middle temporal gyrus</td>
<td>MTG</td>
</tr>
<tr>
<td>294</td>
<td>7.03</td>
<td>R</td>
<td>X = 56, Y = -34, Z = 34</td>
<td>Supramarginal gyrus (SMG)</td>
<td>SMG</td>
</tr>
<tr>
<td>256</td>
<td>6.09</td>
<td>L</td>
<td>X = -32, Y = -38, Z = 54</td>
<td>Primary somatosensory cortex (BA2)</td>
<td>BA2, Postcentral gyrus (BA1, BA3b)</td>
</tr>
<tr>
<td>134</td>
<td>6.85</td>
<td>L</td>
<td>X = -12, Y = -78, Z = 10</td>
<td>Primary visual cortex (V1)</td>
<td>V1 (BA17)</td>
</tr>
<tr>
<td>67</td>
<td>7.59</td>
<td>R</td>
<td>X = 8, Y = -12, Z = -14</td>
<td>Brainstem</td>
<td>Hippocampus (EC), Amygdala (SF)</td>
</tr>
<tr>
<td>66</td>
<td>6.31</td>
<td>R</td>
<td>X = 24, Y = -4, Z = -6</td>
<td>Pallidum</td>
<td>Pallidum, Amygdala (SF/CM)</td>
</tr>
<tr>
<td>62</td>
<td>6.29</td>
<td>R</td>
<td>X = 44, Y = -30, Z = -2</td>
<td>Superior occipital gyrus (SOG)</td>
<td>SOG</td>
</tr>
<tr>
<td>62</td>
<td>5.69</td>
<td>R</td>
<td>X = 28, Y = -78, Z = 40</td>
<td>Superior temporal gyrus (STG)</td>
<td>MTG/STG</td>
</tr>
<tr>
<td>57</td>
<td>5.97</td>
<td>R</td>
<td>X = 38, Y = -30, Z = -10</td>
<td>Hippocampus (CA)</td>
<td>Hippocampus (CA/FD)</td>
</tr>
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<td>6.50</td>
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<td>Superior occipital gyrus</td>
<td>SOG</td>
</tr>
<tr>
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<td>4.69</td>
<td>R</td>
<td>X = 34, Y = -42, Z = 62</td>
<td>Primary somatosensory cortex (BA2)</td>
<td>BA2, BA1, BA3b</td>
</tr>
<tr>
<td>39</td>
<td>5.17</td>
<td>L</td>
<td>X = -10, Y = -4, Z = -16</td>
<td>Brainstem</td>
<td>Brainstem, Amygdala (SF)</td>
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<tr>
<td>38</td>
<td>5.27</td>
<td>L</td>
<td>X = -40, Y = 12, Z = -28</td>
<td>Medial temporal pole</td>
<td>Medial temporal pole</td>
</tr>
<tr>
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<td>5.01</td>
<td>L</td>
<td>X = -64, Y = -32, Z = 32</td>
<td>Supramarginal gyrus</td>
<td>SMG</td>
</tr>
<tr>
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<td>5.30</td>
<td>R</td>
<td>X = 22, Y = -44, Z = -30</td>
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<td>Cerebellum (VI)</td>
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<td>X = 32, Y = 52, Z = 0</td>
<td>Middle frontal gyrus (MFG)</td>
<td>MFG</td>
</tr>
<tr>
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<td>L</td>
<td>X = -46, Y = -40, Z = 2</td>
<td>Middle temporal gyrus</td>
<td>MTG</td>
</tr>
</tbody>
</table>

Emotion (3) x Context (2) x Group (3) at uncorrected p=.001 (F=3.93) and a cluster extent threshold of 20 voxels (all voxels survive FDR-correction, F_{FDR,.05}=3.76). F-values and MNI coordinates correspond to the first maximum of the activated cluster. Column $^a$ reports the functional name or location that belongs to the peak voxel, while column $^b$ lists brain areas that are part of the cluster according to Anatomy Toolbox.
Table S6 Mean differences in brain activity during the observation of each of the six movie types versus baseline

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Ncup

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Group differences for the six different movie types (3 Emotions: Disgust, Neutral, Pleasure, 2 Contexts: Cup, No Cup) using independent sample t-tests at uncorrected \( p=0.001 \) (\( T=3.23 \)) and cluster extent threshold of 20 voxels. T-values and MNI coordinates correspond to the maximum of the activated cluster. In black, clusters for which ASD or Schizophrenia > TD. In dark gray, clusters for which TD > ASD or Schizophrenia. * = clusters survive FDR-correction for DnoCup (\( F_{DnoCup} = 2.88 \)), and partly for Pcup (\( F_{Pcup} = 4.39 \)). STG = Superior Temporal Gyrus, ACC = Anterior Cingulate Cortex, MCC = Middle Cingulate Cortex of, SI = Primary Somatosensory Cortex, SII = Secondary Somatosensory Cortex, MI = Primary Motor Cortex, MOG = Middle Occipital Gyrus, IFG = Inferior Frontal Gyrus, V1 = Primary Visual Cortex
7.7 Supplementary Figures

Figure S1 Main effect of group

Barplots for the main effect of group at uncorrected \(p<.001\) (F=7.75) in peak voxels of clusters that survive a cluster-threshold \(p<.05\). MCC = Middle Cingulate Cortex, ACC = Anterior Cingulate Cortex, SII = Secondary Somatosensory Cortex, ASD = Autism Spectrum Disorder, TD = Typically Developing. * = \(p<.05\), ** = \(p<.01\), *** = \(p<.001\).
Figure S2 Plots in the peaks of the significant Emotion x Group clusters
The interaction effects can be identified by comparing the group’s slopes of the lines that connect two emotion categories. Panels A - F show a reduced difference between pleasure, and disgust and/or neutral in the schizophrenia group versus the TD group, while panel G shows an increase of pleasure relative to disgust/neutral in schizophrenia compared to the other two groups. Panels H and I show that emotion responses are not differentiated in the schizophrenia group. Panels J-N show that compared to the TD group, the ASD group and to a lesser extent the schizophrenia group respond relatively stronger to disgust compared to pleasure and/or neutral. For panels I and N this is only true for the ASD group.
Figure S3 Plots in the peaks of the significant three-way interaction clusters

Middle Temporal Gyrus (r)

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Middle Temporal Gyrus (l)

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Superior Temporal Gyrus (r)

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cup  
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Neural similarities and differences in autism and schizophrenia

Superior Occipital Gyrus (I)

Primary Somatosensory Cortex (I)

Primary Somatosensory Cortex (r)
Chapter 7

Cerebellum (r)

Brainstem/Amygdala (I)

Brainstem/Hippocampus (r)
Neural similarities and differences in autism and schizophrenia

Hippocampus (r)

Parameter Estimates (arbitrary units)

ASD  Schizophrenia  TD
D  N  P  D  N  P  D  N  P

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Pallidum (r)

Parameter Estimates (arbitrary units)

ASD  Schizophrenia  TD
D  N  P  D  N  P  D  N  P

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Superior Occipital Gyrus (r)

Parameter Estimates (arbitrary units)

ASD  Schizophrenia  TD
D  N  P  D  N  P  D  N  P

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The three-way interaction effects (Emotion x Context x Group) can be identified by comparing the differences between the group’s slopes of the lines that connect two emotion categories (Disgust, Neutral, Pleasure) for Cup versus No Cup. Panels A - F show a reduced difference between pleasure (versus disgust and/or neutral) in the Cup context relative to the No Cup context in schizophrenia compared to the TD and ASD groups. Panels G - J show that compared to the No Cup context, there is less emotional differentiation in the Cup context in the schizophrenia group than in the other two groups, who show a stronger response to pleasure than to neutral and/or disgust in the Cup context. Panels B, K, and L show a relative increase in response to disgust (versus pleasure and/or neutral) in the patient groups - in contrast with the TD group - in the No Cup context relative to the Cup context. Panels M and N this pattern for the schizophrenia group compared to the TD group, while panels I and J show this pattern for the ASD group compared to the TD group. Panels O and P show that in the ASD group emotion responses are not modulated by context (as opposed to the schizophrenia and TD groups). Panel Q shows that in the No Cup context emotion patterns are quite similar across groups, while in the Cup context patients do not represent emotions more strongly than the neutral stimuli, which the TD group does.
Figure S4 Group Differences for the Separate Movie Types

Disgust | Neutral | Pleasure
--------|---------|---------
ASD - TD

Disgust | Neutral | Pleasure
--------|---------|---------
Schiz - TD

Group differences in brain activity during the observation of the movie types (Disgust No Cup, Pleasure Cup) versus baseline using independent sample t-tests at punc = .001 (T = 3.23) and a cluster extent threshold (k) of 20 voxels. In orange areas that are more activated by either the ASD or schizophrenia group compared to the TD group. In blue the reverse contrast (TD > ASD or Schiz).
General discussion
By providing a direct link between ourselves and others, mirror mechanisms may play an important role in understanding the actions and emotions of other people. These mechanisms have been implicated in Autism Spectrum Disorder (ASD); a neurodevelopmental disorder characterized by impaired social functioning and difficulties in many important social cognitive skills such as the ability to understand and feel with the emotions of other people. In this thesis, we investigated the workings of mirror mechanisms during emotion perception in typically developing (TD) individuals and individuals with ASD. We believe this endeavor will help improve our understanding of the neurobiology of ASD and provide pointers for the development of more effective therapies. Merely comparing individuals with ASD to TDs cannot, however, establish whether neural abnormalities are specifically associated with ASD or with social dysfunction in general. Therefore, we included a second psychiatric group with marked social deficits: schizophrenia. Social deficits are the most reliable predictor of long-term prognosis in schizophrenia, and are especially profound when negative symptoms are on the foreground. In this thesis, we presented the first studies that compare the two disorders where they resemble each other most, that is in high-functioning individuals with ASD and non-psychotic individuals with chronic schizophrenia. To this end, we used behavioral observation, measures of social cognition and social function, and functional magnetic resonance imaging (fMRI) mainly focused on the perception of dynamic displays of emotion. The combined study of ASD and schizophrenia provides a unique opportunity to gain more information about the underlying neural mechanisms of social (cognitive) dysfunction and the differences between the disorders. In this concluding chapter, we will integrate our findings to form new directions for research and the clinic.

**Mirror mechanisms in emotion processing**

Historically, theories of social cognition have emphasized cognitive processes in identifying the mental states of others. In chapter 2 we reviewed evidence that shows that seeing someone else experiencing an emotion triggers much more in us than a purely theoretical, disembodied interpretation of other people's mental states. Humans activate premotor and parietal cortical areas involved in action execution when they see someone perform a goal-directed action, but also when they see facial expressions (Carr et al., 2003; Hennenlotter et al., 2005; Leslie et al., 2004; van der Gaag et al., 2007; Wicker et al., 2003; Wild, Erb, Eyb, Bartels, & Grodd, 2003). This motor simulation in what became known as the Mirror Neuron System (MNS) is involved in action understanding and may also contribute to understanding the intentions of others (Rizzolatti & Craighero, 2004). Emotion simulation extends beyond the ventral premotor cortex and inferior frontal gyrus (IFG) to somatosensory and affective regions, such as the ACC, anterior insula, and amygdala, all of which are crucial for emotion recognition (Adolphs et al., 2000; Adolphs et al., 2003; Broks et al., 1998; Calder et al., 2000; Sprengelmeyer et al., 1999; Tranel et al., 2006). Embodied simulation thus seems to be a general endowment of the brain, which involves motor, somatosensory, and affective components (Keysers & Gazzola, 2006). There is, however, no one-to-one mapping of particular emotions onto particular brain regions. Rather, emotion simulation seems to involve a network of regions, whose activation strengths depend on the quality of the emotion and its associated output. Signals of fear, for instance, possibly enhance amygdala activity (in particular in stressful situations), because they indicate a potential threat, which increases visual
attention to the outside world. Similarly, visceral responses mediated by the anterior insula are probably more important for disgust, and the anterior middle cingulate cortex (aMCC) with its strong motor connections might be particularly relevant for pain. The precise interaction between the various components and how they relate to more cognitive systems still remains unclear. There are probably many routes to equally many types of emotions, which are processed in various regions of the brain. One route could lead from motor simulation, in the frontal component of the MNS, to trigger emotion simulation in emotional centers of the brain, such as the insular cortex and amygdala (Carr et al., 2003; Jabbi & Keyser, 2008; Keyser & Gazzola, 2006; Niedenthal, 2007). This motor route found some support from findings in the next chapter.

**Seeing, feeling, and imagining disgust**

Chapter 3 illustrated that simulation is a highly integrated process, which is likely to depend on different networks in different conditions. For this study, we scanned healthy control subjects while they experienced, viewed someone else experiencing, and imagined experiencing gustatory emotions through script-driven imagery. We showed that social perception, mental imagery, and personal experience of disgust commonly involve the anterior insula and frontal operculum (IFO), which confirms the role of this structure in the embodiment of feeling states. The IFO involvement in the imagery condition might help explain why reading books and imagining hypothetical experiences can make you experience vivid emotions. This finding is promising for the use of script-driven imagery as an easy and more comfortable way of inducing emotions in experimental settings. Most importantly, effective connectivity analysis demonstrated that, although the IFO is commonly recruited when observing, feeling and even imagining disgust, the involved networks are quite different. During disgust experience, the IFO is embedded into a network composed of somatosensory, gustatory/motivational and motor output regions. During observation, the IFO receives its strongest input from the right inferior frontal gyrus (BA45), which supports the idea presented in chapter 2 that motor simulation of facial expressions could trigger emotion simulation in the anterior insula. During script-driven mental imagery, the IFO is embedded in a network of language processing and semantic memory areas, which is suggestive of a more cognitive route to emotion simulation. The differences in the networks in which shared circuits are engaged may play an important role in distinguishing self and other during social interactions and in the different phenomenology of seeing, feeling, and imaging an emotional event.

**Motor simulation of emotional facial expressions in autism**

As discussed in chapter 2, motor simulation in the frontal part of the MNS may play an important role in many social skills such as imitation and emotion understanding. Hypoactivation of the inferior frontal gyrus (IFG) during the perception of facial expressions has been interpreted as evidence for a deficit of the mirror neuron system in children with autism (Bookheimer et al., 2008; Dapretto et al., 2006; Uddin et al., 2008). In chapter 4, we zoomed in on this structure to examine whether its dysfunction persists in adulthood, and how brain activity in the MNS relates to social functioning outside the laboratory. To this end, we measured brain activity during the observation of dynamic facial expressions in a group of adults with ASD compared to pair-matched controls.
Contrary to findings of IFG hypoactivity in children, on average, we did not find evidence for an underactivated MNS, even when the analysis was restricted to the region of hypoactivity in children. This discrepancy between findings in children and adults is intriguing. Our study demonstrated that age may be a critical factor: IFG activity during the observation of facial expressions increased with age in ASD, but not in controls. In addition, the within-subject variance decreased with age in the ASD group, suggesting that neural ‘noise’ in the IFG decreases and functioning of the MNS improves. The age-related increase in activity was associated with changes in gaze behavior and improvements in social functioning. These age-related neurocognitive improvements were not found in the group of individuals with schizophrenia, who had comparable levels of social functioning. Our cross-sectional study instigates an interesting hypothesis that is important for future (longitudinal) studies on autism to assess: individuals with ASD might have a unique developmental pattern of improving facial simulation abilities during adolescence and early adulthood, which positively impacts social relationships. These results are consistent with the clinical impression of improvements in social functioning and responsiveness to other’s distress in adolescents and adults with ASD (Farley et al., 2009; McGovern & Sigman, 2005; Piven et al., 1996; Seltzer et al., 2003; Shattuck et al., 2007).

Here, it is important to note that these findings should not be taken to suggest that with age all will be normal: even in our older individuals with ASD the levels of social functioning were still below those of the TDs. There might, however, be unexpected room for improvement later in life. We hope that our findings will generate new research on this possibility and will give an impulse to the investigation of interventions directed at emotion simulation or directly targeted at the MNS. Given the plasticity of the MNS and its connections to visual regions, especially early imitation training of facial expressions and explicit training to change gaze behavior might be beneficial (Calvo-Merino et al., 2005; Catmur et al., 2007; Cross et al., 2006; Haslinger et al., 2005; Lahav et al., 2007; Wright et al., 2008). Training methods for individuals with ASD, however, often cope with limited generalization from training material to daily life situations (Golan et al., 2009; Golan & Baron-Cohen, 2006). Possibly, imitation training effects could be enhanced by modifying cortical plasticity in the inferior frontal cortex through Transcranial Magnetic Stimulation (TMS) or transcranial Direct Current Stimulation (tDCS) in those children with ASD, who show abnormal mu wave suppression, theta burst abnormalities, or diminished BOLD responses when perceiving other’s actions or emotions (see chapter 5, Dapretto et al., 2006; Martineau et al., 2008; Oberman et al., 2005). The effects of these noninvasive brain stimulation techniques on cortical plasticity and excitability in ASD (Oberman et al., 2010) and their clinical benefits will be important topics for future research (Fregni & Pascual-Leone, 2007).

**Autism and the mirror neuron system**

The mirror neuron hypothesis of autism is grounded in the idea that a pervasive impairment of the MNS in individuals with autism may lead to a cascade of impairments ranging from impaired imitation and goal inference to poor mentalizing and social abilities (Iacoboni & Dapretto, 2006; Oberman & Ramachandran, 2007; Rizzolatti & Fabbri-Destro, 2008; Rizzolatti et al., 2009; Williams et al., 2001). The fact that IFG activity was not disrupted in (older) adults with ASD during emotion perception (chapter 4), indicates that the role of the MNS in ASD is not that straightforward. Therefore, we further evaluated the MNS hypothesis of ASD in chapter 5 by reviewing studies on
simulation of observed actions and facial expressions.

In the past five years many studies have been conducted in this domain, but overall they have come up with very inconsistent results. The available evidence in the hand action domain suggests that individuals with ASD do not seem to have great difficulty imitating and understanding the goal of other’s actions. Instead, they seem to experience more difficulties when imitation requires understanding the beliefs of someone else (Hamilton et al., 2007) and when the exact imitation of style is required (Hobson & Hobson, 2008; Hobson & Lee, 1999). Moreover, action perception can trigger motor responses in individuals with ASD even though responses are not reported as consistently as in typically developing individuals. The relatively intact basic imitation mechanisms in combination with the comparatively greater theory of mind difficulties form an interesting paradox. It means that, if higher forms of social cognition are underpinned by imitation (Rogers & Pennington, 1991), mind-reading impairments in ASD cannot be reduced to basic abnormalities in MNS functioning (Hamilton et al., 2007). Understanding emotions and other mental states of others also engages structures outside the MNS, among which the medial prefrontal cortex (MPFC), the superior temporal sulcus (STS), the temporal poles, and the temporo-parietal junction (TPJ, Amodio & Frith, 2006; Gallagher & Frith, 2003; Saxe, 2006), which may (also) be affected in ASD (Castelli et al., 2002; Happe et al., 1996).

However, the fact that abnormal MNS functioning cannot on its own explain the whole constellation of clinical features that constitute ASD does not mean that the MNS is unrelated to the social cognitive problems in ASD. In fact, many studies did point to abnormalities in motor simulation, which may be more profound for emotional faces (Beall et al., 2008; Dapretto et al., 2006; McIntosh et al., 2006) than for goal-directed meaningful hand actions (Avikainen et al., 1999; Bernier et al., 2007; Bird et al., 2007; Dinstein et al., 2010; Fan et al., 2010; Raymaekers et al., 2009). Additionally, activity in the IFG during emotion perception has been linked to social competence in both typically developing individuals (Pfeifer et al., 2008) and individuals with ASD (Bastiaansen et al., 2011a; Dapretto et al., 2006).

The involvement of the MNS in ASD depends on several factors, which we are now beginning to uncover (e.g. nature of the stimuli, degree of identification with the actor, heterogeneity in ASD groups within and between studies). Of particular interest is the role of age, because its link to motor simulation and social functioning could mean that promoting the simulation of facial and bodily expressions at an early age improves social functioning in ASD. On the one hand, inconsistencies between studies thus inform us about important factors. On the other hand, they make it difficult to draw firm conclusions about the role of the MNS in the social cognitive difficulties and etiology of ASD and call for further research. One aspect that deserves further attention is the nature of imitation difficulties in ASD. The dissociation between imitation of goal and style (Hobson & Hobson, 2008) and the finding of diffuse facial mimicry in ASD (McIntosh et al., 2006) instigate an interesting yet speculative hypothesis. Possibly, the broadly congruent mirror neurons function sufficiently well to successfully match the goal of observed actions, but the strictly congruent mirror neurons do not function well enough (early in development) for the more vulnerable reproduction of style.
Behavioral similarities in autism and schizophrenia

In chapter 4 we reported that our ASD and schizophrenia groups have similar levels of general social dysfunction. This means that both groups participate less than their typically developing peers in various areas that are crucial to community maintenance, including employment and prosocial activities. In chapter 6 we investigated whether these patients also show similar social behaviors by investigating the psychometric properties of the Autism Diagnostic Observation Schedule (ADOS, Lord et al., 2000). The ADOS is a standardized instrument that assesses social interaction, communication, imagination, and restricted and repetitive behaviors during a semi-structured interaction with an examiner. The instrument has been widely used in clinical as well as research settings to assess the presence of ASD. It has been extensively validated in children, but not yet in adolescents and adults with fluent speech (module 4). Here, we investigated the psychometric properties of module 4 in (a subsample of) four groups of adults that signed up for neuroimaging experiments conducted in our lab and for whom ADOS was administered: ASD\(^1\) and schizophrenia, but also psychopathy and typical development.

In general, our findings show that ADOS module 4 is a reliable instrument that has good predictive value for ASD. We found high specificity (0.82), which means that a high proportion of non-autistic individuals was correctly classified as ‘nonspectrum’. Therefore, if an individual does receive an ADOS classification it is a strong lead for a clinician to at least consider an ASD diagnosis. Specificity is tightly linked to sensitivity, which in this context refers to the proportion of actual clinical cases of ASD that the instrument correctly classified. We found rather low sensitivity (0.61), which indicates that ADOS module 4 failed to classify ASD in a significant proportion of our high-functioning and more mildly affected ASD group. This could be due to compensatory strategies during the semi-structured setting of the interview. As an unfortunate consequence, various motivated individuals that signed up for our fMRI studies could not participate. The aim of the assessment determines whether sensitivity or specificity is most important. High specificity of module 4 is particularly important for research purposes such as ours, because researchers need to be certain that their ASD group comprises only individuals with ASD. When the aim of the assessment is to screen for ASD, high sensitivity is more crucial in order not to miss any potential case. For this purpose, lower thresholds could be considered at the expense of specificity. To prevent overinclusiveness, developmental history and current daily functioning should then be carefully reviewed.

Concerning group differences, we showed that ADOS module 4 can adequately discriminate ASD from psychopathy and typical development in an adult population. The distinction between psychopathy and ASD remained when we only took forensic individuals with ASD into account (although the group size was rather small to perform such an analysis). The finding that ASD and psychopathy are so well-discriminated by means of ADOS scores is promising for forensic psychiatric settings. As expected, discrimination was more difficult with respect to schizophrenia due to behavioral overlap. In the group with schizophrenia the degree of negative symptomatology was positively associated with ADOS scores, especially on items resembling negative symptoms such as

\(^1\) The ASD group in chapter 6 consisted of 38 individuals. Only 21 of them were eligible for participation in the reported fMRI studies, which was primarily based on ADOS classification and the absence of MRI contraindications.
(lack of) directed facial expressions and expressions of shared enjoyment. The ASD and schizophrenia group can be distinguished on some core items, and the revised algorithm of the ADOS seems promising for better discrimination of the two groups. In sum, these findings confirm that there can be overlap in the social behaviors seen in ASD and schizophrenia, which extends the finding of comparable levels of social dysfunction presented in chapter 4.

**Neural similarities and differences in autism and schizophrenia during emotional facial expression processing**

Despite these similarities, the region of interest fMRI analysis in chapter 4 showed that only in the ASD group improvements in social functioning were associated with increased activity in the inferior frontal gyrus. In chapter 7, we zoomed out of the IFG and presented the first whole-brain fMRI study that investigates the similarities and differences in neural dysfunctions in these two groups with a shared social profile. First, we showed that both groups tend to withdraw from social participation in daily life and have similar difficulties with empathy and emotion recognition. Then, we probed social dysfunctions in an ecologically valid fashion, by presenting all subjects with movies of facial expressions that vary in valence (positive, neutral, negative) and social directedness without an explicit task. Based on learning theory, we hypothesized that two processes may play a role in the social withdrawal seen in both disorders. First, increased social avoidance may occur because of increased sensitivity to negative social stimuli or a general bias towards perceiving social stimuli as more negative or excluding. Second, reduced social engagement or approach may occur due to reduced sensitivity/reinforcement by positive social stimuli. The core findings of this chapter are two-fold. First, although the patient groups differed in their overall level of activation -with hyperactivity in the ASD group and a tendency towards hypoactivity in the schizophrenia group- both groups rated the faces more negatively and showed a relative bias towards negative facial expressions in visual brain regions and regions involved in the embodied processing of emotions. Excessive sensitivity to and vicarious sharing of negative emotions of other people may lead to the reported higher levels of personal distress and social withdrawal seen in both ASD and schizophrenia. Second, only the schizophrenia group showed a reduced sensitivity for pleased faces in regions involved in approach behavior and reward processing, which might further reduce social engagement in schizophrenia. Neural responses during emotion processing in patients with ASD and schizophrenia are thus biased in a way that might provide a learning theoretical explanation for their social isolation.

Our findings suggest that therapies aimed at normalizing the balance between sensitivity for positive and negative social information may help both patient groups to re-establish an adaptive equilibrium between social approach and withdrawal. The groups should, however, be approached in different ways. Due to the differences in overall sensitivity, individuals with ASD might benefit most from desensitization to social stimuli in general and negative stimuli in particular (e.g. by reappraisal strategies). Individuals with schizophrenia might benefit most from findings ways to make social interactions more rewarding or perhaps from techniques that directly target brain regions that are hypoactivated. A recent meta-analysis suggests that individuals with schizophrenia and negative symptoms might benefit from repetitive TMS (rTMS) of frontal brain regions (Diabac-de Lange, Knegtering, & Aleman, 2009). RTMS of the (left) prefrontal cortex can increase prefrontal
cortical excitability and may modulate mesolimbic and mesostriatal dopamine release (Pogarell et al., 2007; Strafella, Paus, Barrett, & Dagher, 2001; Strafella, Paus, Fraraccio, & Dagher, 2003). This suggests that TMS might be able to enhance activity in those areas that we found were hypoactive when seeing positive facial expressions (i.e. left frontal cortex, striatum and insula). Improving the negative symptoms by rTMS might, however, worsen positive symptoms (Hajak et al., 2004). Current findings warrant further study of rTMS as a potential treatment for negative symptoms and call for studies combining rTMS with neuroimaging techniques (Dlabac-de Lange et al., 2009; Sparing & Mottaghy, 2008).

**Taking a developmental perspective**

Although most obvious abnormalities of ASD are already present in early childhood, our findings suggest that important developmental processes may continue throughout the lifespan. We reported age-related neurocognitive changes in high-functioning adults with ASD ranging from 18 to 54 years of age. The incremental activity of the IFG could mean that, although in ASD motor simulation of facial expressions is suboptimal in childhood (Dapretto et al., 2006), it improves during adolescence and early adulthood. Premotor activity can be reflected in facial muscle reactions in response to the perception of facial expressions (Schilbach et al., 2008). Together, three cross-sectional studies on facial mimicry provide support for an abnormal developmental pattern of face simulation in ASD. First, in contrast to their typically developing peers, children with ASD between 7 and 12 years of age did not show any muscle-specific reactions to happy and angry facial expressions and showed undifferentiated responses to fearful expressions (Beall et al., 2008). Second, undifferentiated facial muscle responses of normal intensity were found in an ASD group that consisted of older children and adults in a wide age range (13-64 years, McIntosh et al., 2006). Finally, in a study involving only adults, Magnée et al. (2007) found stronger facial muscle responses in the ASD group that were congruent with the displayed happy and fearful emotions. These findings suggest that ASD might be characterized by a delayed developmental trajectory, which moves from a complete absence of simulation in children (Beall et al., 2008; Dapretto et al., 2006), to a pattern of normal overall intensity coupled with a lack of specificity, to normal or even enhanced simulation of facial expressions in adults (Bastiaansen et al., 2011a; Magnée, De Gelder et al., 2007). Importantly, our findings suggest that these changes may have a significant impact on social functioning. Interpretations of cross-sectional findings should be made cautiously, but these studies might provide the dots that longitudinal studies may be able to connect.

Although we did not find age-related changes in our group of individuals with schizophrenia, a developmental perspective seems equally important for the study of schizophrenia. Central to this thesis were the remitted phases of the illness, which are dominated by social impairments. Social impairments and neurodevelopmental abnormalities have, however, also been widely reported before the onset of symptoms in the prodrome period and even in early childhood (Addington, Penn, Woods, Addington, & Perkins, 2008b; Asarnow, 1988; Ballon, Kaur, Marks, & Cadenhead, 2007; Cannon et al., 2008; Feinberg, 1982; Jones, 1997; Mednick & McNeil, 1968; Mirsky, Kugelmass, Ingraham, Frenkel, & Nathan, 1995; Rapoport, Addington, Frangou, & Psych, 2005). Social dysfunction thus seems to be an early-emerging, defining, and persistent characteristic of schizophrenia (Sasson, Pinkham, Carpenter, & Belger, 2011). To better understand the nature and
course of these deficits, longitudinal studies are necessary that follow the development of individuals from childhood to prodrome to chronic schizophrenia.

**Keeping an eye on visual processing**

Another or perhaps complementary explanation for the observed age-related increase in IFG activity could be a change in the way older individuals with ASD look at facial expressions. We analyzed the subjects’ points of regard during the experiment and found that with age the time spent looking at the lower half of the face increased. The associated increases in IFG activity and social functioning suggest that this gaze pattern could be a beneficial strategy. In fact, the lower half of the face contained much relevant information about the emotion displayed in the movie clips (i.e. disgust, neutral, pleasure). Similar to our findings, Klin et al. (2002) have also found that time spent looking at the mouth region is significantly correlated with social competence in adolescents with ASD. Even though our eye tracking analyses were rather rough, they point to the important role of gaze behavior during social perception. Future neuroimaging studies should take into account visual processing in more detail to investigate how differential gaze patterns influence activity throughout the brain.

While looking at the lower face region may be a compensatory strategy later in life, a failure to look at the eyes of others during critical windows of development may have cascading effects on socialization (Jones & Klin, 2009). From early on, children with ASD do not show the normal preferential attention to the eyes of other people (Jones et al., 2008) and they do not automatically orient towards biological motion, but tend to orient towards non-social contingencies instead (Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998; Klin et al., 2009). An early deficit in attention allocation to socially relevant signals likely has negative consequences on the maturation of the posterior STS circuits supporting the processing of biological motion (Blake et al., 2003; Boddart et al., 2004), and consecutively on the development of the mirror neuron system. More research is needed on the developmental trajectory of abnormal social skills in ASD, especially on how early abnormalities in basic mechanisms such as preferential attention to the eyes and biological motion might give rise to problems in more complex social skills such as the ability to understand the intentions of others (Jones & Klin, 2009).

**Face to face: autism and schizophrenia**

There are sizeable independent literatures on social functioning, social cognition, and implicated neural substrates in both ASD and schizophrenia (e.g. see Abdi & Sharma, 2004), which makes it difficult to draw conclusions on where these disorders are similar and different. Studies have, for instance, used a wide range of experimental tasks, different methodologies, and used samples with different characteristics (Sasson et al., 2011). Knowledge on the disorder specific and social cognitive impairment specific pathways and mechanisms will be essential for developing new interventions. Direct comparisons between autism and schizophrenia that can help illuminate what aspects are disorder-specific and what aspects are related to social deficits per se are, however, scarce.

In this thesis, we presented two of the first fMRI studies that compare two relatively large
and homogeneous groups with a shared presentation of social isolation while using the same experimental designs. The unique combination of these groups allowed us to demonstrate behavioral (chapter 6) and neurobiological (chapter 4 & 7) similarities and differences between ASD and schizophrenia. One important finding was that the groups were comparable in terms of social dysfunction, but during emotion perception the degree of social function was only related to activity strength in the MNS in the ASD group. By contrasting the two groups, we may have found evidence for a specific developmental trajectory of motor simulation of facial expressions in ASD. Another finding was that, although both groups were more sensitive to negative social stimuli, only the schizophrenia group showed reduced responses to positive social stimuli in regions related to reward processing. This suggests reward processing may be a particularly important area of disturbance in schizophrenia (Ziaudddeen & Murray, 2010).

There is currently only one other study that has directly compared the neural substrates of social cognition in autism and schizophrenia. Using gray-scale pictures of unemotional faces during a trustworthiness task, Pinkham et al. (2008) found similar abnormalities in predefined regions implicated in social cognitive processing (e.g. amygdala, fusiform face area) in individuals with ASD and paranoid schizophrenia. Interestingly, they found a specific impairment in the posterior STS for individuals with ASD compared to both paranoid and non-paranoid individuals with schizophrenia. Another rare study that directly compared gaze behavior in autism and schizophrenia adds to this that while both patient groups pay less attention to faces, only individuals with ASD fail to orient more rapidly to faces when facial information is present (Sasson et al., 2007). Together with the findings presented in the previous paragraph, this suggests that circuits supporting attention orienting to socially relevant signals, including the posterior STS, are specifically disturbed in individuals with ASD. These and our studies are illustrative of the knowledge that can be gained from studying autism and schizophrenia in combination.

Moving across borders

As discussed in the previous paragraph, contrasting two diagnostic categories can provide important information about the convergence and divergence between disorders. Using diagnostic categories as a starting point does, however, raise two important issues.

First, the current DSM-defined psychiatric categories are very heterogeneous and therefore likely involve multiple brain systems instead of reflecting neurologically discrete phenomena (Gillihan & Parnes, 2011; Insel et al., 2010). In case of schizophrenia, for instance, two individuals with the same classification do not necessarily share one similar symptom (DSM-IV-TR). In our studies, we have tried to reduce this heterogeneity by selecting non-psychotic individuals with schizophrenia on the basis of negative symptomatology and high-functioning individuals with ASD with above cut-off scores on the ADOS. These groups were comparable on important factors as age, sex and general cognitive abilities. As discussed previously, the comparison of two different patient groups with common behavioral characteristics helps illuminate what neurobiological aspects are disorder-specific and what aspects are related to the common characteristics. Selecting subgroups on the basis of behavioral characteristics naturally reduces heterogeneity at the expense of generalizability. Therefore, our findings will be difficult to generalize to, for instance, mentally retarded individuals with ASD and psychotic individuals with schizophrenia. Another important open
question is to what extent our findings apply to women.

The second issue is that the current state of the literature suggests that the genetic and neural correlates of psychopathology are generally not unique to specific diagnoses (Gillihan & Paren, 2011). Similar genes have, for instance, been implicated in ASD and schizophrenia, but also in schizophrenia and bipolar disorder (e.g., Cuthbert & Insel, 2010; Marx, 2007). In chapter 7, we showed that both individuals with ASD and individuals with schizophrenia may have a negative bias, which was reflected in increased activity for negative social stimuli in visual and somatosensory regions. The additional reduced sensitivity to positive social stimuli in the schizophrenia group may also not be disorder specific. In fact, anxiety and depression have also been linked to an attentional bias for negative information and a reduced sensitivity for positive information (Baert, De Raedt, Schacht, & Koster, 2010; Bourke et al., 2010; Winton et al., 1995). This suggests that the neural circuits underlying negative affect and reward processing may be two important brain-behavior domains in the study of psychiatric disorders in general. Therefore, another fruitful approach could be to identify the neural circuitry underlying mental domains such as negative emotionality and reward and then examine how variation relates to behavior. This approach would be in line with a new strategic plan developed by the US National Institute of Mental Health (NIMH), which emphasizes the need to study mental domains across current diagnostic boundaries (www.nimh.nih.gov/research-funding/rdoc.shtml). This could entail a dimensional approach that investigates mental domains such as reward processing and negative affect across diagnostic borders of ASD, schizophrenia, bipolar disorder, major depressive disorder, and social anxiety disorder. The hope is that when behavior-brain relationships (e.g., reward circuitry) can be linked to clinical phenomena (e.g., anhedonia), more specific therapies can be developed to target them. The new strategic plan might eventually lead to a new classification of mental disorders that is based on identifiable neural circuits instead of a constellation of various symptoms.

Concluding remarks

In short, we have shown that motor, affective, and somatosensory representations play a role in the embodied simulation of the emotional expressions of other people. The interaction between these components during emotion perception is still largely unknown, but motor simulation may play an important role in triggering activity in emotional centers of the brain. Abnormal motor simulation in the MNS seems to be related to the social problems in ASD, but cannot explain the whole constellation of clinical features. Additionally, findings on the MNS are too inconsistent to provide for a reliable biological diagnostic marker, but there may be an abnormal developmental trajectory of simulation abilities specific to ASD that needs further investigation. We have also reported that social behaviors of individuals with deficit schizophrenia can resemble those individuals with ASD. In addition, these groups were comparable in terms of social dysfunction, social withdrawal and social cognitive impairments. However, with respect to the underlying neural pathology there seem to be more differences than similarities. We need to pursue a more thorough understanding of the neurobiology of ASD and schizophrenia by taking a developmental and comparative approach, for instance by simultaneously mapping social cognitive development in ASD and schizophrenia starting in early childhood. More detailed knowledge about the similarities and differences is necessary in order to develop more tailored therapies for these patient groups. At present, interventions are
especially lacking for the two patient groups studied throughout this thesis: adults with ASD and individuals with schizophrenia presenting with negative symptoms. As discussed previously, imitation training or TMS treatment might be useful to target core problems of these disorders. Further research into other treatments that can help alleviate comorbid symptoms of distress and depression, such as mindfulness-based stress reduction (MBSR), is also warranted (Bögels, Hoogstad, van Dun, de Schutter, & Restifo, 2008; Spek, van Ham, & van Lieshout, 2010).

Moving on...

The studies discussed in this thesis were conducted in the Social Brain Lab headed by Christian Keysers. In the next years, the autism line of the lab will be further developed by Leonardo Cerliani and Marc Thioux. They will be working on a highly multimodal study that combines fMRI, structural imaging, and connectivity analyses. Of special interest will be how the structures involved in low-level embodied processes are integrated with regions involved in higher-level inference-based processes (Keysers & Gazzola, 2006), such as the TPJ and MPFC (Amodio & Frith, 2006; Gallagher & Frith, 2003; Saxe, 2006). Recent studies indicate that regions involved in mental state attribution and shared sensorimotor representations influence each other and are equally important to come to a complete understanding of the mental states of another person (Cheng et al., 2007; Schippers, Roebroeck, Renken, Nanetti, & Keysers, 2010; Simon et al., 2006; Singer et al., 2006; Zaki et al., 2007). Given the fact that individuals with ASD often experience great difficulties with mentalizing tasks and have shown MPFC abnormalities in a couple of independent fMRI studies (Castelli et al., 2002; Happe et al., 1996), we believe it is time to evaluate the functioning of both the MNS and ToM systems in one experimental design (Thioux & Keysers, in preparation). The strength of the new project is that it will combine measures of functional connectivity within and between these systems with measures of structural connectivity of the white matter structures that interconnect gray matter regions and form neural circuits underlying important (social) cognitive functions.
Bibliography


Bibliography


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**Bibliography**


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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACC</td>
<td>Anterior Cingulate Cortex</td>
</tr>
<tr>
<td>ADOS</td>
<td>Autism Diagnostic Observation Schedule</td>
</tr>
<tr>
<td>AI</td>
<td>Anterior Insula</td>
</tr>
<tr>
<td>ASD</td>
<td>Autism Spectrum Disorder</td>
</tr>
<tr>
<td>BA</td>
<td>Brodmann Area</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood Oxygen Level Dependent</td>
</tr>
<tr>
<td>DSM</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalography</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>FEEST</td>
<td>Ekman 60 Faces Test</td>
</tr>
<tr>
<td>(f)MRI</td>
<td>(functional) Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>GLM</td>
<td>General Linear Model</td>
</tr>
<tr>
<td>IFG</td>
<td>Inferior Frontal Gyrus (BA44/45)</td>
</tr>
<tr>
<td>IFO</td>
<td>Anterior Insula/ Frontal Operculum</td>
</tr>
<tr>
<td>IPL</td>
<td>Inferior Parietal Lobule</td>
</tr>
<tr>
<td>IRI</td>
<td>Interpersonal Reactivity Index</td>
</tr>
<tr>
<td>MI</td>
<td>Primary Motor Cortex</td>
</tr>
<tr>
<td>MEG</td>
<td>Magnetoencephalography</td>
</tr>
<tr>
<td>MEP</td>
<td>Motor Evoked Potential</td>
</tr>
<tr>
<td>MCC</td>
<td>Middle Cingulate Cortex</td>
</tr>
<tr>
<td>MNS</td>
<td>Mirror Neuron System</td>
</tr>
<tr>
<td>MPFC</td>
<td>Medial Prefrontal Cortex</td>
</tr>
<tr>
<td>PPI</td>
<td>Psychophysiological Interaction</td>
</tr>
<tr>
<td>pSTS</td>
<td>posterior Superior Temporal Sulcus</td>
</tr>
<tr>
<td>ROI</td>
<td>Region Of Interest</td>
</tr>
<tr>
<td>SI</td>
<td>Primary Somatosensory Cortex</td>
</tr>
<tr>
<td>SII</td>
<td>Secondary Somatosensory Cortex</td>
</tr>
<tr>
<td>SFS</td>
<td>Social Functioning Scale</td>
</tr>
<tr>
<td>SPM</td>
<td>Statistical Parametric Mapping</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial Magnetic Stimulation</td>
</tr>
<tr>
<td>TPJ</td>
<td>Temporo Parietal Junction</td>
</tr>
<tr>
<td>vPMC</td>
<td>ventral Premotor Cortex (BA6/44)</td>
</tr>
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Publication list


- Thioux, M., Bastiaansen, J.A., & Keysers, C. Autism spectrum disorders and the putative mirror neuron system. *Under review.*
Mini samenvatting

Stel je voor: je ziet dat een vrouw na een hap van haar broodje een afgrijselijke grimas trekt en een hand voor haar mond slaat om het kokhalzen te onderdrukken. Grote kans dat jij in reactie daarop ook je gezicht vertrekt en je wat misselijk gaat voelen. In dit proefschrift laten we zien dat deze verstrijking van emotie waarneming en emotie ervaring ook zichtbaar is op breinniveau. Het zien van andermans emotionele gezichtsuitdrukkingen betrekt namelijk deels dezelfde gebieden als het zelf ervaren van die emoties. Het spiegelen van motorische aspecten in het zogeheten spiegelsysteem speelt daarbij een belangrijke rol. In tegenstelling tot wat eerder werd gedacht, laten we zien dat afwijkingen in het spiegelsysteem niet de hele verscheidenheid aan autistische symptomen kunnen verklaren. Wel zien we dat volwassenen met autisme mogelijk met de jaren beter worden in het spiegelen en dat dit samenhangt met een lichte verbetering in sociaal functioneren. Naast autisme wordt ook schizofrenie vaak gekenmerkt door sociale tekorten. We laten zien dat terwijl het sociale profiel van schizofrenie kan lijken op dat van autisme wanneer negatieve symptomen (zoals affectvervlakking) op de voorgrond staan, er voornamelijk verschillen zijn in de onderliggende neurale profielen. De unieke combinatie van autisme en schizofrenie in de beschreven studies zorgt voor meer kennis over deze stoornissen en over neurobiologische mechanismen die mogelijk ten grondslag liggen aan sociaal disfunctioneren. Deze inzichten kunnen de eerste belangrijke aanwijzingen geven voor de ontwikkeling van meer effectieve therapieën.

Nieuwsgierig geworden?
Lees dan de uitgebreide Nederlandse samenvatting.
Nederlandse samenvatting

Stel je voor: je zit in een lunchroom en er zit een vrouw tegenover je aan tafel. Wanneer ze in haar broodje bijt, trekt ze de meest afgrijzelijke grins en slaat een hand voor haar mond om het kokhalzen te onderdrukken. Grote kans dat jij in reactie daarop ook je gezicht vertrekt en je misschien zelf ook wat misselijk gaat voelen. In dit proefschrift onderzochten we of deze verstregeling van emotie perceptie en emotie ervaring ook zichtbaar is op het niveau van het brein. Spiegelt ons brein de emotionele signalen van andere mensen? En wat gebeurt er bij mensen met autisme en schizofrenie, die vaak moeite hebben met het begrijpen van andermans emoties? In welk opzicht spiegelen deze stoornissen elkaar en in welk opzicht verschillen ze? Om dit te kunnen onderzoeken hebben we gebruik gemaakt van gedragsobservatie, instrumenten die sociale cognitie en sociaal functioneren meten, en van beeldvormend onderzoek. De unieke combinatie van autisme en schizofrenie in de beschreven studies zorgt voor meer kennis over deze stoornissen en over neurobiologische mechanismen die mogelijk ten grondslag liggen aan sociaal disfunctioneren. Deze inzichten kunnen belangrijke aanwijzingen geven voor de ontwikkeling van meer effectieve therapiëën. Voordat we je onder zullen dopmelen in onze bevindingen, zullen we je eerst kort kennis laten maken met de belangrijkste theorie en wetenschappelijke bevindingen waarop ons onderzoek is gebaseerd.

Simulatie theorie

Dit proefschrift neemt je mee naar het onderzoeksgebied van de sociaal cognitieve neurowetenschap. Dit betekent dat we normaal en afwijkend sociaal gedrag proberen te begrijpen vanuit een breinperspectief. Onze speciale aandacht gaat daarbij uit naar hoe we emoties van andere mensen begrijpen. Dit is een belangrijke sociaal cognitieve vaardigheid, dat wil zeggen een vaardigheid die ons in staat stelt ons te handhaven in sociale relaties (Burns, 2006). Een simpel voorbeeld: er komt iemand op je af rennen, maar je weet niet of diegene boos of blij is. Hoe weet je dan of je je armen moet openen om diegene te omarmen of dat je je armen beter kan gebruiken om je gezicht te beschermen? Het begrijpen van de emoties van andere mensen is nodig om op een sociaal gepaste manier te kunnen reageren in de vele sociale rollen die we in het dagelijks leven innemen (bijv. ouder, collega, partner, vriend). Moeilijkheden in het oppikken van emotionele signalen kunnen leiden tot grote problemen in het deelnemen aan het dagelijkse sociale leven (Feldman, Philippot, & Custrini, 1991). Dit zien we bijvoorbeeld bij de stoornissen die in dit proefschrift centraal staan: autisme en schizofrenie (zie kader, Chan, Li, Cheung, & Gong, 2010; Harms, Martin, & Wallace, 2010; Kee, Green, Mintz, & Brekke, 2003; Law Smith, Montagne, Perrett, Gill, & Gallagher, 2010).
Nederlandse samenvatting

Autisme Spectrum Stoornissen (hier afgekort tot autisme)

- Paraplu term voor een groep neuro-ontwikkelingsstoornissen, die zich kenmerkt door stoornissen in sociaal contact en communicatie, en door stereotype gedragingen en interesses.
- Autistische symptomen manifesteren zich gewoonlijk al voor het derde levensjaar.

Schizofrenie

- Een neuro-ontwikkelingsstoornis met een grote verscheidenheid in ernst en symptomen, waaronder “positieve” symptomen zoals onlogische gedachtrapronen, wanen en hallucinaties, en “negatieve” symptomen zoals apathie en sociale terugtrekking.
- Schizofrenie openbaart zich meestal in de adolescentie of vroege volwassenheid.

Psychologische theorieën over hoe we anderen begrijpen hebben van oudsher de nadruk gelegd op theoretische processen. Deze “Theorie” theorieën zeggen dat we bepalen hoe iemand anders zich voelt op basis van weloverwogen, logisch redeneren (Gallese & Goldman, 1998; Goldman & Sripada, 2005). In het geval van de vrouw met het broodje zou je bijvoorbeeld op basis van verschillende waarnemingen (een zieke geur, het vertrokken gezicht van de vrouw, het geluid van haar kokhalzen) en de kennis die je hebt over ondermeer de betekenis van gezichtsuitdrukkingen en de hygiëne in het restaurant, kunnen beredeneren dat deze dame waarschijnlijk walging ervaart. Hoewel redeneren zonder meer belangrijk is voor emotie begrip, bieden dit soort theorieën geen verklaring voor het feit dat mensen vaak intuitief aanvoelen wat de ander doormaakt of het soms zelfs zelf mee lijken te ervaren. Deze processen worden beter verklaard door “Simulatie” theorieën. Deze theorieën gaan er vanuit dat mensen hun eigen mentale systeem gebruiken om het mentale leven van de ander te doorgronden (Goldman & Sripada, 2005; Keysers & Gazzola, 2006). Mensen spiegelen als het ware de ervaringen van een andere persoon (hij/zij doet) in hun eigen eerste persoonsbeleving (ik doe) (Gallese, 2003). De verstrengeling van actie en perceptie is soms duidelijk te zien op gedragsniveau. Het uitvoeren van een bepaalde handeling wordt bijvoorbeeld gemakkelijker wanneer we tegelijkertijd naar een zelfde actie kijken, en wordt juist moeilijker wanneer we tegelijkertijd een tegengestelde actie zien (Brass, Bekkering, Wohlschlager, & Prinz, 2000; Craighero, Bello, Fadiga, & Rizzolatti, 2002; Kilner, Paulignan, & Blakemore, 2003). Probeer maar eens met je wijsvinger op tafel te tikken wanneer degene die tegenover je zit hetzelfde doet en probeer eveneens op tafel te tikken wanneer die ander juist zijn vinger optilt. Je zult zien dat het in het laatste geval lijkt alsof de uitvoering van je eigen beweging stroever gaat. Ook bij emoties zien we die verstrengeling van actie en perceptie terug: als we een lachend gezicht zien, verhogen de spieren die onze eigen mondhoeken doen opkrullen hun activiteitie (Dimberg, Thunberg, & Elmehed, 2000). We bootsen dus als het ware de motoriek van het gezicht dat we zien na (in het Engels: facial mimicry). De directe link tussen jezelf en de ander zou volgens simulatie theorieën als basis kunnen dienen om de ander te begrijpen. De populariteit van simulatie theorieën nam een vlucht door de ontdekking van hersencellen met een hele bijzondere eigenschap: de spiegelneuronen.
Spiegelsysteem

Spiegelleuronen werden in de jaren negentig van de vorige eeuw ontdekt bij makaak apen in een gebied in de ventrale premotorische cortex en gebied in de meer naar achteren gelegen parietale kwab (in figuur 1a zie je de overeenkomstige gebieden bij de mens). Opvallend aan deze hersencellen was dat ze niet alleen vuurden wanneer de aap zelf een object manipuleerde, maar ook wanneer de aap een andere aap of onderzoeker een soortgelijke actie uit zag voeren (voor overzichtsartikelen zie Keysers & Perrett, 2004; Rizzolatti & Craighero, 2004). Spiegelleuronen leggen dus een directe link tussen de handelingen die de aap uitvoert, en de handelingen die de aap anderen ziet doen. Sommige spiegelleuronen, de strikt congruente (ofwel overeenstemmende), zijn daarin heel kieskeurig: ze vuren alleen wanneer de waargenomen actie op dezelfde wijze wordt uitgevoerd. De meerderheid van de spiegelleuronen, de breed congruente, nemen het iets minder nauw en vuren zolang het doel van de actie maar in overeenstemming is. Voor deze neuronen zou het bijvoorbeeld niet uitmaken of de ander een kopje naar de mond brengt met de vingers aan het oortje en de pink in de lucht of dat de ander dit doet met de hand om de kop geklemd, zolang de kop naar de mond gaat om uit gedronken te worden. Met name deze laatste groep spiegelleuronen zou de aap in staat kunnen stellen inzicht te krijgen in de acties van zijn soortgenoten (Gallese, Fadiga, Fogassi, & Rizzolatti, 1996; Kohler et al., 2002; Rizzolatti, Fadiga, Gallese, & Fogassi, 1996; Rizzolatti et al., 2001; Umiltà et al., 2001). Direct na de ontdekking van spiegelleuronen in de makaak aap zijn wetenschappers zich gaan buigen over de vraag of spiegelleuronen ook voorkomen in het menselijk brein. Door middel van beeldvormende technieken is inmiddels aangetoond dat er inderdaad een overlap is in de hersengebieden die actief zijn wanneer wij handelingen uitvoeren en wanneer we soortgelijke handelingen zien of horen bij andere mensen (Buccino et al., 2001; Filimon, Nelson, Hagler, & Sereno, 2007; Gazzola, Rizzolatti, Wicker, & Keysers, 2007;Grèzes, Armony, Rowe, & Passingham, 2003). Recent onderzoeken geven aan dat dit zeer waarschijnlijk ook op celniveau het geval is (Avenanti, Bolognini, Maravita, & Aglioti, 2007; Dinstein, Hasson, Rubin, & Heeger, 2007; Mukamel, Ekstrom, Kaplan, Iacoboni, & Fried, 2010). Actie en perceptie lijken dus daadwerkelijk gekoppeld in het brein. In figuur 1a vind je belangrijke delen van het spiegelsysteem (in het Engels: Mirror Neuron System, afgekort MNS) terug zoals de ventrale premotorische cortex (vPMC: BA6/44) en de inferieure parietale lobule (IPL).

Menselijke emoties worden ook vaak zichtbaar door motorische acties. Denk maar aan onze gezichtsuitdrukkingen. Door voorbijgaande veranderingen in de samentrekkingen van onze gezichtsspieren, zenden we zowel bewust als onbewust allerlei boodschappen uit, die anderen kunnen helpen te begrijpen wat er met ons en onze omgeving aan de hand is. Zoals eerder besproken hebben mensen de neiging de gezichtsuitdrukkingen van de ander na te bootsen; dit naboosten kan de herkenning van de emotie vergemakkelijken, terwijl het blokkeren van je eigen gezichtsbewegingen de herkenning moeilijker kan maken (Niedenthal, 2007; Niedenthal, Brauer, Halberstadt, & Innes-Ker, 2001; Oberman, Winkielman, & Ramachandran, 2007). Belangrijk voor ons onderzoek is dat er niet alleen bij apen spiegelleuronen zijn gevonden die reageren op gezichtsacties (Ferrari, Gallese, Rizzolatti, & Fogassi, 2003), maar dat bij de mens ook bij het zien van andermans emotionele gezichtsuitdrukkingen dat deel van de aPMC actief wordt dat betrokken is bij het bewegen van ons eigen gezicht (Carr, Iacoboni, Dubeau, Mazziotta, & Lenzi, 2003; Hennenlotter et al., 2005; Leslie, Johnson-Frey, & Grafton, 2004; van der Gaag, Minderaa, &
Nederlandse samenvatting

Keysers, 2007; Wicker et al., 2003; Wild, Erb, Eyb, Bartels, & Grodd, 2003). Opvallend is dat activiteit in dit frontale (“vooraangelegen”) deel van het spiegelsysteem samenhangt met hoe sterk een persoon geneigd is mee te leven met een ander (Gazzola, Aziz-Zadeh, & Keysers, 2006; Jabbi, Swart, & Keysers, 2007; Pfeifer, Iacoboni, Mazziotta, & Dapretto, 2008; Saarelä et al., 2007; Schulte-Rüther, Markowitsch, Fink, & Pieffe, 2007). Sterker nog, beschadigingen aan dit gebied kunnen leiden tot beperkingen in empathie en emotie herkenning (Shamay-Tsoory, Aharon-Peretz, & Perry, 2009). Deze studies suggereerden dat het simuleren van de motoriek van gezichtsuitdrukkingen in het spiegelsysteem kan helpen om de ander te begrijpen en met hem of haar mee te leven.

Figuur 1 Anatomische locaties van gebieden die motorische en somatische informatie verwerken

a) Zijaanzicht van een modelvorm van het menselijk brein met de locatie van belangrijke motorische spiegelgebieden: de ventrale premotorische cortex (BA6/BA44) en de inferiure parietale lobule (IPL)
b) Zijaanzicht van een modelvorm van het menselijk brein met de locatie van de primaire (SI) en secundaire (SII) somatosensorische cortex, waar somatische ofwel lichaamsinformatie wordt verwerkt zoals pijnprikkels, informatie vanuit de tastzin, en informatie over de positie en houding van de verschillende lichaamsdelen.

Shared circuits

Als mensen emotionele gezichtsuitdrukkingen van anderen zien, hebben ze niet alleen de neiging die na te bootsen. Zoals we al zagen bij de vrouw met het broodje kan ook de gemoedstoestand van de ander zich naar ons verspreiden (Wild, Erb, & Bartels, 2001). Dit proces wordt emotionele besmetting genoemd (in het Engels: emotional contagion). Op gedragsniveau zien we dat motor simulatie van andermans gezichtsuitdrukkingen en affectieve processen elkaar beïnvloeden. Het aannemen van emotiespecifieke houdingen kan bijvoorbeeld de overeenkomstige emotie opwekken (probeer maar eens de hele maandagochtend te blijven glimlachen en kijk wat dat doet voor je humeur, Ekman, 1992; Strack, Martin, & Stepper, 1988). In hoofdstuk 2 van dit proefschrift lieten we zien dat emotionele signalen van een ander ook in ons brein veel meer oproepen dan een puur theoretische, abstracte interpretatie van zijn of haar geestestoestand. Naast motorische gebieden stelt het zien van andermans emotionele expressies ook affectieve en somatosensorische gebieden (waar o.a. lichaamsinformatie wordt verwerkt) in werking in ons eigen brein. Deze gebieden, die geregeld actief zijn bij zowel emotie perceptie als emotie ervaring, vallen strikt genomen niet onder het (motorische) spiegelsysteem, maar worden “shared circuits” genoemd (Gallese, Keysers,
& Rizzolatti, 2004; Keysers & Gazzola, 2006). Emotie simulatie spreidt zich uit van de vPMC naar somatosensorische en affectieve gebieden zoals de anterieure insula, anterieure cingulate cortex, en amygdala, die allen cruciaal zijn voor emotie herkenning (Adolphs, Damasio, Tranel, Cooper, & Damasio, 2000; Adolphs, Tranel, & Damasio, 2003; Broks et al., 1998; Calder, Keane, Manes, Antoun, & Young, 2000; Sprengelmeyer et al., 1999; Tranel, Gullickson, Koch, & Adolphs, 2006). In figuren 1b en 2 vind je de anatomische locatie van deze gebieden terug. Het is niet zo dat één bepaalde emotie gelynkt is aan één bepaald hersengebied. In plaats daarvan is bij zowel het ervaren als het zien van emoties een heel netwerk van gebieden betrokken. De sterkte van de activatie van die gebieden lijkt daarbij wel samen te hangen met het type emotie en de daarmee geassocieerde output. Signalen van angst verhogen bijvoorbeeld mogelijk activiteit in de amygdala (met name in stressvolle situaties), omdat ze een mogelijke dreiging aangeven en daarom visuele aandacht naar de buitenwereld doen toernen. Lichaamsresponses gemedieerd door de anterieure insula zijn waarschijnlijk belangrijker bij walging, terwijl de anterieure middel cingulate cortex (aMCC) vanwege zijn sterke motorische verbindingen mogelijk bijzonder relevant is voor pijn. De precieze interactie tussen de verschillende simulatie componenten (motorisch, somatosensorisch, affectief) en hoe ze samenhangen met beredeneergebieden is nog onduidelijk. Er zijn waarschijnlijk vele routes naar evenzoveel type emoties, die worden verwerkt in verschillende hersenregio's. We veronderstellen dat één mogelijke route van motorische simulatie in het frontale deel van het spiegelsysteem naar emotie simulatie in affectieve gebieden van het brein loopt (Carr, Iacoboni, Dubeau, Mazziotta, & Lenzi, 2003; Jabbi & Keysers, 2008; Keysers & Gazzola, 2006; Niedenthal, 2007). Deze motorische route vindt steun in hoofdstuk 3 van dit proefschrift.

Figuur 2 Anatomische locaties van hersengebieden met belangrijke affectieve functies

a) Sagitale doorsnede (langs de middenas) van het brein van een onderzoeker met de locatie van de anterieure cingulate cortex (ACC)
b) Coronale doorsnede (van boven naar beneden) van een menselijk brein met de locaties van de insula en amygdala
Het paradigma

Inmiddels heb je vanaf de kant de nodige achtergrondinformatie gekregen: tijd om in het verdere onderzoek te duiken. Want hoe meet je nu eigenlijk “spiegelactiviteit”? Voor de in dit proefschrift beschreven studies hebben we gebruik gemaakt van MRI, *Magnetische Resonantie Imaging* (d.w.z. beeldvorming). Een MRI scanner vind je in elk modern ziekenhuis waar het gebruikt wordt om op hoge resolutie foto’s te maken van structuren in het menselijk lichaam zoals het kniegewricht of de hersenen. Met radiogolven kan het krachtige magneetveld van de scanner gemanipuleerd worden om verschillende soorten informatie te verkrijgen. Belangrijk voor de neurowetenschap is dat een MRI scanner niet alleen gebruikt kan worden voor het visualiseren van hersenstructuren (zie figuur 2), maar ook om hersenprocessen in kaart te brengen (zie pagina’s 65, 74, 122, 124, en 146). *Met functionele MRI* (fMRI) kun je afleiden hoe actief een bepaald gebied is door te kijken naar fluctuaties in het MRI signaal: naarmate een regio actiever is, verandert de verhouding tussen zuurstofrijk en zuurstofarm bloed, wat zorgt voor wijzigingen in het opgepikt signaal. MRI is een goede techniek voor de studie van het menselijk brein, omdat het onschadelijk is voor de deelnemers, een goede spatiële resolutie heeft, en elke 1-3 seconden een beeld kan maken van activiteit in het hele brein. Het is dan nog wel zaak om de juiste taken te kiezen; deze moeten niet alleen de juiste processen meten, maar ook veelvuldig herhaald kunnen worden (vanwege de matige signaal-ruis verhouding), en fysiek haalbaar zijn in de krapte omgeving van de scanner (zie figuur 3b).

Voor de experimentele studie van motorische spiegelmechanismen heb je een conditie nodig waarin proefpersonen gezichtsuitdrukkingen van andere mensen zien én een conditie waarin de proefpersonen zelf gezichtsuitdrukkingen maken. Voor de studie van affectieve spiegelmechanismen of shared circuits heb je naast een conditie waarin proefpersonen de emoties van anderen waarnemen ook een conditie nodig waarin de proefpersonen zelf emoties ervaren. Emotie waarneming kan relatief gemakkelijk worden bestudeerd in de MRI omgeving. Wij hebben ervoor gekozen om daarvoor gebruik te maken van dynamische gezichtsuitdrukkingen (figuur 3a, *hoofdstukken 3,4,7*), terwijl we de oogbewegingen van de proefpersonen volgen met een oogcamera (figuur 3b). Het opwekken van emoties is een meer uitdaginge taak vanwege ethische overwegingen (stel je voor hoe je angst op zou wekken) en de beperkingen die de scanneromgeving oplegt (o.a. beperkte ruimte, bewegingsrestricties). Je zult merken dat wij nogal dol zijn op waalging. Dat komt doordat deze emotie relatief eenvoudig en herhaaldelijk op te roepen is tijdens een experiment zoals in ons geval door het toedienen van onaangename smaken (*hoofdstukken 3,4*) en door gebruik te maken van korte scripts (*hoofdstuk 3*). Figuur 3 geeft je een beeld van de taken die we hebben gebruikt.
**Figuur 3 De experimentele set-up**

![Figuur 3 De experimentele set-up](image)

Het is hartje zomer wanneer je voor één nacht een kamer boekt in een vervallen pension in een afgelegen gebied. Wanneer je op het bed neerploft, zie je een afgetakelde rattenkop meedenen met de beweging van de matras. In een reflex sla je met blote hand het kadaver van de lakens. Je hand schiet naar je mond om het kokhalzen tegen te gaan. Maar wanneer je hand contact maakt met je gezicht, word je nóg misselijk. Je vingers blijven onder een rode smurrie te zitten, bedekt met kroelende maden! Je voelt de plakkerige substantie aan je wang en lippen kleven. Dan voel je hoe de afgrizelijke smaak van bedorven bloed zich in je mond verspreidt....

a) Stilstaand beeld van een filmpje met een walgend gezicht  
b) MRI scanner met aan het voeteneinde de oogcamera. Tijdens de smaaktaak staat de proefleider naast het bed van de scanner om de smaken toe te dienen.  
c) De smaakconstructie: de smaakjes worden door middel van spuitjes aan het eind van het scannerbed in twee meterlange buisjes gespoten, die via een speentje in de mond van de proefpersoon uitkomen.  
d) Ingekort voorbeeld van een script dat we gebruikt hebben om walging op te roepen
Vieze verhaaltjes

Voor de studie in hoofdstuk 3 hebben we een groep studenten gescand, terwijl zij emoties ervaren, iemand anders emoties zien ervaren, en zich inbeelden smaakgerelateerde emoties te ervaren die werden “getriggered” (d.w.z. opgewekt) door scripts (zie figuur 3). In dit hoofdstuk hebben we laten zien dat sociale perceptie, mentale inbeiding én persoonlijke ervaringen van walging vaak de anterieure insula en het frontale operculum (afgekort: IFO) activeren, wat de vermeende rol van deze structuur in de belichaming van gemoedstoestanden bevestigt. De betrokkenheid van de IFO in de inbeeldingsconditie kan helpen verklaren waarom het lezen van boeken en het inbeelden van ervaringen ervoor kunnen zorgen dat je levendige emoties ervaart (misschien wekte het lezen van de zinnen in figuur 3d ook wel wat weerzin bij jou op). Deze bevinding is veelbelovend voor het gebruik van script-gestuurde inbeiding als een makkelijke en comfortabele manier voor het opwekken van emoties in experimentele settings. Meest belangrijk: de effectieve connectiviteitsanalyse toonde aan dat, hoewel de IFO gemeenschappelijk wordt betrokken bij het waarnemen, voelen en zelfs inbeelden van walging, de betrokken netwerken heel verschillend zijn. Tijdens het ervaren van walging, is de IFO ingebouwd in een netwerk bestaande uit gebieden die betrokken zijn bij lichaamssensaties, smaakbeleving, motivatie en het uitvoeren van bewegingen. Tijdens het zien van walging, ontvangt de IFO haar sterkste input van de rechter inferieure frontale gyrus (BA45). Deze bevinding ondersteunt het in hoofdstuk 2 gepresenteerde idee dat motor simulatie van gelaatsuitdrukkingen in het frontale deel van het spiegelsysteem emotie simulatie in de anterieure insula kan “triggeren”. Tijdens script-gestuurde mentale inbeelding, is de IFO ingebouwd in een netwerk van taalgeworden en gebieden die betrokken zijn bij het geheugen van betekenenissen, wat wijst op een meer cognitieve route naar emotie simulatie. De verschillen in de netwerken waarin shared circuits zijn betrokken kan een belangrijke rol spelen bij het onderscheid tussen jezelf en de ander tijdens sociale interacties en in de verschillende fenomenologie (d.w.z. ervaring) van het zien, voelen, en inbeelden van een emotionele gebeurtenis.

Spiegeltje, spiegeltje aan de wand

Vanwege de vermeende rol van het spiegelsysteem in emotie herkenning, empathie en andere sociale vaardigheden zoals het imiteren van anderen (Iacoboni et al., 1999) en het uitvoeren van gezamenlijke acties (Kokal, Gazzola, & Keysers, 2009), hebben verschillende onderzoekers geopperd dat een disfunctioneel spiegelsysteem de kern vormt van autisme (Iacoboni & Dapretto, 2006; Oberman & Ramachandran, 2007; Rizzolatti & Fabbri-Destro, 2008; Rizzolatti, Fabbri-Destro, & Cattaneo, Williams, Witter, Suddendorf & Perrett, 2001). Eerder onderzoek uit de Verenigde Staten (Dapretto et al., 2006) heeft aangetoond dat kinderen met autisme die keken naar andermans emotionele gezichtsuitdrukkingen de inferieure frontale gyrus (BA44) van het spiegelsysteem - in tegenstelling tot hun niet autistische leeftijdgenoten - niet activeerden. In hoofdstuk 4 zoomden we in op deze structuur en lieten we zien dat bij jongvolwassenen met autisme dit gebied inderdaad nog altijd verminderd actief is wanneer ze naar videoclips van gezichtsuitdrukkingen kijken. De activiteit neemt in onze groep echter toe met leeftijd en lijkt rond de 30 jaar op een normaal niveau te zitten. Opvallend is dat deze toename samenhangt met een verbetering in het sociaal functioneren en veranderingen in kijkgedrag. Deze bevindingen komen overeen met de
indruk van clinici dat er in de adolescentie en volwassenheid verbeteringen in sociaal functioneren en een toename in responsiviteit op andermans leed plaats kunnen vinden bij mensen met autisme. Mogelijk vinden mensen met autisme op latere leeftijd -ondanks blijvende autistische symptomen- manieren die hen helpen meer deel te nemen aan het sociale leven. (Farley et al., 2009; McGovern & Sigman, 2005; Piven, Harper, Palmer, & Arndt, 1996; Seltzer et al., 2003; Shattuck et al., 2007). We hopen dat deze resultaten de eerste aanzet zullen vormen voor onderzoek naar deze mogelijkheid, waarbij het van belang zal zijn dezelfde mensen met autisme in verschillende levensfases te meten. Onze hoop is dat kennisvermeerdering over de plasticiteit (“kneedbaarheid”) van het spiegelsysteem en leeftijds effecten in autisme zal leiden tot de ontwikkeling van therapiëén die in zouden kunnen grijpen op het spiegelsysteem. Hierbij kan gedacht worden aan de ontwikkeling van nieuwe interventies gericht op emotie simulatie. Gezien de plasticiteit van het spiegelsysteem en zijn verbindingen met visuele gebieden, zouden vroege imitatie training van gezichtsuitdrukkingen en expliciete training om kijkgedrag te veranderen kunnen baten (Calvo-Merino, Glaser, Grezes, Passingham, & Haggard, 2005; Catmur, Walsh, & Heyes, 2007; Cross, Hamilton, & Grafton, 2006; Haslinger et al., 2005; Lahav, Saltzman, & Schlag, 2007; Wright et al., 2008). Deze trainings effecten zouden mogelijk versterkt kunnen worden door de inferieure frontale gyrus van kinderen die afwijkende activiteit laten zien te stimuleren met technieken als Transcranial Magnetic Stimulation (TMS) en transcranial Direct Current Stimulation (tDCS). Hoewel deze technieken veelbelovend zijn (Fregni & Pascual-Leone, 2007), zal er eerst nog veel onderzoek gedaan moeten worden naar de precieze positieve en negatieve effecten voordat ze in de klinische praktijk kunnen worden ingezet.

Kapotte spiegels?

De spiegelneuronen hypothese van autisme is gebaseerd op de idee dat een diep doordringende storing in het spiegelsysteem bij mensen met autisme kan leiden tot een cascade aan beperkingen, van meer basale processen als imitatie tot meer complexe sociale vaardigheden als het begrijpen van andermans gemoedstoestand (Iacoboni & Dapretto, 2006; Oberman & Ramachandran, 2007; Rizzolatti & Fabbri-Destro, 2008; Rizzolatti, Fabbri-Destro, & Cattaneo, 2009; Williams, Witter, Suddendorf & Perrett, 2001). Hoewel activiteit in het spiegelsysteem samen lijkt te hangen met sociaal functioneren in autisme, laten we in hoofdstuk 5 zien dat de rol van het spiegelsysteem in autisme niet zo helder noch eenvoudig is. Hoewel er in de afgelopen vijf jaar veel studies zijn uitgevoerd in dit domein, zijn ze over het algemeen namelijk met zeer inconsistente resultaten gekomen. Eén belangrijke verklaring voor deze tegenstrijdigheden is dat de betrokkenheid van het spiegelsysteem afhangt van verschillende factoren die we nu pas beginnen te ontdekken (bijv. de aard van de gebruikte stimuli, de mate van identificatie met de acteur, heterogeniteit in de autisme groepen binnen en tussen de studies, en zoals we zagen in het vorige hoofdstuk: de leeftijd van de deelnemers). Problematisch voor de spiegeltorie van autisme is daarnaast dat mensen met autisme geen grote moeilijkheden laten zien in één van de vermeende basale functies van het spiegelsysteem: het imiteren van het doel van andermans acties. De huidige literatuur over de aard van de imitatie problemen zou er wel op kunnen wijzen dat in autisme de breed congruente spiegelneuronen voldoende functioneren om het doel van een actie te spiegelen, maar dat de strikt congruente spiegelneuronen (vroeg in de ontwikkeling) niet goed genoeg werken om de meer
kwetsbare reproductie van de exacte stijl tot stand te brengen. Deze mogelijkheid is echter nog niet specifiek onderzocht. Het feit dat in autisme de meer basale mechanismen die zouden leunen op het spiegelsysteem relatief intact lijken te zijn, maakt het onwaarschijnlijk dat verstoringen in meer complexe sociale processen kunnen worden teruggevoerd op elementaire afwijkingen in de werking van het spiegelsysteem. Bij complexere processen zoals het begrijpen van emoties en andere mentale toestanden van anderen ("mentalizing") zijn ook andere structuren buiten het spiegelsysteem betrokken. Deze gebieden, die soms aangeduid worden als het "redeneersysteem", lijken (ook) te worden aangetast in autisme (Amodio & Frith, 2006; Gallagher & Frith, 2003; Saxe, 2006; Castelli, Frith, Happe & Frith, 2002; Happe et al., 1996). De oorzaak van autisme is dus niet simpelweg terug te brengen tot het hebben van kapotte "spiegels."

**Autisme en schizofrenie: spiegelbeelden?**

Autisme en schizofrenie hebben allebei unieke kenmerken en hun eigen ontwikkeling, maar ernstige moeilijkheden in het sociaal functioneren bestaan in beide (DSM-IV-TR, 2000; Frith, 2003; Goldstein, Minshew, Allen & Seaton, 2002; Kanner, 1943). In *hoofdstuk 6* lieten we zien dat wanneer positieve symptomen in remissie zijn en negatieve symptomen meer op de voorgrond treden, het sociale gedrag van individuen met schizofrenie kan lijken op autistische symptomen (zie kader voor meer details over het gebruikte instrument, de ADOS). In dit proefschrift lieten we zien dat niet alleen het sociale gedrag, maar ook de mate van sociale disfunctie, sociale teruggetrokkenheid, en sociale cognitieve problematiek van individuen met autisme en schizofrenie op elkaar kunnen lijken (hoofdstuk 4 & 7). Directe vergelijkingen van deze groepen met overeenkomsten in hun sociale profielen, wijzen op meer verschillen dan overeenkomsten in de onderliggende neurale profielen. De eerder vermelde leefomgevingen neurocognitieve verbetering in de groep met autisme werd, bijvoorbeeld, niet gevonden in de groep met schizofrenie (hoofdstuk 4). Daarnaast lieten we in hoofdstuk 7 zien dat het algemene niveau van hersenactiviteit tijdens emotie waarneming zeer verschillend is in de patiëntgroepen met bovenmatige of hyperactiviteit in de autisme groep en een tendens naar hypoactiviteit in de schizofrenie groep. Daarnaast leken alleen de mensen met schizofrenie relatief ongevoelig te zijn voor positieve sociale stimuli. Beide groepen beoordeelden de gezichten echter meer negatief en toonden relatief meer activiteit bij het zien van negatieve sociale stimuli in visuele en somatosensorische gebieden (hoofdstuk 7). Deze verhoogde gevoeligheid voor negatieve emoties van andere mensen hangt mogelijk samen met de verhoogde niveaus van persoonlijk ongemak ("distress") die beide groepen rapporteren wanneer ze geconfronteerd worden met andermans ellende. Dit zou één van de gemeenschappelijke factoren kunnen zijn die leidt tot sociale terugtrekking. Deze interessante hypothese behoeft verder onderzoek.

**Laatste bespiegelingen...**

Het is belangrijk om op te merken dat we in dit proefschrift gekeken hebben naar subgroepen van autisme en schizofrenie die gemeenschappelijke gedragskenmerken hebben. Dit hebben we opzettelijk gedaan om zo neurobiologische verschillen en overeenkomstigheden te kunnen duiden. Tegelijkertijd maakt deze selectie het lastig om de resultaten te generaliseren naar bijvoorbeeld
De Autism Diagnostic Observation Schedule (ADOS, Lord et al., 2000) is een gestandaardiseerd gedragsobservatie instrument, dat veel wordt gebruikt in zowel klinische als onderzoeksinstellingen om de aanwezigheid van autisme te beoordelen. De ADOS is uitgebreid gevalideerd bij kinderen, maar nog niet bij adolescenten en volwassenen (module 4). In hoofdstuk 6 onderzochten we de psychometrische eigenschappen van module 4 in vier (relatief kleine) groepen volwassenen: autisme en schizofrenie, maar ook psychopathie en een controlegroep.

De belangrijkste resultaten:
- betrouwbaar instrument met een goede voorspellende waarde voor autisme
- autisme wordt goed onderscheiden van de controle en de psychopathie groep
- autisme en schizofrenie (gekenmerkt door negatieve symptomen) zijn lastiger te onderscheiden door overlappende gedragskenmerken
- het herziene algoritme (Gotham et al., 2007) kan dit onderscheid mogelijk beter maken
- hoge specificiteit: een groot deel van de niet-autistische personen werd correct als “non-spectrum” geclassificeerd
- matige sensitiviteit: een aanzienlijk deel van onze hoog-functionerende autisme groep werd niet als autistisch geclassificeerd (hierbij spelen compensatie strategieën mogelijk een rol)

Sensitiviteit en specificiteit hangen sterk (negatief) samen, waarbij het doel van de afname bepaalt wat het meest belangrijk is. Hoge specificiteit van module 4 is met name belangrijk voor onderzoeksdoeleinden, omdat onderzoekers er zeker van willen zijn dat hun autisme groep uitsluitend personen met autisme omvat. Wanneer het doel van de afname is om te screenen op autisme, is hoge sensitiviteit van cruciaal belang om te voorkomen dat eventuele gevallen worden gemist. In dit geval, zouden lagere drempelwaarden overwogen kunnen worden.

| zwakbegaafde mensen met autisme, mensen met schizofrenie met psychotische kenmerken, en de vrouwelijke populatie. Deze groepen verdienen daarom bijzondere aandacht in vervolgonderzoek. |
| Zoals een vrachtwagenchauffeur altijd goed om zich heen moet blijven kijken, geldt dat ook voor wetenschappers. Als we bijvoorbeeld alleen maar zouden blijven kijken naar het spiegelsysteem zou dat gevaarlijke situaties op kunnen leveren. Andere belangrijke processen zouden dan namelijk in de dode hoek verdwijnen. Toekomstige studies kunnen bijvoorbeeld niet voorbijkomen aan de meer theoretische processen die gelinkt zijn aan het “redeneersysteem”. Recent onderzoek toont aan dat het redeneersysteem en het spiegelsysteem elkaar beïnvloeden en even belangrijk zijn om tot een compleet begrip te komen van de gemoedstoestand van een andere persoon (Cheng et al., 2007; Schippers et al., 2010; Simon et al., 2006; Singer et al., 2006; Zaki et al., 2007). Daarbij ervaren zowel mensen met autisme en schizofrenie vaak problemen op taken die het nadenken over de gemoedstoestand van anderen vergen. Het is dus tijd om in het onderzoek een koppeling te gaan maken tussen deze systemen. In de komende jaren zal de autisme lijn van het Social Brain Lab waar de hier beschreven studies zijn uitgevoerd verder voortgezet worden door mijn collega’s Marc Thioux en Leonardo Cerlani. Zij zullen zich voornamelijk gaan richten op die mogelijk verstoorde interactie tussen het “redeneersysteem” en het “spiegelsysteem” en op de structuren die hersengebieden met elkaar verbinden. |
| Wat ook niet uit het oog verloren mag worden is de rol van kijkgedrag op de rijping én het functioneren van verschillende hersengebieden. Bij autisme zijn bijvoorbeeld al op jonge leeftijd |
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afwijkingen gevonden in de manier van oriënteren op sociale informatie. Dit zal waarschijnlijk niet alleen gevolgen hebben voor de rijping van visuele gebieden, maar ook zijn impact hebben op gebieden die hiermee in verbinding staan, zoals de hersengebieden die onder het spiegelsysteem worden geschaard. Het in kaart brengen van de vroege ontwikkeling van sociale vaardigheden is ook van belang bij schizofrenie. Ook al manifesteert de stoornis zich gewoonlijk pas in de vroege volwassenheid, zijn er namelijk voor die tijd ook al sociale en neurobiologische afwijkingen te vinden. Een vergelijkende aanpak van autisme en schizofrenie met een ontwikkelingsperspectief zou heel vruchtbaar kunnen zijn om meer gedetailleerde kennis te vergaren, die bij zou kunnen dragen aan het ontwikkelen van meer op maat gesneden therapieën. Hierbij zal het niet alleen van belang zijn gelijktijdig de vroege sociale cognitieve ontwikkeling van autisme en schizofrenie in kaart te brengen, maar ook latere levensfasen, omdat effectieve interventies juist missen voor volwassenen met autisme en mensen met schizofrenie en negatieve symptomen. We hopen dat dit proefschrift naast een bescheiden bijdrage aan de ideevorming rond nieuwe therapieën een bijdrage levert aan de wetenschap door het oproepen van nieuwe vragen.
Dankwoord

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Dankwoord

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Dankwoord

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