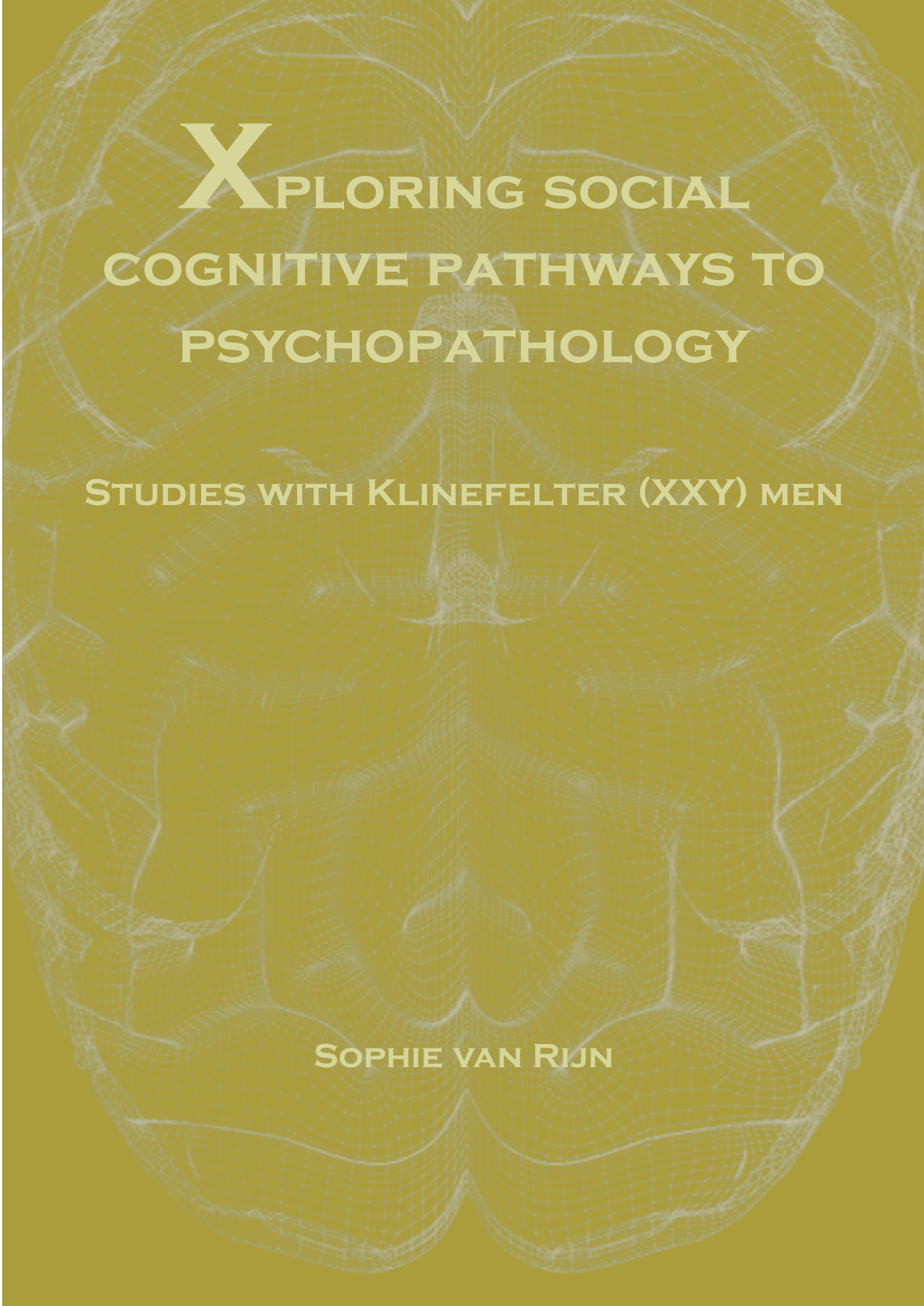

XPLORING SOCIAL COGNITIVE PATHWAYS TO PSYCHOPATHOLOGY

Studies with Klinefelter (XXY) men

Sophie van Rijn

XPLORING SOCIAL COGNITIVE PATHWAYS TO PSYCHOPATHOLOGY

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Een exploratie van sociaal-cognitieve routes naar psychopathologie:
studies met Klinefelter (XXY) mannen
(met een samenvatting in het Nederlands)

Proefschrift

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‘All that we see or seem is but a dream within a dream’

Edgar Allan Poe (Dream within in a dream)

Voor mijn ouders

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Chapter 1

General introduction

Social cognition

Humans are equipped with affective and cognitive capacities that allow them to deal with a complex and dynamic social world. Although social signals can be processed automatically and quickly, in many situations the social significance of expressions, actions, and experiences is not fixed, and depends upon cognitive computations to extract meaning. As a consequence, social skills largely rely on cognitive capacities that are needed to make sense of the incoming socially relevant information (Pinkham et al., 2006).

The cognitive skills required to adapt to our social environment are encompassed in the term social cognition, which has been defined as 'all cognitive processes underlying interaction with conspecifics' or 'the ability to perceive, process en appropriately respond to social signals'. Examples of socio-emotional processes that are included in this 'umbrella' term are perception of faces, decoding affective expressions in faces, decoding social signals from voices and body postures, attribution of mental states (believes, desires and intentions) to others, empathy and regulation of emotions.

Social cognitive capacities appear to be relatively independent from other aspects of intelligence, such as memory or planning. Support for this idea comes from observations of selective impairments in social behavior together with normal general intelligence as is seen after damage to the frontal cortex in the brain (Anderson et al., 1999; Fine et al., 2001). In turn, individuals with William's syndrome are characterized by below-normal intelligence, but are described as 'hypersocial' (Jones et al., 2000). Dissociations between social cognitive abilities and general cognitive capacities have led to the idea that these functions can be dissociated at the neural level and that specific regions devoted to social cognition exist (Adolphs, 2001).

The neural basis of social cognition

Social cognitive capacities rely on neural networks in the brain that include regions specifically dedicated to processing social information as well as regions that are generally involved in complex perceptual or cognitive computations. A body of research has pointed to a set of strongly interconnected key areas in the brain, which are tuned to processing socio-emotional information. The amygdala seems to play a central role as indicated by the high density of incoming and outgoing projections to other brain regions. The amygdala is especially known for its automatic engagement in screening information for emotional and social significance, especially threat-related information (Amaral, 2003; Phelps, 2006). As emotional expressions on faces provide a crucial source

of information needed for decoding social and emotional signals, the amygdala generally activates in response to facial expressions (Adolphs, 2001; Haxby et al., 2002; Phan et al., 2002). A region within the fusiform gyrus, the 'fusiform face area', seems to be specifically tuned to faces. This area appears important for processing the structural, static properties of faces, which are used to determine personal identity (Adolphs, 2001; Haxby et al., 2000). Another region is the insula, which is important for monitoring and organizing physiological (autonomic) changes in the internal milieu, as is seen in reponse to emotion inducing stimuli (Damasio et al., 2000). It is involved in mediating affective responses to emotional incoming information (Adolphs, 2002; Phillips et al., 2003). It is shown that the superior temporal sulcus (STS) is implicated in processing socially salient 'motion' information, such as gaze direction, goal-directed movements and biological motion. It contributes to the detection of other people's goals and intentions and is involved in mentalizing (Frith et al., 1999; Pelphrey et al., 2006; Zilbovicius et al., 2006). The medial prefrontal cortex also plays a role in the detection of intentions as it seems active during mentalizing; that is, attributing mental states, goals and believes to others (Adolphs, 2001; Ochsner, 2004). This region, especially the ventromedial part, also appears to be important for regulation of affective states and behavior (Phillips et al., 2003). Another region that is important for regulation of social behavior is the orbital frontal cortex, which is involved in representation of reward value and ways in which this representation guides social behavior (Phillips et al., 2003). The ventral part of the anterior cingulate gyrus plays a role in emotional and social behavior by integrating sensory, motivational and cognitive information (Bush et al., 2000). This region has been associated with response selection, decision making and volitional behavior.

In sum, a network of brain regions prominently involved in social cognition includes the amygdala, fusiform face area, insula, superior temporal sulcus, medial prefrontal cortex, orbital frontal cortex and anterior cingulate.

Genetic factors in social cognition

Individual variance in social cognitive competence is for a substantial part attributable to genetic factors, as indicated by twin studies. For social cognitive skills, a heritability of 68% has been reported, with shared environment accounting for only a minor part of the variance (5%) (Scourfield et al., 1999). In line with this study, an estimated 60% of the individual variation in understanding the minds, i.e. believes, intentions and goals, of other individuals, seems to be due to genetic factors (Hughes et al., 1999). Shared environment

accounted for only 7% in that study. There is also evidence that social reciprocal behavior is highly heritable in the general population (Constantino et al., 2003a; Constantino et al., 2000). For monozygotic twin boys (who share 100 % of their genes) an 'inter-twin' correlation of 0.73 has been observed for impaired social reciprocal behavior. For dizygotic twin boys (who share on average 50 % of their genes), the intertwin correlation was 0.37.

Socially deviant behavior

Social cognitive competence appears to be a good predictor of social behavior and adaptation. Severe difficulties in social adaptation, accompanied by a detachment from reality and preoccupation with inner thoughts and feelings, have been described for individuals with an autism spectrum disorder or schizophrenia (Bleuler, 1911; Kanner, 1943). For the diagnostic criteria of schizophrenia and autism according to the DSM-IV, the Diagnostic and Statistical Manual of Mental Disorders -fourth edition (1994), see box I and II. Autism spectrum disorders and schizophrenia are neurodevelopmental disorders with a considerable genetic component, as estimated heritability is around 80% for schizophrenia (Gottesman, 1991) and 90% for autism (Santangelo et al., 2005). Both autism and schizophrenia are more prevalent in men. A meta-analysis has reported a risk ratio of 1.42 for men to develop schizophrenia relative to women (Aleman et al., 2003), while autism spectrum disorders are diagnosed approximately four times more often in boys than in girls (Volkmar et al., 1993).

Autism spectrum disorders and schizophrenia share some characteristics, such as clinical phenomena pertaining to affect, communication and social insight (Abdi et al., 2004; Frith, 1992; Goldstein et al., 2002; Konstantareas et al., 2001; Rumsey et al., 1986). Note that clinical criteria (box II.F.) state that 'if there is a history of autistic disorder or another pervasive developmental disorder, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least a month'. Although they are distinct disorders, with the onset of autistic disorders in childhood while schizophrenia is typically diagnosed in late adolescence/early adulthood (DeLisi, 1992; Minshew, 1996), autism spectrum disorders and schizophrenia share social cognitive dysfunctions, including aspects of language and emotion (Abdi et al., 2004; Frith, 1992; Pilowsky et al., 2000; Rumsey et al., 1986).

Box I.

DSM-IV criteria for autism

Autistic disorder

I A total of six (or more) items from (A), (B), and (C), with at least two from (A), and one each from (B) and (C)

- **A.** qualitative impairment in social interaction, as manifested by at least two of the following:
  1. marked impairments in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body posture, and gestures to regulate social interaction
  2. failure to develop peer relationships appropriate to developmental level
  3. a lack of spontaneous seeking to share enjoyment, interests, or achievements with other people, (e.g., by a lack of showing, bringing, or pointing out objects of interest to other people)
  4. lack of social or emotional reciprocity (note: in the description, it gives the following as examples: not actively participating in simple social play or games, preferring solitary activities, or involving others in activities only as tools or "mechanical" aids)
- **B.** qualitative impairments in communication as manifested by at least one of the following:
  1. delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime)
  2. in individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others
  3. stereotyped and repetitive use of language or idiosyncratic language
  4. lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level
- **C.** restricted repetitive and stereotyped patterns of behavior, interests and activities, as manifested by at least two of the following:
  1. encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus
  2. apparently inflexible adherence to specific, nonfunctional routines or rituals
  3. stereotyped and repetitive motor mannerisms (e.g. hand or finger flapping or twisting, or complex whole-body movements)
  4. persistent preoccupation with parts of objects

II Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years:

- **A.** social interaction
- **B.** language as used in social communication
- **C.** symbolic or imaginative play

III The disturbance is not better accounted for by Rett's Disorder or Childhood Disintegrative Disorder

Box II.

DSM-IV criteria for schizophrenia

Schizophrenia

A. Characteristic symptoms: Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated):

1. delusions
2. hallucinations
3. disorganized speech (e.g., frequent derailment or incoherence)
4. grossly disorganized or catatonic behavior
5. negative symptoms, i.e., affective flattening, alogia, or avolition

B. Social/occupational dysfunction: For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning, such as work, interpersonal relations, or self-care, are markedly below the level achieved before the onset. (Or when the onset is in childhood or adolescence, failure to achieve expected level of interpersonal, academic, or occupational achievement).

C. Duration: Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or two or more symptoms listed in criterion A present in an attenuated form (i.e., odd beliefs, unusual perceptual experiences).

D. Schizoaffective and mood disorder exclusion: Schizoaffective disorder and mood disorder with psychotic features have been ruled out because either 1) no major depressive, manic, or mixed episodes have occurred concurrently with the active-phase symptoms; or 2) if mood episodes have occurred during active-phase symptoms, their total duration has been brief relative to the duration of the active and residual periods.

E. Substance/general medical condition exclusion: The disturbance is not due to the direct physiologic effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition.

F. Relationship to a pervasive developmental disorder: If there is a history of autistic disorder or another pervasive developmental disorder, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least a month (or less if successfully treated).

Schizophrenia

Among the clinical manifestations of schizophrenia, significant impairments in social functioning have been consistently reported. Social isolation, impairments in social competence (Penn et al., 1996), deterioration in interpersonal close relationships (Poole et al., 2000), communication-deficits and interpersonal oddity (Hooker et al., 2002) are recognized as characteristics frequently displayed by patients suffering from schizophrenia. Social cognitive impairments have been widely described for these patients, such as impairments in gaze-interpretation, reading of affective facial expressions, picking up emotional signals in tone of voice and the ability to infer and interpret intentions, knowledge and believes of others, as measured in theory-of-mind (mentalizing) tasks (Corcoran et al., 1995; Corrigan et al., 2001; Doody et al., 1998; Edwards et al., 2002; Mazza et al., 2001; Penn et al., 1997; Sarfati et al., 1997).

Deficits in social perception appear to play an important role in social dysfunctioning of schizophrenia patients. General cognitive skills seem to explain between 20% to 60% of the variance in social outcome (Green et al., 2000). It has been suggested that social cognitive performance may help to, partly, explain the remaining 40% to 80%. Indeed, it has been shown that social cognitive capacities can explain significantly more variance (26 %) in social functioning in these patients as compared to general cognitive abilities (15 %) (Pinkham et al., 2006), which is comparable to a report of mentalizing abilities explaining 27 % of the variance in social behavioral problems in schizophrenia patients (Brune, 2003). In line with this, another study revealed that the ability to mentalize (realize that others may have different thoughts, feelings or goals) is among the best cognitive predictors of global social functioning in schizophrenia patients (Roncone et al., 2002).

Autism spectrum

Disabilities in the social domain are considered as the primary symptoms in the autism spectrum (Fein et al., 1986). A triad of impairments is characteristic of this spectrum: atypical development in reciprocal social interactions, atypical communication, and restricted, stereotyped and repetitive behaviors.

The impairments in social interactions can for example take form of difficulties in forming friendships, a lack of social motivation, misinterpretation in communicative intent of others and difficulties in understanding socio-emotional signs and social nuances (Wing et al., 1979). Research devoted to identifying the social cognitive deficits that may contribute to social dysfunction

in the autism spectrum, has revealed abnormalities in eye-gaze processing, interpreting language in social contexts, mentalizing ('theory of mind') abilities and identifying social signals from faces, voices and body postures (Buitelaar et al., 1999; Klin et al., 2002; Ozonoff et al., 1996; Rutherford et al., 2002; Sasson, 2006; Tager-Flusberg, 1999).

The importance of social cognitive capacities in coping with the social world and related mental well-being calls for a search into the origins of social cognition on the level of cognition, neurobiology and genes. Although it is widely acknowledged that there is a genetic basis to the neural and cognitive abnormalities seen in schizophrenia and autism, defining the underlying genetic factors directing these aberrations appears to be a difficult task (Norton et al., 2006).

Factors that complicate the search for genetic origins of socially deviant behavior

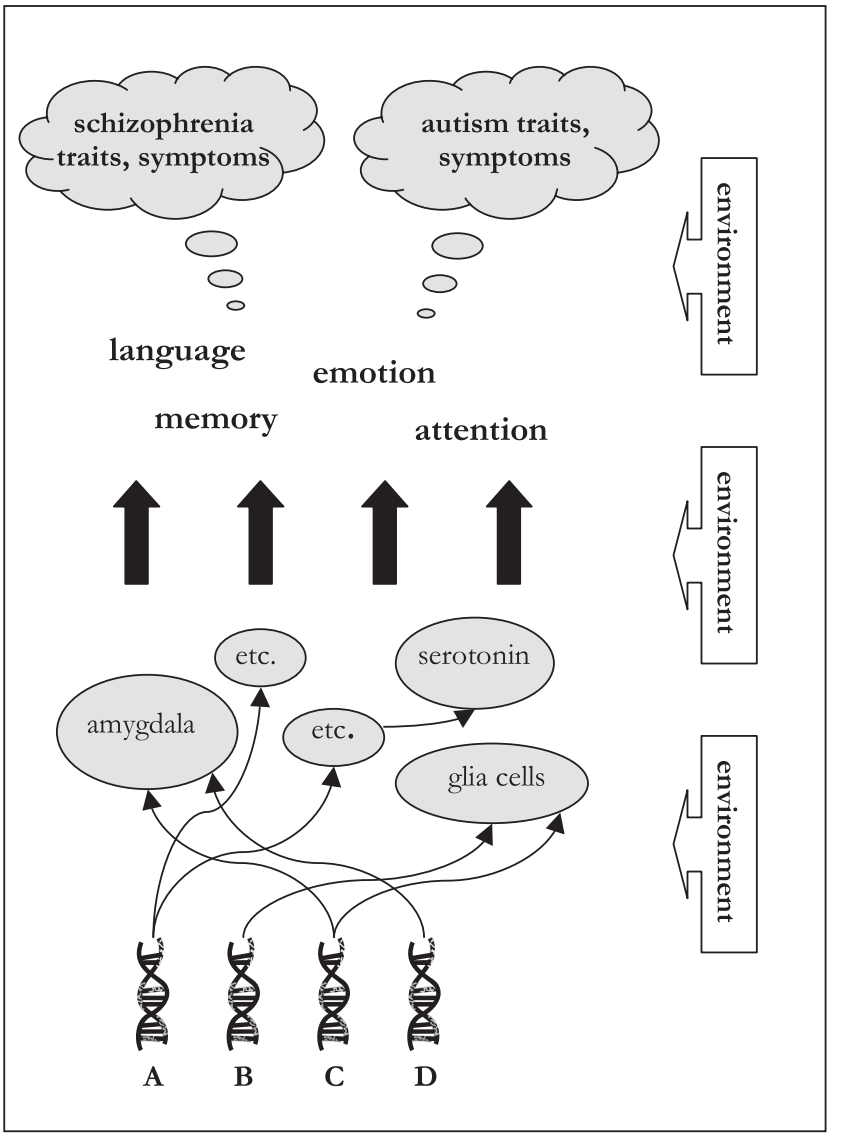
1. Genetic mechanisms are complex

The relations between genes and final phenotypes in behavior are complex. Many forces determine the unique phenotypic expression of an individual's genetic make-up. Genes can have dynamic interactions with environmental factors. These environmental influences not only encompass things as life events, nutrition, aging, and chance, but also endogenous factors such as genes that regulate expression of other genes (epigenetics). Moreover, genes may predispose an individual to be exposed to certain environmental influences, i.e. genotype-environment correlations are present (for a review on gene-environment interactions see (Gottesman et al., 2005). Another reason why the relation between genes and behavior is complex, is that gene expression is dynamic over time. Genes can be turned on and off or can take on different roles during various stages of development (Gottesman et al., 2005).

It is a long road from genotype to final phenotype and many intermediary mechanisms are involved, which are referred to as endophenotypes (see figure 1) (Gottesman et al., 2003). For a behavioral phenotype these intermediary endophenotypes can for example be neurobiological (brain structure) or cognitive (brain function) in nature. The closer a (endo-) phenotype to the genotype is, the less 'noise' from interaction with environmental factors. In case of the brain, these also include biochemical interactions and dynamic

interactions between circuits of cells. Because of this complexity, it has been a difficult challenge to identify gene-brain-behavior pathways leading to social adaptation. In the field of abnormal and non-adaptive behavior in the social domain, such as is seen in psychiatric disorders like autism and schizophrenia, identification of etiological pathways to psychopathology has been hampered by the distant relation between clinical features (the 'molar' level) and genetic abnormalities (the 'molecular' level) together with a lack of knowledge of intermediary mechanisms in the genotype-phenotype pathway (Bearden et al., 2004; Gottesman et al., 2003).

Figure 1
Traits and symptoms from the schizophrenia or autism spectrum may arise from an interplay among various genetic and environmental (including epigenetic) forces, that interactively affect brain development and associated cognitive capacities along the genotype-phenotype pathway. Adversities in brain development or cognitive abilities underlying social behavior are considered as endophenotypes (vulnerability markers) that may be shared by autism and schizophrenia.



2. Behavioral phenotypes are heterogeneous

Genotype-environment interactions allow maximum adaptability to our environment and lead to a heterogeneity in behavioral phenotypes. As a result, behavioral phenotypes may be limited in their use for identifying etiological pathways, including biological and genetic underpinnings, to psychopathology (Gottesman et al., 2003) Despite the presence of standardized criteria (DSM-IV) allowing reliable diagnostic classification of mental disorders based on observed behavior, self-reported symptoms and course, disorders such as autism and schizophrenia are heterogeneous clinical phenomena. (Bearden et al., 2004; Caspi et al., 2006; Jablensky, 2006). Variation in phenotypic expression across development, i.e. temporal inconsistency, as well as individual differences in exposure to medication or other treatments, may result in additional heterogeneity in clinical populations (Jablensky, 2006).

Not only variance at the behavioral or clinical level, but also variance on a genetic level is implicated in the phenotypic variation of brain disorders such as autism or schizophrenia. It is thought that these disorders have a polygenetic origin, i.e. many genes are involved rather than one single gene (Jablensky, 2006; Risch et al., 1999).

The continuum approach

The select set of genes that we carry can direct the development of very complex phenotypes such as is seen in brain structure, brain function and behavior, because of a 'pleiotropic' design; many genes, in different combinations, affect a phenotype. Together with a variety of environmental forces, a spectrum of variation in brain structure, -function and behavior is produced in the general population. These distributions tend to take form of a bell curve, with the majority of individuals represented in the small range around the mean and only a small part of the population in the extremes. Indeed, plots of social competence in the general population appear to be bell curved distributions (Constantino et al., 2003a; Constantino et al., 2000).

Individuals with severe impairments in the social domain, as is seen in autism and schizophrenia, are found at the far end of the spectrum of social abilities. Because of the many factors (genetic or environmental) involved in development of the disorders, variation in phenotypic expression is likely to be distributed along a spectrum. Only in the case of a single factor, such as a single gene, one would expect the distribution to be truly dichotomous giving rise to all-or-none phenomena (Johns et al., 2001).

Phenomena that are part of the clinical phenotype of autism or schizophrenia may be quantitatively distributed along a continuum, rather than being all-or-none, dichotomous disease entities (Johns et al., 2001; Krabbendam et al., 2004). In this view, a clinical definition of autism or schizophrenia may represent only a small part of the total phenotypic continuum, which is not necessarily clinical in nature.

It has been proposed that autism is a 'social brain disorder' that reflects the extreme of a bell-shaped distribution of variation in autism traits, including social competence, in the population (Baron-Cohen et al., 2001; Constantino et al., 2003b; Spiker et al., 2002). Considering the male disadvantage in social cognitive abilities in the general population, the 'extreme male brain theory of autism' postulates that autism represents the male end of a sexually-dimorphic social cognitive continuum (Baron-Cohen, 2002). Similarly, it has been proposed that psychotic or schizophrenia-like traits are distributed along a continuum of severity that ranges from complete absence of schizophrenia-like traits to a severity that is seen in individuals with schizophrenia (Lenzenweger, 1994). For example, some individuals from the general population have magical ideas, whereas individuals with schizophrenia may have severe delusions impacting their behavior and functioning in society (Mullen, 2003). Also, subtle, subclinical signs that parallel the symptoms of the illness may be present in healthy individuals. These signs may be manifested as various schizotypal personality traits, such as unusual perceptual experiences, excentric and desorganized speech and behavior, suspiciousness and social isolation. Subclinical signs may in some individuals progress to symptoms of schizophrenia. In fact, a number of schizotypal traits partially predict schizophrenia at long term follow-up in subjects diagnosed with Schizotypal Personality Disorder (DSM III) (Fenton et al., 1989). Also, the following schizotypal traits in young relatives of patients predict progression to schizophrenia in the following dimensions: social withdrawal, psychotic symptoms and socio-emotional dysfunction (Miller et al., 2002).

The observation that several different personality traits and disorders tend to cluster among biological relatives of individuals suffering from schizophrenia has lead to the hypothesis that a there is spectrum of related phenotypes that includes schizophrenia, as well as less severe phenotypes such as schizotypal personality disorder (DSM IV) and schizotaxia (Cadenhead et al., 2002; Jablensky, 2006; Meehl, 1989; Vollema et al., 1995). Several studies have reported cognitive and neuroanatomical abnormalities in individuals with schizotypal personality disorder that resemble those found in schizophrenia

patients, although the deviations are to a lesser degree (Cadenhead et al., 2002; Siever et al., 2004). The term schizotaxia is proposed to reflect a genetically determined defect in integration in the brain, predisposing to schizophrenia. In this view, only a minority of individuals with this defect decompensate to the point of being diagnosed with schizophrenia based on DSM-IV criteria (Faraone et al., 2001; Meehl, 1989). Also for autism, the concept of a 'broader phenotype' has been introduced to describe the mild features of the clinical autism phenotype that are seen in biological (i.e. genetically related) relatives of individuals with this disorder (Bailey et al., 1998; Bishop et al., 2004). Based on twin studies, it has been suggested that the typical clinical phenotype of autism or schizophrenia as seen in subjects with these disorders and the broader subclinical phenotypes (i.e. autism or schizotypal traits) that are seen in biological relatives, may share a genetic origin (Rutter, 2000; Torgersen et al., 2002).

Relevance of studying genetic disorders

Genetic disorders associated with specific deficits in brain development and cognition may help us to unravel genotype-phenotype relations. Starting at the level of the genotype instead of the phenotype, reversing the typical line of research, may be a complementary approach. Specific genetic conditions may be used as models of cognitive or behavioral disorders and provide insights into neurodevelopmental pathways that may be more difficult to uncover by studying heteregeneous, behaviorally defined populations (Reiss, 2000; Reiss et al., 2000). As such, studying individuals with a genetic abnormality who display social cognitive abnormalities and hence difficulties in coping with social situations may help us understand the mechanisms involved in social behavior. It may especially be useful for understanding etiological pathways to autism- or schizophrenia psychopathology.

Klinefelter (47,XXY) syndrome

One genetic disorder that is associated with abnormal brain development and behavior is Klinefelter syndrome, defined by the presence of an extra X chromosome in males (47,XXY). Klinefelter syndrome is the most common sex chromosomal disorder (Wesner et al., 1973), affecting approximately 1 in 700 males (Bojesen et al., 2003). This sex chromosomal aneuploidy results in a variety of phenotypes including hypogonadism, androgen deficiency and infertility (Lanfranco et al., 2004). Cognitive and behavioral dysfunctions in Klinefelter syndrome have generally been under-appreciated relative to

endocrinological and physical features. Although the primary focus in research has been on reproductive dysfunction of these patients, there is an awareness of behavioral and cognitive abnormalities (Boone et al., 2001; Geschwind et al., 2000b). The most prominent behavioral problems in men with Klinefelter syndrome are found in the social domain, such as social withdrawal, social anxiety, shyness, impulsivity and inappropriate or anti-social behavior (Bender et al., 1999; Geschwind et al., 2000a; Ratcliffe, 1999). In early adulthood they report having few or no friends, little energy and initiative, few or no sparetime interests and poor relations with siblings and parents (Nielsen et al., 1980).

The literature on cognitive mechanisms that may underlie impaired social adaptation in XXY men is scarce. It has been proposed that difficulties in social interactions, and specifically those related to communication, are largely attributable to disabilities in the language domain in Klinefelter syndrome (Rovet et al., 1996). The reported verbal disabilities include impairments in both language production and perception and indicate compromised language functions that are typically associated with the left hemisphere (Samango-Sprouse, 2001). For example, Klinefelter boys or men display disabilities in reading, articulation, phonemic processing, spelling, language expression, verbal memory, language comprehension, understanding words, finding words and verbally expressing their thoughts, all resulting in a verbal IQ that is somewhat lower than their performance IQ (Boone et al., 2001; Geschwind et al., 2000a; Money, 1993).

Compared to what is known about the cognitive mechanisms that contribute to social incompetence in Klinefelter syndrome, even much less is known about the neural mechanisms that are involved. Resting state cerebral blood flow patterns, as measured with SPECT, seem more symmetrical in XXY men as compared to men from the general population (Itti et al., 2003). Higher resting state blood flow in the right hemisphere in men with the XXY karyotype has been related to language impairments. Specific language dysfunctions have also been associated with morphological abnormalities of the temporal lobe (Itti et al., 2006). Furthermore, structural Magnetic Resonance Imaging (sMRI) studies with XXY men have indicated volume reductions in regions that are part of a neural network supporting social cognition, such as the amygdala, insula, anterior cingulate and superior temporal gyrus (DeLisi et al., 2005; Patwardhan et al., 2002; Patwardhan et al., 2000; Shen et al., 2004). The difficulties in social adaptation together with the structural brain abnormalities associated with the XXY karyotype suggest that a genetic mechanism involving genes on the X chromosome might lead to disturbances in development of social cognition in XXY men.

Difficulties in coping with the social world may be reflective of an increased vulnerability to traits and symptoms from the autism or schizophrenia spectrum. Indeed, there is some suggestive evidence for a link between the XXY karyotype and increased psychopathology from the schizophrenia spectrum. The importance of investigation into the cognitive and behavioral phenotypical manifestations of Klinefelter syndrome as a means of understanding a predisposition to schizophrenia, is shown by epidemiological studies reporting an increased incidence of XXY karyotypes in schizophrenia. The prevalence of the XXY karyotype in the general population is 0.1-0.2% (Bojesen et al., 2003). There is suggestive evidence that prevalence of the XXY karyoype in the male schizophrenia population may be 1.6 %, which is several times higher (DeLisi et al., 1994; Kunugi et al., 1999). However, these studies involved relatively small sample sizes (N=60 and N=120) in epidemiological terms and some studies have been unable to replicate these findings (Mors et al., 2001; Toyota et al., 2001). In turn, early studies have indicated an increased risk for schizophrenia and psychotic illnesses among Klinefelter men (Lishman, 1998). A review of mental hospital surveys pointed to a threefold increase in Klinefelter patients compared to the general population, which was mainly due to 'psychotic illnesses of a schizophrenic nature' (Forssman, 1970). Another study showed that 7% of the Klinefelter patients in the psychiatric literature had psychoses with paranoid delusions and 6% suffered from schizophrenia (Nielsen et al., 1969). Recently, a survey of hospital admissions and discharge diagnoses has indicated a significantly increased relative risk of being hospitalized with psychoses (hazard ratio of 4.97) for men with Klinefelter syndrome (Bojesen et al., 2006). In addition, several case reports of Klinefelter men suffering from schizophrenia or psychosis have been described in the literature (Dervaux et al., 2002; Michielsen et al., 2001; Ong et al., 1995; Roy, 1981; Warwick et al., 2003).

Present thesis

Studying socio-emotional information processing in XXY men at a neuropsychological level as well as neurobiological level might reveal a cognitive and neural basis for the difficulties in interpersonal relations and social 'awkwardness' that have been described. Because the XXY chromosomal pattern appears to be associated with difficulties in the social domain, Klinefelter syndrome might also prove to be a useful model for studying gene-brain-behavior pathways to socially deviant behavior and associated traits from the autism- or schizophrenia spectrum. Importantly, not only is the XXY

population narrowly defined by the presence of an extra X chromosome, individuals with the XXY pattern are generally not mentally retarded (in contrast to many other X chromosomal disorders) which allows the study of specific cognitive disabilities, and underlying neural mechanisms, without the confound of general intellectual decline.

In this thesis, I will focus on socially deviant behavior in adult XXY men on a behavioral, cognitive and neuroanatomical level. In **chapter two,** we aim to refine the social behavioral phenotype in XXY men. We will examine frequency of social behavior and distress during social interactions in men with the XXY karyotype. In addition, we will assess the degree to which features of the autism phenotype, as expressed in autism traits, are present in XXY men. In **chapter three,** we will explore evidence for increased schizophrenia spectrum pathology in XXY men. We will report on clinical measures of schizophrenia symptoms as well as measures of schizotypal personality traits.

I will continue with four chapters that deal with social cognitive abilities in XXY men. In **chapter four** we will examine socio-emotional processing in Klinefelter syndrome. Several domains of social cognition will be discussed, reflecting aspects of social-emotional information processing on levels of perception, experience and expression: labeling of facial expressions of emotion, emotion-cognition interactions in decision making and emotion regulation, that refers to subjective experience and identification of emotional arousal as well as verbal expression of emotions. **Chapter five** focuses on 'social intuition' in XXY men. The ability to quickly and automatically process basic social cues, such as gaze direction and implied biological motion, is thought to be a prerequisite for establishing successful social interactions and especially for construing a sense of 'social intuition'. We report on the extent to which social cues are processed effortlessly and implicitly in three different groups characterized by the presence of traits or symptoms from the schizophrenia spectrum, i.e. patients with schizophrenia, first-degree relatives of patients with schizophrenia and individuals with Klinefelter syndrome (47,XXY). Performance in those groups will be compared to matched controls from the general population. In **chapter six,** we test the hypothesis of the importance of the right hemisphere for specific pragmatic aspects of language in individuals from the general population. We will examine the effects of transcranial magnetic stimulation (TMS) over the right hemisphere on detection of emotions in tone of voice, a pragmatic aspect of language, in contrast to discrimination of emotions in verbal content, a semantic aspect of language

which has been associated with the left hemisphere. **Chapter seven** describes a first exploration of evidence for such pragmatic language impairments in in Klinefelter syndrome. By assessing the ability to discriminate emotions in speech we are able to examine the capacity to perceive and understand social signals in the auditory modality. We will contrast perception of emotional prosody (tone of voice), which is a pragmatic aspect of language thought to be lateralized to the right hemisphere, with perception of emotions in verbal content, which is lateralized to the left hemisphere.

The following three chapters deal with neuroanatomical mechanisms that may underlie social cognitive capacities in Klinefelter syndrome. In **chapter eight,** a possible neural mechanism underlying language disabilites in XXY men is explored. By using fMRI we are able to reveal the effects of an extra X chromosome on lateralization of neural activation during language processing. This technique allows us to identify functional asymmetries in specific brain regions as well as to determine whether reduced lateralization, if found, is secondary to decreased function of the left- or increased activity in the right hemisphere. We will explore the relation between loss of language lateralization and mental functioning in these men, with special interest in clinical phenomena of disorganization of thought and language.

As the amygdala is considered as a key brain area in socio-emotional processing, in **chapter nine** I will review evidence for structural abnormalities of the amygdala in Klinefelter syndrome based on findings in the literature. Findings will be compared to what is known of abnormalities of the amygdala in populations with increased vulnerability to schizophrenia: individuals from the general population displaying subclinical signs of schizophrenia and biological relatives of schizophrenia patients who may carry a genetic predisposition for the disorder. Not only volume of the amygdala, also functioning of the amygdala will be considered. **Chapter ten** deals with findings from a funtional MRI study (fMRI) focused on the neural mechanisms underlying social cognition in XXY men. In this chapter the functional contributions of a neural circuit comprising the amygdala, insula, fusiform gyrus and superior temporal gyrus, to social judgements of faces will be discussed.

Finally, in **chapter eleven,** I will evaluate all the presented evidence regarding social behavior, autism and schizophrenia spectrum traits, social cognitive disabilities and underlying neural mechanisms in XXY men. Besides describing the social cognitive phenotype at the level of behavior, cognition and brain structure and -function of this X chromosomal disorder, I will discuss the

potential implications of a link between the X chromosome and disturbances in development of social cognition and underlying neural networks for understanding gene-brain-behavior pathways to neuropsychiatric disorders such as autism or schizophrenia.

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Part I

Social behavior and psychopathology

Chapter 2

Social behavior and autism traits in a sex chromosomal disorder: Klinefelter (47XXY) syndrome

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Submitted for publication

Abstract

The XXY chromosomal pattern has been associated with difficulties in psychosocial functioning. Our aim was to examine frequency of participation in social interactions, distress during social interactions and autism traits in Klinefelter syndrome.

Scores of 31 XXY men on the Scale for Interpersonal Behavior and the Autism Spectrum Questionnaire were compared to 24 and 20 control men respectively.

XXY men reported increased distress during social interactions and less engagement of specific social behaviors. Overall rates of autism traits were significantly higher in XXY men.

These findings call for a clinical investigation of vulnerability to autism in Klinefelter syndrome. Klinefelter syndrome might serve as a model for studying a role of the X chromosome in social behavioral dysfunction and autism-like behavior.

Introduction

Klinefelter syndrome affects approximately 1 in 700 men and is the most commom sex chromosomal disorder. Men with this syndrome have an extra X chromosome, giving rise to the XXY chromosomal pattern. This sex chromosomal aneuploidy results in a variety of phenotypes including hypogonadism, androgen deficiency and infertility (Lanfranco et al., 2004). Cognitive and behavioral dysfunctions in Klinefelter syndrome have generally been under-appreciated relative to endocrinological and physical features. However, there is an awareness of behavioral and cognitive abnormalities (Boone et al., 2001; Geschwind et al., 2000b). Prominent behavioral problems in men with Klinefelter syndrome are found in the social domain, such as social withdrawal, social anxiety, shyness, impulsivity and inappropriate social behavior (Bender et al., 1999; Geschwind et al., 2000a; Geschwind et al., 2004; Ratcliffe, 1999). In early adulthood XXY men report having few or no friends, poor relations with siblings and parents, little energy and initiative, and few or no sparetime interests (Nielsen et al., 1980). Difficulties in social functioning have been attributed to language based learning difficulties (Geschwind et al., 2000a), social cognitive impairments (van Rijn et al., 2006) and verbal disabilities (Rovet et al., 1996) that have been observed in Klinefelter syndrome.

In previous studies, social adjustment in Klinefelter syndrome has primarily been described from a psychosocial perspective. Psychosocial competence has been measured using psychiatric interviews or parental questionnaires focused at, for example, the quality of relationships with familymembers, self-esteem and coping with stressors (Bender et al., 1995; Ratcliffe, 1999). Impairments in social adjustment, communication and social cognition might reflect an increased liability for neurodevelopmental disorders such as autism. Therefore, a refinement of the social behavioral phenotype in individuals with the XXY karyotype is warranted.

In this study, we measured frequency of participation in social interactions and distress during social behavior in adult XXY men. To explore the extend to which social disabilities reflect increased levels of features that belong to the autism phenotype, we included quantative measures of autism traits. We used this dimensional, rather than categorical, approach as it has been proposed that autism is a disorder of social behavior that reflects the extreme of a bell-shaped distribution of variation in autism traits, including social competence, in the population (Baron-Cohen et al., 2001; Constantino et al., 2003; Spiker et al., 2002).

Methods

Subjects

We included 31 men with Klinefelter syndrome (mean age 41.3, SD 10.0) with help from the Dutch Klinefelter Association. Diagnosis of Klinefelter syndrome was confirmed by karyotyping, using standard procedures. Twenty-four men were treated with testosterone supplements, with a mean age of treatment onset of 26.9 years (SD 7.6).

We compared autism traits in XXY men with 20 men from the general population (control group I), who were recruited using advertisments in local newspapers. Mean age in this group was 39.2 years (SD 13.1). Social behavior in XXY men was compared with 24 men from the general population (control group II). Mean age in this group was 35.7 (SD 8.5). There were no significant differences in age between the three groups, as indicated by a multivariate ANOVA as well as post-hoc tests ($F(2,72)=1.8$, $p=0.16$). None of the control subjects had a history of psychiatric illness as confirmed with the Mini International Neuropsychiatric Interview plus (MINI) (Sheehan *et al.*, 1998).
The study was approved by the local ethics committee and written informed consent was obtained according to the declaration of Helsinki.

Intellectual ability

Raven's Advanced Progressive Matrices (short form)

This test is commonly accepted as a measure of general intelligence and has been shown to correlate with a number of other standardized intelligence tests (Lezak, 1995; Raven *et al.*, 1993). Subjects are shown 12 pictures of matrices (i.e., related patterns), each of which is a figural design with a part removed. The subject must choose the correct missing part from eight options.

National Adult Reading Test (NART)

The Dutch translation of the NART (Nelson, 1982; Schmand et al., 1991) provides an estimate of verbal IQ and is based on the high correlation between reading ability, specifically of irregular words, with intelligence in the normal population. Subjects are required to read 50 irregular words aloud, and, on the basis of the number of errors made in pronunciation a reliable estimate of WAIS-R IQ can be calculated (Willshire *et al.*, 1991).

Social behavior

Social behavior was evaluated using the Scale for Interpersonal Behavior (SIB) (Arrindell, 1985). The SIB is a reliable and valid self-report measure of the frequency of engagement in specific social behaviors as well as the experienced distress it is accompanied by (Arrindell et al., 2001). Besides an overall measure of *frequency* of social behavior and *distress* during social behavior, there are four factorially-derived subscales: (I) Display of negative feelings (negative assertion), such as refusing a request or standing up for one's rights in a public situation, (II) Expression of and dealing with personal limitations, such as ability to deal with criticism or requesting attention/help, (III) Initiating assertiveness, such as starting a conversation with strangers or expressing one's own opinion and (IV) Praising others and the ability to deal with compliments/praise of others (positive assertion), such as giving and receiving compliments. Scores that are obtained with the SIB represent mean item-scores for each dimension of social behavior, on a scale from one (high frequency or low distress) to five (low frequency or high distress).

Autism traits

The Autism-spectrum Quotient (ASQ) (Baron-Cohen *et al.*, 2001) is a self-administered questionnaire for adults that assesses the degree to which any individual adult of normal intelligence might have features of the core autistic phenotype. It has good test-retest reliability and good discriminative validity for Asperger syndrome at a cut-off score of 26 (Woodbury-Smith *et al.*, 2005). Scores on the ASQ have shown to be normally distributed in the general population. Five subscales cover personality traits associated with the autistic spectrum; social skills, communication, imagination, attention to detail, and attention switching.

Results

Intellectual ability

Mean score on the Raven's Advanced Progressive Matrices was not significantly different between the groups ($F(2.72)=1.3$, $p=0.27$) as indicated by a multivariate ANOVA. Post-hoc tests also showed no significant differences between XXY men and the control groups ($p=0.18$ and $p=0.16$). Mean scores were 105.2 (SD 9.1), 109.4 (SD 8.8) and 109.6 (SD 14.6) for the Klinefelter group and control group I and II respectively.

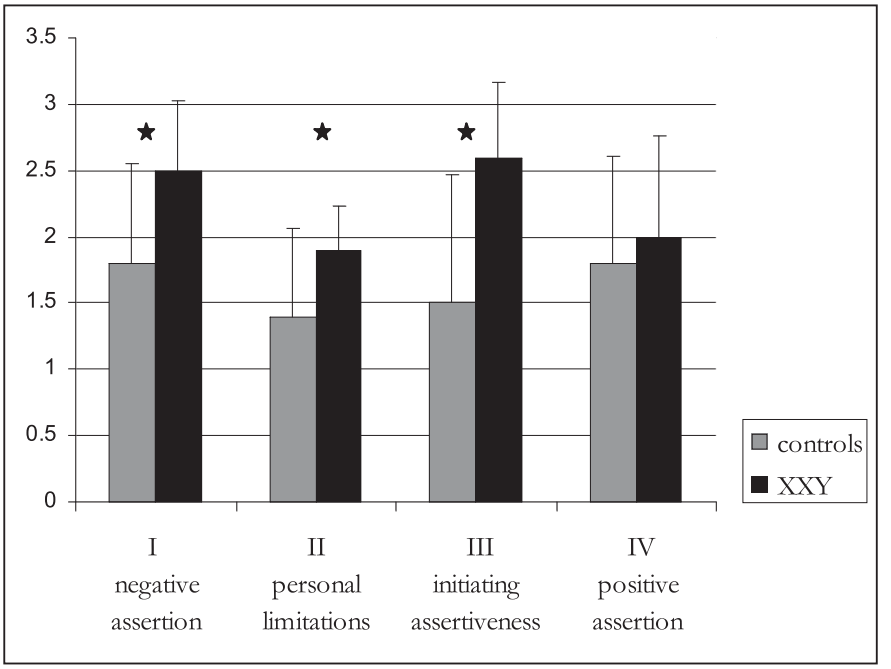
On the NART, mean score of the Klinefelter men did not significantly differ from the control groups ($F(2,72)=0.7$, $p=0.48$). Post-hoc tests also showed no significant differences between XXY men and the control groups ($p=0.46$ and $p=0.58$). Mean score in de Klinefelter group was 108.6 (SD 13.5), for the control group I and II it was 110.0 (SD 5.3) and 107.1 (SD 7.8) respectively.

Social behavior

Overall distress during social interactions was significantly higher in the XXY group as compared to men from the general population. Mean score in the XXY group was 2.2 (SD 0.67) and in the control group 1.6 (SD 0.49), which was significantly different ($F(1,52)=13.2$, $p=0.001$). Significantly higher scores, i.e. more distress, in XXY men were observed in the subscales 'negative assertion' ($F(1,52)=13.9$, $p<0.001$), 'personal limitations' ($F(1,52)= 12.1$, $p=0.001$) and 'initiation assertiveness' ($F(1,52)=20.5$, $p<0.001$). Mean item scores for distress in each dimension of social behavior are presented in figure 1.

Although overall frequency score of social behavior was not significantly different between XXY men and control men, the XXY group reported to less frequently display negative assertion, such as refusing a request or standing up for one's rights in a public situation. Mean frequency score in this domain of social behavior was 2.9 (SD 0.66) in the XXY group and 3.5 (SD 1.1) in the control group, which was significantly different ($F(1,52)=6.2$, $p=0.01$).

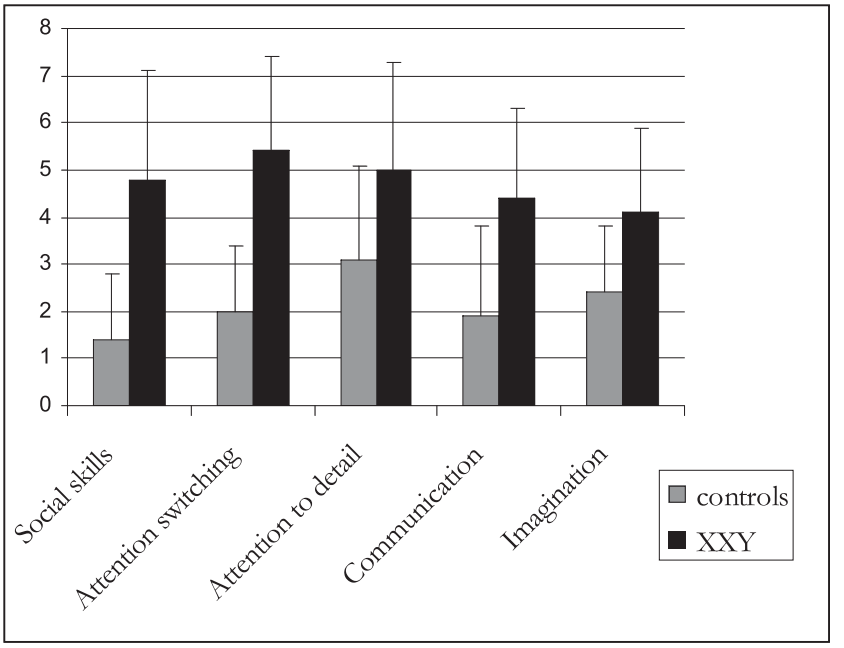
Figure 1
Self reported distress in XXY men and men from the general population during social interactions in four domains of social behavior: (I) Display of negative feelings (negative assertion), (II) Expression of and dealing with personal limitations, (III) Initiating assertiveness and (IV) Praising others and the ability to deal with compliments/praise of others (positive assertion).
* significantly different at p≤0.001



Autism traits

Mean total ASQ score and all subscales separately were significantly higher in Klinefelter men as compared to controls. Total score in the XXY group was 23.8 (SD 6.6) and in the control group 11.0 (SD 5.1), $F(1,57)=57.8$, $p<0.001$). As compared to men from the general population, XXY men displayed more autism traits in all domains; social skills ($F1,57)=32.3$, $p<0.001$), attention switching ($F1,57)=38.9$, $p<0.001$, attention to detail ($F1,57)=8.3$, $p=0.006$), communication ($F1,57)=19.6$, $p<0.001$) and imagination ($F(1,57)=12.7$, $p=0.001$. See figure 2 for mean scores and SD's on the five subscales of the ASQ.

Figure 2
Levels of autism traits in XXY men and men from the general population as measured with the Autism Spectrum Questionnaire. Scores in all individual dimensions were significantly higher in XXY men.



Discussion

The aim of this study was to refine the social behavioral phenotype in Klinefelter syndrome (47, XXY), a sex chromosomal disorder. We measured frequency of social behavior and distress during social interactions in adult men with the XXY karyotype. In addition, this study was the first exploration of features of the autism phenotype in Klinefelter syndrome.

As compared to men from the general population, XXY men reported increased levels of distress during social interactions in various domains of social behavior, namely: expressing of negative emotions to others, expressing and dealing with personal limitations and initiating contact with others. Although overall frequency of social behavior in XXY men was not different from men from the general population, significant differences in specific domains of social behavior were observed. XXY men reported to less often

engage in social behavior dealing with expression of negative emotions, such as refusing a request or standing up for one's rights in a public situation. In addition, high rates of autistic-like traits were observed in XXY men, across all dimensions of the autism phenotype, namely: difficulties in social skills, attention switching, imagination, communication and increased attention to details.

Our findings of difficulties in coping with social situations in XXY men, especially high levels of distress during social interactions, are consistent with reports of social anxiety, social withdrawal and shyness in individuals with the XXY karyotype (Bender et al., 1999; Ratcliffe, 1999). Difficulties in social adjustment have primarily been reported for children or adolescents with Klinefelter syndrome. Our data suggest that social difficulties may persist into adulthood, with social distress more prominent than a general reduction in engagement of social behavior. The use of self-report measures might have biased the degree of social disabilities in Klinefelter syndrome. However, studies showing that XXY men tend to overrate, rather than underrate, their own social adjustment (Bender et al., 1999) suggest that our findings might rather be an underestimation than overestimation of social disabilities in Klinefelter syndrome.

The high levels of autism traits in XXY men that were observed across all dimensions of the autism phenotype, suggest that the impairments in social interactions and communication parallel autism-like features that characterizes individuals at increased risk for autism. Increased levels of autism traits have also been found in biological relatives of subject with autism (Bishop et al., 2004). Our findings fit with the concept of a 'broad phenotype' of autism, which refers to the mild autistic-like features that are seen in individuals that are genetically related to an individual with autism (Bailey et al., 1998; Bishop et al., 2004). Similar to biological relatives of individuals with autism, autism-like features in XXY men were observed in the face of spared verbal- and general intellectual abilities (Bishop et al., 2004).

Based on twin studies, it has been suggested that the typical clinical phenotype of autism as seen in subjects with the disorder and the broader subclinical phenotype of autism as seen in biological relatives may share a genetic origin (Rutter, 2000). Although speculative, this might suggest that the X chromosome might be one of many genetic factors that play a role in the etiology of autism-like behaviors. Our findings are in line with studies in Turner syndrome, another X chromosomal disorder characterized by a partial or complete absence of one of the X chromosomes in females (45,XO). Turner

females also display difficulties in the social domain and the estimated risk of autism spectrum disorders may be several times higher as compared to women from the general population (Creswell et al., 1999; Mazzocco et al., 1998; McCauley et al., 2006; Skuse, 2000).

A hypothesized role of genetic mechanisms involving the X chromosome in social behavior fits with the notion that several genetic factors in autism might operate on components of the disorder, rather than the syndrome as a whole (Rutter, 2000). Influence of genes on the X chromosome on the development of autism-like features would fit with the male preponderance in autism spectrum disorders (Volkmar et al., 1993).

Taken together, as compared to men from the general population, XXY men reported increased levels of distress during social interactions and less engagement in those aspects of social behavior that deal with display of negative emotions. The increased levels of autism traits that we observed in XXY men call for a more thorough clinical investigation of vulnerability to autism in Klinefelter syndrome in a larger and more representative sample in epidemiological terms. Although our findings require replication, Klinefelter syndrome might prove to serve as a useful model for studying a role of the X chromosome in social behavioral dysfunction and autism-like behavior.

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Chapter 3

Klinefelter's syndrome (karyotype 47,XXY) and schizophrenia-spectrum pathology

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British Journal of Psychiatry, 2006, 189 (5), 459-461

Abstract

Klinefelter syndrome, characterized by a 47,XXY chromosomal pattern, has largely been associated with physical abnormalities. Here, we report high levels of schizophrenia spectrum pathology in 32 Klinefelter men in comparison to 26 healthy controls. Schizophrenia spectrum pathology was measured with the Schizotypal Personality Questionnaire (SPQ) and Positive and Negative Syndrome Scale (PANSS).

Our findings may have important implications for treatment of Klinefelter syndrome. In addition, these results suggest that the X chromosome may be critically involved in the aetiology of schizophrenia.

Introduction

Klinefelter syndrome is the most common sex chromosome disorder, affecting approximately 1 in 400 to 800 males. Individuals with this syndrome are characterized by an additional X chromosome, leading to the 47,XXY karyotype. This sex chromosomal aneuploidy results in a variety of phenotypes including hypogonadism, androgen deficiency and infertility (Lanfranco et al., 2004). Although the primary focus in clinical research has been on physical phenotypes of these men, there is a an awareness of neuro-anatomical, cognitive and behavioral abnormalities (Lanfranco et al., 2004; Shen et al., 2004). Specific impairments on measures of verbal skills, high incidence of dyslexia and social dysfunctioning are among the most consistentlty reported behavioral phenotypes (Geschwind et al., 2000). On the other hand, in a recent review on Klinefelter syndrome the authors conclude that it still remains unclear whether Klinefelter syndrome can be associated with psychiatric disturbances (Lanfranco et al., 2004). Interestingly however, many of the abnormalities in Klinefelter syndrome resemble those found in schizophrenia.

For example, structural MRI studies have reported smaller whole brain volumes, enlarged lateral ventricles and volume reductions of the superior temporal gyrus (STG), amygdala, hippocampus, insula and cingulate in Klinefelter men (Shen et al., 2004). A comparison of these findings to structural MRI studies in schizophrenia shows that all these regions are also affected in schizophrenia. Support for the hypothesis that sex chromosomes may play a role in the development of schizophrenia is derived from studies showing that males are more often affected by the disease than females and have an earlier age of onset (Aleman et al., 2003).

Case studies have been published describing Klinefelter subjects suffering from schizophrenia and higher rates of Klinefelter syndrome among samples of schizophrenia patients (DeLisi et al., 1994). Studies investigating psychiatric pathology in Klinefelter syndrome have been limited in that they described Klinefelter men in psychiatric care and mental hospitals, or recorded mental hospital admissions. However, there have been no systematic reports of levels of schizophrenia psychopathology in a large sample of Klinefelter subjects unselected for psychiatric disorders. In addition, a biological-genetic vulnerability to schizophrenia may not only be investigated using dichotomous, diagnostic outcomes, but also using dimensional measures of schizophrenia spectrum pathology, which are more sensitive measures of vulnerability to schizophrenia. Schizophrenia spectrum phenotypes share common cognitive, neuro-anatomical and genetic characteristics with the severe schizophrenia

phenotype. The present study was designed to test the hypothesis of increased levels of of schizophrenia spectrum pathology in subjects with Klinefelter syndrome.

Methods

32 Klinefelter men (mean age 38.8, SD 8.1) and 26 healthy controls (mean age 35.0, SD 9.0), matched for age, sex, years of education and intellectual ability, were included in the study. Klinefelter subjects were recruited from the Dutch Klinefelter Association and not selected for psychological or behavioral abnormalities. Also, the psychiatry department was not mentioned during the recruitment process. The diagnosis of Klinefelter syndrome (47,XXY karyotype) was confirmed by karyotyping using standard techniques. In all Klinefelter males, all 16 cells that were screened showed a 47,XXY karyotype, indicating non-mosaicism in this group. 80% Of the Klinefelter men received testosterone supplementation. Mean age of onset of treatment was 27.8 years (SD 7.6). Control subjects were recruited by advertisements. None of the controls met criteria for an Axis-I psychiatric disorder, as shown by screening with the MINI-Plus. After complete description of the study to the subjects, written informed consent was obtained.

Schizophrenia spectrum pathology was measured with the Schizotypal Personality Questionnaire (SPQ). The SPQ is regarded as an indicator of the genetic vulnerability to schizophrenia, since there is a gradient increase in schizotypal traits in relatives of schizophrenia patients that is in proportion to the risk for schizophrenia associated with the degree of kinship with the schizophrenic family member (Vollema et al., 2002). Factor analytical studies have revealed three dimensions of schizotypy; *Positive schizotypy* (for example referential thinking and delusional atmosphere), *Negative schizotypy* (for example constricted affect and social anxiety) and *Disorganization* (odd speech and eccentric behavior).

In addition, the Positive and Negative Syndrome Scale (PANSS) was included. This is a widely used structured interview to assess symptom profiles in schizophrenia that are present in the week prior to the interview. The PANSS allows categorization of negative-, positive-, and general symptoms.

Intellectual ability was measured with the National Adult Reading Test and Raven's Advanced Progressive Matrices , an estimator of verbal I.Q. and performance I.Q. respectively.

Group differences were tested using analysis of variance (ANOVA). Effect sizes were presented as Cohen's d.

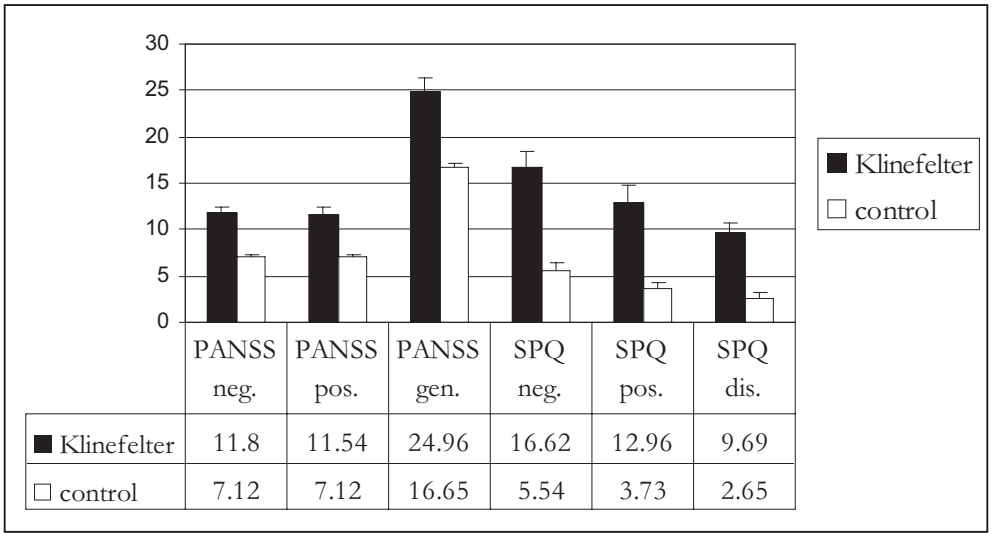
Results

In the Klinefelter group, the mean level of schizotypal traits, measured with the SPQ, was significantly higher than in healthy controls ($F(1,56)=36.67$, $p<0.0001$). Scores on all individual subscales were significantly increased (see table 1, presented as supplementary data at http://bjp.rcpsych.org/). Effect sizes were 1.43 for the negative dimension, 1.31 for the positive dimension and 1.81 for the disorganized dimension. The impact of these findings is illustrated by findings in schizophrenia. A study including 93 schizophrenia patients and 172 healthy controls, also using the SPQ, showed that the effects size (cohen's d) for mean total SPQ score was 1.95, for positive schizotypy 1.86, for negative schizotypy 1.83 and for disorganized schizotypy 1.45 (Rossi & Daneluzzo, 2002).

Similarly, PANSS scores showed increased levels of schizophrenia symptoms in the Klinefelter group ($F(1,56)=48.80$, $p<0.0001$). All symptom categories contributed to this effect. Effect sizes of 1.60 were observed for negative symptoms, 1.45 for positive symptoms and 1.66 for general psychopathology.

Results are presented in Figure 1. No significant group differences were observed for the estimators of verbal- and performance I.Q.

Figure 1
Schizophrenia spectrum pathology scores in Klinefelter syndrome (mean, SE).



(PANSS neg.= negative, pos.= positive, gen.=general;
SPQ neg.=negative, pos.=positive, dis.= disorganised)

Table 1
Scores on each of the individual subscales of the Schizotypal Personality Questionnaire were significantly increased in the Klinefelter group compared to controls (mean, SD).

	Klinefelter	**control**
Ideas of reference	2.7 (1.8)	1.3 (1.5)
Delusional ideas	1.0 (1.2)	0.3 (0.4)
Excessive social anxiety	4.1 (2.7)	1.1 (1.7)
Magical thinking	2.5 (2.2)	0.7 (1.2)
Unusual perceptual experiences	3.1 (3.3)	0.6 (1.7)
Odd or eccentric behavior	2.1 (2.2)	0.7 (1.6)
No close friends	4.5 (3.1)	1.3 (1.7)
Odd speech	7.8 (3.7)	2.0 (2.1)
Constricted affect	2.5 (2.0)	1.0 (1.2)
Suspiciousness	3.0 (2.5)	1.0 (1.0)

Discussion

The present study shows that the 47,XXY karyotype is strongly associated with high levels of schizophrenia spectrum pathology. This was evident in dimensional measures of schizotypal traits (SPQ) as well as actual schizophrenia symptoms (PANSS). Notably, magnitudes of the effect sizes of schizotypy levels approached those from a recent study with schizophrenia patients (Rossi et al., 2002; Vollema et al., 2002). Although healthy first degree relatives of patients with schizophrenia also have increased schizotypy scores when compared to individuals from the general population, schizotypy levels of relatives are substantially lower than those in schizophrenia patients (Vollema et al., 2002). Thus, the liability for schizophrenia might be higher in Klinefelter subjects than in relatives of schizophrenia patients. The presence of schizophrenia spectrum pathology in Klinefelter syndrome might have important implications for treatment. Whereas treatment is currently focused at medical problems, our data suggest it to be important to screen Klinefelter men for mental illnesses, in particular schizophrenia spectrum disorders.

Furthermore, our findings suggest a link between a X-chromosomal abnormality and liability to schizophrenia. This might provide a useful heuristic in the search for the genetic aetiology of schizophrenia. Indeed, a crucial role for X chromosome abnormalities in the aetiology of schizophrenia has been proposed (Lishman, 1998). Specifically, it has been argued that abnormal cerebral lateralisation may contribute to the development of schizophrenia, possibly involving abnormal expression of a gene on the X chromosome directing development of cerebral asymmetry (Crow, 2002). It is interesting in this regard that abnormal cerebral asymmetry has also been reported in Klinefelter syndrome . Additional support for a link between X-chromosomal abnormalities and liability to schizophrenia comes from two studies investigating the presence of 47,XXY karyotypes in a sample male schizophrenia patients. Whereas the prevalence of Klinefelter Syndrome in the general population is 0.1-0.2% (Lanfranco et al., 2004), these studies indicate that the prevalence of Klinefelter Syndrome in the schizophrenia population might be several times higher (DeLisi et al., 1994; Kunugi et al., 1999). Also, the present findings are consistent with a very recent study that reported auditory hallucinations in four out of eleven Klinefelter men (DeLisi et al., 2005).

Research with Klinefelter subjects, who are at increased risk for schizophrenia, may reveal specific genotype-phenotype associations. Endophenotypes in schizophrenia, i.e. expressions of a genetic predisposition at a neural or cognitive level, that are shared by Klinefelter syndrome and

schizophrenia may be the result of a X-chromosomal abnormality.

Finally, we are aware of some limitations of the present study. As many men with Klinefelter syndrome remain undiagnosed, our sample may not be completely representative. In spite of this, we believe that the present effect sizes convincingly indicate a relationship between Klinefelter and schizophrenia spectrum pathology, although the possibility that effect sizes might be attenuated in a representative sample from the general population can not be excluded.

In sum, Klinefelter syndrome can be associated with high levels of schizophrenia spectrum pathology. This suggests that the X chromosome may be critically involved in the aetiology of schizophrenia. Studying the genetics of Klinefelter syndrome may help localizing genes that are involved in the development of schizophrenia.

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Part II

Social cognition

Chapter 4

X Chromosomal effects on social cognitive processing and emotion regulation: a study with Klinefelter men (47,XXY)

Sophie van Rijn, Hanna Swaab, André Aleman and René S. Kahn

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Abstract

Studying Klinefelter syndrome (47,XXY), a genetically defined disorder characterized by the presence of an additional X chromosome, can reveal insights into genotype-phenotype associations. Increased vulnerability to psychiatric disorders characterised by difficulties in social interactions, such as schizophrenia and autism, has been reported for this population. The reported social difficulties in 47,XXY men may arise as a consequence of impairments in the processing of social and emotional information. The present study is the first investigation of social-emotional information processing in this X-chromosomal disorder.

32 Klinefelter men and 26 men from the general population, with the groups matched for age, educational level and I.Q., participated in the study. Several tasks were included, reflecting aspects of social-emotional information processing on levels of perception, experience and expression: labeling of facial expressions of emotion, emotion-cognition interactions in decision making and emotion regulation, that refers to subjective experience and identification of emotional arousal as well as verbal expression of emotions.

A discrepancy between cognitive appraisal of emotions and emotional arousal was observed in Klinefelter syndrome. Taken together, Klinefelter men seem less accurate in perception of socio-emotional cues such as angry facial expressions, they are less able to identify and verbalize their emotions, but experience increased levels of emotional arousal, in comparison to the general population. Besides describing the social-emotional phenotype of this X-chromosomal disorder, the present data may prove to be an important contribution to the development of more general models describing pathways to neuropsychiatric disorders characterized by social cognitive disturbances.

Introduction

Studying genetically defined syndromes can reveal insights into gene-brain-behavior mechanisms. This may lead to a better understanding of complex psychiatric diseases, such as schizophrenia. In this regard, an interesting genetic condition that has been associated with an increased vulnerability to schizophrenia, is Klinefelter syndrome (Bojesen et al., 2006; L.E. DeLisi et al., 1994; L. E. DeLisi et al., 2005; Kunugi et al., 1999; van Rijn et al., in press).

Klinefelter syndrome is characterised by the presence of an additional X chromosome in men. The 47,XXY chromosomal pattern has been associated with a variety of physical and neuropsychological abnormalities (Lanfranco et al., 2004). For example, infertility, lowered testosterone levels and language disabilities have been reported. At a behavioral level, Klinefelter men have been reported to have difficulties in social functioning and have been described as being introvert, anxious, impulsive, quiet, subassertive and socially withdrawn (D. H. Geschwind et al., 2000a). Socially inappropriate behavior as well as anti-social behavior have also been documented (Gotz et al., 1999; Ratcliffe, 1999).

Social difficulties in Klinefelter syndrome may be dismissed as a consequence of daily life struggles associated with the disorder, but they may also arise from deficits in social cognitive processing. Social cognitive processing, i.e. the ability to perceive, understand and express social signals, covers aspects of intelligence other than general cognitive abilities such as language and attention, that have an independent contribution to social functioning (Amy E. Pinkham et al., 2003). In the general population, social competence seems to be under substantial genetic control (Scourfield et al., 1999).

Findings from MRI studies showing structural abnormalities of brain regions that form part of a neural network subserving social cognition and emotion call for a more thorough investigation of social cognitive processing in Klinefelter syndrome. For example, volume reductions of the amygdala, insula, anterior cingulate and superior temporal gyrus, all of which have been shown to play an important role in social cognition and emotion, have been observed in this syndrome (Shen et al., 2004).

Social cognitive processing is thought to mediate between the molecular, genetic level and the behavioral, or even clinical, level and thereby may provide insight into gene-brain-behavior linkages underlying psychopathology (Van Rijn et al., 2005). Interestingly, as Klinefelter syndrome has been associated with increased levels of schizophrenia spectrum pathology and autistic features (L.E. DeLisi et al., 1994; L. E. DeLisi et al., 2005; Gillberg, 1995; Swaab et al., in prep.;

van Rijn et al., in press), studying social cognitive processing in Klinefelter syndrome may prove to be an important contribution to the development of more general models describing pathways to neuropsychiatric disorders characterized by social cognitive disturbances (Van Rijn et al., 2005).

The present study is, to the best of our knowledge, the first investigation of social cognitive processing in a large sample of Klinefelter men. Therefore, a broad range of aspects of social cognitive processing was investigated. Several tasks were included, reflecting aspects of social cognitive processing on levels of perception, experience and expression: labeling of facial expressions of emotion, emotion-cognition interactions in decision making and emotion regulation, that refers to subjective experience and identification of emotional arousal as well as verbal expression of emotions. The tasks in this study have been used in previous studies of social cognitive processing in schizophrenia and/or autism, and for all tasks neural correlates have been defined (A. Aleman, in press; R. Gur et al., 2002a; Sanfey et al., 2003).

Methods

Subjects

32 Men with Klinefelter syndrome (mean age 38.8, SD 8.3) were studied. The participants were recruited from the Dutch Klinefelter Association, and were not selected for psychological, behavioral or cognitive abnormalities. Diagnosis of Klinefelter syndrome was confirmed by karyotyping, using standard procedures. In this group, 28 men were treated with testosterone supplements, with a mean age of treatment onset of 26.2 years (SD 7.9).

26 Male controls from the general population (mean age 35.2, SD 9.1) were included in the study. They were recruited using advertisements in local newspapers or were drawn from an already existing database in our department. There were no significant differences in age ($F(1,50)=2.4$, $p=0.12$) and years of education ($F(1,56)=0.4$, $p=0.52$) between the groups. None of the control subjects had a history of psychiatric illness as confirmed with the Mini International Neuropsychiatric Interview plus (MINI) (Sheehan et al., 1998).

Exclusion criteria for both Klinefelter men and controls were neurological conditions or history of head injury with loss of consciousness, recent history of substance abuse and mental retardation. After complete description of the study to the subjects, written informed consent was obtained.

Intellectual ability

Raven's Advanced Progressive Matrices (short form)

This test is commonly accepted as a measure of general intelligence and has been shown to correlate with a number of standardized intelligence tests (Lezak, 1995; Raven et al., 1993). Subjects are shown 12 pictures of matrices (i.e., related patterns), each of which is a figural design with a part removed. The subject must choose the correct missing part from eight options.

National Adult Reading Test (NART)

Since Klinefelter syndrome has been associated with verbal deficits, we included a measure of reading skills in addition to general I.Q. The Dutch translation of the NART (Nelson, 1982; Schmand et al., 1991) provides an estimate of verbal IQ and is based on the high correlation between reading ability, specifically of irregular words, with intelligence in the normal population. The test is composed of a list of 50 irregular words (i.e., pronunciation does not follow the normal phonetic rules) printed in order of increasing difficulty. Subjects are required to read these words aloud, and, on the basis of the number of errors made in pronunciation a reliable estimate of WAIS-R IQ can be calculated (Willshire et al., 1991).

General cognition

General face recognition

The Benton and Van Allen Test of Facial Recognition, Short Form (Benton & Van Allen, 1973), comprises a series of sheets containing a single photographed target face to be matched to a set of six face photographs. In the first six trials, the identical face has to be selected out of six options. In the remaining six trials, three different views (changed in orientation or lighting conditions compared to the target photograph) have to be discriminated from three incorrect alternatives. All faces are physically similar, not showing glasses or hair.

Social cognitive processing

Facial Affect Recognition

This task is a measure of explicit facial affect recognition of degraded faces, that has been used in earlier studies on emotion processing (van 't Wout et al., 2004). Photographs of four different actors, two male and two female, were used (Frigerio et al., 2002). Sixty-four trials were presented, consisting of 16 face presentations in each of four conditions: angry, happy, fearful and neutral. In each condition, 8 trials displayed 100% emotional intensity and the other 8 trials

displayed 75% emotional intensity. All photographs of the faces were passed through a filter that reduced visual contrast by 30%, in order to avoid local- and stimulate global processing. Subjects were asked to indicate the expression of each emotion by clicking with the mouse on one of the four emotion labels depicted on the computer screen. They were asked to work as accurately and rapidly as possible.

Emotion in decision making

The Ultimatum Game is an extensively studied paradigm originating from the field of strategic decision-making. It provides a measure of cognition-emotion interactions in strategic decision making, by inducing conflict between acceptance (cognitive motive) and rejection (emotional motive) of unfair financial offers (Sanfey et al., 2003).

In the Ultimatum Game two players were given the opportunity to split fictitious a sum of money, ?5. A fictitious proposer (20 times another person and 20 times the computer) made an offer as to how this money should be split between the two. The responder (participant) could either accept or reject this offer. If it was accepted, the money was split as proposed, but if the responder rejected the offer, then neither player received anything. In either event, the next offer appeared. 20 times the money was fairly split (5,00 versus 5,00) and in another 20 rounds the offer was unfair. There were three different unfair offers, i.e. twice 9,00 versus 1,00; twice 8,00 versus 2,- and once 7,00 versus 3,00. This resulted in a total of 40 rounds. The different offers (fair, unfair, human, computer) were assigned in a random order.

Emotion regulation

Alexithymia refers to a personality trait implying an inability or reduction to identify, experience, describe and reflect on one's own emotions (Lane et al., 1997; Sifneos, 1973).

We used the Bermond-Vorst Alexithymia Questionnaire to measure alexithymia, i.e. emotion-regulation (Morera et al., 2005; Vorst & Bermond, 2001). The BVAQ has good psychometric properties, and consists of five subscales: *Emotionalizing*: the degree to which someone is emotionally aroused by emotion inducing events. *Fantasizing*: the degree to which someone in inclined to fantasize, imagine, daydream, etc. *Identifying*: the degree to which one is able to define one's arousal states. *Analyzing*: the degree to which one seeks out explanations of one's own emotional reactions. *Verbalizing*: the degree to which one is able or inclined to describe or communicate about one's own

emotional reactions. Each of the subscales consists of eight items. Answers are scored on a 5-point scale (1=certainly does not apply to me, up to 5=certainly applies to me). The calculated scores were substracted from 50, resulting in lower scores reflecting lower capacity or performance.The latter three BVAQ-subscales have substantial overlap (correlation of .80) with the more widely used Toronto Alexithymia Scale 20 (TAS-20) (Bagby et al., 1994). However, the BVAQ also includes two measures of emotional experience, fantasizing and emotionalizing, which form part of the original definition of Alexithymia introduced by Sifneos (Sifneos, 1973).

Statistical analyses

Separate t-tests were conducted on the demographic data, Raven's Advanced Progressive Matrices, NART and the Benton and Van Allen test of Facial Recognition.

Data of the social cognitive tasks were analyzed using GLM repeated measures analysis, with a fixed factor Group (Klinefelter and control) and a variable factor reflecting the conditions within the tasks. When the multivariate tests indicated significant differences, the differences were analyzed with subsequent post-hoc ANOVA's. When potential confounding variables (i.e. general face recognition and intellectual ability) differed between the groups, then these were included as a covariate in the analysis. Alpha was set at 0.05.

Results

Intellectual ability

Mean score on the Raven's Advanced Progressive Matrices was not significantly different between the groups ($t(1,55)=1.0$, $p=0.32$). Mean scores were 107.2 (SD 14.2) and 110.4 (SD 8.6) for the Klinefelter group and control group respectively.

On the NART, mean score of the Klinefelter men did not significantly differ from controls ($t(1,54)=1.78$, $p=0.08$). Mean score in de Klinefelter group was 103.5 (SD 9.8), for the control group it was 108.0 (SD 9.1).

General cognition

General face recognition

In general, no ceiling effects were observed in the Benton and van Allen test. Mean percentage of correct responses was 76.25 % and 80.0 % in the Klinefelter and control group respectively. No group differences were present

in general face recognition performance, t(1,55)= 1.83, p=0.07. Mean number of correct responses was 21.35 (SD 2.3) for Klinefelter men and 22.42 (SD 2.0) for control participants.

Social cognitive processing

Facial Affect Recognition

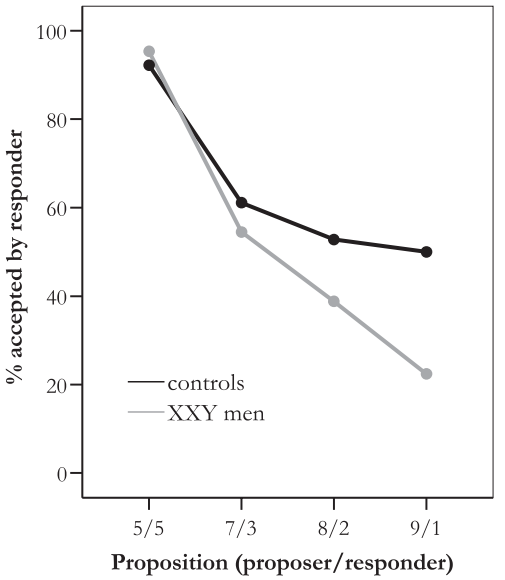
In general, performance on degraded facial affect recognition was well beyond chance level for each emotion. In the control group percentage correct was 84.4 for neutral faces, 89.7 for happy faces, 60.6 for fearful faces and 78.6 for angry faces. Percentage correct in the Klinefelter sample was 75.8 for neutral faces, 91.7 for happy faces, 58.8 for fearful faces and 67.7 for angry faces.

A GLM repeated measures analysis showed an interaction between Group (Klinefelter, control) and Emotional Expression (neutral, fear, anger, happy), F(1,53)=3.11, p=0.03. Post-hoc ANOVA's demonstrated a significant difference between the groups in the recognition of anger, F(1,55)=5.98, p=0.018, i.e. Klinefelter men were less accurate as compared to control subjects (mean number correct responses: 10.8 and 12.6 respectively). This deficit appeared not to depend on intensity of the emotional expressions, as group differences were present in both the 75% anger condition (F(1,55)=4.57, p=0.037) and the 100% anger condition (F(1,55)=4.79, p=0.033). There was no specific bias of Klinefelter men in type of errors when erroneously labeling anger. A GLM repeated measures analysis with factor Group (Klinefelter, control) and factor error-type (% happy, fear and neutral with error anger) demonstrated no group differences in the error pattern, F(2,48)=0.73, p=0.48.

Emotion in decision-making

Although the UItimatum Game was not performed by 8 of the controls and 3 of the Klinefelter men, exclusion of these subjects did not affect matching of the groups on age, years of education, verbal I.Q and performal I.Q. A significant (within-subjects contrasts) interaction between Group and Percentage accepted offers was observed when the Ultimatum Game was played with a human proposer (F(1,45)=6.45, p=0.015). Post-hoc ANOVA's demonstrated a significant difference between the groups in the proportion accepted offers in the most unfair (9/1) condition (F(1,45)=5.93, p=0.019), i.e. Klinefelter men rejected significantly more offers compared to control subjects. Results are presented in figure 1. No significant differences between the groups were observed when the Ultimatum Game was played with a computer.

Figure 1
When the Ultimatum game was played with a human proposer, Klinefelter males rejected significantly more offers compared to control subjects in the most unfair (€9:€1) condition.



Emotion regulation
A significant interaction was observed between Group and the alexithymia dimensions ($F(1,54)=6.32$, $p<0.001$). Klinefelter men showed increased scores compared to controls, indicating more problems, on the subscales *Identifying* ($F(1,54)=8.18$, $p=0.006$) and *Verbalizing* ($F(1,54)=4.73$, $p=0.034$). In addition, lower scores on the subscale *Emotionalizing* ($F(1,54)=10.88$, $p=0.002$) were observed in the Klinefelter group, indicating that Klinefelter men are more easily emotionally aroused. See table 1 for mean scores and standard deviations.

Table 1
In the Alexithymia questionnaire, Klinefelter males reported significantly more difficulties (i.e. higher scores) in *identifying* as well as in *verbalizing* their emotions and reported to be more easily emotionally aroused as indicated by lower scores on the subscale *emotionalizing* (*p<.05).

Dimensions of Alexithymia	Controls (mean, SD)	Klinefelter men (mean, SD)
Verbalizing emotions	20.2 (6.2)	24.6 (8.3) *
Fantasizing	24.6 (8.2)	21.1 (8.0)
Identifying emotions	15.6 (4.0)	20.2 (7.0) *
Emotionalizing	26.2 (6.3)	20.7 (5.9) *
Analyzing emotions	18.6 (6.1)	19.2 (7.7)

Conclusion

The current study investigated various aspects of social cognitive processing and emotion regulation in Klinefelter syndrome, a genetic disorder characterized by an XXY chromosomal pattern. Our findings suggest disturbances in perception, experience and expression of social cognitive information in this population. Specific deficits in perception of facial expressions of anger were observed. Abnormal experience of emotions in Klinefelter men was suggested by both explicit and implicit measures. Klinefelter men explicitly reported increased emotional arousal in response to emotion-inducing events and were more influenced by their emotions in a strategic decision making game. Difficulties in identification and expression of one's own emotions were also found in this group, as indicated by the alexithymia measures. Taken together, Klinefelter men seem less accurate in perception of social-emotional cues, experience increased levels of emotional arousal, but are less able to identify and verbalize the emotions they experience, in comparison to the general population.

These deficits in social cognition may explain, in part, the social difficulties that have been described in Klinefelter syndrome. First, we found Klinefelter men to be impaired in recognizing facial expressions of anger, independent of the intensity of the emotional expression. Misperception of angry facial expressions may contribute to problems in social interactions, as non-verbal signals may convey crucial information about the emotional state of the sender. Various studies have revealed significant relationships between facial affect recognition performance and social functioning (Christine Hooker & Park, 2002).

Regarding the experience of emotions, Klinefelter men rejected more often unfair financial offers from human proposers as compared to the control group in the Ultimatum game. This pattern of performance is shown to reflect the influence of emotion, in response to the offer being unfair, on strategic decision making (Sanfey et al., 2003). A recent fMRI study has shown that rejection of unfair offers could be significantly predicted on the basis of insular activity (Sanfey et al., 2003), a brain region involved in processing emotions such as disgust (Wicker et al., 2003), and monitoring of the internal milieu, for example arousal (Augustine, 1996). In line with this, a recent study revealed that autonomic, emotional arousal, as measured by skin conductance activity, is higher for unfair offers and associated with the rejection of unfair offers in this particular paradigm (van 't Wout et al., in press). Interestingly, in Klinefelter syndrome, structural abnormalities of the insula and amygdala have been

reported which may, in part, contribute to this pattern of performance indicating increased emotional arousal (Shen et al., 2004).

Autistic adults, like Klinefelter men, also accept very little in this paradigm and it has been proposed that impairments in reading intentions and beliefs from others play a role in rejecting unfair offers (Camerer, 2003). Although speculative, our finding that Klinefelter men only rejected more offers when proposed by a human individual, and not when proposed by a computer, tentatively suggests that Klinefelter men have problems in reading intentions of other people. The present results support our hypothesis that *social* cognition in Klinefelter syndrome is disturbed, which is expressed predominantly in social contexts such as interpersonal relations. The hypothesis regarding theory of mind capabilites in Klinefelter syndrome should be tested in future research.

Besides this implicit emotional task, increased emotional experience was also observed in an explicit, self-report measure of emotional experience, i.e. the alexithymia questionnaire. Klinefelter men reported increased emotional arousal in response to emotion-inducing events, as indicated by the subscale 'emotionalizing' in the Alexithymia questionnaire. In contrast to apparent *hyperfunctional* emotional -experience and -reactivity, identifying and verbalizing these emotions appears to be *hypofunctional* in Klinefelter syndrome. The latter finding can be considered as a possible risk factor for both medical and psychiatric disorders (Bagby & Taylor, 1997). Interestingly, the specific alexithymia profile observed in Klinefelter syndrome has recently also been described for schizophrenia patients and their relatives, indicating that it may be an expression of the genetic vulnerability for the disease (van 't Wout et al., in prep.). The finding that alexithymia seems to be under considerable genetic control fits this picture (Valera & Berenbaum, 2001).

Recently, a model has been proposed that can explain the two seemingly contradictory features in schizophrenia that we also find in Klinefelter syndrome; deficits in perception and experience on the one hand and increased emotional arousal on the other hand (A. Aleman & Kahn, 2005a). This neuroanatomical model describes amygdala dysfunctions that arise from structural abnormalities of the amygdala in combination with an imbalance in dopamine systems, with specific effects on intra-amygdaloid processing. Indeed, Klinefelter syndrome, as well as other populations at increased risk for schizophrenia, has been associated with structural abnormalities of the amygdala (Van Rijn et al., 2005). Interestingly, such a dissociation between cognitive appraisal of emotions on the one hand and autonomic, emotional arousal on the other hand has also been reported for females with Turner

syndrome, characterised by X monosomy (45,X) and a 200-fold increased risk for autism. Turner syndrome subjects display (left amygdala mediated) impairments in labeling of facial expressions of both anger and fear, while their (right amygdala mediated) somatic responses are enhanced in reponse to emotional faces (D. Skuse et al., 2005). Thus, X-linked genes seem important for functional integration of autonomic, emotional arousal with cognitive appraisal of emotions.

Altogether, the present study reveals the behavioral/functional consequences of a compromised neural network for social cognitive processing. A central issue concerns the degree to which these disturbances in social cognitive processing are related to the X chromosomal abnormality. Evidently, neural and behavioral phenotypes in Klinefelter syndrome can all be traced back to the additional X chromosome. In this regard, studies with individuals with Turner syndrome (45,X) may provide some insight. In one of these studies, a dosage-sensitive locus on the X chromosome has been identified that directs development of the amygdala (Good et al., 2003). As in Klinefelter syndrome, individuals with Turner syndrome have social adjustment problems and poor social skills (Lesniak-Karpiak et al., 1999; Mazzocco et al., 1998). These parallels in brain architecture and socio-behavioral phenotype support the idea that (mal)development of this area in Klinefelter syndrome may indeed be under control of genes on the X chromosome.

Unfortunately, the exact pathway from X chromosome abnormalities to social cognitive impairments in Klinefelter syndrome remains unclear. One of the issues deserving attention is the role of abnormal testosterone levels in Klinefelter syndrome, which become apparent in puberty. It is thought that during development circulating gonadal hormones can modify brain structure and -function of target areas. As shown by animal studies, one of the major target areas of testosterone is the amygdala (Simerly et al., 1990). However, the relation between testosterone levels and behavior is complex; timing of exposure, sensitivity to testosterone reflected in androgen receptor density and modulation by environmental factors are important determinants in the behavioral effects of testosterone (Craig et al., 2004). Furthermore, gonadal hormones may be one of many mechanisms by which sex chromosomes exert their influence on brain development. Recent animal studies have pointed to direct, non-hormonal, effects of sex-chromosomes on brain maturation (Dewing et al., 2003).

Reports of increased levels of schizophrenia spectrum pathology and autistic features in Klinefelter men point to a role of the X chromosome in

psychiatric disorders with predominant social cognitive impairments (L.E. DeLisi et al., 1994; L. E. DeLisi et al., 2005; Gillberg, 1995; Swaab et al., in prep.; van Rijn et al., in press; Van Rijn et al., 2005). Although speculative, X-related disturbances in social cognitive processing may be one of many pathways to psychiatric disorders such as schizophrenia and autism. As these disorders are more common in males compared to females (A. Aleman et al., 2003; D. H. Skuse, 2000), it will be interesting in future studies to investigate if and how the widely described sex differences in social cognition can be related to genes on the X chromosome and whether such genes are implicated in these psychiatric disorders (S. Baron-Cohen et al., 2005; Scourfield et al., 1997). Also, more studies are needed to explore the role of testosterone in social cognitive impairments in Klinefelter syndrome as well as autism and schizophrenia, especially since there are findings pointing to abnormal testosterone levels in these latter disorders (S. Baron-Cohen et al., 2005; Goyal et al., 2004).

In a recent review it has been argued that the amygdala abnormalities seen in schizophrenia may be an X-linked endophenotype that can account for the social cognitive processing deficits observed in patients and their relatives (Van Rijn et al., 2005). Also, it has been proposed that dysfunctional neural circuits underlying social cognitive impairments in autism may be phenotypes of X-linked genes, that may be sexually dimorphic in expression (S. Baron-Cohen et al., 2005; D. Skuse, 2003). Support for this hypothesis also comes from a review of all chromosomal studies in autism suggesting that the X chromosome, chromosome 15 and 17 are the most consistently reported chromosomes to show abnormalities in the field of autism spectrum disorders (S Baron-Cohen, 2004).

A crucial question is how Klinefelter syndrome may serve as a model for investigating the potential role of X-linked genes for susceptibility to mental disorders, most notably schizophrenia. In contrast to schizophrenia, the genetic pathology underlying deficits in Klinefelter syndrome is known. Common endophenotypes that are shared by both disorders, such as deficits in social cognition, point to a role of the X chromosome in social cognitive dysfunction in the schizophrenia spectrum. Subsequently, one can narrow the search for genetic pathology involved in schizophrenia, with the hypothesis that genes on the X chromosome may code for abnormal social cognition in schizophrenia. Other possibilities may be to compare and study genetic pathology of subgroups of Klinefelter men: with low versus high levels of schizophrenia spectrum pathology, or poor versus relatively spared social cognition. This might lead us to more specific underlying genetic mechanisms such as genomic

imprinting or degree of X inactivation. Of course, these strategies apply to all endophenotypes that are shared by schizophrenia, which is clinically defined, and Klinefelter syndrome, which is genetically defined.

Finally, we are aware of some limitations of the present study. As many men with Klinefelter syndrome remain undiagnosed (Bojesen et al., 2003), our sample may not be completely representative. In spite of this, the present findings describe social cognitive deficits, that may have important implications for daily life functioning, in at least a specific population of Klinefelter men.
In summary, the disturbances in perception, experience and expression of social cognitive information that were observed in Klinefelter men may explain the social difficulties that have been reported in this syndrome. Besides describing the social cognitive phenotype of this specific genetic disorder, the present data encourage the development of a general model describing gene-brain-behavior pathways underlying neuropsychiatric disorders characterized by social cognitive disturbances.

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Chapter 5

Social information processing in the schizophrenia spectrum: implicit attention to social signals

Mascha van 't Wout, Sophie van Rijn, Tjeerd Jellema, René S. Kahn, André Aleman

Abstract

Schizophrenia is characterized by disturbances in social functioning. In the search for determinants of social dysfunction in schizophrenia patients, social cognitive capacities appear to be of crucial importance. The ability to process basic social cues, such as gaze direction and biological motion direction, quickly and automatically is thought to be a prerequisite for establishing successful social interactions and especially for construing a sense of 'social intuition'. However, studies that address the ability to automatically process such basic social cues in schizophrenia are lacking. We used a new visual illusion measuring the extent in which social cues are processed effortlessly and implicitly in three different groups characterized by the presence of traits or symptoms from the schizophrenia spectrum, i.e. 33 patients with schizophrenia, 32 siblings of patients with schizophrenia and 32 individuals with Klinefelter syndrome (47,XXY). These groups were compared to 50 age-, sex- and education-matched healthy control subjects. Results indicated that, in contrast to control subjects, patients with schizophrenia showed insensitivity to social cues. This was particularly pronounced in patients with negative symptoms. The reduced influence of social cues was also observed in first-degree relatives of patients with schizophrenia as well as in Klinefelter subjects. We suggest that the insensitivity for social cues is a cognitive aspect of schizophrenia that may be seen as an endophenotype as it appears to be present both in relatives who are at increased genetic risk and a genetic disorder associated with schizophrenia spectrum psychopathology. These social cue-processing deficits could contribute, in part, to the difficulties in higher order social cognitive tasks and hence decreased social competence that have been observed in these groups.

Introduction

One of the cardinal dysfunctions associated with the schizophrenia phenotype concerns disturbances in social functioning (DSM-IV American Psychiatric Association) (1994). Although some researchers have argued that this might be a consequence of severe psychopathology, others have demonstrated that social dysfunction is relatively independent of symptomatology (Lenzenweger et al., 1996). This view is further supported by findings that disturbances in social functioning are already present in early adolescence and often precede the onset of psychosis (Baum et al., 1995; Hans et al., 1992; Walker, 1994). In the search for determinants of social dysfunction in schizophrenia patients, cognitive capacities appear to be of crucial importance. In the last decade, there is a growing body of research demonstrating deficits in the cognitive processing of social information in schizophrenia (Pinkham et al., 2003), such as difficulties in emotion recognition (Edwards et al., 2002; Kohler et al., 2004) and the inability to understand and manipulate other people's behavior in terms of their mental states, also called Theory of Mind (Frith, 1992). Furthermore, schizophrenia patients have difficulties in the recognition of abstract social signals, such as inferences regarding actors' affect and goals (Corrigan et al., 1993). These deficits seem to be independent of intelligence, i.e. not attributable to a generalized performance deficit (Corrigan, 1994), but are related to negative symptoms such as withdrawal (Corrigan et al., 1994) and skills to receive, process, and send social signs (Corrigan et al., 1995).

An important underlying characteristic of successful social interaction is the ability to quickly process social information (Frith et al., 1999). Basic elements of a social signal that are processed fast and automatic are for example gaze direction, head orientation and body postures (Jellema et al., 2005). These cues can give clues about someone's intentions, goals and believes (Perrett, 1999). Usually, these basic social cues are largely automatically processed, which is necessary to continuously infer the meaning of the rapidly changing social signals. Moreover, it is suggested that the ability to process these basic social cues quickly and automatically is a prerequisite for establishing successful social interactions and communication (Frith et al., 1999). It may be especially relevant for construing a sense of 'social intuition', as such implicit learning processes form the basis of social intuition (Lieberman, 2000). Intuitions have been described as following: "intuitions are fast and take into account nonconsciously generated information, gathered from experience, about the probabilistic structure of the cues and variables relevant to one's judgments, decisions, and behavior" (Bruner, 1960). Although schizophrenia patients seem to fail in areas

of social intuition and automaticity in social interactions as observed in their social behavior (Bellack et al., 1990), studies that address the ability to automatically process such basic social cues in schizophrenia are lacking.

Therefore, we used a new paradigm involving a bias in the judgment of the distance between two agents induced by the automatic processing of social cues conveyed by these agents (Jellema et al., 2004). The social cues consisted of the direction of attention and implied goal-directed actions. Typically, these social cues induce the sensation of people (dis-)engaging in social interaction when their gaze or body postures are attended towards (or away from) each other. Therefore, the social cues of gaze direction and implied biological motion used in the present paradigm results in people judging the persons as closer together compared to reference objects whilst this is not the case objectively.

In addition to patients with schizophrenia and healthy controls, we also included 2 other groups in the study: a) individuals at increased genetic risk for schizophrenia, i.e. sibling of schizophrenia patients and b) individuals with an X chromosomal disorder and high levels of schizotypal traits, i.e. men with Klinefelter syndrome. Biological siblings of patients have been shown to be at significantly higher risk for the development of schizophrenia (Gottesman, 1991) and display cognitive deficits that are also seen in schizophrenia patients, although to a lesser degree (Sitskoorn et al., 2003). Inclusion of the sibling group enables the study of social cognitive deficits related to a genetic vulnerability to schizophrenia without confounding environmental influences as hospitalization, medication and psychopathology. Support favoring the role of genetic mechanisms in social cognitive deficits is derived from studies demonstrating abnormalities in the processing of social-emotional cues in biological relatives of patients with schizophrenia (Loughland et al., 2004; Toomey et al., 1999). This fits with the finding that social skills are under considerable genetic control in the general population. Men with Klinefelter syndrome have an extra X chromosome (47,XXY chromosomal pattern), and display abnormal brain development (Shen et al., 2004), social cognitive impairments (van Rijn et al., 2006b) and psychopathology that is also seen in patients with schizophrenia (Van Rijn et al., 2006a). An additional advantage arising from studying Klinefelter men is knowledge of the precise genetic etiology of this syndrome, in contrast to what is known of the genetic underpinnings of social cognitive impairments in schizophrenia. Including XXY men might point to a role of the X chromosome in development of cognitive systems that are important for processing basic social signals (cf. Skuse, 2005). Involvement of sex chromosomes might explain, in part, the sex

differences that have been observed in social cognitive skills in the general population as well as in schizophrenia populations (Hampson et al., 2006; McClure, 2000; Scholten et al., 2005).

The aim of the present study was to investigate sensitivity to basic social cues in individuals with schizophrenia, individuals with an increased genetic risk for schizophrenia and individuals with a genetic disorder and high levels of schizotypal traits. To this end we used a social distance judgment task that included two social cues, social attention (gaze direction) and implied biological motion (Jellema et al., 2004). It was hypothesized that patients with schizophrenia would demonstrate difficulties in the automatic processing of social cues compared to control participants, i.e. patients may show no response bias congruent with the direction of the social cues. Furthermore, the relationship between symptomatology and social cue processing is investigated. We predicted that the problems in social cue processing would be especially prevalent in patients with negative symptoms, since patients with negative symptoms are characterized by social-emotional disturbances. We hypothesized that siblings of patients with schizophrenia as well as XXY men would show a deviant pattern comparable to patients, although to a lesser extent. This would indicate that deficient processing of basic social cues forms part of the genetic vulnerability for the disease, possibly involving genes on the X chromosome, rather than environmental factors such as medication and potential toxic effects of psychosis.

Methods

Participants

33 Patients (23 men, 10 women) with a diagnosis of schizophrenia were recruited at the University Medical Center Utrecht. All patients met the DSM-IV criteria for schizophrenia, as confirmed by the Comprehensive Assessment of Symptoms and History interview (CASH) (Andreasen et al., 1992) administered by a psychiatrist. Patients were also screened for affective disorders, i.e. depression and mania, substance-related disorders by the CASH. Most patients were diagnoses with paranoid schizophrenia (n=22), one with disorganized type, one with residual type, six with undifferentiated type and three with schizophreniform disorder. Most patients were clinically stable and in residual state and four patients were inpatients and 29 were outpatients. Patients were all clinically stable, 31 patients received medication (30 patients only antipsychotics, such as leponex (n=13), quetiapine (n=4), olanzapine (n=6),

risperidone (n=8) and one patient also received oxazepam). Symptoms and severity were independently rated by two raters with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Raters were trained by a qualified trainer and followed interrater reliability training every six months. Mean positive symptoms was 14.22 (SD 5.22, range 7-27), negative symptoms 14.84 (SD 5.78, range 7-29) and general psychopathology 26.66 (SD 6.84, range 17-47). Most patients were in remission, residual state and were outpatients (29 outpatients and 4 inpatients). Mean duration of illness was 9.44 years (SD 8.01) and mean age of onset was 23.83 years (SD 5.45).

32 Siblings of patients with schizophrenia (12 men, 20 women) were recruited through advertisements at the Ypsilon website, which is a website dedicated to relatives of patients with schizophrenia. The diagnosis of schizophrenia for the affected sibling was confirmed with a CASH interview (Andreasen et al., 1992). However, due to ethical reasons we were unable to verify the diagnosis of schizophrenia for 12 affected siblings with the CASH interview.

32 Men with Klinefelter syndrome (47,XXY) were studied. The participants were recruited from the Dutch Klinefelter Association, and were not selected for psychological, behavioral or cognitive abnormalities. Diagnosis of Klinefelter syndrome was confirmed by karyotyping, using standard procedures. 50 Non-psychiatric control participants (31 men, 19 women) were drawn from the general population via advertisements in local newspapers.

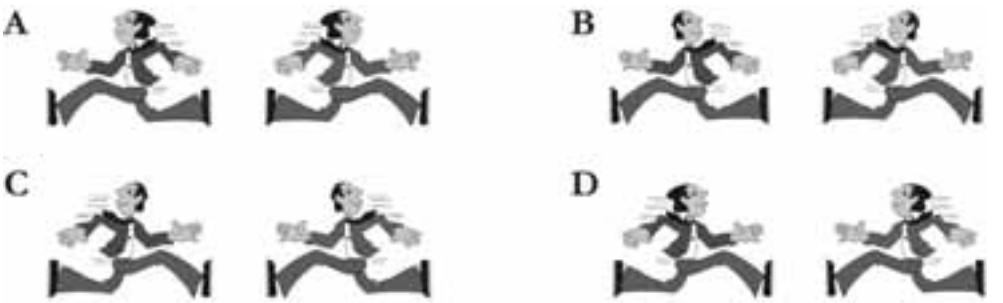
Inclusion criteria for all participants were age between 18 and 65 years and good physical health. Exclusion criteria were neurological conditions, history of head injury with loss of consciousness, recent history of alcohol and substance abuse, or mental retardation. None of the control participants and siblings had a history of psychiatric illness or use of psychiatric medication confirmed with the Mini International Neuropsychiatric Interview plus (MINI) (Sheehan et al., 1998). The local ethics committee approved the study and all subjects provided written informed consent after the procedure had been fully explained, according to Declaration of Helsinki. The Dutch translation of the National Adult Reading Test (NART) (Schmand et al., 1991) and Raven's Advanced Progressive Matrices (Raven et al., 1993) were used to match the groups on estimates of verbal and performance intelligence level, respectively (Lezak, 1995).

Social Distance Judgment Task

The Social Distance Judgment Task measures the illusion of de- or increasing distance caused by the automatic processing of social cues (Jellema et al., 2004). It is hypothesized that the perceived distance between the agents will be influenced by the social cues conveyed by the agents, resulting in a response bias paralleling the strength of social cues, which has been confirmed by pilot data from our lab. Stimuli were pairs of two cartoon figures shown in running postures conveying two different social cues: gaze direction (figures looking away or towards each other) and implied biological motion (figures running away or towards each other). Head and body of the cartoon figures were pointing in the same direction, or in opposite directions, amounting to a total of four different compositions of cartoon figures, see figure 1.

Figure 1

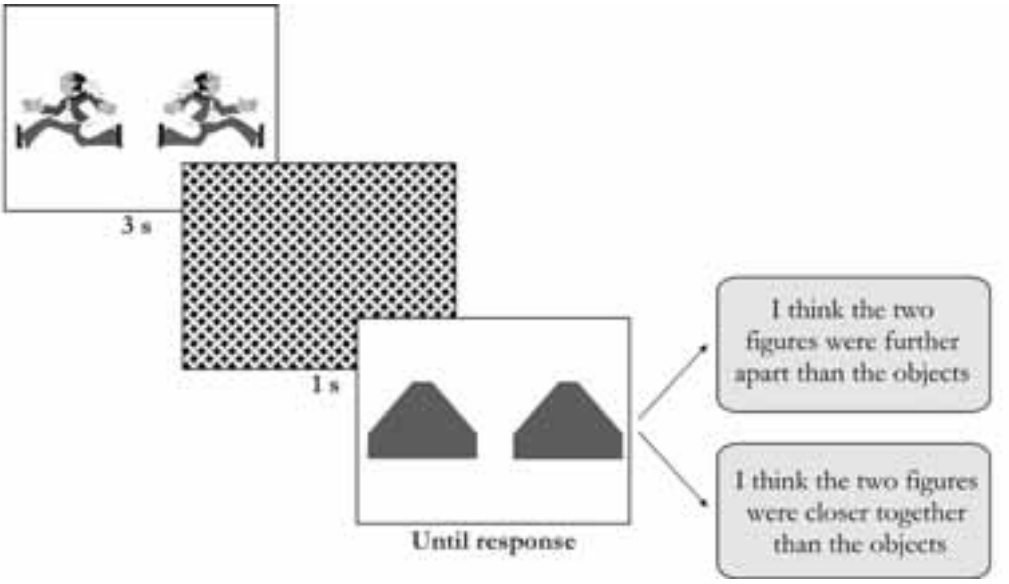
From left to right: increasing strength of social cues leading to underestimation of the distance between the cartoon figures, i.e. the response: 'I think the two cartoon figures were closer together than the two geometrical objects'.



A pair of cartoon figures was presented for 3 s, after which a mask of 1 s was shown, followed by a pair of geometrical figures (see figure 2 for an example of a trial). Participants had to choose one of two possible responses: (1) 'I think the two cartoon figures were closer together than the two geometrical objects', and (2) 'I think the two cartoon figures were further away from each other than the two geometrical objects'.

Except for the catch trials, the distance between the geometrical figures was always the same as the distance between the cartoon figures and three different distances were randomly presented: 2, 3 and 4 cm. In the catch trials the distance between the geometrical figures was different (2 cm) from the distance between the cartoon figures. The catch-trials were used to allow exclusion of those participants from analysis who did not pay proper attention to the task. Participants who made more than two errors in the catch-trials were excluded from the analyses.

Figure 2
Example of a single trial.



Results

As only males are affected with Klinefelter syndrome, two separate analyses were performed. One for schizophrenia patients and relatives, including both males and females, and one for Klinefelter men, including XXY men and male controls. 5 Patients with schizophrenia, 3 siblings, 3 Klinefelter men and 4 control participants made more than two errors in the catch-trials and were not included in further analysis.

Schizophrenia patients and relatives

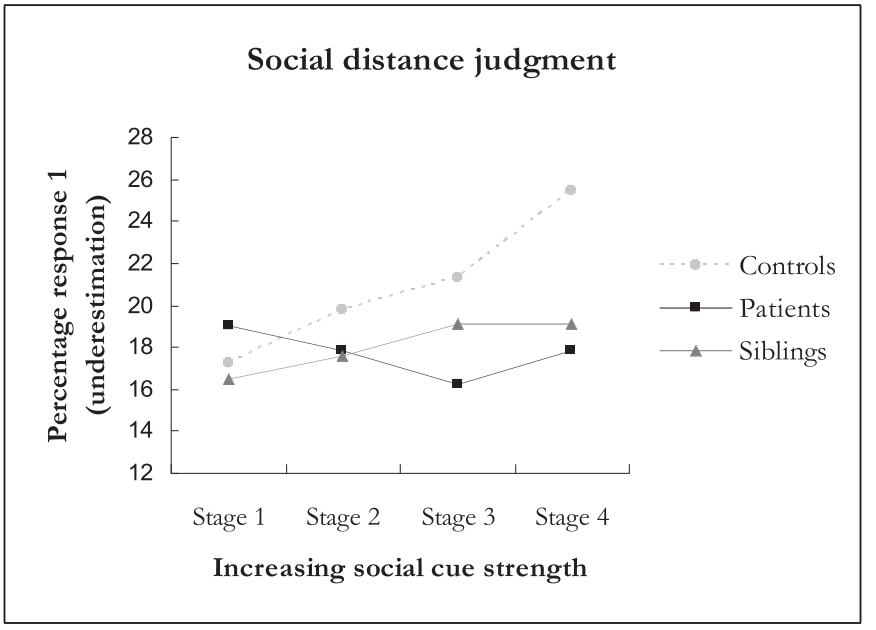
28 Patients with schizophrenia, 29 siblings of patients with schizophrenia and 46 control participants were included in the analyses, see table 1 for demographic data. A GLM repeated measures test of within subject contrasts with increasing social cue strength as within subjects variable (with 4 strength levels) revealed a significant linear increase in percentage of response 1 ("I think the two figures are closer together than the two geometrical figures") in the control group, $F(1,45)=14.27$, $p=0.0005$. In contrast, percentage response 1 did not change with increasing social cue strength of in the patient group, $F(1,27)=0.34$, $p=0.56$. Remarkably, absence of a response bias was also found in the sibling group , $F(1,28)=0.77$, $p=0.39$. The sensitivity for social cues differed significantly between patients with schizophrenia, siblings and control subjects, revealed by different patterns of percentage response 1 with increasing strength of social cues, $F(2,100)= 3.79$, $p=0.026$ (figure 3). Post-hoc tests revealed the control group to differ significantly from the patient group in sensitivity for social cues ($F(1,72)=8.06$, $p=0.006$). The sibling group did not differ from the control group ($F(1,73)=2.21$, $p=0.14$), nor from the patient group ($F(1,55)=1.09$, $p=0.30$).

Table 1
Demographic data of 28 patients with schizophrenia, 29 siblings of patients with schizophrenia and 46 healthy control participants included in the Social Distance Judgment Task.

Variable	Patients	Siblings	Control subjects	P
Age in years (SD)	32.43 (7.51)	34.62 (10.72)	31.89 (9.19)	0.45
Male:female ratio	18:10	11:18	27:20	0.11
Education in years (SD)	14.29 (2.80)	16.21 (1.93)	14.89 (2.58)	0.01*
Parental education in years (SD)	13.97 (2.98)	14.67 (2.68)	13.20 (2.89)	0.27
NART (SD)	103.56 (8.16)	104.54 (8.11)	107.63 (9.54)	0.13
Raven's (SD)	NA	109.21 (9.93)	108.40 (13.75)	0.79

- P<0.05, Between-groups comparisons with Student's t-tests, except Male:female ratio is analyzed with non-parametric Kruskal Wallis test, df =100; NA= Not available

Figure 3
Increasing social cue strength resulted in a linear increase in response 1 ('I think the two cartoon figures were closer together than the two geometrical objects') in healthy control subjects, but not in patients or sibling of patients.



Social distance judgment and symptomatology in schizophrenia patients

There was a significant negative correlation between the response bias due to social cue strength and negative symptoms of schizophrenia as measured with the Positive and Negative Syndrome Scale (PANSS), r=-0.39, p=0.04. This suggests that patients with more negative symptoms are less influenced by social cues. There were no correlations between positive symptoms or general psychopathology as measured with the PANSS and influence of social cues.

Klinefelter men

A group of 29 Klinefelter men was compared to 25 control men, see table 2 for demographic variables. A GLM repeated measures test of within subject contrasts revealed that in the control group, the social cues did elicit a response bias congruent with the directions of the social cues. We observed a significant linear increase in underestimations (i.e. increase in percentage response 1) of the perceived distance as strength of the social cues would increase, $F(1,24)=13.54$, $p=0.001$. Sensitivity for social cues differed significantly between the Klinefelter men and controls, as reflected by different patterns of percentage response 1 over the four conditions (group effect in GLM repeated measures of within subject contrasts; $F(1,52)=4.4$, $p=0.04$). Although strength of the social cues increased, percentage response 1 remained at the same level in the Klinefelter group, $F(1,28)=0.001$, $p=0.98$. The absence of a response bias congruent with direction of the social cues indicated that the distance judgment performance in this group was not influenced by social interpretation and thus more accurate compared to controls. Results are presented in figure 4.

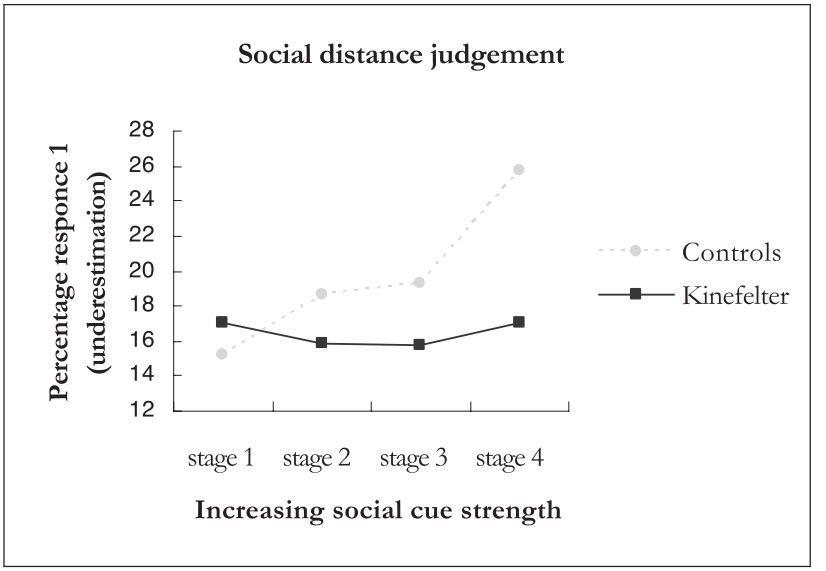
Table 2

Demographic data of 29 Klinefelter men and 25 control men.

Variable	Klinefelter men	Control men	P
Age (in years)	38.07 (8.47)	33.84 (8.90)	0.08
Education (in years)	13.92 (2.65)	14.36 (2.66)	0.56
NART (SD)	102.67 (8.61)	107.20 (10.03)	0.09
Raven's (SD)	107.68 (14.37)	111.71 (9.20)	0.24

P: Between-groups comparisons with Student's t-tests, df =52

Figure 4
Increasing social cue strength resulted in a linear increase in response 1 ('I think the two cartoon figures were closer together than the two geometrical objects') in healthy control subjects, but not in Klinefelter men.



Discussion

This study examined automatic processing of basic social cues, i.e. implied biological motion and gaze direction in three different groups: a) schizophrenia patients, b) individuals at increased genetic risk for schizophrenia, i.e. sibling of schizophrenia patients and c) individuals with an X chromosomal disorder and high levels of schizotypal traits, i.e. men with Klinefelter syndrome.

In healthy controls, an increasing strength of social cues in the stimuli was accompanied by an increasing illusion of the perceived distance between the stimuli, indicating that social cues affected decision making as we expected. In contrast, in schizophrenia patients, siblings of patients and Klinefelter men, an increasing strength of social cues in the stimuli did not have any effect on the perceived distance between the stimuli, indicating that social cues were not incorporated in the process of judging the stimuli. When considering the groups separately, schizophrenia patients and Klinefelter men showed to be less sensitive to social cues compared to controls. Performance of the siblings of patients was in between patients and control participants, that is siblings did not

differ significantly from either controls or patients. The differences can not be ascribed to differences in general cognitive function, as the groups were matched on education and intelligence estimates. Furthermore, the subjects included in the analysis understood the task and were able to perform the task correctly, as is evidenced from their lack of errors on the catch trials.

These results suggest that patients with schizophrenia demonstrated a lack of sensitivity to even basic, simple social cues, instead of deficits only in more abstract, higher-order social cue recognition (Corrigan et al., 1993). A failure to automatically and quickly process these basic social cues may contribute to difficulties in social intuition and hence coping with social situations in these patients. Also, because less basic social information is available, more widespread effects on ('upstream'-) higher-order social cognitive processing can be expected. The observed insensitivity to social cues may underly social cognitive deficits and social dysfunction in schizophrenia. The ability to process social cues automatically is thought to be especially important for theory of mind, i.e. deducing someone else's intentions, goals and believes (Perrett, 1999) and deficits in the automatic processing of these social cues might lead to disturbances in the attribution of mental states to others (Frith et al., 1999). Indeed, a recently published study demonstrates that patients with schizophrenia were impaired in using appropriate language to describe Theory of Mind animations (Russell et al., 2006).

Our results showed that especially patients with negative symptoms, which comprise social and emotional withdrawal, were insensitive to the influence of the social cues in their judgments. Patients with negative symptoms typically show problematic social functioning (Dickerson et al., 1996, 1999; Van Der Does et al., 1996), but also deficits in other social emotional tasks (Kohler et al., 2000; Mandal et al., 1998; Martin et al., 2005; Schneider et al., 1995). Thus, these results corroborate previous research demonstrating that patients with schizophrenia show deficits in the processing of social information (Pinkham et al., 2003), with more severe impairments in patients with negative symptoms (Corcoran et al., 1995; Kohler et al., 2000; Leitman et al., 2005; Mandal et al., 1999). However, this study extends previous research in demonstrating deficits in the effortless processing of simple social cues.

Interestingly, the absence of influence of the social cues on distance judgments was also observed in individuals at increased genetic risk for schizophrenia (relatives of patients) and individuals with a genetic disorder associated with increased schizophrenia spectrum pathology (Klinefelter syndrome). Based on these findings three important conclusions can be drawn.

First, siblings as well as the Klinefelter men were not clinically psychotic and did not use antipsychotic medication. The lack of sensitivity for social cues could thus not be due to the effects of illness or the medication use. In that way, these results validate the observed results in patients. Second, we propose that the observed lack of sensitivity for social cues is related to a genetic vulnerability to schizophrenia. The results showed that there were no differences between patients and siblings in distance judgment, suggesting that siblings resemble patients in an absence of automatic processing of social cues. However, one could also argue that siblings perform normally, as they also did not differ from controls. When taking the within group analysis into account we demonstrated that siblings, in contrast to normal controls, did not show a linear increase in underestimations, i.e. their distance judgments were not influenced by the social cues of human figures running towards each other or looking towards each other. Thus, our findings imply that performance of siblings resembles the lack of sensitivity to social cues observed in schizophrenia patients, albeit to a lesser extent. Moreover, our results mirror and extend previous studies demonstrating impairments in other types of social emotional cue processing in relatives of patients with schizophrenia such as recognizing emotional facial expressions (Loughland et al., 2004; Toomey et al., 1999), suggesting that insensitivity for social cues might be regarded as a genetic vulnerability to schizophrenia. Third, additional evidence for a genetic loading on social cue processing comes from the findings in individuals with a genetic disorder. Performance in the social cue task was indistinguishable between schizophrenia patients and Klinefelter men. As this disorder is defined by an X chromosomal abnormality, impaired cognitive processing of social cues in this group can be regarded as the expression of X-linked genetic pathology. Klinefelter men also display impairments in higher order social cognitive processing, such as recognition of facial expressions (van Rijn et al., 2006b). As insensitivity to social cues seems an endophenotype that is shared by schizophrenia patients and Klinefelter men, this deficit may have a common genetic (X-linked) origin in both syndromes.

Notably, individuals with autism, a disorder of the 'social brain' as indicated by deficits in theory of mind, facial affect recognition and reciprocal social behavior, also demonstrate an insensitivity to social cues using this task (Jellema et al., 2004). Hence, this study gives converging evidence that attention to basic, typically effortlessly processed social cues may belong to the fundamental cognitive operations needed for successful social behavior. With regard to the neural correlates involved in the processing of biological motion and social attention, the superior temporal gyrus, medial prefrontal cortex and anterior

cingulate have been implied (Jellema et al., 2005). Both in schizophrenia patients as well as relatives, abnormalities in these regions have been reported (Ashton et al., 2000; Dolan et al., 1995; Fletcher et al., 1999; Mitelman et al., 2005; Rajarethinam et al., 2000; Shenton et al., 2001; Takahashi et al., 2004). Interestingly, structural abnormalities in the anterior cingulate and the superior temporal gyrus have been found in Klinefelter syndrome as well (Shen et al., 2004). Additional evidence for a role of the X chromosome in development of the superior temporal gyrus comes from studies with individuals with X monosomy, showing that volume of this region is dependent on parental origin of the X chromosome (Kesler et al., 2003). It is suggested that these neural correlates also underlie Theory of Mind capabilities (Frith et al., 1999; Siegal et al., 2002).

Future studies should relate neural substrates of social cue processing in schizophrenia and relatives together with measures of social functioning. This would elucidate the relationship between the ability to process social cues and social behavior and its underlying brain pathology in schizophrenia and provide more insight into the biological vulnerability to schizophrenia. Such research should also take into account the role of the amygdala, which has been implied in social information processing (Adolphs et al., 1998). Abnormalities of the amygdala have been documented in patients with schizophrenia (Aleman et al., 2005) as well as their relatives and Klinefelter patients (Van Rijn et al., 2005).

In summary, this study allowed the investigation of sensitivity to simple, basic social cues that are usually effortlessly processed, i.e. implied biological motion and gaze direction, in individuals with a) a diagnosis of schizophrenia b) an increased risk for schizophrenia (relatives of patients) and c) with a genetic disorder associated with increased schizophrenia spectrum pathology (Klinefelter syndrome). Results showed that patients with schizophrenia, siblings of patients with schizophrenia and Klinefelter men (47, XXY) did not process these social cues automatically compared to healthy controls. Within the schizophrenia group, this was especially the case in patients with more severe negative symptoms, i.e. patients that show additional social emotional disturbances. Hence, social cue processing deficits seem related to the vulnerability to schizophrenia, instead of illness in general and with a potential involvement of genes on the X chromosome. These basic social cue processing deficits might underlie impairments in other aspects of social cognition and social functioning. Future research should investigate further the relationship between insensitivity to social cues, social functioning and neurobiological substrates in schizophrenia.

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Chapter 6

What is said or *how* it is said makes a difference: role of the right fronto-parietal operculum in emotional prosody as revealed by repetitive TMS

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Abstract

Emotional signals in spoken language can be conveyed by semantic as well as prosodic cues. We investigated the role of the fronto-parietal operculum, a somatosensory area where the lips, tongue and jaw are represented, in the right hemisphere to detection of emotion in prosody versus semantics. 14 Healthy volunteers participated in the present experiment, which involved Transcranial Magnetic Stimulation (TMS) in combination with frameless stereotaxy. As predicted, compared to sham stimulation, TMS over the right fronto-parietal operculum differentially affected reaction times for detection of emotional prosody versus emotional semantics, showing that there is a dissociation at a neuroanatomical level. Detection of withdrawal emotions (fear and sadness) in prosody was significantly delayed by TMS. No effects of TMS were observed for approach emotions (happiness and anger). We propose that the right fronto-parietal operculum is not globally involved in emotion evaluation, but sensitive to specific forms of emotional discrimination and emotion types.

Introduction

In order to understand messages in verbal communication, it is important to pay attention not only to what is said but also to how it is said. Besides the semantic (linguistic) meaning of words, features such as intonation and loudness in speech also may convey crucial information. The term prosody has been introduced to describe these non-semantic cues in spoken language. Prosodic cues can have linguistic- as well as affective functions. Linguistic functions are, for example, emphasizing important parts of the message or presenting information as a statement or a question. Affective functions of prosody are also important for understanding intentions of others, because variations in tone of voice, such as intonation and loudness, provide information about the emotional state of the speaker. Thus, the emotional relevance of a spoken message may be not only be conveyed by meaning of words (i.e. emotional semantics), but also expressed in emotional prosody. The present study is focused on processing of affective information conveyed by these different aspects of spoken language, i.e. emotional semantics versus emotional prosody.

These two different communicative channels of information about the emotional relevance of a spoken message, i.e. semantic and prosodic, may be dissociable at a neuroanatomical level. A range of studies have suggested that the left hemisphere seems to be specialized for most aspects of language processing (Binder, 1999), including linguistic functions of prosody (e.g, stress evaluation,(A. Aleman et al., 2004)). However, the right hemisphere (RH) appears to be involved in non-linguistic components, such as emotional prosody (Baum & Pell, 1999).

The best evidence regarding RH involvement in affective prosody comes from studies directly contrasting emotional semantics with emotional prosody. In contrast to processing of emotional semantics, emotional prosody involves the right hemisphere as is shown in imaging studies (Baum & Pell, 1999; R. L. Mitchell et al., 2003; Vingerhoets et al., 2003). These findings suggest that the role of the right hemisphere in emotional prosody is not a specialization in emotion detection in general, but that RH involvement depends on the communication channel through which emotional relevance is presented.

In addition to type of communication channel, involvement of the right hemisphere may also depend on type of emotion. A widely used categorisation of emotions is based on broader constructs of behavioral inhibition and activation (Sutton & Davidson, 1997). Whereas withdrawal emotions such as fear and sadness are accompanied by inhibitory motivational tendencies, appraoch emotions such as anger and happiness are accompanied by

activational motivational tendencies. Right hemisphere damaged patients show specific deficits in processing withdrawal emotions, i.e. fear and sadness, in contrast to normal processing of approach emotions such as happiness and anger (A. K. Anderson et al., 2000; M. K. Mandal et al., 1999). Also, it has been proposed that whereas the left frontal cortical region is important for expression and experience of approach emotions, the right frontal cortical region is involved in expression and experience of withdrawal emotions (Harmon-Jones, 2003; Sutton & Davidson, 1997).

Recently, Vingerhoets et al. (Vingerhoets et al., 2003) have measured blood flow velocity (BFV) with functional transcranial doppler ultrasonography (fTCD) to study the contribution of the right and left hemisphere to the detection of emotion in prosody versus detection of emotion in semantics of spoken language. fTDC is a non-invasive tool with high temporal resolution that allows a continuous monitoring of blood flow velocity in the basal cerebral arteries, thereby reflecting changes in cerebral metabolism that accompanies mental activity. During detection of emotion in semantics a significant left-hemispheric lateralization of BFV was observed. This lateralization effect disappeared when attention was shifted to discriminating emotion in prosody, due to a rise in right hemispheric BFV. Although metabolism in the right hemisphere is correlated with detection of emotional prosody in this study, the question remains whether the RH is causally involved in emotional prosody. In addition, is not clear which brain region within the right hemisphere is critically involved in emotional prosody discrimination and whether such a region is sensitive to specific types of emotions.

As an extension of the study of Vingerhoets et al. (Vingerhoets et al., 2003), we used Transcranial Magnetic Stimulation (TMS) in order to answer these questions. TMS delivers short magnetic pulses that penetrate the skull and disrupt neural processing in a non-invasive, reversible way (Walsh & Pascual-Leone, 2003). This virtual lesion technique allows investigation of the causal relation between neural activity and performance at a behavioural level. In addition, specific brain regions can be targeted, depending on the type of coil used for stimulation. Ideally, such regions should be determined on the basis of lesion data in human patients. Recently, a comprehensive 3-D lesion study investigating the neural correlates of emotional prosody has been published (Adolphs et al., 2002). In a three-dimensional reconstruction of all the lesions in focal brain-damaged patients, density of lesion-overlap was analysed as a function of task performance. The most robust finding was a relation between damage to the right fronto-parietal operculum and compromised detection of emotion from prosody.

Our prediction was that, when compared to sham stimulation, TMS over the right fronto-parietal operculum differentially affects reaction times for detection of emotional prosody when compared to emotional semantics, showing that a) distinct neuroanatomical networks underlie attention to emotional prosody versus emotional semantics and b) the right fronto-parietal operculum is sensitive to specific forms of emotional discrimination (semantic/prosodic) and emotion types (withdrawal/approach emotions), rather then globally involved in emotion evaluation.

Methods

Subjects

Fourteen healthy adult subjects participated in the study after giving written informed consent (19-27 years, mean age 23 years, 8 m/6 f). Handedness was measured with the Edinburgh Handedness Inventory (-24= exclusively left handed, 0=no preference, 24= exclusively right handed) (Oldfield, 1971). All participants were right-handed (mean 21.3, S.D.= 2.6). Subjects were screened for contraindications to TMS, neurological and medical problems. The experiment was conducted in accordance to the Declaration of Helsinki and local ethics board approval (University Medical Center Utrecht).

Apparatus

For TMS, we used a MagStim Rapid magnetic stimulator (MagStim Co, Whitland, Wales) with a figure-of-eight magnetic coil with a diameter of 70 mm for each loop.

Experimental protocol

The off-line TMS experiment comprised two conditions, TMS and sham, each followed by two tasks, emotional semantics and emotional prosody. Order of both the conditions (stimulation over the right and left fronto-parietal operculum for real TMS and sham, respectively) and the tasks was counterbalanced over subjects. Minimum time interval between TMS and sham was 30 minutes to prevent carry-over effects (c.f. (Kosslyn et al., 1999; Oliveri et al., 2004)).

TMS over the fronto-parietal operculum

First, the Motor Threshold (MT) was determined for each subject, defined as the lowest stimulation intensity that induced visible finger movements in at least 5 out of 10 trials when TMS was applied to the motor cortex (Pridmore et al., 1998).

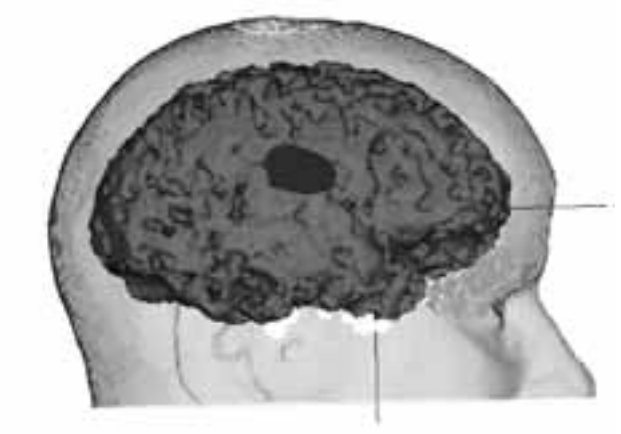
Second, localization of the right fronto-parietal operculum was accomplished individually using structural MRI and neuronavigation by frameless stereotaxy (NeNa) (Neggers et al., 2004). Anatomical T1-weighted MRI scans were used to delineate the fronto-parietal operculum bilaterally in each participant. The fronto-parietal operculum was defined as the inferior peri-central sulcus area (see figure 2) and delineated independently by two separate raters as a Region of Interest (ROI) of approximately 1 to 2 centimeters in diameter, conform the size of the cortical region TMS typically affects (Walsh & Cowey, 2000). Size and location of the ROI was based on the regions with highest lesion-overlap values of the fronto-parietal operculum from the 3-D lesion study by Adolphs et al. (Adolphs et al., 2002).

The structural MRI scan as well as the Region of Interest (ROI) maps, representing the fronto-parietal operculum bilaterally, were loaded into NeNa before the experiment. Six anatomical landmarks were set on the skin rendering. The participant would then be seated, the head fixed in a head support, and a rubber head cap placed over his head. The positions of the same anatomical landmarks were measured with the MiniBIRD position-tracker probe, directly at the head of the participant, and the mapping between 3D space and MRI space was calculated. After co-registration, the fronto-parietal operculum was targeted by 'looking' through the NeNa probe to the renderings of the skin and ROI on the screen and the position directly overlying the ROI was marked on the head-cap. For more details on localization using NeNA system, see (Neggers et al., 2004).

In the stimulation condition, subjects were then stimulated with 1 Hz TMS during 12 min. over the right fronto-parietal operculum, marked at the head-cap, at 90% of the Motor Threshold. These parameters have been applied in earlier studies of cognitive TMS (for an overview see (Robertson et al., 2003)) and have been shown to affect brain metabolism (Mottaghy et al., 2003). The coil
was held manually with the handle pointing backwards and kept tangential to the subject's scalp. In the stimulation condition, subjects were then stimulated with 1 Hz TMS during 12 min. over the right fronto-parietal operculum, marked at the head-cap, at 90% of the Motor Threshold. These parameters have been applied in earlier studies of cognitive TMS (for an overview see (Robertson et

al., 2003)) and have been shown to affect brain metabolism (Mottaghy et al., 2003). In the control condition, sham stimulation was applied during 12 min. over the left fronto-parietal operculum by rotating the coil with 90 degrees, in order to direct the magnetic field away from the brain, but controlling for the characteristic 'click' and sensation on the scalp. Because concerns have been raised that this sham method could still affect brain activity (when rotated with 45 degrees) (Loo et al., 2000), we stimulated at a lower intensity of 35% of stimulator output in the sham condition, and over the left hemisphere in order to avoid weakening of the manipulation, resulting in a null effect.

Figure 2
The fronto-parietal operculum (in black) as a region of interest (ROI), based on anatomical T1 weighted MRI images, projected on a rendering of an individual brain.



Emotion discrimination tasks

Immediately after (sham) stimulation, subjects were required to identify the emotion conveyed by prosody or semantics of a number of sentences. We used the tasks designed by Vingerhoets et al. (Vingerhoets et al., 2003). Each task (prosody and semantics) was assessed twice (after TMS and after sham stimulation), with a different list of stimuli. Each task presentation was preceded by 4 practice sentences. Order of the tasks was counterbalanced over subjects.

Of the 24 sentences in each task, 6 were happy, 6 were sad, 6 were angry and 6 were fearful. Sentences were of approximately equal length and were articulated by two professional actors, one male and one female. The digitized stimuli were presented binaurally through earphones. During listening, the emotions to be discriminated were presented on the computer screen. In the prosody task, affective discrimination was based on the affective tone of voice. In this task, the content of the sentences was not affective (i.e. always neutral). In the semantic task, affective discrimination was based on the semantic content of the sentences. In this task, tone of voice was not affective (i.e always neutral). As soon as they identified the emotion expressed in the sentence, either based on content or tone of voice, subjects were required to pronounce that particular emotion in a microphone that was connected to the computer. Their responses, both selected emotion and reaction time for detection, were collected. Total duration for completing one of either tasks was 7 minutes.

Statistical analyses

Statistical analyses were performed using Statistical Package for the Social Sciences 11.5.0 (2002). Accuracy and reaction times (RT) for detection of emotion in semantics and in prosody were chosen as dependent variables. Reaction times for incorrect responses were excluded from analyses. 2x2 GLM repeated measures analyses with TMS (stimulation, sham) and Task (prosody, semantics) as independent factors were used to test the effects of TMS on the two tasks. Post-hoc analyses were performed using paired T-tests. P values were set at 0.05, two tailed.

Results

Mean Motor-Threshold was 49.7% (SD=3.7%) of stimulator output.

Accuracy

No significant TMS by Task interaction was observed for accuracy in detection of emotion in all four conditions ($F(1,13)=0.42$, $p=0.53$). At baseline (sham condition), participants were significantly more accurate in identifying emotion in semantics compared to prosody ($t(1,13)=2.98$, $p=0.01$).

Regarding the different types of emotions, again no TMS by Task interaction was observed for withdrawal emotions, i.e. fearful and sad, ($F(1,13)=0.11$, $p=0.74$) and approach emotions, i.e. anger and happy, ($F(1,13)=0.92$, $p=0.35$). At baseline (sham condition), paired t-tests showed that participants were significantly more accurate at identifying approach emotions when compared to withdrawal emotions in both the prosody task ($t(1,13)=4.74$, p,0.001) and the semantics task ($t(1,13)=3.48$, $p=0.004$), which is in accordance with other studies. Accuracy scores are presented in table 1.

Table 1

Percentage correct identified emotions in all conditions (mean, SD).

	% correct all emotions	% correct withdrawal emotions	% correct approach emotions
Sham prosody	84.2 (8.2)	73.2 (15.4)	94.6 (5.3)
TMS prosody	81.5 (9.7)	70.8 (14.5)	92.3 (7.6)
Sham semantics	93.7 (4.8)	89.9 (8.1)	97.6 (3.9)
TMS semantics	94.0 (4.8)	89.8 (7.4)	98.2 (3.5)

Reaction times

Only reaction times for correct responses were included in the analyses.

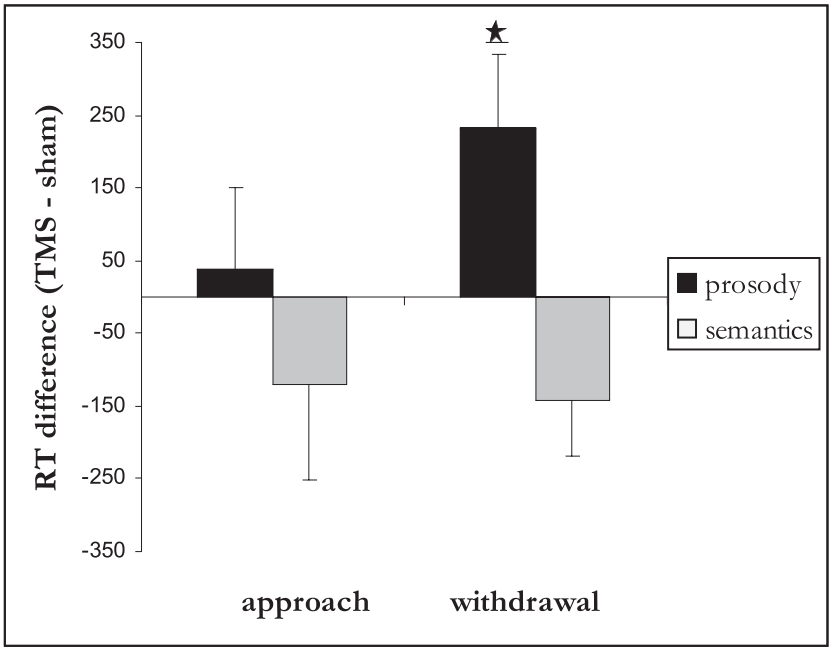
First, we explored TMS effects on emotional prosody versus emotional semantics when all emotions (happy, sad, anger, fear) were taken together. As expected, reaction times for detection of emotion conveyed by prosody were shorter when compared to emotion in semantics ($t=-3.7$, $p=0.002$). Emotion in tone of voice can be detected well before completion of the sentence, whereas critical words can be positioned at the end of a sentence when relying on semantic information.

Regarding reaction times for detection of emotion in all four conditions, a significant TMS by Task interaction was present ($F(1,13)=9.31$, $p=0.009$). Post-hoc tests failed to reach significance ($p=0.32$ and $p=0.06$, for prosody and semantics respectively), which implies that the effect is driven by a combination of both a decrease in semantic reaction times and an increase in prosody reaction times.

In the crucial analysis, the four types of emotions were grouped into two different categories; withdrawal (fearful and sad) versus approach (anger and happy). For withdrawal emotions, again a significant TMS by Task interaction appeared ($F(1,13)=9.13$, $p=0.01$). Post hoc tests revealed a significant effect of TMS on reaction times for detection of withdrawal emotions in the prosody condition ($t(1,13)=2.34$, $p=0.03$). The mean reaction times increased from 3554 ms. (SD=576) at sham to 3788 ms. (SD=813) after TMS. In the semantics condition, the effects of TMS on reaction times for detection of withdrawal emotions did not reach significance ($t(1,13)=-1.87$, $p=0.08$). In this condition the mean reaction times decreased from 3808 ms. (SD= 356) at sham to 3664 ms. (SD=344) after TMS.

Interestingly, for approach emotions, happy and anger, no significant interaction between TMS and Task was observed ($p=0.26$). Results are presented in figure 1.

Figure 1
TMS effects on detection of approach emotions (happy, anger) and withdrawal emotions (fear, sad) in prosody versus semantics (mean, SE).
(★ t(1,13)=2.34, p=0.036)



Discussion

The present study used Transcranial Magnetic Stimulation (TMS) to investigate the contribution of the fronto-parietal operculum in the right hemisphere to discrimination of emotions from prosodic and semantic information in spoken language. As predicted, TMS over the right fronto-parietal operculum differentially affected reaction times for detection of emotional prosody when compared to emotional semantics, indicating that at least partly separable neural networks are involved. Specifically, in the prosody condition detection of withdrawal emotions (fear and sadness), but not approach emotions (happiness and anger), was delayed by TMS over the right fronto-parietal operculum, suggesting that this region is crucial for detection of withdrawal emotions in prosody.

The neuroanatomical dissociation as revealed by the present study, is consistent with lesion, and neuroimaging studies, indicating that processing of emotional information from prosody and semantics may be differentially

localized in the brain (Baum & Pell, 1999; R. L. Mitchell et al., 2003; Vingerhoets et al., 2003). Whereas these studies are in essence correlational in nature, TMS allows a causal interpretation of the data. By using TMS in combination with frameless stereotaxy, we were not only able to attribute the changes in performance to disruptions in neural processing caused by TMS, but also link performance to a specific, well defined area in the brain. As shown in the present study, the fronto-parietal operculum in the right hemisphere appears to be part of a neural network selectively involved in detection of emotion, depending both on emotion type (i.e. withdrawal emotions) and whether information is conveyed by semantics or prosody.

The present evidence that emotion perception might be mediated by different neural substrates, depending on the communicative channel through which it is presented, is at odds with the 'right hemisphere dominance hypothesis', postulating that the right hemisphere is specialized for emotion evaluation, regardless of processing mode, such as lexical or prosodic (Blonder et al., 1991; Borod et al., 1993). It has been argued that right somatosensory areas are important understanding emotions of others and contribute to recognizing emotions by creating somatosensory representations based on internal simulation (Adolphs et al., 2000; Damasio, 1994). The fronto-parietal operculum forms part of the secondary somatosensory cortex where the lips, jaw and tongue are represented. Although speculative, specific knowledge of the emotional state of a speaker may be retrieved by reactivating a neural pattern in the fronto-parietal operculum that simulates emotion expressed in non-verbal cues in spoken language.

More specifically, it has been proposed that the somatosensory cortices may be an 'affective convergence zone', where emotion representations are formed not only irrespective of modality, but also aspecific for discrete emotions (Adam K. Anderson & Phelps, 2000). Findings from a recent study of patients with localized brain lesions shows that the somatosensory cortices are critical for understanding a broad range of emotional states from facial expressions (Adolphs et al., 2000). However, the present study shows specific effects for different emotion categories, suggesting that involvement of somatosensory areas may depend on specific emotion types. Indeed, there is evidence from studies with right hemisphere damaged patients who show specific deficits in detection of withdrawal emotions on faces, such as fear and sadness, in contrast to normal processing of approach emotions (A. K. Anderson et al., 2000; M. K. Mandal et al., 1999). A very recent TMS study has measured the effects of TMS over the right somatosensory cortex on perception of facial expressions of fear

(withdrawal emotion) and happiness (approach emotion) (Pourtois et al., 2004). Interestingly, only perception of fear, and not happiness, was disrupted by TMS over the right somatosensory cortex. Our findings are in line with this study, supporting the hypothesis that right somatosensory areas are important for perception of withdrawal emotions both in facial expressions and spoken language.

Not only visual perception, also experience and expression of withdrawal emotions, but not approach emotions, seem to be mediated by the right hemisphere. PET (Positron Emission Tomography) and EEG (Electroencephalograph) studies have revealed that induced negative affective states that are withdrawal-related, as well as production of facial poses of withdrawal emotions, are associated with increased activation in cortical regions in the right hemisphere (Coan et al., 2001; Davidson et al., 2000). Thus, in addition to a right hemispheric specialization in *expression* and *perception* of facial withdrawal emotions as well as *experience* of withdrawal emotions as suggested by these studies, the present data suggest the right hemisphere is crucially involved in *prosodic perception* of withdrawal emotions.

Interestingly, a trend in decreasing reaction times for *semantic perception* of (withdrawal) emotions was observed after TMS to the right fronto-parietal operculum. Although this trend effect should be interpreted with caution, it may point to facilitation of semantic processing after RH TMS. Facilitation effects in previous TMS studies have been explained by disinhibition of areas that are connected to the region exposed to TMS or loss of competition between two brain areas after TMS (Walsh et al., 1999). In the present study, the decrease in reaction times for detecting emotion in semantics after TMS to the right fronto-parietal operculum, may for example result from loss of competition between this area and the right ventrolateral prefrontal cortex (PFC) that is involved in linguistic aspects of emotion (Hariri et al., 2000). Hence, the ventro-lateral PFC may process semantic information more efficiently. An alternative explanation may be that subjects are automatically screening both semantic and prosodic aspects of spoken language. When processing of prosodic information is disrupted by TMS, subjects may have more processing capacity for analysing semantic information.

An important issue regarding our TMS effects concerns the potential contribution of differences in difficulty between approach and withdrawal emotions. Consistent with the literature, withdrawal emotions were more difficult to detect than approach emotions (REF), which might yield them more sensitive to the effects of TMS. However, whereas this higher difficulty of

withdrawal emotions applies to both semantic and prosodic tasks, TMS effects were only observed on reaction times in the prosodic task. Moreover, in the sham condition, faster response times were observed in the prosody condition compared to the semantics condition, suggesting that by this index prosody was easier and thus should be less influenced by TMS disruption. Both findings suggest a pattern of TMS effects that is the opposite of what was observed if difficulty is confounded with TMS disruption. With this in mind, it may be important to note that reaction times analyses were limited to correct responses, so any effects of speed-accuracy trade-off were eliminated from the analyses. Although we cannot completely exclude that difficulty might play a role, as this is inherent to the approach-withdrawal dichotomy, our results are consistent with evidence from lesion-studies (A. K. Anderson et al., 2000; M. K. Mandal et al., 1999), which lends credence to our interpretation. The present study shows that right hemisphere TMS selectively interferes with processing of withdrawal emotions, which might imply that approach emotions are less mediated by the right hemisphere. This interpretation is consistent with a recent meta-analysis of 106 imaging studies of emotion that revealed a significant difference in the spatial distributions associated with withdrawal and approach emotions (Murphy et al., 2003). Processing of approach emotions was stronger associated with left hemisphere activation compared to the right, supporting the idea that differences in functional neuro-anatomy, rather than differences in difficulty level, can explain our results regarding approach and withdrawal emotions.

A related issue is that TMS was applied to the right hemisphere only, which is a limitation of the present study. Future studies should incorporate a more complex experimental design including TMS over the right as well as left hemisphere. This design would allow conclusions regarding *lateralization* of a) emotional prosody and emotional semantics and b) withdrawal and approach emotions.

Another concern could be that our effects of TMS on prosodic perception of emotions could be due to stimulation of the nearby auditory cortex. Although we can not exclude that unintended stimulation of auditory cortex can have affected our findings, the fact that we found specific effects for withdrawal emotions only is not consistent with this explanation. If TMS would have disrupted auditory analysis as a result of stimulation of the auditory cortex, we would have expected uniform TMS effects on prosodic perception across all types of emotions. Besides this argument regarding functional specificity, we would also like to point out that localisation of stimulation site

using an MRI-guided neuronavigator has been shown to have a high anatomical specificity,(i.e. of 5 mm, (Neggers et al., 2004)). The targeted area was always above the STG (superior temporal gyrus).

In sum, the present findings indicate that at the level of the somatosensory cortex, evaluation of emotional meaning appears to rely on distinct neural networks that are sensitive to a) the communicatieve channel through which emotional information is presented and b) types of emotions. Our data suggest that at least part of the somatosensory cortex, the right fronto-parietal operculum, may not serve a global function in comprehension of emotional states of others, but appears to be part of a modality-specific neural network. These findings may contribute to our understanding of how socially relevant information is processed at different levels of organization in the brain.

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Chapter 7

What is said versus *how* it is said: Comprehension of affective prosody in men with Klinefelter (47,XXY) syndrome

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Abstract

Difficulties in social communication in individuals with Klinefelter syndrome (XXY chromosomal pattern) have largely been attributed to deficits in left hemisphere mediated, language functions. This study examined the ability of XXY men to decode emotions from tone of voice, a pragmatic aspect of social communication that may be associated with right hemisphere functioning.

26 XXY men and 20 men from the general population completed tasks involving emotion discrimination in speech, based on verbal content or tone of voice.

The XXY group displayed relative difficulties in discriminating emotions in tone of voice, and, to a lesser extend, in verbal content.

This suggests that the XXY chromosomal pattern may not only be associated with difficulties in semantic aspects of language, but with prosodic aspects, as well. Our findings may contribute to the development of more comprehensive models addressing the role of the X chromosome in normal and abnormal development of social communication.

Introduction

Klinefelter syndrome is a genetic disorder that affects approximately 1 in 700 men. Men with this disorder have an extra X chromosome, creating the 47,XXY chromosomal pattern. Although general intelligence is within the normal range, XXY men display specific deficits in brain development and cognition. Behavioral problems in men with Klinefelter syndrome are found in the social domain, such as social withdrawal, social anxiety, shyness, impulsivity and inappropriate social behavior (Bender et al., 1999; Geschwind et al., 2000; Ratcliffe, 1999).

It has been proposed that difficulties in social interactions, and specifically those related to communication, are largely attributable to disabilities in the language domain that have been observed in XXY boys and men (Rovet et al., 1996). The reported verbal disabilities in Klinefelter syndrome include impairments in both language production and perception, and indicate compromised semantic language functions that are typically associated with the left hemisphere (Boone et al., 2001; Rovet et al., 1996). For example, Klinefelter boys and men may display disabilities in reading, articulation, phonemic processing, spelling, language expression, verbal memory, language comprehension, understanding words, finding words and verbally expressing their thoughts, all resulting in a verbal IQ that is somewhat lower than their performance IQ (Boone et al., 2001; Geschwind et al., 2000). Some of these language dysfunctions have been related to left hemisphere abnormalities in Klinefelter syndrome (Itti et al., 2006).

Although deficits in *semantic* language functions have been widely described for XXY men and boys, it is not yet clear whether deficits in understanding *affective prosodic* aspects of language are present. The term prosody has been introduced to describe non-semantic cues in spoken language. Prosodic cues can have linguistic- as well as affective functions. Linguistic functions are, for example, emphasizing important parts of the message or presenting information as a statement or a question. Affective functions of prosody are also important for understanding intentions of others, because variations in tone of voice, such as intonation and loudness, provide information about the emotional state of the speaker. Thus, the emotional relevance of a spoken message may not only be conveyed by the meaning of words (i.e. emotional semantics), but also expressed in emotional prosody. Although semantic processing may be more lateralized to the left hemisphere, affective prosody represents a pragmatic aspect of language that may be lateralized to the right hemisphere (Mitchell et al., 2003; Ross et al., 1997). Indeed, deficits in

perceiving and understanding emotional prosodic aspects of language are often seen after damage to the right hemisphere and may lead to difficulties in social communication (for an overview see Joanette et al., 1990). In a functional magnetic resonance imaging (fMRI) study, Mitchell et al. (2003) found that attention to semantics increased activation in widespread left hemisphere regions, whereas attention to emotional prosody increased activation in generally right-sided brain regions. Importantly, subtracting the activation associated with semantic processing from the activation during affective prosody processing resulted in a pattern of almost exclusively right sided activation. Vingerhoets et al. (2003) have measured blood flow velocity (BFV) with functional transcranial doppler ultrasonography (fTCD) to study the contribution of the right and left hemisphere to the detection of emotion in prosody versus detection of emotion in semantics of spoken language. During detection of emotion in semantics a significant left-hemispheric lateralization of BFV was observed. This lateralization effect disappeared when attention was shifted to discriminating emotion in prosody, due to a rise in right hemispheric BFV. Using identical tasks, (Van Rijn et al., 2005) we have been able to provide evidence that the right hemisphere plays a crucial role in processing affective prosody. In that study we examined the effects of transcranial magnetic stimulation (TMS) over the fronto-parietal operculum in the right hemisphere on affective prosody detection. Temporarily disrupting neural processing in the right hemisphere delayed reaction times in detecting specific emotions in prosody but not in verbal content, which suggests that this region within the right hemisphere is critically involved in processing emotional prosody.

The current study is the first exploration of affective prosody performance in men with the XXY karyotype. We contrasted the ability to discriminate emotions in prosody with the capacity to discriminate emotions based on verbal content (semantics) in both XXY men and men from the general population. We used affective prosodic and affective semantic stimuli designed by Vingerhoets et al. (2003). Findings from the abovementioned TMS, fTDC, fMRI and lesion studies, which used tasks identical or very similar to ours, suggest that the task we used for assessing emotion detection in prosodic cues versus semantic cues, may be considered as task constrasting language functions that are either more lateralized to the right or the left hemisphere, respectively. In sum, we investigated the ability of XXY men and control men from the general population to label emotions based on prosodic versus semantic cues in spoken language. In addition, we assessed performance in a spatial, 3D mental rotation, task that allowed us to compare performance of both groups in a

cognitive task outside the language domain. We hypothesized that in Klinefelter syndrome, deficits are present in discriminating emotions not only based on verbal content, but also on tone of voice.

Methods

Subjects

Twenty-six men with Klinefelter syndrome (mean age 42.7, SD 8.7) were recruited from the Dutch Klinefelter Association. Diagnosis of Klinefelter syndrome was confirmed by karyotyping, using standard procedures. Twenty-two men were treated with testosterone supplements, with a mean age of treatment onset of 26.9 years (SD 6.8).

Twenty male controls from the general population (mean age 38.6, SD 13.3) were recruited using advertisements in local newspapers or were drawn from an already existing database in our department. None of the control subjects had a history of psychiatric illness as confirmed with the Mini International Neuropsychiatric Interview plus (MINI) (Sheehan et al., 1998).

There were no significant differences in age between the groups ($t(1,43)=-1.1$, $p=0.26$). All subjects in the study were right handed. Mean handedness score, as indicated by the Edinburgh Handedness Inventory (Oldfield, 1971), in the Klinefelter group (17.6, SD 12.8) did not significantly differ from that in controls (17.8, SD 13.3) ($t(1,43)=0.23$, $p=0.98$). Scores can range from -24 (completely left handed) to 24 (completely right handed).

The study was approved by the local ethics committee and written informed consent was obtained according to the declaration of Helsinki.

General Intelligence

Raven's Advanced Progressive Matrices (short form)

This test is commonly accepted as a measure of general intelligence and has been shown to correlate with a number of standardized intelligence tests (Lezak, 1995). Subjects are shown 12 pictures of matrices (i.e., related patterns), each of which is a figural design with a part removed. The subject must choose the correct missing part from eight options.

National Adult Reading Test (NART)

The Dutch translation of the NART (Nelson, 1982; Schmand et al., 1991) provides an estimate of verbal IQ and is based on the high correlation between reading ability, specifically of irregular words, with intelligence in the normal

population. Subjects are required to read 50 irregular words aloud, and, on the basis of the number of errors made in pronunciation a reliable estimate of WAIS-R IQ can be calculated (Willshire et al., 1991).

Experimental tasks

Emotion discrimination in speech

Subjects were required to identify the emotion conveyed by prosody or semantics of a number of sentences. We used an emotion discrimination task with 4 conditions designed by Vingerhoets et al. (2003). In the 2 prosody conditions, subjects were required to discriminate emotions based on the affective tone of voice and ignore the semantic content. In the simple version of the prosody task, the semantic content was always neutral. In the complex version of the prosody task, the semantic content was affective and always incongruent with the prosodic emotion. In the 2 semantic conditions, subjects were required to discriminate emotions based on the semantic content of the sentences and ignore the tone of voice. In the simple version of the semantics task, tone of voice was always neutral. In the complex version of the semantics task, tone of voice was affective and always incongruent with the semantic emotional content. See table 1 for exemples of sentences in the four conditions. Subjects were asked to orally report the perceived emotion as fast as possible. Voice onset times were collected with a microphone and selected emotion was recorded. Of the 24 sentences in each task, 6 were happy, 6 were sad, 6 were angry and 6 were fearful. Each task presentation was preceded by 4 practice sentences. Order of the four conditions was quasi-randomized. Time for completion of one of the four conditions was approximately 7 minutes.

Table 1

Examples of sentences in the four conditions of the emotion discrimination task.

Emotion discrimination condition	Examples of sentences	Correct answer
Prosody simple	'The battery will charge automatically' (angry tone of voice)	angry
Prosody complex	'He misses her especially in the evenings' (happy tone of voice)	happy
Semantics simple	'Her husbant gave her a nice present' (neutral tone of voice)	happy
Semantics complex	"His best friend is in a deep coma' (happy tone of voice)	sad

Mental rotation task

The mental rotation test was an adaptation of original test by Shepard and Metzler (1971). In this computer task, participants were asked to compare two simultaneously presented objects composed of 10 cubes, and determine whether they were identical or not. All objects were two-dimensional representations of three-dimensional objects, rotated at different angles. In 50 % of the trials the objects were identical. Participants were given 6 min. to complete 20 of these items. Number of correct responses was collected.

Results

General Intelligence

Mean estimated IQ, as measured with the Raven's Advanced Progressive Matrices (short form), in the Klinefelter group (102.9, SD 11.3) did not significantly differ from that in the control group (109.6, SD 14.3).

Mean estimated verbal IQ, as measured with the National Adult Reading Test (NART), also did not significantly differ between the Klinefelter group (107.4, SD 15.6) and the control group (110.8, SD 5.1).

Experimental tasks

Emotion discrimination in speech

Significant group differences were present as shown by a General Linear Model (GLM) multivariate test including all four conditions of the emotion discrimination task ($F(2,41)=7.7$, $p=.001$).

Significant group differences were present in the number of errors made in the simple prosody condition ($F(1,42)=13.2$, $p=.001$). Klinefelter men made more errors than controls. Also in the complex prosody condition, Klinefelter men made more errors in discriminating emotions than controls ($F(1,42)=15.4$, $p<.001$). When corrected for performance in the semantics task (using MANOVA), the group difference in performance in the prosody conditions remained significant ($F(1,43)=9.8$, $p=0.003$ and $F(1,43)=9.6$, $p=0.003$ for the simple and complex prosody conditions respectively).

Furthermore, in the simple semantics condition as well as in the complex semantics condition, Klinefelter men made more errors as compared to controls ($F(1,42)=4.5$, $p=.04$ and $F(1,42)=7.4$, $p=.009$ respectively). All means and SD's are presented in Table 2.

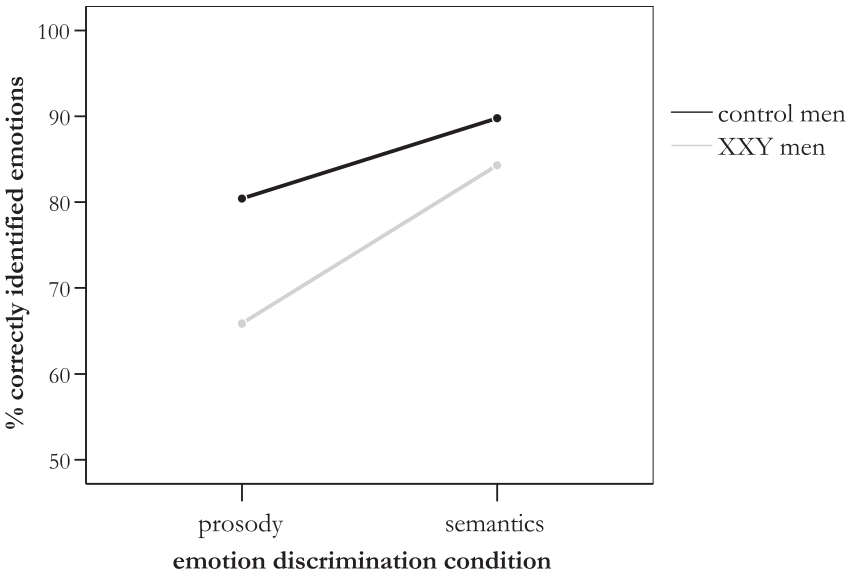
Reaction times in the Klinefelter group were significantly higher in all four conditions ($F(4,41)=4.4$, $p=.005$) as indicated by a GLM multivariate test.

Table 2
Percentages correct responses (mean, S.D.) in the four different conditions of the task measuring emotion discrimination in speech.

Emotion discrimination condition	Klinefelter (mean % correct , S.D.)	Controls (mean % correct , S.D.)
Prosody simple	65.8 (15.8)	80.4 (9.9)
Prosody complex	59.6 (19.7)	78.9 (9.9)
Semantics simple	84.3 (8.6)	89.8 (9.6)
Semantics complex	75. 8 (25.3)	93.7 (13.0)

Interactions
A GLM repeated measures analysis revealed a significant group (Klinefelter, control) by condition (simple prosody, simple semantics) interaction ($F(1,44)=4.0$, $p=.05$), showing that, although Klinefelter men showed deficits in both conditions, they were more impaired in the simple prosody condition than in the simple semantics condition (see figure 1). A GLM repeated measures analyses showed there were no group (Klinefelter, control) by condition complex ('simple', 'complex') interactions when attending to emotional prosody or -semantics. This indicates that the difference in performance between the simple and complex conditions was not different between XXY men and controls.

Figure 1
There was a significant group by condition (prosody, semantics) interaction (F(1,44)=4.0, p=.05) showing that although Klinefelter men showed deficits in both conditions of the emotion discrimination task, they were more impaired in detection of emotions based on tone of voice than based on verbal content.



Mental Rotation task
No significant group differences were present in performance on the mental rotation task. Mean percentage correct in the Klinefelter group was 66.4 % (SD 19.4), in the control group 72.2 % (SD 17.5).

Discussion

This is the first study in men with the XXY karyotype that explores the understanding of pragmatic aspects of language. We compared comprehension of emotional prosody, i.e. aspects of speech such as loudness and intonation that convey information about the emotional state of the speaker, between men with the XXY karyotype and men from the general population. We contrasted the ability to discriminate emotions in prosody, which may be more lateralized to the right hemisphere, with the capacity to discriminate emotions based on verbal content (semantics), which is more lateralized to left hemisphere. Our findings show that XXY men have difficulties in discriminating emotions in verbal content of speech and, even more so, in tone of voice.

The XXY group showed deficits in identifying emotions from prosodic cues as well as from semantic cues. These impairments were observed in the simple versions of the task, where either the semantic content or the tone of voice was neutral, as well as in the complex versions of the task, where semantic content and tone of voice were emotional as well as incongruent. The finding that XXY men display semantic deficits in our study was expected considering the literature on language deficits in Klinefelter syndrome. However, both the condition by group interaction and the finding that XXY men made significantly more errors in the affective prosody condition even when corrected for their performance in the semantics condition, suggested that on top of this semantic deficit, even more profound deficits are present in the prosody task. In the study of Vingerhoets et al. (2003), the observed significant bilateral increase in BFV during the complex versions of this tasks as compared to the simple versions were prosposed to reflect a general increase in attentional demand as the level of BFV change is thought to be associated with task demand and reflects the attentional capacity necessary to perform a task (Vingerhoets et al., 2001). As no effects of group on the difference in performance between the simple and complex tasks were present, this might suggest that the specific deficits observed in XXY men were not due to a general difficulty in attending to the stimuli. Importantly, as performance on a spatial, 3D mental rotation task was not impaired in the XXY group, the language deficits may not be attributable to a generalized reduction in cognitive performance.

Difficulties in picking up pragmatic communicative cues in conversation might have an impact on how well XXY men cope with social situations, as these aspects of language are important for understanding interpersonal intentions and responding to those in an appropriate way. The present findings show that Klinefelter syndrome may not only be associated with difficulties in identifying emotions based on facial expressions (Van Rijn et al., 2006), but also when expressed in tone of voice. Besides providing insight in social cognitive impairments that might underlie difficulties in social communication and coping with a social environment, the present findings may have several potential implications.

First, our findings suggest that not only language functions typically mediated by the left hemisphere, but also prosodic aspects of language which may be associated with the right hemisphere, are affected in Klinefelter syndrome. Although speculative, this would be in line with the reported abnormalities in language-related functioning of the right hemisphere. A

SPECT study (single-photon emission computed tomography) has shown reduced hemispheric asymmetry in cerebral flood flow in XXY men, with increased cerebral blood flow in the right hemisphere related to difficulties in verbal skills (Itti et al., 2003). Also, a functional neuroimaging study with XXY men has revealed reduced lateralization of brain activation in language regions during verbal tasks, due to increased activation in the right hemisphere, rather than decreased activation in the left hemisphere (Van Rijn et al., submitted). However, an alternative explanation might be that defects in integrated bi-hemispheric processing underlie the observed deficts in discrimination affective prosody in Klinefelter syndrome. Imaging studies are needed to explore the exact neural underpinnings of affective prosody deficits in XXY men.

Second, as Klinefelter syndrome is characterised by an X chromosomal abnormality, we might extrapolate from these findings that the X chromosome influences some aspects of language, semantic aspects that are associated with the left hemisphere as well as emotional prosodic aspects that are associated with the right hemisphere. Support for a role of the X chromosome in development of the ability to decode emotional prosody is derived from studies with females with Turner syndrome, another X chromosomal disorder that is characterised by the presence of only one X chromosome in females (i.e. the X0 karyotype). Similar to men with the XXY chromosomal pattern, females with X monosomy also display impairments in perception of emotions in tone of voice (Ross et al., 1995).

In sum, this study has shown that the XXY chromosomal pattern may be associated with difficulties in discriminating emotions in verbal content of speech and, even more, in tone of voice. Besides revealing prosodic deficits that might underlie communicative difficulties and social dysfunction in XXY men, our findings may contribute to the development of more comprehensive models addressing the role of the X chromosome in normal and abnormal development of social communication.

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Part III

Neural basis of social cognition

Chapter 8

Effects of an extra X chromosome on language lateralization: an fMRI study with Klinefelter (XXY) men

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Abstract

De novo occurring genetic variations provide an opportunity to study the effects of genes on structure and function of the brain. In this regard, Klinefelter syndrome, characterized by a XXY chromosomal pattern, is of significant interest. Although general intelligence is average in XXY men, prominent effects of the extra X chromosome on cognition are observed in the language domain. One possible neural mechanism underlying these language deficits is reduced hemispheric specialization for language. However, there has been no study of brain activity patterns underlying language processing in XXY men. Also, the consequences for mental functioning in XXY men are as yet unresolved. The clinical relevance of exploring mental consequences in Klinefelter syndrome is illustrated by the finding that Klinefelter men manifest symptoms that are seen in schizophrenia. A possible commonality between these disorders is that both are characterized by language deficits.

We used functional Magnetic Resonance Imaging (fMRI) to reveal the effects of an extra X chromosome on language lateralization. This technique allows us to identify functional asymmetries in specific brain regions as well as to determine whether reduced lateralization, if found, is secondary to decreased function of the left- or increased activity in the right hemisphere. We explored the relation between loss of language lateralization and mental functioning in these men, with special interest in clinical phenomena of disorganization of thought and language.

Hemispheric dominance for language was assessed in 15 XXY men and 14 control men using fMRI. In each hemisphere, activity in five different language regions was analyzed: Broca's area, superior temporal gyrus, middle temporal gyrus, angular gyrus and supramarginal gyrus. Psychopathology was measured using the Positive and Negative Syndromes Scale for measuring schizophrenia symptoms and a schizotypal personality questionnaire.

Compared to controls, the XXY group showed reduced hemispheric specialization for language. This was due to increased activity in the language areas of the right hemisphere rather than reduced activity in the left hemisphere. Decreased functional asymmetry was most prominent in the superior temporal gyrus and correlated with symptoms of disorganization.

These findings may suggest that a genetic mechanism involving the X chromosome contributes to hemispheric specialization for language, since loss of language lateralization, most prominent in the superior temporal gyrus, was observed in this X chromosomal disorder. Moreover, loss in hemispheric specialization for language processing may have important consequences for mental functioning, as it was associated with dysfunctions in organization of thought and language.

Introduction

The study of de novo occurring genetic variations that are associated with neural, cognitive and behavioral abnormalities may increase our understanding of complex gene-brain-behavior relations. In this regard, Klinefelter syndrome, which is defined by the presence of an additional X chromosome in men, is of significant interest. Although general intelligence is average in XXY men, prominent effects of the extra X chromosome on cognition have been observed, particularly in the language domain (D. H. Geschwind et al., 2000a). The reported verbal disabilities include impairments in both language production and perception and indicate compromised language functions that are typically associated with the left hemisphere (Samango-Sprouse, 2001). For example, Klinefelter boys or men have difficulty understanding words, finding words and to verbally express their thoughts, all resulting in a verbal IQ that is lower than their performance IQ. As language is a crucial part of social communication, it has been proposed that these language impairments may contribute to the observed difficulties in social functioning in Klinefelter men (Samango-Sprouse, 2001; van Rijn et al., 2006b).

Although language impairments in Klinefelter syndrome are well-documented, underlying neurocognitive mechanisms are not well understood (D. H. Geschwind et al., 2000a). One possible neural mechanism involved in the verbal disabilities in Klinefelter syndrome may be abnormal hemispheric involvement in language processing. Although the left hemisphere is dominant for processing verbal information in right-handed individuals from the general population, there are indications that this dominance is diminished in XXY men. First, decreased language lateralization has been shown in a study of 32 XXY boys using a dichotic listening paradigm (Netley & Rovet, 1984). Second, in a more recent SPECT study, resting state blood flow patterns were more symmetrical in nine XXY men as compared to nine healthy controls (Itti et al., 2003). In that study, increased right temporal lobe blood flow was related to verbal impairments as measured with neuropsychological tasks assessed in the same week as the SPECT-session. Although these studies provide some indication that loss of hemispheric asymmetry in the processing of language may be a characteristic of the XXY phenotype, it is unclear whether the decreased language lateralization is secondary to decreased function of the left or increased activity in the right hemisphere. Moreover, it remains unresolved which specific language regions within the hemispheres are more bilaterally involved in processing language in XXY men.

Another reason for studying language lateralization in Klinefelter syndrome is that the consequences for behavioral and psychological or mental functioning in XXY men are as yet unresolved. The importance of considering this issue is illustrated by reports of reduced hemispheric specialization for language in patients with schizophrenia. In these patients, loss of language lateralization is thought to be related to language impairments as well as clinical symptoms associated with language, such as auditory hallucinations ('voices') or disorganization of language and thought (Kircher et al., 2002; I. E. Sommer et al., 2001b). Indeed, Klinefelter men manifest symptoms that are also observed in schizophrenia patients (van Rijn et al., in press), including disorganization and thought disorder. Moreover, the frequency of the XXY chromosomal pattern has been reported to be increased in patients with schizophrenia as compared to the general population (L.E. DeLisi et al., 1994).

To assess hemispheric specialization for language in XXY men, we used functional Magnetic Resonance Imaging (fMRI). By using fMRI we are able to investigate lateralization of neural activity during language processing in XXY men. In addition, this technique allows us to identify specific language regions in which lateralization is diminished. Our second aim was to investigate the link between loss of language lateralization and mental functioning in these men, with special interest in those symptoms and personality traits pertaining to organization of thought and language, that are also seen in the schizophrenia spectrum.

Methods

Subjects

15 XXY men (mean age 36.9, SD 11.8) and 14 healthy control men (mean age 35.5, SD 9.5) participated in the fMRI study. XXY men were recruited from the Dutch Klinefelter Association, and were not selected for psychological, behavioral or cognitive abnormalities. Diagnosis of Klinefelter syndrome was confirmed by genetic analysis (i.e. karyotyping) using standard procedures. Of the XXY men, 14 were treated with testosterone supplements (mean age of treatment onset of 23.9, SD 7.1 years).

Controls were recruited using advertisements in local newspapers or were drawn from a database in our department. None of the control subjects had a history of psychiatric illness as confirmed with the Mini International Neuropsychiatric Interview plus (MINI) (Sheehan et al., 1998).

The primary language of all participants was Dutch. There were no significant

differences in age (t(1,27)=-1.1, p=0.26) or years of education between the groups (Klinefelter group 15.8 (SD 2.0), control group 15.4 (SD 1.8), t(1,27)=0.41, p=0.68). All participants were right-handed. Mean handedness score, as indicated by the Edinburgh Handedness Inventory (Oldfield, 1971), in the Klinefelter group (21.21, SD 3.1) did not significantly differ from that in controls (22.1, SD 2.1) (t(1,27)=0.85, p=0.40).

Exclusion criteria for both Klinefelter men and controls were neurological conditions or history of head injury with loss of consciousness, recent history of substance abuse and mental retardation. After complete description of the study to the subjects, written informed consent was obtained according to the declaration of Helsinki.

Schizophrenia psychopathology

Schizophrenia spectrum pathology was only measured in the Klinefelter group. Schizophrenia spectrum pathology in a larger population of Klinefelter men at our department as compared to healthy controls has been described elsewhere (van Rijn et al., in press). Schizotypal traits were measured using the Schizotypal Personality Questionnaire (SPQ) (Raine, 1991). The SPQ is a self-report measure of schizotypal personality traits, which have shown to be normally distributed in the general population. It is regarded as an indicator of the genetic vulnerability to schizophrenia, since there is a gradient increase in schizotypal traits in relatives of schizophrenia patients that is in proportion to the risk for schizophrenia associated with the degree of kinship with the schizophrenic family member (Vollema et al., 2002). Factor analytical studies (Vollema & Hoijtink, 2000) have revealed three dimensions of schizotypy, being (a) *Positive schizotypy* (for example referential thinking and delusional atmosphere), (b) *Negative schizotypy* (for example constricted affect and social anxiety), and (c) *Disorganization* (odd speech and eccentric behavior).

A clinical measure of schizophrenia symptoms was also included. The Positive and Negative Syndromes Scale (PANSS) (Kay et al., 1987) is a widely used structured interview to assess symptom profiles in schizophrenia patients that are present in the week prior to the interview. The PANSS allows categorization of negative, positive, and general symptoms.

Language tasks

Language tasks were adopted from Ramsey et al. (2001). In the MRI scanner subjects completed three different tasks that have been shown to activate language areas in the brain, being a paced verb generation task, an antonym

generation task, and a semantic decision task. Difficulty of the tasks was set at a level that would allow Klinefelter men to perform comparable to healthy controls. All subjects practiced the tasks with a different set of stimuli before the scan session. In all tasks, a word was presented visually every 3 seconds and consisted of four to eight characters. Silent vocalization (i.e. without overt articulation) was used to avoid head motion. Each task was performed during 5 blocks of 29 s, alternated with rest periods of 29 s. A total of 360 functional scans were collected for each individual.

In the verb generation task, subjects were instructed to repeat a visually presented noun silently and subsequently silently vocalize an appropriate verb for the presented noun. In the antonym generation task, subjects had to think of a word that was of opposite meaning in response to the visually presented word. In the control condition for these tasks a number of dots, equal to the number of characters in the presented words, appeared on the screen. Finally, in the semantic decision task, subjects had to indicate, by pushing a button, whether the visually presented word was an animal. The control task also included button presses, which were cued by the presence of five dots (either three or five dots appeared). Performance was registered with a computer. Performance in the verb- and antonym generation tasks was assessed outside the scanner. Each task included 45 words, which were different from those presented during scanning, but presented with an identical interstimulus interval.

Scans

Scanning technique was adopted from Ramsey et al. (2001). Functional scans were acquired with a Phillips ACS-NT 1.5-T clinical scanner, using the blood-oxygen-level dependent sensitive, navigated 3D PRESTO pulse sequence (N.F. Ramsey et al., 1998), with the following parameter settings: TE/TR 35/24 ms, flip angle 9º, FOV 225x180x77 mm3 , matrix 64x52x26, voxel size 4 mm isotropic, scan time per volume 2.4 s. Following the fMRI procedure, an anatomical scan was acquired.

fMRI analysis

Functional MRI data preprocessing and analysis was done using SPM2 (Wellcome Department of Imaging Neuroscience, London, England; www.fil.ion.ucl.ac.uk).

Brain activity maps were obtained by analyzing the fMRI scans during all three tasks conjointly. It has previously been shown that such an analysis improves reliability of the subsequently computed laterality index, as compared to that obtained with individual task analysis (N. F. Ramsey et al., 2001). The rationale for conjoint analysis is that it improves sensitivity for brain activity that is present in all tasks, while reducing contribution of activity that is specific for individual language tasks.

All functional scans were registered to the last volume of the last block and coregistered to the anatomical scan. Next, all functional images were registered to an MNI standard brain, to enable group-wise comparisons. For each subject, a statistical map (i.e. T-map) was obtained from a general linear model regression analysis using a factor matrix that contained one factor modeling task activation for all three tasks combined. Eight discrete cosine functions were included to correct for low-frequency drifts.

Significant activation was then determined in each voxel by applying a threshold. The threshold corresponded to a p value of 0.001, uncorrected for multiple comparisons, and amounted to a T-value of 3.12. This relatively low threshold was applied to reduce the chance of a type II error (i.e. the failure to find a difference while there is a true difference between the conditions). Also, by setting the threshold at this T-value, one increases the likelihood that active voxels are present in both the left and the right hemisphere in each individual, allowing calculation of a more reliable laterality index.

Regions of interest

A map including five regions of interest (ROI), based on nine Brodmann areas (BA), was created using the WFU Pickatlas tool for SPM (Maldjian et al., 2003). This map was dilated with one voxel isotropic and coregistered with the T-maps. The advantage of using Brodmann areas is that volumes of the ROI's are of equal size across the hemispheres and across the groups. The following ROI's were created for the hemispheres separately: Broca's area and the homotopical region in the right hemisphere (BA 44, 45), superior temporal gyrus (BA 22, 38, 41, 42), middle temporal gyrus (BA 21), angular gyrus (BA 39) and supramarginal gyrus (BA 40).

Lateralization index

For each individual, the number of voxels above a threshold of T=3.12 ($p < 0.001$, uncorrected for multiple comparisons) was counted for all ROI's separately. A lateralization index for each ROI and all ROI's combined was calculated using the following algorithm:

$$\frac{\text{Active voxels left - active voxels right}}{\text{Total active voxels}}$$

The index ranges from –1 (complete right hemispheric dominance) to +1 (complete left hemispheric dominance). The advantage of using this index over absolute values is that this measure reflects hemispheric dominance within an individual, and is relative to overall brain activity in each individual. Individual lateralization indices for each language region, and all regions combined, were entered in an ANOVA.

Language lateralization

The mean lateralization index (for all language regions included) in the XXY group was 0.45 (SD 0.30), which was significantly lower than the index of 0.74 (SD 0.23) in the control group, $F(1,27)= 7.6$, $p=.01$. See figure 1 for the distribution of lateralization indices in the groups. The lower lateralization index in the XXY group was due to increased language related activity in the right hemisphere as compared to controls ($F(1,27)=6.0$, $p=.02$), rather than group-differences in left hemispheric language-related activity ($F(1,27)=1.7$, $p=.20$). More specifically, the mean lateralization index in the Superior Temporal Gyrus (STG, Brodmann area 22, 38, 41, 42) was significantly lower in XXY men (0.27, SD 0.39) as compared to controls (0.69, SD 0.36), $F(1,27)=8.2$, $p=.008$). See table 1 for the lateralization indices for each language region and figure 2 for activation patterns in a typical XXY subject and a typical control subject.

Analysis of the button presses during scanning indicated that performance in the semantic decision task was not significantly different between Klinefelter men and controls ($t(1,27)=-0.38$, $p=0.71$). Mean percentages correct were 90.6 (SD 8.8) and 89.3 (SD 8.4) respectively. Also, we observed no significant group differences in mean correct responses in the antonym-generation task (control group 42.1 (SD 3.4), Klinefelter group 41.2 (SD 3.8), $t(1,24)=0.52$, $p=0.61$) and verb-generation task (control group 40.4 (SD 3.2), Klinefelter group 34.6 (SD 9.0), $t(1,24)=1.8$, $p=0.08$) outside the scanner. Language lateralization in the XXY group did not correlate with handedness or age at which testosterone supplementation was started.

Figure 1
Distribution (and mean) of individual lateralization indices in the XXY and control group. Data are presented for all language regions combined (A) and for the superior temporal gyrus (STG) separately (B). In plot B, 7 controls have a lateralization index of 1.0.

Figuur 1

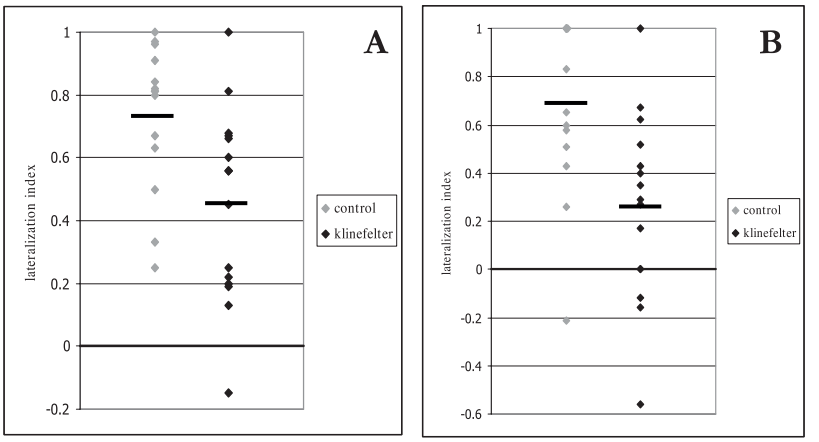
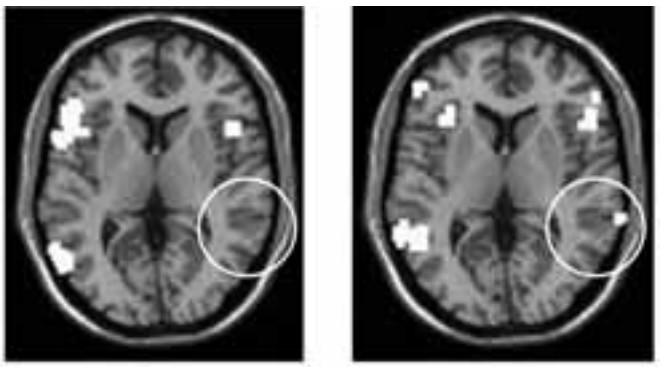


Table 1
Mean lateralization indices for each language region in XXY men and controls. (n.s.= not signficant)

Language region (Brodmann area)	Lateralization index control group (mean, SD)	Lateralization index XXY group (mean, SD)	P-values
All language regions included	0.74 (0.23)	0.45 (0.30)	0.01
Superior temporal gyrus (22, 38, 41, 42)	0.69 (0.36)	0.27 (0.39)	0.008
Middle temporal gyrus ((21)	0.71 (0.42)	0.42 (0.39)	n.s.
Angular gyrus (39)	0.70 (0.55)	0.52 (0.45)	n.s.
Supramarginal gyrus (40)	0.68 (0.49)	0.38 (0.55)	n.s.
Broca (44, 45)	0.73 (0.30)	0.61 (0.35)	n.s.

Figure 2
Examples of brain activity patterns during language processing in a control subject (with an overall lateralization index of 0.82) and an XXY subject (with an overall lateralization index of 0.45), that illustrate the following findings in XXY men as compared to controls; *a)* more bilateral activity, *b)* more activity in the right hemisphere, rather than less activity in the left hemisphere and *c)* more bilateral activity only in the superior temporal gyrus (STG) (see circle). The slices are transaxial (z=6) through Broca's area and the STG in MNI space and show voxels (in white) with a T value above threshold in language regions.



control subject XXY subject

Psychopathology

In the Klinefelter group mean total score on the Schizotypal Personality Questionnaire was 46.0 (SD 37.3), with a mean score of 14.0 (SD 14.0) for the positive dimension, 20.0 (SD 17.3) for the negative dimension and 12.0 (SD 7.2) for the disorganized dimension. Mean total score on the Positive and Negative Syndromes Scale (PANSS) was 46.5 (SD 9.7), with a mean score of 12.0 (SD 4.8) for the positive dimension, 10.3 (SD 2.9) for the negative dimension and 23.0 (SD 4.0) for the general symptoms dimension. For more details on schizophrenia spectrum pathology in a larger population of Klinefelter men at our department (of which the present is a subsample) as compared to healthy controls, see (van Rijn et al., in press).

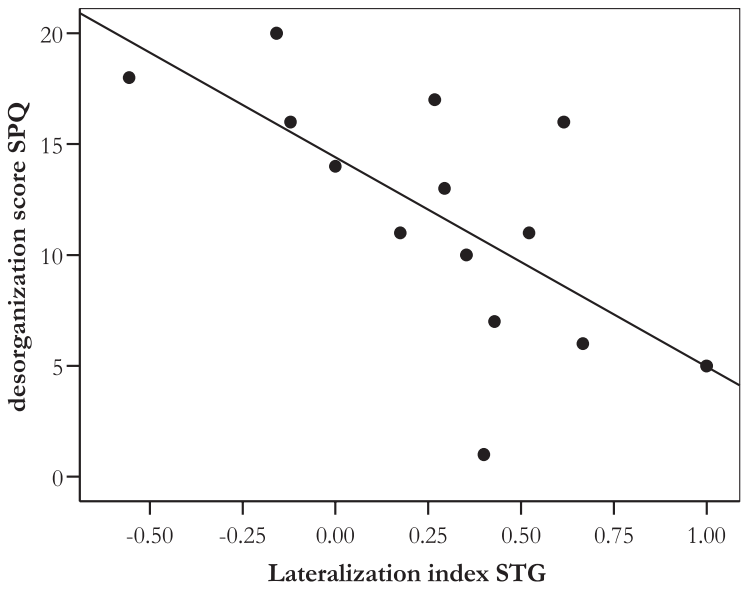
Language lateralization and psychopathology

Within the Klinefelter group, the relation between language lateralization and psychopathology was assessed (N=14). Only the overall lateralization index and the lateralization index for the STG were analyzed, since only these were

significantly different between XXY men and controls. In both cases, the index was correlated with six measures of psychopathology (the positive, negative and general dimensions of the PANSS and the positive, negative and disorganized dimensions of the SPQ). After correction for multiple comparisons (Bonferroni), the p-level for significance was 0.008.

The overall lateralization index (including all language areas) did not correlate significantly with any psychopathology dimension, although the sumscore of all three dimensions of the PANSS did (spearman's r=-.80, p=.001). Functional lateralization in the STG, the specific language region in which lateralization was lower in XXY men as compared to controls, correlated significantly with the SPQ disorganization dimension (spearman's r=-0.72, p=0.003) (see Figure 3). The correlation was inverse, indicating that a decrease in lateralization in the STG was associated with more disorganization of thought and language.

Figure 3
Correlation between lateralization index for the superior temporal gyrus (STG) and score on the disorganization dimension of the Schizotypal Personality Questionnaire in the XXY group (spearman's r=-0.72, p=.003).



Discussion

Using functional Magnetic Resonance Imaging (fMRI) we were able to measure, for the first time, patterns of brain activity during language processing in 47,XXY men. By measuring the relative contribution of the right and left hemisphere in each individual it was shown that language activity in the brain was less lateralized in the XXY group as compared to healthy males. Loss of asymmetric processing of language was due to increased activity in the right hemisphere rather than reduced activity in the left hemisphere. Analysis of functional asymmetry within different language regions indicated that the superior temporal gyrus (STG) was the only region in which language was less lateralized in the XXY group. This regional loss of language laterality was highly correlated with the degree of disorganization in these subjects.

Reduced hemispheric specialization for language may contribute to the widely reported language deficits in XXY adults and children (Boone et al., 2001; D. H. Geschwind et al., 2000a; Money, 1993; Samango-Sprouse, 2001). These include disabilities in reading, articulation, phonemic processing, word finding, spelling, language expression, verbal memory, language comprehension and verbal fluency. The reported patterns of language impairments seem similar to those observed in developmental language disorders, for example dyslexia (D. H. Geschwind et al., 2000a). A proposed neurocognitive basis for the language profile in developmental language disorders is reduced hemispheric specialization for language in the brain. Findings of less cerebral asymmetry, both functional (Ors et al., 2005) and structural (Herbert et al., 2005), in developmental language disorders illustrate the contribution of cerebral lateralization to the development of language capabilities. Our fMRI study shows that specifically an increase in right hemispheric involvement in language may contribute to language impairments, which fits with findings from a SPECT-study (Itti et al., 2003) in which increased resting state blood flow in the STG in the right hemisphere was observed in nine XXY men. They observed that abnormal resting state activity in XXY men was correlated with verbal skills as assessed in a neuropsychological session.

The use of fMRI allowed us to identify specific areas in the brain underlying the loss of hemispheric specialization for language in Klinefelter syndrome. One of our key findings was that loss of functional asymmetry in the STG was the most prominent contributor to reduced language laterality. Our finding of functional abnormalities in the STG is consistent with studies reporting structural abnormalities in the temporal lobes in Klinefelter syndrome. An MRI study including 10 XXY men showed asymmetric reduction in volume of the

temporal lobes, with a smaller volume of the left temporal lobe in these men (Patwardhan et al., 2000). In a larger sample of 34 XXY men, Shen et al. (2004) found that more regions in the left, as compared to the right, temporal lobe were smaller in the XXY group in comparison to 62 healthy men. Two other studies have reported bilateral volume reductions of (regions in) the temporal lobe. DeLisi et al. (2005) observed smaller volumes of the STG bilaterally in 11 XXY men. Itti et al. (2006) measured smaller volumes of the temporal lobes bilaterally in 9 XXY men, but only volume reductions in the left hemisphere correlated with verbal skills.

Decreased functional and structural asymmetry of language area's in the brain in XXY men may be secondary to abnormal X chromosomal (in-)activation, since a pseudo-autosomal region on the X chromosome is thought to direct abnormal development of asymmetry in XXY men (D.H. Geschwind et al., 1998). Genes in the pseudo-autosomal region escape inactivation resulting in expression of both copies of the genes in XX women and XY men and all three copies in XXY men (i.e. overexpression). This dosage mechanism makes genes in this region prime candidates for abnormal brain development, including cerebral asymmetry, and cognition in XXY (D.H. Geschwind et al., 1998). For example, one candidate gene in the pseudo-autosomal (X-Y homologous) region that has been shown to escape inactivation resulting in expression of all three copies in Klinefelter syndrome is Protocadherin11XY (PCDH11XY), which is involved in axonal guidance in the brain (T. J. Crow, 2002; N. L. J. Ross et al., 2006).

Support for X chromosomal effects on structural asymmetry of the superior temporal area in the brain is also provided by studies on brain volumes in individuals with Turner syndrome (46 XO, i.e. X monosomy). Decreased volume of the superior temporal sulcus in the left hemisphere as well as increased volume of the superior temporal gyrus in the right hemisphere (Kesler et al., 2003) have been observed in these subjects. In this study, increased right STG volume was related to lower verbal intelligence scores in Turner subjects (but not in controls). Involvement of the X chromosome in abnormal development of the STG, at least in Turner syndrome, was indicated by the observed imprinting effects. (Imprinting refers to differential expression of a gene depending on whether the gene is passed through from the mother or the father.) Only STG volumes in Turner subjects with the single X chromosome derived form the mother, and not those with an X chromosome from paternal origin, were different from controls. The findings that reduced asymmetry, either secondary to increased right hemisphere volume as in Turner syndrome

or decreased left hemisphere volume as in Klinefelter syndrome, can be accompanied by language dysfunctions, implies that reduced asymmetry may be a key abnormality in language processing deficits.

Decreased functional asymmetry in the STG in Klinefelter men correlated with the disorganization dimension of the schizotypy measure. Both items of this dimension, 'odd speech' (vague or over-inclusive) and 'odd/eccentric behavior', also separately correlated inversely with language lateralization in the STG. This is consistent with studies finding structural (Matsumoto et al., 2001; Menon et al., 1995; Rajarethinam et al., 2000; Rossi et al., 1994a; Shenton et al., 1992) and functional (Kircher et al., 2002) abnormalities of the STG to be related to disorganization symptoms such as thought disorder, in a another disorder, i.e. schizophrenia. Specifically, in a functional MRI study with schizophrenia patients with formal thought disorder, higher levels of thought disorder were related to increased STG activity in the right hemisphere (Kircher et al., 2002). Interestingly, in schizophrenia, sex differences have been reported in the volume of the STG (Flaum et al., 1995; R.E. Gur et al., 2000). A genetic mechanism involving the sex chromosomes might explain this finding. An X-Y homologous, pseudo-autosomal genetic region directing development of asymmetry in the brain has been proposed (T.J. Crow, 2004) to underlie the observed sex differences in verbal abilities, handedness, relative rates of hemispheric growth and both ages of onset and prevalence of schizophrenia. The idea that reduced cerebral asymmetry and, more specifically, loss of language lateralization is one of the core developmental brain abnormalities underlying schizophrenia symptoms (Bhati, 2005; T.J. Crow, 2004) is supported by a range of language laterality studies in schizophrenia (Artiges et al., 2000; Dollfus et al., 2005; Kircher et al., 2002; I. Sommer et al., 2001a; Weiss et al., 2006). One of these studies not only assessed the degree of lateralization, but also examined whether this was secondary to increased right hemisphere activation or decreased left hemisphere activation (I. E. Sommer et al., 2001b). Similar to what we observed in the Klinefelter men, decreased lateralization of language in schizophrenia patients was due to increased activity in the right hemisphere, rather than decreased activity in the left hemisphere.

A proposed mechanism by which abnormal functional lateralization of the STG may contribute to disorganization and thought disorder, incorporates the differences in semantic processing between the left and right auditory association areas (including the STG). Semantic fields are groups of words and concepts that are closely related in meaning, often subsumed under a general term, forming a category (such as 'animals'). In the left hemisphere, words are

associated with other words and concepts within small, focal semantic fields, whereas in the right hemisphere they are associated within large, diffuse semantic fields (Beeman & Chiarello, 1998). Therefore, the right hemisphere appears to be biased to aspects of language that require 'global' semantic processing, such as forming semantic relations that are only loosely coupled in meaning, generation of multiple meanings of words and the interpretation of whole sentences, stories or metaphors. In contrast, the left hemisphere seems to be biased to aspects of language that require 'local' semantic processing, such as single interpretations for each word. In the left hemisphere contextually inappropriate meanings of words are inhibited and one single meaning is selected. It is an imbalance in these complementary processes that is proposed to underlie disorganization symptoms and thought disorder (Kircher et al., 2002). An imbalance, created by increased right hemispheric activity leading to generation of distantly related meanings together with reduced left hemispheric activity resulting in loss of inhibition and selection of meaning, may explain the increased levels of disorganization in XXY men.

The degree to which functional asymmetries in the XXY brain represent the effects of testosterone- deficits or supplementation remains unclear. The relationship between testosterone levels and behavior is complex; timing of exposure, sensitivity to testosterone reflected in androgen receptor density and modulation by environmental factors are important determinants in the effects of testosterone (Craig et al., 2004). Furthermore, gonadal hormones may be one of many mechanisms by which sex chromosomes exert their influence on brain development. Recent animal studies have pointed to direct, non-hormonal, effects of sex-chromosomes on brain maturation (Dewing et al., 2003). In line with this, a recent longitudinal study dealing with the effects of testosterone treatment on language lateralization in transsexuals showed that language laterality is highly stable and not affected by hormonal interventions (I. E. C. Sommer et al., in prep).

In sum, the present study provides evidence for a loss of language lateralization, most prominent in the superior temporal gyrus, in XXY men. Moreover, we found that the loss in hemispheric specialization for language processing may have important consequences for mental functioning, as it was associated with dysfunctions in organization of thought and language. This suggests that a genetic mechanism involving the X chromosome is involved in the development of hemispheric specialization for language. Although speculative, such a mechanism may involve genes that are overexpressed as a result of escaping inactivation. Alternatively, it might be an epi-genetic

mechanism, involving an environmentally (i.e. non-genetic) induced change in gene function, or epi-static, involving suppression of expression of other genes. Future genetic studies with XXY men may provide insight into the mechanisms by which genes on the X chromosome direct development of functional asymmetry in the brain.

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Chapter 9

Neurobiology of emotion and high risk for schizophrenia: Role of the amygdala and the X chromosome

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Abstract

Abnormalities in emotion processing and in structure of the amygdala have consistently been documented in schizophrenia. A major question is whether amygdala abnormalities reflect a genetic vulnerability for the disease.

In the present paper, we reviewed Magnetic Resonance Imaging (MRI) studies that reported amygdala measures in several high risk populations: subjects from the general population with subclinical schizophrenia symptoms and relatives of schizophrenia patients. In addition, we reviewed the evidence regarding Klinefelter syndrome (characterised by an additional X chromosome), which has also been related to an increased risk for schizophrenia.

Overall, the evidence points to structural abnormalities of the amygdala in individuals at increased risk for schizophrenia. Although the genetic basis of amygdala deficits remains unclear, abnormalities (of genes) on the X chromosome might play a role as suggested by the evidence from individuals with sex chromosome aneuploidies. We propose that amygdala abnormalities are an endophenotype in schizophrenia and may account for subtle emotional processing deficits that have been described in these high risk groups.

Introduction

There is ample evidence that the amygdala plays a central role in emotional information processing (Aggleton, 2000; Aggleton et al., 2000; Baxter et al., 2002). The amygdala appears to be important, among other functions, for evaluation of the emotional valence of stimuli in the very early phases of sensory processing, linkage of perceptual representation to emotional memories, and regulation of autonomic responses (Aggleton, 2000; Aggleton et al., 2000). In humans, the amygdala appears to be especially important for processing of emotional information in a social context. Facial affect recognition, for example, is shown to depend on the integrity of the amygdala (Adolphs et al., 1994; Adolphs et al., 1999; Calder et al., 1996; Gur et al., 2002a; Morris et al., 1998; Young et al., 1995). Not only is the amygdala involved in processing information about basic facial emotions, but also about complex social judgements, such as trustworthiness judgements of faces (Adolphs, 2002).

Schizophrenia is a serious psychiatric disorder characterised by positive symptoms, reflecting the presence of abnormal behavior such as delusions and hallucinations, and negative symptoms, representing the absence of normal behavior. Besides impairments in the cognitive domain (Aleman et al., 1999; Heinrichs et al., 1998), individuals with schizophrenia show deficits in emotion processing, as indicated by a markedly reduced ability to perceive, process and express facial emotions (Mandal et al., 1998; Morrison et al., 1988; Mueser et al., 1996; Streit et al., 2001). These difficulties have a major impact on social dysfunction in these patients (Edwards et al., 2002). Deficits in facial affect recognition, for example, have been associated with dysregulation of social behaviours and deterioration of interpersonal relations in schizophrenia (Mueser et al., 1996; Poole et al., 2000).

Although a broad range of brain regions seems to be affected in patients with schizophrenia, structural and functional MRI (Magnetic Resonance Imaging) studies have suggested that abnormalities in the amygdala may account for deficits in emotional processing. Structural MRI studies have shown reduced volume and reduced gray matter density of the amygdala in these patients (Breier et al., 1992; Bryant et al., 1999; Gur et al., 2000; Hulshoff-Pol et al., 2001; Lawrie et al., 2003; Wright et al., 2000). Recent fMRI-studies with schizophrenia patients have shown less functional activation of this structure in response to emotional salient stimuli, such as faces (Gur et al., 2002b; Schneider et al., 1998; Taylor et al., 2002). As revealed by a review of studies focused at facial affect recognition in schizophrenia (Mandal et al., 1998), recognition of facial

expressions of fear and anger seems to be specifically impaired in this illness. These patterns of emotion processing deficits parallel those found in both humans and primates with damage to the amygdala; the processing of fear and anger is an almost invariable consequence of amygdala lesions (Skuse et al., 2003). Not only the recognition of basic facial emotions, also creation of complex social judgements such as trustworthiness of faces, which involves the amygdala as evidenced by functional neuroimaging- as well as focal brain lesion studies, appears to be impaired in schizophrenia (Hall et al., 2004). More support for dysfunction of the amygdala in schizophrenia comes from impairments of patients in aversive conditional avoidance learning (Hofer et al., 2001; Kosmidis et al., 1999), in which the amygdala is critically involved (Maren, 2003).

Evidence for a specific contribution of morphological abnormalities of the amygdala to deficient emotional processing in schizophrenia is given by a study showing that volume of the amygdala can significantly predict performance in an emotional learning task in schizophrenia (Exner et al., In Press). Besides specific contributions to behavioral impairments, effects of amygdala abnormalities may also extend to a clinical level in schizophrenia. Processing of emotional faces is impaired in schizophrenia and associated with problems in social behavior (Hooker et al., 2002). Social dysfunction is one of the hallmarks of schizophrenia and social impairments are often already present before actual onset of the disease (Pinkham et al., 2003), which underlines the importance of studying neural networks, with a key role for the amygdala, that underlie social cognition in schizophrenia.

Interestingly, it has been argued that in schizophrenia early amygdala damage can lead to dysfunction in other brain areas (Grossberg, 2000), for example prefrontal regions. Indeed, using an animal model for neurodevelopmental psychopathological disorders it has been shown that amygdala lesions early in life can result in a restricted pattern of innervation of the prefrontal cortex later in adulthood (Bouwmeester et al., 2002).

Although emotion deficits in schizophrenia have been widely reported, the degree to which these deficits are present in individuals with a genetic vulnerability for the disease has received less attention. Therefore, we will address the issue whether abnormalities of the amygdala form part of the liability to schizophrenia. We will review evidence of amygdala dysfunction with regard to several distinct populations who are at risk for schizophrenia; subjects from the general population with subclinical schizophrenia symptoms and relatives of schizophrenia patients. Furthermore, we review the evidence

regarding individuals with Klinefelter syndrome, characterised by an additional X chromosome and cognitive and emotional deficits that parallel those associated with schizophrenia. Klinefelter Syndrome has also been related to an increased risk of schizophrenia (Lishman, 1998).

The study of these individuals with an increased risk for developing the illness is important for several reasons. First, these studies may help distinguish the latent abnormalities that are related to genetic vulnerability of the illness apart from the illness itself, because in these studies no confounding effects of hospitalisation, antipsychotic drug treatment and potential neurotoxic consequences of psychosis are present. Second, these studies may lead to the identification of biobehavioural markers of the illness, which are important for genetic research because these markers may be more reliable and valid descriptors of a vulnerability to schizophrenia compared to the clinical, DSM-IV-based, phenotypes.

High risk paradigm

There is a general consensus that both environmental- and genetic factors underlie the structural and functional brain abnormalities found in schizophrenia (Hirsch et al., 2003). The genetic predisposition to schizophrenia can be expressed in a variety of manifestations, ranging from mild schizotypal personality traits to severe schizophrenia, as reflected in a schizophrenia phenotype continuum (Faraone et al., 1995; Johns et al., 2001; Keefe et al., 1991; Lenzenweger, 1994). Accordingly, neurobiological markers of a predisposition to schizophrenia are not only present in schizophrenia patients, but to some degree also in healthy individuals carrying a vulnerability for the disease. One reason why it may be interesting to focus on structural brain abnormalities in these individuals, is that brain volumes are highly heritable. To illustrate, several twin-studies have reported heritabilities of 0.94 (Bartley et al., 1997), 0.92 (Carmelli et al., 1998) and 0.90 (Baare et al., 2001) for total brain volume.

Table 1. lists characteristics and findings of studies into structural amygdala abnormalities in high-risk groups, which are discussed in the present review.

Table 1.
Overview of Magnetic Resonance Imaging (MRI) studies that compared amygdala or AHC measures between healthy controls and the following populations who are at risk for schizophrenia; relatives of schizophrenia patients, subjects from the general population with subclinical symptoms and individuals suffering from Klinefelter syndrome (characterised by an additional X chromosome).

Authors	High risk group	Method	Findings
Hendren et al. 1995	Children with mild symptoms (n= 20) Healthy controls (n=12)	MRI, ROI analysis	Volume reduction left and right amygdala left < right
Yeo et al. 1997	Children with mild symptoms (n=20) Healthy controls (n=20)	MRI, ROI analysis	Volume reduction left and right amygdala Reductions more prominent in children with mild symptoms and developmental delay
Keshavan et al. 1997	Adolescent relatives (n=11) Healthy controls (n=12)	MRI, ROI analysis	Volume reduction left amygdala
Seidman et al. 1997	Adult relatives (n=6) Healthy controls (n=11)	MRI, ROI analysis	Volume reduction right amygdala
Marcelis et al. 2003	Adult relatives (n=32) Schizophrenia patients (n=31) Healthy controls (n=27)	MRI, VBM analysis	Reduced grey matter density in amygdala in patients, not in relatives (but no significant differences between patients and relatives either)
Staal et al. 2000	Adult relatives (n=32) Schizophrenia patients (n=32) Healthy controls (n=32)	MRI, ROI analysis	No differences in amygdala volumes between patients, controls and relatives
Seidman et al. 1999	Adult relatives (n=28) Healthy controls (n=26)	MRI, ROI analysis	Volume reduction AHC
Lawrie et al. 2001	Adolescent relatives (n=147) Schizophrenia patients (n=34) Healthy controls (n=36)	MRI, ROI analysis	Reduction AHC volume patients < high risk < controls
Keshavan et al. 2002	Adolescent relatives (n=17) Healthy controls (n=22)	MRI, ROI analysis	Volume reduction anterior AHC, increased leftward asymmetry in AHC
Schreiber et al. 1999	Adolescent relatives (n=15) Healthy controls (n=15)	MRI, ROI analysis	Volume reduction right amygdala, smaller right to left ratio in amygdala
O'Driscoll et al. 2001	Adult relatives (n=20) Healthy controls (n=20)	MRI, ROI analysis	Volume reduction left and right amygdala-anterior-hippocampus
Steel et al. 2002	Adult obligate carriers (n=6) Nonaffected/noncarriers (n=6) Schizophrenia patients (n=6)	MRI, ROI analysis	Volume reduction AHC complex in obligate carriers and schizophrenia patients patients = obligate carriers < nonaffected/noncarriers
Warwick et al. 1999	Adult klinefelter patients (n=10) Healthy controls (n=25)	MRI, ROI analysis	No differences in AHC volume
Patwardhan et al. 2002	Adult Klinefelter patients (n=10) Healthy male controls (n=10) Healthy female controls (n=10)	MRI, ROI analysis	Volume reduction amygdala healthy females = Klinefelter patients < healthy males
Shen et al. 2004	Adolescent Klinefelter patients (n=34) Adolescent healthy controls (n=62)	MRI, VBM analysis	Volume reduction right amygdala

MRI: Magnetic Resonance Imaging, ROI: region of interest, VBM: voxel based morphometry, AHC: amygdala hippocampal complex

Subclinical signs in the general population

One approach to investigate vulnerability to schizophrenia is to identify subtle, subclinical signs in healthy individuals that parallel the symptoms of the illness. These signs, often manifested as various schizotypal personality traits, have been suggested to be continuously distributed in the general population (Aleman et al., 2001; Claridge et al., 1996; Jessimer et al., 1997; Johns et al., 2001). Examples of schizotypal traits are referential and magical thinking, suspiciousness and social isolation.

Subclinical signs may in some individuals progress to symptoms of schizophrenia. In fact, the abovementioned schizotypal traits partially predict schizophrenia at long term follow-up in subjects diagnosed with Schizotypal Personality Disorder (DSM III) (Fenton et al., 1989). Also, schizotypal traits in young relatives of patients predict progression to schizophrenia in the following dimensions: social withdrawal, psychotic symptoms and socio-emotional dysfunction (Miller et al., 2002).

Subclinical signs in the general population: abnormalities of the amygdala

Some of the structural neuroimaging studies have relied on clinical criteria when defining vulnerability for the disease. Hendren and colleagues (Hendren et al., 1995) have examined amygdala volumes of twelve children between 8 and 12 years, who displayed mild symptoms of (early-onset) schizophrenia and/or schizotypal personality disorder. When compared to twelve age-, sex- and socio-economic status- paired matched controls, a significant reduction in amygdala volume was present in the symptomatic group. Measurements were adjusted for the effects of age and total brain volume. Group differences were more prominent for the left than the right amygdala, a finding that has also been reported in studies with chronic schizophrenia patients (Rossi et al., 1994; Shenton et al., 2001).

Yeo and al. (Yeo et al., 1997) have been able to partially replicate this result in a group of twenty children, aged 9 to 12, characterised by schizophrenia-like symptoms, but who did not meet all the diagnostic criteria for schizophrenia. Again, compared to paired matched controls, a reduction in the amygdala volume was found using MRI, but no differences between the right and left amygdala were present. When the symptomatic group was split up in children with and without a history of developmental delay in motor or language function, a comparison of volumetric measurements showed a significantly smaller amygdala volume in the group who did have a history of developmental

delay. All measurements were controlled for age and total brain volume. As the authors noted, these results point to a possible role of the amygdala in the neural development of schizophrenia.

Thus, already in pre-adolescence schizophrenia-like symptoms may be associated with abnormalities in the development of the amygdala. Functions of the amygdala may become more relevant with age, since it is important for recognizing and learning the emotional, and possibly survival-related, meaning of stimuli in the environment and producing appropriate behavioural responses. Indeed, findings from structural MRI studies suggest that there is an age- and gender related increase in amygdala volume during childhood and adolescence (Giedd et al., 1996; Sowell et al., 1998). We speculate that the behavioural effects of dysmaturations of the amygdala may become apparent as high risk children approach adolescence, a period in which maturational processes are dynamic and strengthening of neural connections between the amygdala and for example frontal areas take place ((Benes, 1998; Walker et al., 2002). Although the degree to which these dysmaturations are a direct expression of a genetic predisposition to schizophrenia remains unclear, the parallels in symptoms between these children and schizophrenia patients indicate that they also may share genetic pathology.

Relatives

Sharing a substantial amount of genes, relatives of patients suffering from schizophrenia are at increased risk for the disease, with the risk increasing with the degree of kinship (Gottesman, 1991). Schizophrenia as well as schizotypal traits are more often identified in relatives of patients suffering from schizophrenia when compared to the general population (Kendler et al., 1995; Kety et al., 1994; Kremen et al., 1998; Vollema et al., 2002; Yaralian et al., 2000). When rates of schizotypal personality traits are substantially increased, criteria for Schizotypal Personality Disorder (SPD) may be met. Whereas the prevalence of this disorder is 3% in the general population, 10 to 15% of the relatives suffer from SPD.

But even in the absence of schizophrenia-like symptoms or schizotypal personality traits, biological relatives of schizophrenia patients are at substantial risk for a range of biobehavioral and neurobiological abnormalities that are related to a genetic vulnerability (McDonald et al., 2002; Seidman et al., 1997; Seidman et al., 1999; Staal et al., 2000). In addition, healthy relatives of patients show abnormalities at a neuropsychological level that parallel the deficits seen in schizophrenia patients, although they are more subtle (Sitskoorn et al., 2003).

All in all, relatives of schizophrenia patients provide an opportunity for studying amygdala abnormalities as a neurobiological marker of the disease.

Relatives: abnormalities of the amygdala

The most widely used approach to identify liability to schizophrenia is to establish the genetic risk of subjects based on a family tree describing affected and unaffected family members. In some family trees unaffected family members have been further specified in obligate carriers, subjects having transmitted schizophrenia while remaining healthy themselves, and non-carriers who have not passed any pathological genes on to their offspring. Besides adolescents with a familial risk for schizophrenia, adult relatives of patients have also been subject of investigation. Several studies have been able to show amygdala- or amygdala-hippocampal-complex (AHC) abnormalities in individuals at familial risk.

Amygdala measurements

An MRI study conducted by Keshavan et al. (Keshavan et al., 1997) included eleven high-risk adolescents, aged 12 to 18, who had a parent suffering from schizophrenia. Neither the high-risk adolescents, nor the healthy controls had a history of psychotic disorders. After correction for whole brain volumes, offspring of patients were characterised by reduced left amygdala volume.

One of the first studies that systematically examined brain volumes in adult relatives of patients compared 6 female relatives with 11 controls (Seidman et al., 1997). None of the subjects had suffered from psychosis or schizophrenia spectrum disorders. Among a range of brain volume abnormalities, a mean reduction of the right amygdala was present in the relatives group. Although this reduction was present in spite of the small sample size, no correction for multiple comparisons had been included. When the sample size was extended to 28 relatives, both male and female, and 26 controls, the volume reduction of the amygdala was not statistically significant. However, the amygdala-hippocampal complex as a whole appeared to be significantly reduced in relatives (Seidman et al., 1999).

The apparent inconsistencies in their results might be related to heterogeneity in their sample. As proposed by the authors, schizophrenia may arise from a single pool of environmental and genetic factors in which small effects of the individual factors can add up to result in a vulnerability for the disease. As a consequence, relatives may be characterised by different, probably partially overlapping, sets of vulnerability markers. One way to deal with this

heterogeneity is to select large sample sizes. Their sample of 28 relatives was further extended to 45 and factor analysis, a psychometric approach, was employed. Although no main effects were present for the left and right amygdala combined, the left amygdala, among other brain regions, contributed to the only factor that discriminated relatives from controls (Faraone et al., 2003). This reduction in left amygdala volume is consistent with findings from Keshavan as described above (Keshavan et al., 1997).

There are imaging studies, however, in which no reduction of the amygdala in relatives has been shown. A recent MRI study used computational morphometry to compare grey matter densities on a clustered-voxel basis in 31 schizophrenia patients, 32 relatives and 27 healthy controls (Marcelis et al., 2003). Grey matter density deficits in the amygdala were present in patients, but not in relatives, when compared to controls. However, grey matter density in the amygdala did not significantly differ between relatives and patients, making it difficult to interpret the findings in this study regarding amygdala abnormalities in relatives. In study by Staal et al. (Staal et al., 2000), 16 schizophrenia patients were compared to their healthy siblings and 16 healthy control subjects. Amygdala volume did not differ between the healthy siblings and controls. However, compared to healthy controls, schizophrenia patients did not show a reduction of the amygdala, which makes it difficult to draw conclusions from this study regarding amygdala abnormalities as a vulnerability marker in healthy siblings.

Amygdala-hippocampal complex (AHC) measurements

Because the amygdala is notoriously difficult to measure reliably (Brierley et al., 2002), several studies try to solve the problem by measuring the amygdala and hippocampus as a whole; the amygdala-hippocampal complex (AHC). Some studies divide the AHC in a posterior and anterior portion, with the latter portion representing the amygdala.

As part of the Edinburgh High Risk Study (EHRS), a series of MRI studies by Lawrie and colleagues have explored abnormalities of the amygdala-hippocampal complex as an expression of a genetic liability to schizophrenia. First, they have compared AHC volumes of 100 adolescents characterised by two or more affected relatives, with 20 first episode schizophrenia patients and 30 healthy controls (Lawrie et al., 1999). AHC volume in the high-risk group appeared to be midway between that of healthy controls and first episode patients, with the latter group showing the smallest mean AHC volume. When the high risk sample was extended to 147 subjects, and compared to 34 first

episode patients and 36 healthy controls, the same pattern of results appeared (Lawrie et al., 2001). Although AHC volumes in the high risk group were not related to the number of affected relatives which may reflect a measure of genetic liability, this gradient in AHC volume provides evidence for the idea that AHC abnormalities in schizophrenia are largely genetically mediated. The findings were only partially replicated when using automated Voxel Based Morphometry (VBM), allowing comparisons of grey matter densities, instead of volumetric Region of Interest (ROI) measurements. Although patients did show a reduction in grey matter density in the amygdala when compared to controls, differences in amygdala volume between healthy controls and high risk subjects disappeared when this VBM analysis was applied to the dataset of the Edinburgh High Risk Study (Job et al., 2003).

In the Edinburgh High Risk Study, abnormalities in AHC complex were not related to the presence of psychotic symptoms in the high risk group. A subgroup of 28% of the high risk adolescents characterized by psychotic symptoms did not differ in mean AHC volume from the high risk adolescents without psychotic symptoms. This finding underlines that AHC abnormalities may reflect an underlying genetic vulnerability to schizophrenia, and may not predict the presence or development of psychotic symptoms which in turn may be triggered by environmental factors and/or other neurodevelopmental processes.

This hypothesis is supported by findings in a two-year follow-up study (Lawrie et al., 2003), which was included allowing investigation of the relation between symptom development and brain volume changes. 20 Healthy subjects and 66 high-risk adolescents were re-scanned and symptoms were re-evaluated. No significant time by group interaction was present for the AHC, meaning that change in AHC volume over time did not differ between high risk adolescents and controls. When comparing high-risk subjects displaying symptoms at follow-up with high-risk adolescents without symptoms, no significant time by group interaction was found for the AHC. This finding has been replicated in a longitudinal imaging study focused at the amygdala as a separate region. Adolescents at high-risk for development of psychosis showed no differences in gray-matter volume of the amygdala between those who subsequently developed a psychotic illness (n=23) and those who did not (n=52) (Pantelis et al., 2003).

Other investigators have also performed measurements of AHC volumes in adolescent relatives of schizophrenia patients. Schreiber et al., for example, focused at brain abnormalities in offspring of schizophrenia patients. 15 High-

risk adolescents and 15 controls were between 11 and 19 years of age, and individually matched for age, sex, education and social background. A mean volume reduction of the right AHC was present in high-risk adolescents. In addition, they were characterized by a smaller right-to-left ratio of the AHC when compared to controls (Schreiber et al., 1999).

Keshavan et al. included seventeen high risk adolescents (offspring) in their MRI study and were able to demonstrate volume reductions of about 25% in the left anterior amygdala-hippocampal complex (AHC) (Keshavan et al., 2002). This anterior part of the AHC largely comprises the amygdala. Significant reductions in the right anterior AHC were also present although not as prominent as in the left hemisphere, indicating an increased leftward asymmetry of the anterior AHC.

Another study in which the AHC was split up in an anterior and posterior part focused at adult individuals at familial risk for schizophrenia. O'Driscoll et al. (O'Driscoll et al., 2001) have compared AHC volumes of 20 first-degree relatives with 20 healthy controls using MRI. All subjects were between 18 and 50 years and were similar on demographic variables. None of the subjects had a history of any DSM axis I disorder or met the criteria for Schizotypal Personality Disorder. The area of the amygdala-hippocampal complex was divided in two regions of interest; the amygdala-anterior hippocampus and the posterior hippocampus. Relatives showed a volume reduction of the left- as well as right amygdala-anterior hippocampus, which comprises the amygdala, the pres hippocampus and the anterior portion of the subiculum.

Although the risk of relatives for developing schizophrenia is increased, only a subgroup will actually carry pathological genes. More evidence for a genetic loading of amygdala abnormalities in schizophrenia comes from experimental designs with multiple affected families in which affected subjects, non-carriers (no affected offspring) and obligate carriers (affected offspring) are identified. In addition to an increase in power, such a design allows a clear separation of the contribution of pathological genes from the effects of the illness itself.

In the Edinburgh High Risk Study, sib-ships in high risk families were identified including one affected, one non-affected/non-carrier and one non-affected/carrier (obligate carrier) familymember (Steel et al., 2002). AHC volumes (right and left combined) in subjects with schizophrenia and obligate carriers were significantly smaller when compared to non-affected/non-carriers, which implies a genetic contribution in AHC pathology in schizophrenia. Thus, although AHC abnormalities were not related to a genetic liability index as

measured by the number of affected relatives and whether these are first-degree or second degree relatives, carriers of pathological genes do show reductions of the AHC. Because cortical changes in relatives were not found, the authors concluded that in a framework of complex genetic mechanisms it could be possible that some vulnerability genes, in this case causing AHC abnormalities, are inherited by obligate carriers, whereas other vulnerability genes, responsible for cortical changes for example, are not.

In summary, both adolescents and adult relatives of schizophrenia patients show abnormalities of the amygdala, either measured as the amygdala region exclusively or as part of the amygdala-hippocampal-complex. Since these relatives share a substantial amount of genes with an affected family member, a shared genetic pathology may account for these amygdala abnormalities. The finding that AHC volumes in obligate carriers of pathological genes are similar to AHC volumes in patients, but different from non-affected/non-carriers supports this hypothesis. AHC volumes in relatives seem not to be related to the presence of symptoms. Because AHC abnormalities are thought to reflect a genetic liability for schizophrenia and not the actual expression of the illness (Steel et al., 2002), this is not unexpected.

Klinefelter syndrome

One interesting neurodevelopmental disorder that has been associated with an increased risk for schizophrenia is Klinefelter Syndrome, a genetic disorder characterised by sex chromosome abnormalities. Klinefelter patients have an additional X chromosome, leading to the XXY karyotype. Although the primary focus in clinical studies on Klinefelter Syndrome has been on reproductive dysfunction of these patients, there is an awareness of behavioral and cognitive abnormalities, including socio-emotional disturbances (Boone et al., 2001; Geschwind et al., 2000; Simpson et al., 2003; Swaab et al., submitted). The importance of investigation into the cognitive and behavioral phenotypical manifestations of Klinefelter syndrome as a means of understanding a predisposition to schizophrenia, is shown by epidemiological studies reporting an increased incidence of XXY karyotypes in schizophrenia. The prevalence of Klinefelter Syndrome in the general population is 0.1-0.2% (Bojesen et al., 2003). The prevalence of Klinefelter Syndrome in the schizophrenia population is 1.6 %, which is several times higher (DeLisi et al., 1994; Kunugi et al., 1999). In turn, early studies have indicated an increased risk for schizophrenia and psychotic illnesses among Klinefelter patients (Lishman, 1998). A review of mental hospital surveys pointed to a threefold increase in Klinefelter patients

compared to the general population, which was mainly due to 'psychotic illnesses of a schizophrenic nature' (Forssman, 1970). Another study showed that 7% of the Klinefelter patients in the psychiatric literature had psychoses with paranoid delusions and 6% suffered from schizophrenia (Nielsen et al., 1969). In addition, Klinefelter syndrome has recently been associated with high levels of schizophrenia spectrum pathology (van Rijn et al., submitted). Compared to 26 healthy males, a group of 26 Klinefelter patients were characterised by elevated levels of schizophrenia symptoms as well as schizotypal traits. Effect sizes, the difference between the groups expressed in standard deviations, in this study paralleled those reported for patients suffering from schizophrenia. From the 26 Klinefelter males, 7 individuals (27%) met diagnostic criteria for a psychotic disorder.

Studying Klinefelter patients present an opportunity to investigate whether amygdala abnormalities can be associated with a predisposition to schizophrenia. An additional advantage over studying relatives is the knowledge of the precise genetic aetiology of Klinefelter syndrome in contrast to what is known of the genetic pathology in schizophrenia.

Klinefelter syndrome: abnormalities of the amygdala

Although AHC abnormalities were not found in an explorative MRI study with Klinefelter patients (Warwick et al., 1999), specific amygdala reductions were present in an MRI study focusing at temporal lobe structures in Klinefelter patients (Patwardhan et al., 2002). 10 XXY males were recruited from a cohort newborns screened for sex chromosome abnormalities and compared to 10 individually matched healthy males and healthy 10 females. Approximately half of the patients had been exposed to testosterone supplements. Amygdala volumes of Klinefelter patients were comparable to those of healthy females. Interestingly, Klinefelter patients suffering from schizophrenia show several parallels with females suffering from schizophrenia; similar ages of onset that differ from male schizophrenia patients and a predominance of positive symptoms over negative symptoms (Pinabel et al., 1997). In an important study, using voxel-based morphometry, Shan et al. observed pronounced volume reductions of the right amygdala in 34 subjects with Klinefelter syndrome (mean age 12.6 years) compared to 62 healthy matched controls (Shen et al., 2004). Compared to previous findings documented in the literature, findings from this study provide a better spatial localization of the affected regions (other affected regions are the insula, cingulate, hippocampus, temporal gyri and occipital gyri).

X-linked genes appear to be involved in the functional as well as structural development of the amygdala (Good et al., 2003). Several studies have shown amygdala volume to be inversely correlated with the number of X chromosomes (Goldstein et al., 2001; Good et al., 2003). For example, males (46,XY) have larger amygdalae than females (46,XX). Patients suffering from Turner syndrome (45,X), who are characterised by a partial or complete deletion of the X chromosome, have even larger amygdala volumes when compared to healthy males. On a behavioural level, these patients are impaired in facial affect recognition while showing normal configural processing (Lawrence et al., 2003). This deficit in emotion recognition appears to be most pronounced for fear, a finding that parallels data from studies with schizophrenia patients (Kohler et al., 2003).

Although speculative, the idea that amygdala abnormalities in schizophrenia may somehow be related to X chromosomes deserves further investigation. Especially in the light of sex-differences in the onset and course of the disease.

Conclusion and discussion

The present review explored whether an increased risk for schizophrenia is associated with structural abnormalities of the amygdala. Structural neuroimaging studies with three distinct populations at risk for schizophrenia have provided indications that reductions in amygdala volume indeed may be associated with an increased risk for schizophrenia. First, individuals from the general population with subclinical signs of schizophrenia show reductions in amygdala volume. Second, volume reductions of the amygdala and amygdala-hippocampal-complex (AHC) have been found in relatives of schizophrenia patients. Third, Klinefelter patients, characterised by an XXY chromosomal pattern, also show reduced amygdala volumes.

The fact that amygdala abnormalities, which have consistently been reported in patients with schizophrenia (Breier et al., 1992; Bryant et al., 1999; Gur et al., 2000; Hulshoff-Pol et al., 2001; Lawrie et al., 2003; Wright et al., 2000), are also observed in high risk populations, is of considerable importance. Since volume reductions of the amygdala seem to be present already in pre-adolescents with subclinical signs of the illness, abnormalities of the amygdala in schizophrenia might have a developmental origin. Indications that these amygdala abnormalities are an expression of a genetic predisposition to schizophrenia are primarily derived from studies with healthy relatives of patients, who besides sharing genes also share amygdala or AHC abnormalities. Consequently, abnormalities of the amygdala or AHC might belong to the

endophenotype of schizophrenia, a concept which is introduced to refer to brain structures or cognitive functions that mediate between the molecular, genetic level and the molar, clinical level of the disease (Gottesman et al., 2003). This would imply that antipsychotic medication, hospitalisation and neurotoxic effects of psychosis may not fully account for volume reductions of the amygdala in schizophrenia, since reductions have been found in subjects with an increased risk for the disease, but who did not have a history of pharmacological treatment, psychosis or hospitalisation. This is supported by an imaging study with medication-naive schizophrenia patients, who show volume reductions of the amygdala (Joyal et al., 2003). In the same study, neither duration of illness, nor symptom severity correlated with amygdala volume.

Amygdala abnormalities may be one of many endophenotypes in schizophrenia, each one being an expression of a particular genetic pathology (Gottesman et al., 2003). Although most attempts to link the general schizophrenia phenotype to aberrations of the X chromosome have produced contradictory results (DeLisi, 1997; DeLisi et al., 2000), recent genome screens have pointed to a possible locus on the X chromosome (Paterson, 1999). However, in these studies the magnitude of the effects regarding the X chromosome is smaller than the most positive loci. We argue that amygdala abnormalities, a specific phenotype, in schizophrenia might be related to X-linked genetic pathology. Support for this idea comes from studies with individuals suffering from X chromosome abnormalities. For example, individuals with Klinefelter syndrome (47,XXY), who have almost an approximately ten-fold increased risk for schizophrenia when compared to the healthy population, show reductions in amygdala volume. Furthermore, support for the hypothesis that amygdala abnormalities may be directed by genes on the X–chromosome, comes from a study with subjects with X chromosome abnormalities, showing that a dosage sensitive genetic locus on the X chromosome appears to play a key role in the functional and structural development of the amygdala (Good et al., 2003). In addition, several lines of research have indicated that the number of X chromosomes is inversely correlated with amygdala volume (Goldstein et al., 2001; Good et al., 2003). For example, males (46,XY) have larger amygdalae than females (46,XX). Males and females do not only differ at the level of X chromosomes and amygdala volumes, at a clinical level gender differences are also present. A recent meta-analysis of 38 studies that reported sex-specific incidences of schizophrenia has provided evidence for a sex difference in the risk for developing schizophrenia (Aleman et al., 2003; Phillips et al., 2004). The reported risk ratio for males to

develop schizophrenia relative to females was 1.42 (95% CI 1.30–1.56). In addition, a meta-analysis on sex differences in deficit schizophrenia (which involves larger emotional deficits) even reported a ratio of 1.7 (Roy et al., 2001). Males not only suffer more often from schizophrenia when compared to females, men also appear to have an earlier age of onset and a more severe course of the illness (Gur et al., 1996). Interestingly, Klinefelter patients suffering from schizophrenia show several parallels with women suffering from schizophrenia; similar ages of onset, later than male schizophrenia patients, and a predominance of positive symptoms over negative symptoms (Pinabel et al., 1997).

X chromosome abnormalities may co-direct amygdala volumes partly through abnormal testosterone levels, because sex of the gonads that produce gonadal hormones is determined by genes on the sex chromosomes (Arnold et al., 2004). However, testosterone levels by itself seem not enough to explain the amygdala abnormalities in individuals with sex chromosome aneuploidies. For example, although females with Turner syndrome, who are missing one X chromosome, are also characterised by a reduction in testosterone levels (Hojbjerg Gravholt et al., 1999), these patients show an increase in amygdala volume rather than a reduction (Kesler et al., 2004). Differences in amygdala volume between Turner and Klinefelter patients appear to be stronger related to the number of X chromosomes, which differentiates the two groups, than to testosterone levels, which are reduced in both syndromes. Alternatively, abnormal testosterone levels may result from X chromosome linked amygdala abnormalities, since it has been shown that the amygdala is involved in the production of testosterone (Banczerowski et al., 2003). In conclusion, although the amygdala, testosterone levels and X chromosomes appear to strongly inter-related, the exact nature of the relationship still remains unclear.

We propose that in schizophrenia, X-linked genetic pathology, either directly or indirectly via testosterone, might result in a endophenotype characterised by amygdala abnormalities. Amygdala abnormalities as an endophenotype of putatively X-linked pathology may not be specific for schizophrenia. This endophenotype may also be present in other psychiatric disorders, for example autism, which has also been associated with structural abnormalities of the amygdala (Brambilla et al., 2003; Palmen et al., 2004). Interestingly, in autism males (46, XY) are affected four-fold more often than females (46, XX). Moreover, females suffering from Turner syndrome (45,X), who are missing one X chromosome, have a 200-fold increased risk of autism (Creswell et al., 1999). These findings support the idea that amygdala

abnormalities in autism may be linked to the X chromosome. If we in turn focus at psychiatric disorders characterised by X-linked genetic pathology, abnormalities of the amygdala have been consistently reported. Not only in the population of Klinefelter patients who are at increased risk for schizophrenia, also in Turner syndrome (complete or partial deletion of X chromosome) and Fragile X syndrome (mutations on X chromosome) abnormalities of the amygdala have been observed (Good et al., 2003; Hessl et al., 2004; Kesler et al., 2004). In contrast to Klinefelter Syndrome, these disorders have been associated with an increase in amygdala volume (Good et al., 2003; Hessl et al., 2004). Interestingly, it has been reported that Turner syndrome occurs approximately three-fold more frequently in schizophrenic females when compared to the general female population (Prior et al., 2000). Thus, amygdala abnormalities as an endophenotype may be present in several psychiatric disorders. This review has provided support that schizophrenia is one of these disorders. In addition, we propose that individuals with Klinefelter Syndrome, associated with X chromosome abnormalities, share this endophenotype with schizophrenia.

A clinically relevant question that arises from these speculations would be: How may amygdala abnormalities at an endophenotypical level extend to a phenotypical, behavioural level? Since the amygdala is strongly involved in emotion processing and appears to be part of a neural network underlying social cognition, one would expect emotional-and social difficulties to be present in the populations that have been described here. A review of the literature tentatively supports this idea. As observed in schizophrenia patients, relatives of patients show deficits in facial affect recognition, although they are more subtle (Toomey et al., 1999). Amygdala abnormalities as an endophenotype may result in behavioural deficits which, again, need not necessarily be specific for schizophrenia. In Autism and Turner Syndrome, deficits in facial affect recognition and impairments in social cognition have been reported (Baron-Cohen et al., 2000; Good et al., 2003; Klin et al., 2002; Lawrence et al., 2003). Interestingly, parallel to schizophrenia patients and amygdala damaged patients, individuals with autism and Turner syndrome show selective impairments in the recognition of facial expressions of fear (Howard et al., 2000; Lawrence et al., 2003).

Regarding high risk populations, there is a shortage of behavioural studies focused at emotion processing. Recently, our department has shown a range of emotional processing abnormalities in a non-clinical psychosis-prone sample (van 't Wout et al., 2004). In this population, unusual perceptual experiences, a positive schizotypal trait, correlated with more problems in identifying

emotions. Verbalizing and analysing emotions, as well as emotional arousability, correlated with constricted affect, a negative schizotypal trait.

Future research should investigate the functional consequences of amygdala abnormalities in genetically predisposed subjects using functional MRI and behavioural measurements. Specific effects of normal brain maturation, illness and medication on the amygdala should be examined in these subjects in longitudinal designs. Twin studies or designs in which multiplex families are included and obligate carriers are identified will provide unique opportunities for disentangling genetic from environmental effects on the development of schizophrenia.

It should be noted that several limitations have become apparent in the present review of studies. A number of limitations associated with structural measurement of the amygdala may contribute to the heterogeneity in findings in MRI-studies focused at high-risk populations. The amygdala is located in proximity of the nasal cavity, which may lead to an increase of MRI susceptibility artefacts. In addition, low scan resolution and limited number of slices through the amygdala make it more difficult to reliably estimate its volume and separate it from the hippocampus. Future studies should follow the guidelines given by Brierley et al. (Brierley et al., 2002; David et al., 2002) in order to enhance methodological rigor. In addition, in several MRI studies reported in this paper, the amygdala is measured as part of a complex (AHC) and not as a separate region of interest, which is a major limitation. However, although the amygdala and the hippocampus are anatomically dissociable, the AHC may be perceived as a unitary complex at some level, since they sustain functions that pertain to emotion processing (Phillips et al., 2003). The hippocampus has direct connections with the amygdala and plays a key role in context modulation of emotional behaviour (Davidson et al., 2000). Finally, the range of MRI studies focused at populations at high risk for developing schizophrenia is somewhat limited and not all studies find amygdala abnormalities. However, the majority of studies are indicative of a role of the amygdala or AHC abnormalities.

In summary, this review focused at structural abnormalities of the amygdala in various populations a high risk for developing schizophrenia. Studies with individuals from the general population showing subclinical signs of the illness and studies with relatives of schizophrenia patients indicate that amygdala abnormalities may form part of the genetic predisposition to schizophrenia. Amygdala abnormalities may be one of the endophenotypes in schizophrenia and might be X chromosome linked, as suggested by converging evidence from

individuals with X chromosome abnormalities. We therefore suggest that amygdala abnormalities might be an X-linked endophenotype in psychiatric disorders, especially in schizophrenia and populations at high risk for schizophrenia, and may account for emotional en social processing deficits that have been described in these populations.

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Chapter 10

Functional neuroimaging evidence for abnormal brain activation during social perception in a sex chromosomal disorder (Klinefelter syndrome, 47XXY)

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Abstract

Klinefelter syndome is a sex chromosomal disorder (47, XXY) that may help us to unravel genotype-phenotype relations. The XXY karyotype has been associated with behavioral problems in the social domain and deficits in social cognition have been observed. Here we used functional MRI to reveal the neural correlates of social perception in XXY men.

Eigtheen healthy men and thirteen XXY men were scanned during social evaluation of faces. In an event-related design, we measured activation in a neural network comprising the amygdala, insula, superior temporal sulcus (STS) and fusiform gyrus during judgments of faces with regard to trustworthiness. Judging faces as untrustworthy was associated with less activation in each of the four brain areas in XXY men as compared to men from the general population. To our knowledge, this is the first study of brain mechanisms underlying social perception in Klinefelter syndrome. Reduced engagement of brain areas important for social perception may explain some of the deficits in social cognition and in social behavior that have been observed in XXY men. Our findings suggest a link between the X chromosome and abnormal development of the neural networks involved in social perception in Klinefelter syndrome. Besides revealing a neural basis for social dysfunctions in this specific genetic disorder, the data show that Klinefelter syndrome may serve as a model for studying the genetic basis of disturbances in the development of social cognition, which are considered core abnormalities in severe psychiatric disorders such as autism and schizophrenia.

Introduction

Genetic disorders associated with specific deficits in brain development and cognition may help us to unravel genotype-phenotype relations. One such disorder is Klinefelter syndrome, defined by the presence of an extra X chromosome in men (47,XXY). Unlike many other X chromosomal disorders, XXY men do not display general intellectual deficits, which allows the study of specific cognitive abilities and their neural mechanisms without the confound of a general intellectual deficit.

Many boys and men with Klinefelter syndrome suffer from behavioral problems in the social domain, such as social withdrawal, social anxiety, shyness, impulsivity and inappropriate social behavior (Boone et al., 2001; Geschwind et al., 2000; Ratcliffe, 1999). Data on cognitive mechanisms that may underlie the impaired social adaptation in XXY men is scarce. It is generally thought that the social difficulties, particularly those regarding communication are attributable to the verbal disabilites that have been found consistently in Klinefelter syndrome (see Samango-Sprouse, 2001). However, other deficits, such as abnormal facial affect recognition, that were also observed in XXY men (Van Rijn et al., 2006b) may play an equally important role in explaining some of the social problems in Klinefelter syndrome.

Compared to what is known about the cognitive deficits that may contribute to social difficulties in Klinefelter syndrome, much less is known about the neural mechanisms that are involved. Interestingly, abnormalities in structural development of brain regions important for processing social information in XXY men are suggested by structural Magnetic Resonance Imaging (MRI) finding volume reductions the amygdala, insula, anterior cingulate and superior temporal gyrus in XXY men (DeLisi et al., 2005; Patwardhan et al., 2002; Shen et al., 2004). As Klinefelter syndrome is defined by the presence of an additional X chromosome, the structural abnormalities may result from genetic mechanisms involving the X chromosome. Support for the importance of the X chromosome for both structural and functional development of neural networks subserving social cognition is provided by neuroimaging studies with females with Turner syndrome, another X chromosomal disorder that is characterised by the presence of only one X chromosome in females (the X0 karyotype). X monosomy has been associated with abnormalities of the amygdala, superior temporal gyrus and -sulcus, anterior cingulate, orbitofrontal cortex and insula, which are brain regions involved in processing social information (Kesler et al., 2003; Kesler et al., 2004; Molko et al., 2004; Murphy et al., 1997; Skuse et al., 2005).

Although reductions in volume of brain areas important for social cognitive processing have been found in XXY men, it remains unclear whether and to what degree these neural networks are dysfunctional. In this study, we investigated the effects of an additional X chromosome on functioning of key brain regions involved in social perception (Adolphs, 2001). To our knowledge, this is the first study of functional brain mechanisms underlying social perception in Klinefelter syndrome. Using functional Magnetic Resonance Imaging (fMRI) we measured activity in a neural network including the amygdala, fusiform face area, insula and superior temporal sulcus during trustworthiness evaluations of faces. These regions were selected based on their involvement in trustworthiness evaluations in the general population (Winston et al., 2002).

The presence of dysfunctions in neural networks underlying social cognition in men with the XXY karyotype may have several potential implications. First, it would provide a neural basis for the social cognitive dysfunctions that have been observed in men with Klinefelter syndrome. Second, it would suggest that the X chromosome may play an important role in the development of some of the brain areas subserving social cognition in Klinefelter syndrome and possibly also in the general population. Third, we might speculate that the X chromosome plays a role in some of the brain abnormalities associated with social cognitive dysfunction in other disorders, such as autism and schizophrenia, which are more prevalent in men.

Methods

Subjects

Thirteen XXY men (mean age 39.0, SD 10.3) and 18 control men from the general population (mean age 32.2, SD 9.4) participated in the fMRI study. XXY men were recruited via the Dutch Klinefelter Association, and were not selected for psychological, behavioral or cognitive abnormalities. Diagnosis of Klinefelter syndrome was confirmed by genetic analysis (i.e. karyotyping) using standard procedures. Of the 13 XXY men, 11 were treated with testosterone supplements (mean age of treatment onset of 23.4, SD 6.8 years). Data on intellectual abilities as measured with the WAIS-III were available in our database for 11 XXY men (data on intelligence were collected within one year of the fMRI session). Mean general intelligence in these XXY men was 94.4 (SD 14.35), with a score of 96.3 (SD 14.9) for verbal intelligence and 93.7 (SD 12.0) for performance intelligence. There were no significant differences in age

(t(1,29)=3.5, p=0.07) or years of education between the groups (Klinefelter group 14.9 (SD 2.1), control group 15.9 (SD1.8), t(1,29)=1.29, p=0.18). None of the control subjects had a history of psychiatric illness as confirmed with the Mini International Neuropsychiatric Interview plus (MINI) (Sheehan et al., 1998). Exclusion criteria for both Klinefelter men and controls were neurological conditions or history of head injury with loss of consciousness, recent history of substance abuse and mental retardation. After complete description of the study (which was approved by the local ethical board) to the subjects, written informed consent was obtained according to the declaration of Helsinki.

Social judgment task

In the MRI scanner subjects completed a task (adapted from Winston et. al.) that involved judgments based on facial appearance. Subjects were presented with 16 task blocks of 45 s., in which every 3 s. a face was presented, alternated with rest periods of 45 s., during which a fixation cross was presented. Neural correlates of both explicit social evaluations as well implicit, indirect attention to social signals from faces was measured. In the explicit condition (eight task blocks) a trustworthiness judgment was required, i.e. untrustworthy or trustworthy. In the implicit condition (eight task blocks) an age judgment was required, i.e. older or younger than 30 years. Explicit and implicit task blocks were alternated. Decisions were indicated by button presses. Each face was presented once.

Scans

Functional scans were aquired with a Phillips ACS-NT 1.5-T clinical scanner, using a blood-oxygen-level dependent sensitive (BOLD), gradient-echo echoplanar T2*-weighted sequence, with the following parameter settings: echo time 40 ms; repetition time 76 ms; flip angle 90 degrees; field of view 192 x 192 x 99 mm. Each volume comprised 33 x 2.2 mm axial scans with 3-mm in-plane resolution, and volumes were continuously acquired every 2.5 s in an interleaved fashion (bottom slice first). Each run was preceded by 6 'dummy' scans (which were not used in further analyses) to allow for T1 equilibration effects. Finally, a T1 weighted structural image was acquired.

Regions of interest

Regions of interest (ROI's) were selected based on the findings in the fMRI study of Winston et al. (2002), showing that the amygdala, insula, fusiform gyrus and superior temporal sulcus (STS) are key brain regions involved in judging trustworthiness from faces. Using the WFU Pickatlas tool for SPM (Maldjian et al., 2003), ROI's for the fusiform area, insula and STS were formed based on the talairach atlas (in MNI space) and a ROI for the amygdala was formed based on the AAL atlas (in MNI space). Size of the ROI's was 162 voxels for the amygdala, 1029 voxels for the fusiform gyrus, 1436 voxels for the insula and 3872 voxels for the STS.

fMRI analysis

Functional MRI data preprocessing and analysis was done using SPM2 (Wellcome Department of Imaging Neuroscience, London, England; www.fil.ion.ucl.ac.uk). After slice-timing correction, all functional scans were registered to the last volume of the last block and coregistered to the anatomical scan. Next, all functional images were registered to an MNI standard brain, to enable group-wise comparisons. All volumes were then smoothed with a 6 mm full-width half-maximum isotropic Gaussian kernel. Time series were high-pass filtered with a cut-off of 128 sec. to remove low frequency signal changes.

In this event-related design, brain activity maps were obtained by analysing the fMRI scans categorised according to individual judgments of trustworthiness and age. For each subject, a statistical map (i.e. t-map) was obtained from a general linear model regression analysis using a factor matrix that contained a regressor modeling the onsets of faces that were judged to be untrustworthy, a regressor modeling the onsets of faces that were judged to be trustworthy and a regressor which modeled the onsets of faces that were judged during the age condition.

First, a whole brain analysis was performed (for the groups seperately) on activation related to trustworthiness decisions (all faces) versus baseline. P value was set at 0.001, uncorrected, with an extend threshold of 10 voxels. Second, significant activation in each region of interest was determined in each voxel by applying a statistical treshold. The threshold corresponded to a p value of 0.05, FWE-corrected for multiple comparisons and resulted in the control group to a t-value of 3.70 for the amygdala, 4.80 for the fusiform gyrus, 5.22 for the insula and 5.73 for the STS. To enhance the interpretability of the within-group activation patterns, the thresholds that we applied in the XXY group were identical to those in the control group. Note however, that group differences in

brain activation were explicitly tested in two-sample T-tests. Several averaged contrasts maps were creasted for both groups, namely: trustworthy minus base-line, untrustworthy minus base-line and untrustworthy minus trustworthy. The averaged contrast maps for each group (XXY men and controls) were subsequently entered in a two-sample t-test and differential task activation was defined as differential activation above a significance threshold of $P<0.001$, uncorrected.

Results

Behavioral

A repeated measures analysis indicated no significant interactions between group and trustworthiness judgments ($F1,29)=0.03$, $p=0.87$). Within each group, no significant differences between the mean number of decisions of 'trustworthy' and 'untrustworthy' were observed, as indicated by paired sample t-tests ($t(1,12)=1.21$, $p=0.25$ for XXY men and $t(1,17)=1.70$, $p=0.11$ for controls).

Neural activations associated with judging trustworthiness of faces

Results of the whole-brain analysis are presented in table 1 for controls and table 2 for XXY men. With regard to our regions of interest, within-group analyses indicated significant activation in the fusiform gyrus in both groups when faces were judged *trustworthy* compared to baseline. In the control group the t value was above threshold in 68 voxels (t max.=7.9; x,y,z= -39, -69, -21), whereas in the XXY group this was 17 voxels (t max.=8.2, x,y,z= 45, -57, -24). Indeed, a subsequent two-sample t-test indicated slightly, but significantly, more activation in the fusiform gyrus in controls as compared to XXY men (1 voxel, t max.=3.6, $p<0.001$, x,y,z= 30, -60, -15). No significant activation in the amygdala, STS or insula was observed in both groups. The crucial comparison was the difference in brain activation between the groups when faces were judged *untrustworthy* (versus baseline). Again, within group analyses of activation in our ROI's indicated significant activation in the fusiform gyrus in the control group (62 voxels, t max.=7.8; x,y,z= 39, -69, -18) and the XXY group (1 voxel, t=4.9; x,y,z= 45, -57, -24). In addition, significant activation in the amygdala (bilaterally) was observed in the control group (16 voxels, t max.=4.2; x,y,z= 21,-3,-18), but not in the XXY group. A two sample t-test not only showed significantly more activation in the fusiform gyrus (17 voxels, t max.=4.7,

p<0.001, x,y,z= -30, -33, -24), including the fusiform face area (FFA, see Grill-Spector et al., 2004 for coordinates), but also in the left amygdala (3 voxels, t max.=4.77, p<0.001, x,y,z=-21, 3, -18) in the control group as compared to the XXY group. In addition, small but significant group differences were observed in the insula (1 voxels, t max.=3.6, p=0.001, x,y,z= 27, 12, -21), with reduced activation in the XXY group.

Neural activations associated with judging age of faces

When judging the age of the presented faces, significant activation (as compared to baseline) in ROI's important for social perception was observed in both groups. In controls, significant activation in the fusiform gyrus (143 voxels, t max.=10.2, x,y,z=-39, -48, -24), insula (12 voxels, t max.=6.6, x,y,z= -33, 18, 9) and amygdala (2 voxels, t max.=4.9, x,y,z= 21, -3, -12) was observed. In XXY men, significant activation was found in the fusiform gyrus (30 voxels, t max.=7.8, x,y,z= 42, -54, -21) and slight activation in the insula (1 voxel, t=6.1, x,y,z= -39, 12, 15) and amygdala (1 voxel, t=3.9, x,y,z= -24, -3, -27), but not in the STS. A two-sample t-test showed that activation in the fusiform gyrus (11 voxels, t max.= 4.3, p<0.001, x,y,z= -30, -66, -15) and STS (12 voxels, t max.= 4.05, p<0.001, x,y,z= -45, -54, 12), but not in the insula or amygdala, was significantly stronger in in the control group than in the XXY group.

Table 1
Areas with significant activation associated with trustworthiness judgments of faces in controls as indicated by a whole-brain analysis.

Region	Max. t value	Max. z value	Nr. of voxels	x,y,z peak activation
Middle occipital gyrus	8.99	5.39	528	-48, -78, -12
Lingual gyrus (region includes fusiform gyrus)	8.47	5.23	1704	-3, -75, 0
Inferior frontal gyrus	7.19	4.81	234	48, 6, 36
	6.85	4.68	81	30, 30, -3
	4.75	3.74	17	51, 36, -6
Medial frontal gyrus	6.88	4.69	90	-6, 6, 48
Insula	6.53	4.56	61	-33, 21, 0
Cingulate gyrus	6.53	4.56	18	9, 24, 30
Caudate nucleus	6.11	4.38	21	-12, 9, 3
Thalamus	6.04	4.36	77	18, -24, 12
	5.11	3.92	38	-6, -18, 9
Parahippocampal gyrus	5.75	4.23	23	-15, -3, 15
Middle frontal gyrus	5.27	4.00	26	36, 42, 27
	5.04	3.89	10	42, 42, 15
Amygdala	4.89	3.81	14	18, -6, -18
Cerebellum	4.38	3.54	11	24, 9, -9

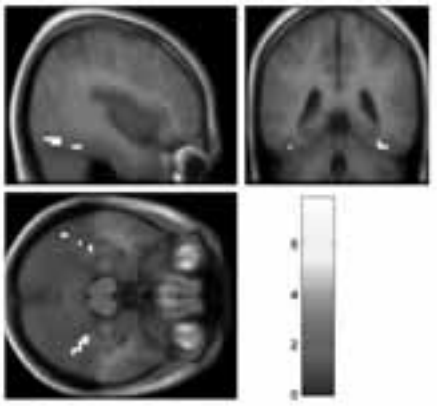
Table 2
Areas with significant activation associated with trustworthiness judgments of faces in XXY men as indicated by a whole-brain analysis.

Region	Max. t value	Max. z value	Nr. of voxels	X,y,z peak activation
Cuneus (occipital)	9.80	5.05	17	21, -93, 18
Fusiform gyrus	8.93	4.86	64	45, -57, -24
	5.28	3.73	26	33, -75, -18
Lingual gyrus	6.21	4.08	63	12, -87, -3
Cerebellum	6.15	4.06	14	-18, -81, -21
	4.96	3.59	16	-42, -57, -27
Middle frontal gyrus	6.05	4.02	17	-39, 45, 12

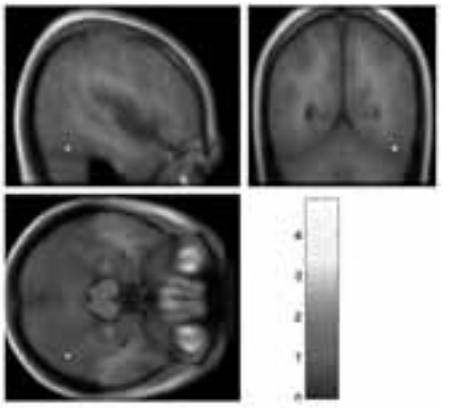
Figure 1

T-maps showing voxels in the fusiform gyrus and amygdala with a t-value above threshold ($p<0.05$, FWE corrected for multiple comparisons) during untrustworthy judgments of faces in the control group and XXY group. 1A) Significant activation in the fusiform gyrus, including the fusiform face area, in control men (62 voxels, maximal voxel (x,y,z) 39,-69,-18, T=7.8). 1B) Significant activation in the fusiform gyrus in XXY men (1 voxel, (x,y,z) 45, -57, -24, T=4.9). 1C) Significant activation in the amygdala in control men (16 voxels, maximal voxel (x,y,z) 21,-3,-18, T=4.2). No significant activation in the amygdala was observed in XXY men.

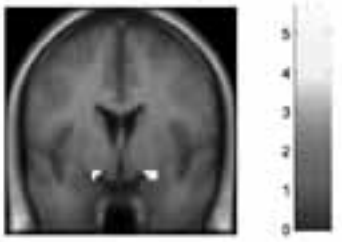
Two-sample t-tests confirmed that the XXY group had significantly less activation in the fusiform gyrus (including the fusiform face area) (17 voxels, t max.=4.7, $p<0.001$, x,y,z= -30, -33, -24) and amygdala (3 voxels, t max.=4.8, $p<0.001$, x,y,z=-21, 3, -18). In addition, small group differences were observed in the the insula (1 voxels, t max.=3.6, $p=0.001$, x,y,z= 27, 12, -21), with reduced activation in the XXY group.



1A. Fusiform gyrus activation in control men



1B. Fusiform gyrus activation in XXY men



1C. Amygdala activation in control men

Discussion

This fMRI study examined brain activation patterns underlying social evaluation of faces in men with an extra X chromosome (XXY chromosomal pattern). Compared to men from the general population, evaluation of faces as untrustworthy was associated with decreased activation in the amygdala, insula and fusiform face area, which are key neural structures subserving social perception. In addition, we observed small, but significant, group differences in brain activation in the insula, with lower activation in XXY men. When faces were evaluated with regard to age, during which faces may be implicitly screened for social significance as shown by Winston et al. (2002), we again observed less activation in the fusiform gyrus and insula, but not in the STS and amygdala, in XXY men as compared to men from the general population.

The finding of decreased involvement of brain areas that process socio-emotional signals in XXY men provides, for the first time, a putative neural explanation for the reported social cognitive deficits (Van Rijn et al., 2006b) in Klinefelter syndrome. Using an event-related design, brain activation was analysed according to subjective evaluation of untrustworthiness. Because the distribution of trustworthiness decisions was not different between XXY men and controls, we may assume that differences in brain activation patterns did not reflect differences in behavioral performance. Therefore, our findings of less activation in the amygdala and fusiform face area during untrustworthy faces in XXY men as compared to men from the general population, can be interpreted as abnormal functioning of these brain areas in Klinefelter syndrome.

The amygdala plays a central role in the neural circuit subserving social cognition, as indicated by the high density of incoming and outgoing projections to other brain regions (Aggleton, 2000). The amygdala is especially known for its engagement in screening information for emotional and social significance, especially threat-related information (Amaral, 2003; Phelps, 2006). As emotional expressions on faces provide a crucial source of information needed for decoding social and emotional signals, the amygdala generally activates in response to facial expressions, especially to angry or fearful faces (Adolphs, 2001; Haxby et al., 2002; Phan et al., 2002). Indeed, in men from the general population, we observed in this study significant activation in the amygdala when judging faces as untrustworthy as well as an increase in amygdala activation during untrustworthy- as compared to trustworthy decisions. In contrast, no significant activation in the amygdala was seen in de XXY group when judging trustworthiness of faces. Interestingly, the functional abnormalities of the amygdala in XXY men as found in this study are in line

with volume reductions of this area that have been observed in structural MRI studies in Klinefelter syndrome (Patwardhan et al., 2002; Shen et al., 2004).

One of the areas that receives input from the amygdala is the fusiform gyrus, which includes the 'fusiform face area' (FFA) (Puce et al., 1996). This area appears to be important for visual processing of the structural, static properties of faces, which are used to determine personal identity (Adolphs, 2001; Haxby et al., 2000). It has been shown that activation in the fusiform gyrus can be modulated by activation in the amygdala (Morris et al., 1998). Increasing threat-related social significance as processed in the amygdala may elicit re-allocation of attentional resources to allow detailed visual analysis of a socially significant stimulus. We speculate that lower levels of activation in the fusiform face area during judgment of faces as untrustworthy might, in part, result from reduced activation in the amygdala.

The insula is another brain area that is closely connected to the amygdala and is thought to be subject to attentional modulation by the amygdala (Adolphs et al., 2006). This somatosensory area is important for monitoring and organizing physiological (autonomic) changes in the internal milieu, as is seen in response to emotion-inducing stimuli (Damasio et al., 2000). It appears to be especially involved in mediating affective responses to aversive, including disgust-related, incoming information (Adolphs, 2002; Phillips et al., 2003a). The latter is consistent with a role of this region in judging faces as untrustworthy. XXY men displayed less activation associated with (un)trustworthiness in the insula as compared to controls. Significantly, the functional abnormalities of this region as found in this study are in line with structural abnormalities, i.e. smaller volumes, of the insula that have been reported for XXY men (Shen et al., 2004).

It has been shown by Winston et al. (2002) that not only during trustworthiness judgments, but also during evaluation of faces with regard to age, the fusiform face area, amygdala and insula are active. This suggests that faces may be automatically screened for social significance. Indeed, in controls we observed activation in these areas during age-judgments of faces. Similar to the trustworthiness condition, XXY men displayed less activation in the insula and fusiform gyrus during age-evaluations as compared to control men. However, no significant group-differences were present in activation in the amygdala, suggesting that even though explicit screening for social relevance by the amygdala seems to be diminished in Klinefelter syndrome, implicit screening for social relevance may be intact.

Our observations of disturbed development of some regions in a neural network supporting social cognition in XXY men may have several implications. First, abnormal engagement of a neural network subserving social perception may underlie some of the social cognitive dysfunctions and impaired social adaptation that have been described in XXY men (Van Rijn et al., 2006b). In other words, it provides a neuro-anatomical basis for the social behavioral phenotype in Klinefelter syndrome. Second, we can extrapolate from these findings that the X chromosome may play an important role in the development of some of the brain areas subserving social cognition. This is also supported by findings of abnormal development of brain areas involved in social cognitive processing in Turner syndrome, another X chromosomal disorder that is characterised by the presence of only one X chromosome in females (i.e. the X0 karyotype) (Mazzocco et al., 1998; McCauley et al., 2006; Ross et al., 2000). Similar to XXY men, individuals with X monosomy display impairments in social cognition, such as facial affect recognition (Lawrence et al., 2003; McCauley et al., 1987). Interestingly, imaging studies have revealed structural and functional abnormalities in the amygdala and insula in females with Turner syndrome, regions that we also found to be abnormal in XXY men (Molko et al., 2004; Murphy et al., 1997; Skuse et al., 2005). Although speculative, involvement of one of the sex chromosomes in the development of social cognitive abilities would also be in line with the observed sex differences in social cognitive skills in the general population, with performance of men generally somewhat lower than that of women (Hall, 1984; Hampson et al., 2006; McClure, 2000). Third, we might consider a role the X chromosome in some aspects of social cognitive dysfunction and underlying neuroanatomical abnormalities in other disorders, such as autism or schizophrenia. Deviant social behavior and impairments in social cognitive functions are considered core abnormalities in these neurodevelopmental disorders (Abdi et al., 2004; Corrigan et al., 2001; Fein et al., 1986). Neuroimaging studies have consistently shown abnormalities of the amygdala, insula and fusiform face area in both autism and schizophrenia (Grelotti et al., 2002; Phillips et al., 2003b; Quintana et al., 2003). Interestingly, these disorders are more prevalent in men. The reported risk ratio for men to develop schizophrenia relative to women is 1.42 (Aleman et al., 2003), while autism is diagnosed approximately four times more often in boys than in girls (Volkmar et al., 1993). This fact has led others to propose that dysfunctional neural circuits underlying social cognitive impairments in autism may be related to genes on the X chromosome, that are differentially expressed in men and women (Baron-Cohen et al., 2005; Skuse,

2000). Interestingly, a study on sex differences in social perception in schizophrenia has shown that the female superiority in reading social signals from faces seems not to be affected by schizophrenia, in contrast to males with the disorder who perform significantly worse as compared to healthy men (Scholten et al., 2005).

Both Turner syndrome and Klinefelter syndrome have been associated with increased symptoms and personality traits that resemble features from the autism- or schizophrenia spectrum. The estimated risk of autism spectrum disorders may be several times higher in women with X-monosomy (3 %) as compared to women from the general population (0.01 %) (Creswell et al., 1999). In Klinefelter syndrome, we observed increased levels of autism traits, schizotypal personality traits and schizophrenia-like symptoms (Van Rijn et al., 2006a; Van Rijn et al., submitted). In addition, a survey of hospital admissions and discharge diagnoses has indicated a significantly increased relative risk of being hospitalized with psychoses (hazard ratio of 4.97) for men with Klinefelter syndrome (Bojesen et al., 2006). In turn, there is suggestive evidence that prevalence of the XXY karyoype in the male schizophrenia population may be several times higher as compared to the prevalence of Klinefelter syndrome in the general population (DeLisi et al., 1994; Kunugi et al., 1999). Although speculative, observations of increased autism- or schizophrenia spectrum pathology in Turner syndrome and Klinefelter syndrome fit with the hypothesis that the X chromosome may play a role in some of the developmental abnormalities of the neural structures for social information processing, such as the amygdala, insula or fusiform face area, in autism or schizophrenia.

In sum, this study has revealed reduced engagement of the amygdala, insula and fusiform face area during social perception in XXY men as compared to men from the general population. Compromised function of these areas may underlie some of the deficits in social cognition and social behavior that have been observed in XXY men. In addition, as Klinefelter syndrome is defined by an X chromosomal abnormality, our findings suggest a link between one of the sex chromosomes and development of some of the neural regions supporting social cognition. In addition to revealing a neural basis for social dysfunctions in this specific disorder, our data suggest that Klinefelter syndrome may serve as a more general model for studying the genetic basis of developmental abnormalities in social cognition. This may be particularly relevant since these deficits are considered core abnormalities in severe psychiatric disorders such as autism and schizophrenia.

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Chapter 11

General discussion

Social cognitive capacities are crucial for flexible navigation through a social world. The severe impact of impaired social cognition is illustrated by disorders such as autism or schizophrenia. In these neurodevelopmental disorders, social cognition is among the core domains of deficits (Corrigan et al., 2001; Fein et al., 1986). The importance of social cognitive capacities in coping with the social world and related mental well-being has called for a search into the origins of social cognition on the level of cognition, neurobiology and genes.

Klinefelter syndrome is an X chromosomal disorder known for social dysfunction and specific deficits in brain development and cognition, which can help us to unravel genotype-phenotype relations that are relevant for social behavior. Klinefelter syndrome might be used as a model for disorders of social behavior to reveal insight into involvement of the X chromosome in social cognitive pathways to psychopathology which may be more difficult to uncover by studying heterogeneous, behaviorally defined populations (Reiss, 2000; Reiss et al., 2000).

In the present thesis, we investigated the effects of an extra X chromosome on social cognition and the underlying neural basis. Besides providing insight into the cognitive and neural basis of social dysfunctions in this specific genetic disorder, another aim of the present thesis was to explore whether Klinefelter syndrome may serve as a model for social cognitive disturbances in autism or schizophrenia. We have focused on socially deviant behavior in adult XXY men on a behavioral, cognitive and neuroanatomical level and reviewed evidence for autism and schizophrenia spectrum traits, social dysfunction, social cognitive disabilites and underlying dysfunctional neural mechanisms in XXY men.

Social behavior and psychopathology

As described in **chapter two** and **three**, we assessed social difficulties, reflected in frequency of participation in social interactions and distress during these interactions, as well as vulnerability to disorders of social behavior, such as autism and schizophrenia, in XXY men. Vulnerability to neurodevelopmental disorders associated with difficulties in social adaptation was investigated from a dimensional, symptom oriented perspective, rather than a dichotomal, all-or-none, approach. Dimensional measures allow quantification of a number of traits associated with the autism or schizophrenia spectrum. In addition, we used a measure of schizophrenia symptoms to quantify symptoms at a clinical level. Measures of social behavior and psychopathology in XXY men were compared to those of men from the general population.

Men with Klinefelter syndrome reported to less often engage in social behavior and to experience more distress during social interactions as compared to men from the general population. In addition, XXY men displayed significantly increased levels of schizophrenia spectrum traits and symptoms as compared to controls. Scores were higher on all traits, with effect sizes (cohen's d) of 1.43 for the negative dimension (for example constricted affect and social anxiety), 1.31 for the positive dimension (for example referential thinking and delusional atmosphere) and 1.81 for the disorganized dimension (vague/overinclusive speech and eccentric behavior). Also on a clinical level, higher levels of schizophrenia symptoms were observed in XXY men, with effect sizes of 1.60 for negative symptoms, 1.45 for positive symptoms and 1.66 for general psychopathology. These findings provide evidence for increased vulnerability to schizophrenia psychopathology in Klinefelter syndrome and are in agreement with reports of increased prevalence of the XXY karyotype among schizophrenia populations (DeLisi et al., 1994; Kunugi et al., 1999) and a survey of hospital admissions and discharge diagnoses that has indicated a significantly increased relative risk of being hospitalized with psychoses (hazard ratio of 4.97) for men with Klinefelter syndrome (Bojesen et al., 2006). Similarly, levels of autism traits, such as impaired social skills, communication, imagination, attention switching and increased attention to details, were all higher in XXY men as compared to controls. This is the first report of increased levels of autism traits in Klinefelter syndrome.

These findings suggest that men with Klinefelter syndrome less often engage in social behavior and experience more distress during social interactions as compared to men from the general population. Our findings of difficulties in coping with social situations in XXY men, especially the high levels of distress during social interactions, are consistent with reports of social anxiety, social withdrawal and shyness in individuals with the XXY karyotype (Bender et al., 1999; Ratcliffe, 1999). Difficulties in social adjustment have mostly been reported for children or adolescents with Klinefelter syndrome. Our data suggest that social difficulties may persist into adulthood, with social distress more prominent than a general reduction in engagement in social behavior.

The high levels of autism traits and schizotypal traits in XXY men that were observed across all dimensions of the autism and schizophrenia phenotype, suggest that some aspects of the XXY phenotype parallel the phenotype that characterizes individuals at increased risk for autism or schizophrenia. For example, biological relatives of subjects with these disorders also display increased levels of schizotypal- or autism traits (Bishop et al., 2004; Vollema et

al., 2002). This hypothesis is in agreement with the concept of 'broad phenotypes' of autism or schizophrenia, which refers to mild features of the clinical autism- or schizophrenia phenotype that are seen in biological (i.e. genetically related) relatives of individuals with these disorders (Bailey et al., 1998; Bishop et al., 2004; Gottesman et al., 2003; Jablensky, 2006).

Thus, mild features of the autism or schizophrenia phenotype may not only be found in genetically related relatives of individuals with these disorders, but also in men with an extra X chromosome.

Social cognition

Next, we investigated the effects of an extra X chromosome on social cognition and explored whether social cognitive dysfunction in Klinefelter syndrome may be an underlying cognitive mechanism contributing to difficulties in coping with social environments as reflected in social distress and increased autism or schizophrenia traits. Several aspects of social cognitive processing were assesed at the level of perception, experience and expression, namely: labeling of facial expressions of emotion, emotion-cognition interactions in decision making, automatic processing of social cues such as gaze direction and implied biological motion, discriminating emotions in tone-of-voice, and emotion regulation, i.e. subjective experience and identification of emotional arousal as well as verbal expression of emotions.

Perception

As described in **chapter four**, we found XXY men to be impaired in recognizing facial expressions of anger, independent of the intensity of the emotional expression. This deficit was not attributable to a generalized impairment in analyzing faces, as performance in a face identity matching task was intact. Misperception of angry facial expressions may contribute to difficulties in social interactions, as non-verbal signals can convey crucial information about the emotional state of the sender. Various studies have revealed significant relationships between facial affect recognition performance and social functioning (Hooker et al., 2002).

The XXY group not only displayed deficits in processing social signals in the visual domain, but also in the auditory domain. Besides facial expressions of emotions, affective prosody in speech is also important for understanding intentions of others, because variations in tone of voice, such as intonation and loudness, provide information about the emotional state of the speaker. In social exchange, it is not only important to understand the content of words and

sentences, i.e. what is said, but it may even be more crucial to consider the (social) context of words and sentences, i.e. how it is said. Lesion- and fMRI studies indicate that whereas the left hemisphere is specialized for processing linguistic (semantic) information, the right hemipshere may be specialized for analyzing pragmatic aspects of language, such as understanding metaphors, irony, discourse, indirect requests and analyzing emotional prosody. In **chapter six**, we provided evidence for a causal involvement of the right hemisphere, more specifically the fronto-parietal operculum, in processing affective prosodic information in individuals from the general population. Applying inhibitory Transcranial Magnetic Stimulation (TMS) to the right hemisphere temporarily disrupted recognition of emotions in prosodic cues in speech, but not semantic (linguistic) cues. These findings extend an earlier study of Vingerhoets et al. (Vingerhoets et al., 2003) who have measured blood flow velocity (BFV) with functional transcranial doppler ultrasonography (fTCD) to study the contribution of the right and left hemisphere to the detection of emotion in prosody versus semantics of spoken language using this particular task. During detection of emotion in semantics they observed a significant left-hemispheric lateralization of BFV. This lateralization effect disappeared when attention was shifted to discriminating emotion in prosody, due to a rise in right hemispheric BFV. Our findings provide evidence that the right hemisphere is not only associated with, but also causally involved in emotional prosody.

As described in **chapter seven**, discrimination of emotions in prosodic cues was impaired in XXY men. Although recognizing emotions in semantic cues was also impaired in the XXY group, which is in line with the typical left hemisphere mediated language dysfunctions in Klinefelter syndrome, recognizing emotions in prosody, a pragmatic, right hemisphere mediated aspect of language, was more affected. Difficulties in picking up pragmatic communicative cues in conversation might have an impact on how well XXY men cope with social situations, as these aspects of language are important for understanding interpersonal intentions and responding to those in an appropriate way.

Another important underlying characteristic of successful social interaction is the ability to quickly and automatically process basic elements of a social signal such as direction of gaze, head orientation and body postures (Frith et al., 1999; Jellema et al., 2005). These cues can give clues about someone's intentions, goals and believes (Perrett, 1999). It is suggested that the ability to process these basic social cues automatically is a prerequisite for establishing successful social interactions and communication (Frith and Frith, 1999). We have shown

(**chapter five**) that in contrast to men from the general population, XXY men have difficulties in automatically attending to, and process, basic social cues such as gaze direction and implied biological motion. Reduced availability of basic social information may contribute to more widespread effects on ('upstream'-) higher-order social cognitive processing. Although speculative, this reduced sensitivity to basic elements of a social signal might, in part, explain social awkwardness and reduced social intuition and which have been described in XXY men. We not only compared performance of XXY men with matched controls, but we were also able to include patients with schizophrenia as well as biological relatives of schizophrenia patients. Interestingly, performance in processing these social cues was indistinguishable between Klinefelter men and schizophrenia patients. Moreover, performance of biological relatives of schizophrenia patients resembled the lack of sensitivity to social cues observed in schizophrenia patients and XXY men, albeit to a lesser extent, indicating that it may be an expression of genetic vulnerability for the disease.

Experience and expression

Not only perception, but also disturbances in experience and expression of emotions were observed in XXY men (**chapter four**). Regarding the experience of emotions, Klinefelter men rejected more often unfair financial offers from human proposers as compared to the control group in the Ultimatum game. This pattern of performance is shown to reflect the influence of emotion, in response to the offer being unfair, on strategic decision making (Sanfey et al., 2003; van 't Wout et al., 2006). Besides this implicit emotional task, increased emotional experience was also observed in an explicit, self-report measure of emotional experience, i.e. the alexithymia questionnaire. Alexithymia is a personality trait implying an inability or reduction to identify, experience, describe and reflect on one's own emotions (Lane et al., 1997; Sifneos, 1973). In contrast to apparent *hyperfunctional* emotional -experience and -reactivity, identifying and verbalizing one's own emotions appeared to be *hypofunctional* in Klinefelter syndrome. This dissociation has been termed type II alexithymia, which contrasts with type I alexithymia (a general reduction in processing emotions). This emotion regulation profile of increased experience but decreased expression of emotions can be considered as a possible risk factor for both medical and psychiatric disorders (Bagby et al., 1997).

Taken together, Klinefelter men seem less accurate in perception of social signals, both from facial expressions and tone-of-voice, and have reduced automatic attention to basic social cues, such as gaze direction and implied

biological motion. Moreover, they are less able to identify and verbally describe their emotions, but experience increased levels of emotional arousal, in comparison to men from the general population. Although it has been suggested in the literature that the reported deficits in typical left hemisphere mediated language functions largely explain social dysfunction in XXY men, the present thesis identified dysfunctions in processing social signals, emotions and right hemisphere mediated pragmatic language as mechanisms that may also contribute to social dysfunction and related mental well-being.

Social cognition: neural basis

Language

As it has been proposed that language impairments, with language being a crucial part of social communication, may contribute to the observed difficulties in social functioning in Klinefelter men (Samango-Sprouse, 2001), we studied the neural basis of language processing in XXY men. Although language impairments in Klinefelter syndrome are well-documented, and are not restricted to the typical left hemisphere language deficits but also include right hemisphere language dysfunctions as pointed out in the present thesis, underlying neural mechanisms are not well understood (Geschwind et al., 2000). One possible neural mechanism involved in the verbal disabilities in Klinefelter syndrome that we investigated was abnormal hemispheric involvement in language processing.

By using functional Magnetic Resonance Imaging (fMRI) we were able to assess lateralization of activation in specific brain areas during language processing in XXY men (**chapter eight**). By measuring the relative contribution of the right and left hemisphere in each individual it was shown that language activity in the brain was indeed less lateralized in the XXY group as compared to control men. Loss of asymmetric processing of language was due to increased activity in the right hemisphere rather than reduced activity in the left hemisphere. Analysis of functional asymmetry within different language regions indicated that loss of functional asymmetry in the superior temporal gyrus (STG) was the most prominent contributor to reduced language laterality in XXY men. This regional loss of language laterality was highly correlated with the degree of disorganization (vague or overinclusive speech and odd/eccentric behavior) in the XXY group. This is consistent with studies finding structural (Matsumoto et al., 2001; Menon et al., 1995; Rajarethinam et al., 2000; Rossi et al., 1994; Shenton et al., 1992) and functional (Kircher et al., 2002) abnormalities

of the STG to be related to disorganization symptoms such as thought disorder in schizophrenia.

Social perception

The present thesis revealed that not only some aspects of language but also perception of social signals, another core function within the domain of social cognition, may be disturbed in XXY men. We explored the effects of an extra X chromosome on the neural basis of social cognition and reviewed structural neuroimaging findings in Klinefelter syndrome, as well as investigated neural networks supporting social perception in XXY men using functional neuroimaging.

The amygdala seems to play a central role in social perception and is especially known for its engagement in screening information for emotional and social significance (Amaral, 2003; Phelps, 2006). In **chapter nine** we have reviewed evidence for structural abnormalities of the amygdala in Klinefelter syndrome based on findings in the literature. Findings were compared to what is known of abnormalities of the amygdala in populations with increased vulnerability to schizophrenia: individuals from the general population displaying subclinical signs of schizophrenia and biological relatives of schizophrenia patients who may carry a genetic predisposition for the disorder. Reductions in volume of the amygdala were found to be present both in Klinefelter syndrome and the populations at high risk for schizophrenia, i.e. across the spectrum of schizophrenia spectrum vulnerability. Morphological abnormalities of the amygdala provide a putative neuro-anatomical basis for the deficits in social perception, such as impaired facial affect recognition, that we have observed in XXY men. In addition, the fact that amygdala abnormalities are not only consistently reported in patients with schizophrenia (Breier et al., 1992; Bryant et al., 1999; Gur et al., 2000; Hulshoff-Pol et al., 2001; Lawrie et al., 2003; Wright et al., 2000) but are also observed in populations with an increased liability for schizophrenia is of considerable importance. Abnormalities of the amygdala in the schizophrenia spectrum may be neurodevelopmental in origin and might reflect a genetic vulnerability for the disease rather than being the consequence of antipsychotic medication, hospitalisation and neurotoxic effects of psychosis.

Structural abnormalities of the amygdala in Klinefelter syndrome suggest abnormal functioning of this brain area, possibly extending to a neural network for social perception this region is part of. By using fMRI we were able to study functioning of such a neural network during social evaluation of faces

(**chapter ten**). We measured brain activity during trustworthiness evaluations of faces, with special interest in the amygdala, fusiform face area, superior temporal gyrus and insula. These regions are highly interconnected brain regions involved in social perception. We selected those regions based on their involvement in trustworthiness evaluations of faces in the general population (Winston et al., 2002). Activity in the amygdala during trustworthiness evaluation of faces may represent screening of social relevance and especially threat. Involvement of the fusiform face area may indicate processing of the structural, static properties of faces that are used for identification. Activation in the superior temporal sulcus may reflect efforts to detect goals and intentions based on eye-gaze and other signals in faces. Finally, insula activity may represent monitoring and organizing physiological (autonomic) changes in the internal milieu in response to the degree of trustworthiness of faces.

As predicted, we observed less involvement of region in this key neural network subserving social cognition during untrustworthy faces as compared to controls. Significantly lower levels of activation were observed in the amygdala, insula and fusiform face area in XXY men as compared to controls. The findings of less involvement of key brain areas in processing socio-emotional signals in XXY men offer, for the first time, insight into the neural basis of social cognition in Klinefelter syndrome. Abnormal engagement of a neural network subserving social perception may contribute to social cognitive dysfunctions and impaired social adaptation that have been described for men with Klinefelter syndrome.

Taken together, we observed reduced hemispheric specialization for language and less engagement of a neural network processing social information in XXY men. These findings, for the first time, may provide a neural explanation for some of the social cognitive impairments in Klinefelter syndrome.

The X chromosome and social cognition

As Klinefelter syndrome is defined by an X chromosomal abnormality, our findings suggest that the X chromosome plays a role in the development of some aspects of social cognition and neural mechanisms involved in social information processing in Klinefelter syndrome. Additional support for a putative role of the X chromosome in the development of social information processing is derived from observations of social dysfunction and social cognitive disabilites in Turner syndrome (45,X0), another X chromosomal disorder characterised by a partial or complete absense of one of the X

chromosomes in females (Mazzocco et al., 1998; McCauley et al., 2006; Ross et al., 2000). Similar to XXY men, individuals with X monosomy display impairments in facial affect recognition (Lawrence et al., 2003b; McCauley et al., 1987). Also, deficits in gaze direction processing and impairments in judging mental states from eyes have been reported (Elgar et al., 2002; Lawrence et al., 2003a). Difficulties in picking up social signals are not only observed in the visual domain, but also in the auditory domain. Comparable to men with the XXY pattern, women with one X chromsome display impairments in decoding emotions from tone of voice, i.e. emotional prosody (Ross et al., 1995).

Similar to XXY men, abnormal development of brain regions that are part of a neural network supporting social cognition have been observed in 45,X0 females. These include the amygdala, superior temporal sulcus and -gyrus, anterior cingulate, orbitofrontal cortex and insula (Kesler et al., 2003; Kesler et al., 2004; Molko et al., 2004; Murphy et al., 1997; Skuse et al., 2005). Specifically interesting is a functional neuroimaging study with Turner females revealing a loss of correlation between activation in the left amygdala and left fusiform gyrus underlying impairments in labeling of facial expressions of both anger and fear, while right amygdala mediated somatic responses were enhanced in reponse to emotional faces (Skuse et al., 2005). This finding is in line with our observation of a dissociation between reduced cognitive labeling of emotions and increased emotional arousability in XXY men.

The extra X chromosome may also affect neural systems involved in language processing. Genetic mechanisms involving the X chromosome may influence the development of hemispheric specialization for language, as reduced language lateralization was observed in XXY men using fMRI. A putative link between the X chromosome and reduced language lateralization is in line with a proposed role of the X chromosome in the development of asymmetry in the brain. It is thought that a pseudo-autosomal region on the X chromosome is involved in abnormal development of brain asymmetry in XXY men, as indicated by increased left-handedness and neuropsychological evidence of anomalous hemispheric dominance (Geschwind et al., 1998). Interestingly, also in Turner syndrome (45,X0) reduced lateralization of verbal information processing has been reported, which supports the hypothesis that the X chromosome may be important for the development of hemispheric specialization for verbal information (Netley et al., 1982).

However, the exact genetic mechanism that leads to the XXY phenotype remains unclear. In healthy females, who have two X chromosomes, certain genes on the X chromosome are inactivated, while others escape inactivation. In

Klinefelter syndrome, genes on the X chromosome that that escape inactivation and have a homologue on the Y chromosome and, may be overexpressed. Is has been proposed that phenotype in Klinefelter syndrome, at a neural, cognitive and behavioral level, might originate from overexpression of such genes (Crow, 1988; DeLisi et al., 2005; Geschwind et al., 2000). Alternatively, genomic imprinting may play a role. Genomic imprinting refers to differential expression of a gene, dependent of the parental origin of that gene. It has been shown that Turner females with a maternally derived X chromosome display more impairments in social skills as compared to those with the X chromosome from the father (Skuse et al., 1997). The authors proposed an X-chromosomal locus for social cognition, which is imprinted and not expressed by the maternally derived X chromosome. In Klinefelter syndrome, the extra X chromosome is derived from the mother in over half of the cases (Thomas et al., 2003). A recent study has pointed to putative imprinting effects in 54 XXY men. Impairments in speech and motor development were more often found in subjects with a paternal (extra) X chromosome as compared to those with a maternal (extra) X chromosome (Stemkens et al., 2006). Taken together, abnormal expression of genes on the X chromosome in Klinefelter syndrome may affect development of the brain resulting in social, emotional and language dysfunctions. Although speculative, an involvement of sex chromosomes in socio-emotional brain functions would be in line with the observed sex differences in social cognitive skills in the general population, with performance of men generally somewhat below that of women (Hall, 1984; Hampson et al., 2006; McClure, 2000; Montagne et al., 2005). However, the exact genetic mechanisms that lead to the XXY phenotype, which may for example include overexpression, abnormal inactivation, genomic imprinting or gene-gene interactions, are as yet unclear. Future genetic studies are needed to identify candidate genetic mechanisms involving the X chromosome that can explain the social cognitive deficits in XXY men, sex differences in social cognition in the general population and the male preponderance in disorders of social cognition.

The role of testosterone deficits

The degree to which the observed cognitive and neurobiological deficits in XXY men represent the effects of testosterone- deficits that become apparent in puberty and subsequent testosterone supplementation, is unclear. The relationship between testosterone levels and brain development is complex; timing of exposure, sensitivity to testosterone reflected in androgen receptor density and modulation by environmental factors are important determinants in

the effects of testosterone (Craig et al., 2004). Although speculative, abnormal testosterone levels might be part of the mechanism by which X chromosomal abnormalities lead to disturbances in development of neural systems supporting social cognition. However, gonadal hormones may be one of many mechanisms by which sex chromosomes exert their influence on brain development. Recent animal studies have pointed to direct, non-hormonal effects of sex-chromosomes on brain maturation (Dewing et al., 2003).

Although speculative, support for the hypothesis that brain function and cognition in XXY men may represent the effects of X chromosomal genetic pathology rather than low testosterone levels by itself, comes from individuals with X chromosomal aneuploidies that are not associated with low testosterone levels. For example, although females with the XXX karyotype show typical hormone levels, they do display impairments in the language domain and have decreased social adjustment (Bender et al., 1999; Harmon et al., 1998). Interestingly, there have been reports of increased psychopathology, such as conduct disorders and affective disorders, in XXX females (Harmon et al., 1998).

Furthermore, there is no evidence for an effect of testosterone supplementation on language lateralization. A recent longitudinal study dealing with the effects of testosterone treatment on language lateralization in transsexuals showed that language laterality is highly stable and not affected by hormonal interventions (Sommer et al., in prep). Also, testosterone levels by itself seem not enough to, for example, explain the amygdala abnormalities in individuals with sex chromosome aneuploidies. Although females with Turner syndrome, who are missing one X-chromosome, are also characterised by a reduction in testosterone levels (Hojbjerg Gravholt et al., 1999), these patients show an increase in amygdala volume rather than a reduction (Kesler et al., 2004). Although speculative, differences in amygdala volume between Turner and Klinefelter patients might be stronger related to the number of X-chromosomes, which differentiates the two groups, than to testosterone levels, which are reduced in both syndromes. Alternatively, abnormal testosterone levels may result from X-chromosome linked amygdala abnormalities, since it has been shown that the amygdala is involved in the production of testosterone (Banczerowski et al., 2003).

In conclusion, the exact nature of the effects of testosterone deficits and testosterone supplementation on development of social cognition and neural substrates in Klinefelter syndrome remains unclear.

Potential implications for the study of autism and schizophrenia

Our findings may have potential implications for the study of neurodevelopmental disorders associated with impaired social adaptation. We might consider a role of genetic mechanisms involving the X chromosome in some aspects of social cognitive dysfunction and underlying neuroanatomical abnormalities in the autism or schizophrenia spectrum. Tentative support for this hypothesis comes from observations of increased autism spectrum psychopathology in Turner syndrome and both autism and schizophrenia spectrum traits in Klinefelter syndrome. The estimated risk for autism spectrum disorders may be several times higher in women with X-monosomy (3 %) as compared to women from the general population (0.01 %) (Creswell et al., 1999). In Klinefelter syndrome, we observed increased levels of autism traits, schizotypal personality traits and schizophrenia-like symptoms (Van Rijn et al., 2006; Van Rijn et al., in preparation). In addition, a survey of hospital admissions and discharge diagnoses has indicated a significantly increased relative risk of being hospitalized with psychoses (hazard ratio of 4.97) for men with Klinefelter syndrome (Bojesen et al., 2006). In turn, there is suggestive evidence that prevalence of the XXY karyoype in the male schizophrenia population may be several times higher as compared to the prevalence of Klinefelter syndrome in the general population (DeLisi et al., 1994; Kunugi et al., 1999).

The increased levels of autism- and schizotypal traits that we observed in XXY men parallel the schizotypal or autism-like features that characterize individuals at increased risk for autism or schizophrenia. Biological relatives of individuals with autism or schizophrenia also display increased levels of traits or mild features of the disorder (Bishop et al., 2004; Vollema et al., 2002). Based on twin studies, it has been suggested that the typical clinical phenotype of autism or schizophrenia as seen in subjects with these disorders and the broader subclinical phenotypes (i.e. autism or schizotypal traits) as seen in biological relatives, may share a genetic origin (Rutter, 2000; Torgersen et al., 2002). Although speculative, this might suggest that the X chromosome may play a role in the etiology of some of the traits that are part of the broad autism or schizophrenia spectrum.

Difficulties in the domains of language, social adaptation and emotion are thought be among the core abnormalities in autism and schizophrenia (Aleman et al., 2005; Crow, 2004; Fein et al., 1986). Some of the social cognitive impairments in individuals with autism or schizophrenia resemble our findings in men with Klinefelter syndrome. I will now discuss parallels between XXY

men and individuals with autism or schizophrenia with regard to socio-emotional processing and language.

Socio-emotional processing

Our studies in Klinefelter syndrome indicated that XXY men have difficulties in analyzing emotional expressions on faces. A body of research has demonstrated that individuals with autism or schizophrenia also have difficulties in analyzing social cues from faces and hence are less able to use this crucial source of information in social interactions (Pinkham et al., 2006; Sigman et al., 2004). These deficits are not only present in decoding of basic emotions (Gross, 2004; Kohler et al., 2004), but also observed when complex social evaluations of faces are required (Adolphs et al., 2001; Hall et al., 2004). In one of our studies we were able to compare social information processing abilities in XXY men with schizophrenia patients and relatives of schizophrenia patients. Interestingly, performance in a task which measured the degree to which social cues (such as direction of gaze and implied biological motion) are processed automatically, was indistinguishable between XXY men and patients with schizophrenia. Biological relatives of schizophrenia patients were also impaired, although to a lesser extent, suggesting that this deficit may be expressed to different degrees across a schizophrenia vulnerability continuum. However, this social cognitive deficit seems not confined to the schizophrenia spectrum. Individuals from the autism spectrum also display reduced automatization in attending to social cues, such as gaze direction and implied biological motion, as measured with the same task as we used (Jellema et al., 2004).

Not only in perception of socio-emotional signals, also at the level of experience and expression of emotions some parallels between XXY men and individuals with autism or schizophrenia are present. Cognitive processing of one's own emotions, which is neccessary for regulation of emotions, also seems to be impaired in these neurodevelopmental disorders. Specifically, deficits in identifying and describing one's own emotions have been reported for individuals with autism or schizophrenia, and also in male biological relatives of schizophrenia patients. (Hill et al., 2004; van 't Wout et al., 2007). Increased emotional experience of emotions is also seen in patients with schizophrenia and their male biological relatives (van 't Wout et al., 2007). The presence of such disturbances in emotional processing in biological relatives of patients with schizophrenia, who share part of their genes, suggests that this may be part of the genetic vulnerability for the disease and possibly be expressed to different degrees across the phenotypic continuum of schizophrenia. The finding that

alexithymia seems to be under considerable genetic control fits this picture (Valera et al., 2001).

Not only our observations in Klinefelter syndrome at the level of cognition, also our findings at the level of neural mechanisms involved in social behavior might be extrapolated to other disorders. If we focus on structural and functional abnormalities of brain regions that are part of a neural network supporting social cognitive functions, some similarities among individuals with autism or schizophrenia and XXY men can be observed. Structural neuroimaging findings that we reviewed suggest that morphological abnormalities of the amygdala might not only be present in individuals with schizophrenia, but may also be an endophenotype both in Klinefelter syndrome and in individuals at increased risk for schizophrenia (because they have a family member suffering from the disease or display subclinical signs of the disease). We might extrapolate from these findings that in patients with schizophrenia and individuals at increased risk for schizophrenia, the observed structural abnormalities of the amygdala might in part originate from genetic mechanisms involving the X chromosome. This endophenotype may also be present in other psychiatric disorders, for example autism, which has also been associated with structural abnormalities of the amygdala (Brambilla et al., 2003; Palmen et al., 2004).

The observed functional abnormalities of the amygdala, fusiform face area and insula in Klinefelter syndrome, also show some resemblence with neuroimaging findings in autism and schizophrenia. Functional MRI studies have situated social cognitive deficits in schizophrenia in a neural network that includes, besides other brain regions, the amygdala, fusiform face area and insula (Phillips et al., 2003; Quintana et al., 2003). Functional abnormalities in these regions have also been reported for individuals with an autism spectrum disorder. Reduced involvement of the amygdala in processing social information has been widely reported and is thought to be among the core neurodevelopmental abnormalities in autism (Baron-Cohen et al., 2000). It has been proposed that amygdala deficits may be related to reduced activation of the fusiform face area in response to faces, which has consistently been observed in autism spectrum disorders (Grelotti et al., 2002).

Language

As language is a crucial part of social communication, it has been proposed that the well documented language impairments in Klinefelter syndrome may contribute to the observed difficulties in social functioning. The reported

language disabilities include impairments in both language production and perception and indicate compromised language functions that are typically associated with the left hemisphere (Samango-Sprouse, 2001). However, data in this thesis suggest that XXY may also have impairments in decoding emotional prosody, which is a pragmatic aspects of language lateralized to the right hemisphere. Deficits in decoding emotional prosody may also play an important role in social behavioral dysfunction in Klinefelter syndrome. In the search for determinants of impaired social communication in autism, impairments in the perception of pragmatic aspects of language, such as emotional prosody, have also been revealed (Lindner et al., 2006). Similarly, in schizophrenia, understanding pragmatic language appears to be affected (Mitchell et al., 2005) and difficulties in analyzing emotional cues in tone of voice have been observed (Edwards et al., 2002). It has even been argued that, at least for language and communication, right hemispheric functions are more affected than those mediated by the left hemisphere in schizophrenia (Ross et al., 2001).

One possible neural mechanism that may underlie language disorders in Klinefelter syndrome is reduced lateralization of language in the brain. Reduced hemispheric specialization for language may contribute to disorders of language and communication that we not only see in men with the XXY karyotype, but also in patients with schizophrenia or autism. Recent studies have suggested that autism spectrum disorders may be associated with abnormal development of hemispheric specialization for language. A magnetoencephalogram (MEG) study has revealed increasing rightward language dominance with age in autistic children in contrast to normally developing children, who display an increasing leftward lateralization (Flagg et al., 2005). The importance of gaining insight into the genetic and neural basis of language lateralization is also illustrated by findings in schizophrenia patients. Reduced cerebral asymmetry and, more specifically, loss of language lateralization is considered to be among the core developmental brain abnormalities underlying schizophrenia symptoms (Bhati, 2005; Crow, 2004). A range of language laterality studies in schizophrenia have supported this (Artiges et al., 2000; Dollfus et al., 2005; Kircher et al., 2002; Sommer et al., 2001a; Weiss et al., 2006). One of these studies not only assessed the degree of lateralization, but also examined whether this was secondary to increased right hemisphere activation or decreased left hemisphere activation (Sommer et al., 2001b). Similar to what we observed in Klinefelter men, decreased lateralization of language in schizophrenia patients was due to increased activity in the right hemisphere, rather than decreased activity in the left hemisphere. For schizophrenia, it has been suggested that deficits in typical

right hemispheric language functions, such as in processing emotional prosody, might arise as a consequence of a shift from left hemisphere dominance to more bilateral control of language processing (Mitchell et al., 2005). The hypothesis that reduced language lateralization may also be an endophenotype in the 'broad' schizophrenia spectrum is tentitavely supported by our findings in XXY men suggesting that reduced language lateralization in the superior temporal gyrus is associated with increased schizotypal traits related to disorganization of thought and language, such as vague or overinclusive speech and odd or eccentric behavior.

Although speculative, similarities between XXY men and individuals from the autism- or schizophrenia spectrum suggest that we might consider a role of the X chomosome in some of the neurobiological and social cognitive abnormalities that are present in these neurodevelopmental disorders. This hypothesis fits with the notion that genetic factors in autism or schizophrenia might operate on components of the disorders, rather than the syndrome as a whole (Gottesman et al., 2003; Jablensky, 2006; Rutter, 2000). It has recently been suggested that some of the relations between genotypes and endophenotypes in schizophrenia may not be specific nor confined to one disorder (Weiser et al., 2005). Weiser et al. (2005) proposed that studying endophenotypes at the level of cognitive, emotional and social functioning in other disorders may help elucidating the etiology of schizophrenia and psychiatric morbidity as a whole.

Sex chromosomal effects on brain development and cognition may be especially relevant for the study of autism and schizophrenia because of the male preponderance in these psychiatric disorders. Schizophrenia is diagnosed approximately 1.5 times and autism spectrum disorders approximately 4 times more often in males than in females (Aleman et al., 2003; Volkmar et al., 1993). Observations that males are more often affected have led others to propose that dysfunctional neural circuits underlying social cognitive impairments in autism may be related to genes on the X chromosome, that are differentially expressed in men and women (Baron-Cohen et al., 2005; Skuse, 2000). Studies focused at the genetic underpinnings of abnormal language lateralization in schizophrenia have also suggested abnormal expression of a X-Y homologous genetic locus directing development of brain asymmetry (Crow, 2004). Crow proposes that such a gene would underlie the observed sex differences in language abilities, handedness, relative rates of hemispheric growth and both ages of onset and prevalence of schizophrenia.

Taken together, the extra X chromosome in Klinefelter syndrome may play a role in abnormal development of some of the brain mechanisms involved in language, emotion and social behavior, which are considered core domains of disabilities in autism and schizophrenia. Although speculative, studying X-linked genetic mechanisms in Klinefelter syndrome might help us understand pathways from genes to psychopathology in the autism- and schizophrenia spectrum.

Concluding remarks and future perspectives

This thesis has presented Klinefelter syndrome as a genetic disorder that may advance our understanding of the biological basis of social cognition, i.e. human capacities that are crucial for adapting to complex social environments. As severe difficulties in social adaptation have been described for neurodevelopmental disorders such as autism or schizophrenia, our findings may also have potential implications for understanding some of the gene-brain-behavior pathways to such forms of psychopathology. Relevance for the study of neurodevelopmental psychiatric disorders is also illustrated by reports suggesting an increased risk for psychopathology in men with Klinefelter syndrome and our observations of increased autism- and schizotypal traits, as well as schizophrenia symptoms, in these men. However, clinical research is needed to more precisely define the vulnerability to autism and schizophrenia in Klinefelter syndrome in a larger and more representative sample in epidemiological terms. The effects of testosterone treatment on the cognitive and neural development in Klinefelter syndrome may be investigated in longitudinal studies during adolescence. Also, developmental studies in Klinefelter syndrome may reveal whether impaired social cognitive functioning is also present in early infancy, before testosterone deficits become apparent. Future genetic studies are warranted to help us understand the mechanisms by which an extra X chromosome affects brain development and cognition in Klinefelter syndrome. In addition, an interesting issue for genetic research concerns the interactions between X chromosomal genetic pathology and environmental factors, such as stress, that may interactively direct brain development, social cognitive abilities and mental well-being. Also, there is a need for studies in which neural and cognitive endophenotypes related to social behavior are directly compared between individuals with autism or schizophrenia and individuals with Klinefelter syndrome. Although speculative, putative overlap in endophenotypes between Klinefelter syndrome and these neurodevelopmental disorders might point to a common genetic origin on the X chromosome.

Besides revealing a putative neural and cognitive basis for social dysfunctions in this specific genetic disorder, the present thesis shows that Klinefelter syndrome may serve as a more general model for studying the genetic basis of disturbances in the development of social cognition, which are considered core abnormalities in severe psychiatric disorders such as autism and schizophrenia.

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Summary

Humans are equipped with affective and cognitive capacities that allow them to deal with a complex and dynamic social world. Although social signals can be processed automatically and quickly, in many situations the social significance of expressions, actions, and experiences is not fixed, and depends upon cognitive computations to extract meaning. As a consequence, social skills largely rely on social cognitive capacities that are needed to make sense of the incoming socially relevant information.

The severe impact of impaired social cognition is illustrated by disorders such as autism or schizophrenia. Autism spectrum disorders and schizophrenia share some characteristics, both clinical phenomena pertaining to affect, communication and social insight (Abdi et al., 2004; Frith, 1992; Goldstein et al., 2002; Konstantareas et al., 2001; Rumsey et al., 1986) as well as cognitive dysfunctions in the domain of social information processing, language and emotion (Abdi et al., 2004; Frith, 1992; Pilowsky et al., 2000; Rumsey et al., 1986).

The importance of social cognitive capacities in coping with the social world and related mental well-being has called for a search into the origins of social cognition on the level of cognition, neurobiology and genes. Klinefelter syndrome is an X chromosomal disorder known for social dysfunction and specific deficits in brain development and cognition, which can help us to unravel genotype-phenotype relations that are relevant for social behavior. Klinefelter syndrome might be used as a model for disorders of social behavior to reveal insight into involvement of the X chromosome in social cognitive pathways to psychopathology. These pathways may be more difficult to uncover by studying heterogeneous, behaviorally defined populations (Reiss, 2000; Reiss et al., 2000).

In the present thesis, we investigated the effects of an extra X chromosome on social cognition and the underlying neural basis. Besides providing insight into the cognitive and neural basis of social dysfunctions associated with the XXY karyotype, the aim of the present thesis was to explore whether Klinefelter syndrome may serve as a model for social cognitive disturbances in autism or schizophrenia. We have focused on socially deviant behavior in adult XXY men on a behavioral, cognitive and neuroanatomical level and reviewed evidence for autism and schizophrenia spectrum traits, social dysfunction, social cognitive disabilites and underlying dysfunctional neural mechanisms in XXY men.

Social behavior and psychopathology

As described in **chapter two** and **three**, we assessed social difficulties, reflected in frequency of participation in social interactions and distress during these interactions, as well as vulnerability to disorders of social behavior, such as autism and schizophrenia, in XXY men. Vulnerability to autism or schizophrenia was investigated from a dimensional, symptom oriented perspective, rather than a dichotomal, all-or-none, approach.

Men with Klinefelter syndrome reported to less often engage in social behavior and to experience more distress during social interactions as compared to men from the general population. In addition, scores were higher on traits accross all dimensions of the autism- and schizophrenia phenotype. Also on a clinical level, higher levels of schizophrenia symptoms were observed in XXY men.

Our findings of difficulties in coping with social situations in XXY men, especially the high levels of distress during social interactions, are consistent with reports of social anxiety, social withdrawal and shyness in individuals with the XXY karyotype (Bender et al., 1999; Ratcliffe, 1999). Difficulties in social adjustment have mostly been reported for children or adolescents with Klinefelter syndrome. Our data suggest that social difficulties may persist into adulthood, with social distress more prominent than a general reduction in participation in social interactions. The high levels of autism traits and schizotypal traits in XXY men suggest that some aspects of the XXY phenotype parallel the phenotype that characterizes individuals at increased risk for autism or schizophrenia. This hypothesis fits with the concept of 'broad phenotypes' of autism or schizophrenia, which refers to mild features of the clinical autism- or schizophrenia phenotype that are seen in biological (i.e. genetically related) relatives of individuals with these disorders. Findings in this thesis suggest that mild features of the autism or schizophrenia phenotype may not only be found in genetically related relatives of individuals with these disorders, but also in men with an extra X chromosome.

Social cognition

Next, we explored various aspects of social cognitive functioning in Klinefelter syndrome. Deficits in social cognition might contribute to the difficulties in coping with social environments as reflected in increased social distress and increased autism or schizophrenia traits.

Klinefelter men appeared less accurate in perception of socio-emotional signals. As described in **chapter four**, we found XXY men to be impaired in recognizing facial expressions of anger, independent of the intensity of the

emotional expression. This deficit was not attributable to a generalized impairment in analyzing faces, as performance in a face identity matching task was intact. The XXY group not only displayed deficits in processing social cues in the visual domain, but also in the auditory domain. Besides facial expressions of emotions, af fective prosody (tone of voice) in speech is also important for understanding intentions of others, because variations in tone of voice provide information about the emotional state of the speaker. Lesion- and MRI studies have suggested that in contrast to linguistic information, which is largely lateralized to the left hemisphere, pragmatic aspects of language, such as emotional prosody, are lateralized to the right hemisphere. Indeed, by using Transcranial Magnetic Stimulation (**chapter six**), we provided evidence for a causal involvement of the right hemisphere in processing affective prosodic information in individuals from the general population. As described in **chapter seven**, recognition of emotions in prosodic cues was impaired in XXY men. Although recognizing emotions in semantic cues was also impaired in the XXY group, which is in line with the typical left hemisphere mediated language dysfunctions in Klinefelter syndrome, recognizing emotions in prosody was more affected.

Another important underlying characteristic of successful social interaction is the ability to quickly and automatically process basic elements of a social signal such as direction of gaze, head orientation and body postures (Frith et al., 1999; Jellema et al., 2005). We have shown (**chapter five**) that in contrast to men from the general population, XXY men have difficulties in automatically attending to, and processing, basic social cues such as gaze direction and implied biological motion. Interestingly, performance in processing these social cues was indistinguishable between Klinefelter men and schizophrenia patients. Moreover, performance of biological relatives of schizophrenia patients resembled the lack of sensitivity to social cues observed in schizophrenia patients and XXY men, albeit to a lesser extent, indicating that it may be an expression of genetic vulnerability for the disease.

Not only perception, but also disturbances in experience and expression of emotions were observed in XXY men (**chapter four**). XXY men were less able to identify and verbally describe their emotions, but reported to experience increased levels of emotional arousal, in comparison to men from the general population. Increased emotional experience as reported by XXY men fitted with our observation that XXY men more often rejected financial offers in a strategic decision game (**chapter four**). Their pattern of performance in this game suggested an increased influence of emotions on strategic decision making.

It has been proposed that the reported deficits in typical left hemisphere mediated language functions, such as understanding and finding words, largely explain social dysfunction in XXY men. However, the present thesis has pointed to dysfunctions in processing affective facial expressions, decoding affective tone of voice, emotion regulation and automatic processing of basic social cues (such as gaze direction and implied biological motion), which may also contribute to social dysfunction and related mental well-being.

Social cognition: neural basis

As it is thought that language impairments, with language being a crucial part of social communication, may contribute to the observed difficulties in social functioning in Klinefelter men (Samango-Sprouse, 2001), we studied the neural basis of language processing in XXY men (**chapter eight**). By using functional Magnetic Resonance Imaging (fMRI) we were able to assess lateralization of activation in specific brain areas during language processing in XXY men. By measuring the relative contribution of the right and left hemisphere in each individual it was shown that language activity in the brain was indeed less lateralized in the XXY group as compared to control men. Loss of asymmetric processing of language was due to increased activity in the right hemisphere rather than reduced activity in the left hemisphere. Loss of functional asymmetry in the superior temporal gyrus (STG) was the most prominent contributor to reduced language laterality in XXY men. This regional reduction of language laterality was highly correlated with the degree of disorganization (vague or overinclusive speech and odd/eccentric behavior) in the XXY group. These findings fit with a proposed role of the X chromosome in development of asymmetry in the brain. In addition, these data suggest that reduced language lateralization might underlie disorders of thought and language in Klinefelter syndrome.

We also explored the effects of an extra X chromosome on the neural basis of social perception and reviewed structural neuroimaging findings in Klinefelter syndrome, as well as investigated neural networks supporting social perception in XXY men using functional neuroimaging. Because the amygdala plays a crucial role in social perception and social behavior, we reviewed evidence for structural abnormalities of the amygdala in Klinefelter syndrome based on findings in the literature (**chapter nine**). Findings were compared to what is known of abnormalities of the amygdala in populations with increased vulnerability to schizophrenia, namely: individuals from the general population displaying subclinical signs of schizophrenia and biological relatives of

schizophrenia patients who may carry a genetic predisposition for the disorder. Reductions in volume of the amygdala were found to be present both in Klinefelter syndrome and the populations at high risk for schizophrenia, i.e. across the broad spectrum of vulnerability for schizophrenia psychopathology. These findings suggest that abnormal development of the amygdala may be an endophenotype that is not only present in patients with the clinical schizophrenia phenotype, but also in individuals displaying traits from the broad (milder) schizophrenia phenotype.

Structural abnormalities of the amygdala in Klinefelter syndrome suggest abnormal functioning of this brain area, possibly extending to a neural network for social perception. By using fMRI we were able to study functioning of such a neural network during social evaluation of faces (**chapter ten**). We measured brain activity during trustworthiness evaluations of faces, with special interest in the amygdala, fusiform face area, superior temporal gyrus and insula. In the XXY group, we observed signifianctly lower levels of activation in the amygdala, insula and fusiform face area during untrustworthy faces as compared to controls. The amygdala is escpecially involved in screening the environment for threat signals en has strong connections with the insula, which is involved in emotional arousal and experience, and fusiform face area, which is involved in analyzing the structural properties of faces in detail. These findings, for the first time, may provide a neural explanation for some of the impairments in social cognition in Klinefelter syndrome.

The X chromosome and social cognition

As Klinefelter syndrome is defined by an X chromosomal abnormality, our findings suggest that the X chromosome may play a role in the development of some aspects of social cognition and neural mechanisms involved in social information processing in Klinefelter syndrome. Additional support for a putative role of the X chromosome in the development of social information processing is derived from observations of social dysfunction and social cognitive disabilites in Turner syndrome (45,X0), another X chromosomal disorder characterised by a partial or complete absense of one of the X chromosomes in females (Mazzocco et al., 1998; McCauley et al., 2006; Ross et al., 2000). Also in Turner syndrome, abnormalities in brain regions important for social cognition have been found. These include the amygdala, superior temporal sulcus and -gyrus, anterior cingulate, orbitofrontal cortex and insula (Kesler et al., 2003; Kesler et al., 2004; Molko et al., 2004; Murphy et al., 1997; Skuse et al., 2005).

The extra X chromosome may also affect neural systems involved in language processing. Genetic mechanisms involving the X chromosome may influence the development of hemispheric specialization for language, as reduced language lateralization was observed in XXY men using fMRI. A putative link between the X chromosome and reduced language lateralization is in line with a proposed role of the X chromosome in the development of asymmetry in the brain, as indicated by increased left-handedness and neuropsychological evidence of anomalous hemispheric dominance in Klinefelter syndrome (Geschwind et al., 1998). Interestingly, also in Turner syndrome (45,X0) reduced lateralization of verbal information processing has been reported, which supports the hypothesis that the X chromosome may be important for the development of hemispheric specialization for verbal information.

Taken together, the X chromosome might play an important role in the development of brain regions that support social social perception and language processing. However, the exact genetic mechanisms that lead to the XXY phenotype are as yet unclear. Such mechanisms might include overexpression or abnormal inactivation of genes on the X chromosome, genomic imprinting or complex gene-gene interactions.

The role of testosterone deficits

The degree to which the observed cognitive and neurobiological deficits in XXY men represent the effects of testosterone- deficits that become apparent in puberty and subsequent testosterone supplementation, is unclear. The relationship between testosterone levels and brain development is complex; timing of exposure, sensitivity to testosterone reflected in androgen receptor density and modulation by environmental factors are important determinants in the effects of testosterone (Craig et al., 2004). Abnormal testosterone levels might be part of the mechanism by which X chromosomal abnormalities lead to disturbances in development of neural systems supporting social cognition. However, gonadal hormones may be one of many mechanisms by which sex chromosomes exert their influence on brain development. Recent animal studies have pointed to direct, non-hormonal effects of sex-chromosomes on brain maturation (Dewing et al., 2003). Indeed, females with the XXX karyotype display impairments in the language domain and have decreased social adjustment, in the face of typical hormone levels (Bender et al., 1999; Harmon et al., 1998).

Potential implications for the study of autism and schizophrenia

Our findings may have potential implications for the study of neurodevelopmental disorders associated with impaired social adaptation. We might consider a role of genetic mechanisms involving the X chromosome in some aspects of social cognitive dysfunction and underlying neuroanatomical abnormalities in the autism or schizophrenia spectrum. Tentative support for this hypothesis comes from observations of increased autism spectrum psychopathology in Turner syndrome and both autism and schizophrenia spectrum traits in Klinefelter syndrome. The increased levels of autism- and schizotypal traits that we observed in XXY men parallel the schizotypal or autism-like features that characterize individuals at increased risk for autism or schizophrenia. Based on twin studies, it has been suggested that the typical clinical phenotype of autism or schizophrenia (as seen in subjects with these disorders) and the broader subclinical phenotype, i.e. autism or schizotypal traits, may share a genetic origin (Rutter, 2000; Torgersen et al., 2002). Although speculative, this might suggest that the X chromosome may play a role in the etiology of some of the traits that are part of the broad autism or schizophrenia spectrum.

Difficulties in the domains of language, social adaptation and emotion are thought be among the core abnormalities in autism and schizophrenia (Aleman et al., 2005; Crow, 2004; Fein et al., 1986). Some of the impairments socio-emotional processing and language that are found in individuals with autism or schizophrenia resemble our findings in men with Klinefelter syndrome. Paralells are also observed at the level of neural networks that support social perception and language. Although speculative, similarities between XXY men and individuals from the autism- or schizophrenia spectrum suggest that we might consider a role of the X chomosome in some of the neurobiological and social cognitive abnormalities that are present in these neurodevelopmental disorders. This hypothesis fits with the notion that genetic factors in autism or schizophrenia might operate on components of the disorders, rather than the syndrome as a whole (Gottesman et al., 2003; Jablensky, 2006; Rutter, 2000). Sex chromosomal effects on brain development and cognition may be especially relevant for the study of autism and schizophrenia because of the male preponderance in these psychiatric disorders.

Taken together, the extra X chromosome in Klinefelter syndrome may play a role in abnormal development of some of the brain mechanisms involved in language, emotion and social behavior, which are considered core domains of disabilities in autism and schizophrenia. Although speculative, studying X-linked

genetic mechanisms in Klinefelter syndrome might help us understand pathways from genes to psychopathology in the autism- and schizophrenia spectrum.

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Nederlandse samenvatting

Mensen zijn uitgerust met cognitieve capaciteiten die hen helpen te navigeren in een complexe en dynamische sociale wereld. Ook al kunnen sociale signalen automatisch en onbewust verwerkt worden, in veel situaties staat de sociale betekenis niet vast en is afhankelijk van cognitieve computaties om betekenis te onttrekken. Als gevolg hiervan zijn sociale vaardigheden sterk afhankelijk van sociaal cognitieve capaciteiten die nodig zijn om sociaal relevante informatie te begrijpen.

De impact van verstoorde sociale cognitie wordt geïllustreerd door psychiatrische stoornissen zoals autisme of schizofrenie. Deze ontwikkelingsstoornissen laten een overlap zien in zowel klinische fenomenen die te maken hebben met affect, communicatie en sociaal inzicht (Abdi et al., 2004; Frith, 1992; Goldstein et al., 2002; Konstantareas et al., 2001; Rumsey et al., 1986), alswel onderliggende cognitieve disfuncties in het domein van de sociale informatieverwerking zoals beperkingen in het begrijpen van emoties, taal en sociale signalen (Abdi et al., 2004; Frith, 1992; Pilowsky et al., 2000; Rumsey et al., 1986).

Het belang van sociaal cognitieve vaardigheden voor het flexibel kunnen afstemmen op de sociale omgeving en de ernstige effecten die verstoorde ontwikkeling van deze vaardigheden kan hebben op mentale gezondheid, hebben aangezet tot een zoektocht naar genetische, neurobiologische en cognitieve factoren die een rol spelen in verstoorde sociale adaptatie. In deze context kan het bestuderen van een genetisch gedefinieerde populatie mogelijk unieke inzichten bieden ten opzichte van het bestuderen van psychiatrische populaties die gedefinieerd zijn op basis van stoornissen in sociaal gedrag (Reiss, 2000; Reiss et al., 2000). Deze psychiatrische populaties, zoals autisme of schizofrenie, zijn namelijk lastiger te definiëren en heterogener. Bovendien kunnen gedragskenmerken op basis waarvan deze populaties gedefinieerd worden afhankelijk zijn van leeftijd en blootstelling aan farmacologische- of gedragstherapieën.

Het Klinefelter syndroom is een X chromosomale aandoening (47,XXY) die ongeveer bij 1 op de 700 mannen voorkomt. Het XXY chromosomaal patroon wordt onder andere geassocieerd met moeilijkheden in sociale contacten en specifieke stoornissen in de ontwikkeling van de hersenen en cognitieve functies. Het bestuderen van genetische, neurobiologische en cognitieve mechanismen in het Klinefelter syndroom kan ons mogelijk iets leren over hoe sociaal aangepast gedrag ontstaat en wat de oorzaak kan zijn van beperkingen hierin. Dit proefschrift beschrijft een serie studies gericht op sociaal cognitieve vaardigheden en onderliggende neurale mechanismen in

mannen met het Klinefelter syndroom. Naast het bieden van inzichten in mogelijke cognitieve en neurobiologische verklaringen voor de moeilijkheden in sociale contacten bij mannen met dit syndroom, was een doel van dit proefschrift om te onderzoeken of het Klinefelter syndroom gebruikt kan worden als model voor mechanismen die betrokken zijn bij verstoorde sociale cognitie in autisme en schizofrenie. Wij hebben ons gericht op sociaal gedrag in volwassen XXY mannen en onderzoek gedaan op het niveau van neuroanatomie, cognitie, gedrag en psychopathologie. We hebben onderzocht of er bij dit syndroom sprake is van verhoogde kwetsbaarheid voor autisme en schizofrenie, stoornissen in sociaal gedrag, sociaal cognitieve disfuncties en afwijkingen in hersengebieden die een belangrijke rol spelen in het verwerken van sociale informatie.

Zoals beschreven in **hoofdstuk twee** en **drie**, hebben we stoornissen in sociaal gedrag geïnventariseerd in XXY mannen. Hiertoe hebben we de frequentie van participatie in sociaal gedrag alswel de spanning die ervaren wordt in sociale situaties vergeleken tussen XXY mannen en mannen uit de algemene populatie. Ook beschrijven deze hoofdstukken de onderzoeken naar kwetsbaarheid voor autisme en schizofrenie in deze populatie. Dit is gedaan vanuit een dimensioneel, symptoom georiënteerd perspectief, in plaats van gebruik te maken van een dichotome, diagnostische benadering.

XXY mannen rapporteerden minder vaak deel te nemen aan sociale interacties en meer spanning te ervaren tijdens deze sociale situaties. Bovendien lieten de XXY mannen hoge niveaus van autistische en schizotypische persoonlijkheidskenmerken zien en vertoonden zij klinische symptomen en klachten uit het schizofrenie spectrum.

Deze resultaten zijn consistent met eerdere bevindingen van sociale angst, sociaal terugtrekkingsgedrag en verlegenheid bij mensen met het Klinefelter syndroom (Bender et al., 1999; Ratcliffe, 1999). Moeilijkheden in sociale interacties zijn in de literatuur vrijwel uitsluitend beschreven voor jongens en jong volwassenen met het Klinefelter syndroom. Onze bevindingen geven aan dat beperkingen in het sociale verkeer, vooral spanning tijdens sociale contacten, mogelijk kunnen aanhouden tot in de volwassenheid. De hoge mate van milde autisme- en schizofrenie kenmerken in de XXY groep suggereert dat sommige aspecten van het XXY fenotype lijken op het fenotype van mensen met een verhoogd risico op autisme of schizofrenie. Deze hypothese is in overeenkomst met het concept van 'brede fenotypen' van autisme en schizofrenie, wat verwijst naar de milde kenmerken van het klinische fenotype in biologische familieleden

van mensen met autisme of schizofrenie. Deze familieleden delen genen met iemand die de stoornis heeft en hebben daardoor een verhoogd risico op de stoornis. Onze bevindingen geven aan dat milde vormen van symptomen uit het autisme en schizofrenie spectrum niet alleen kunnen voorkomen bij genetische verwanten van mensen met deze stoornissen, maar ook bij mannen met een extra X chromosoom.

Sociale cognitie

Vervolgens hebben we verschillende aspecten van sociaal cognitief functioneren bij mannen met het Klinefelter syndroom vergeleken met mannen uit de algemene populatie. Sociaal cognitieve disfuncties zouden kunnen bijdragen aan moeilijkheden in het sociale verkeer zoals gereflecteerd in verhoogde spanning in sociale situaties en gedragskenmerken uit het autisme en schizofrenie spectrum.

Onze onderzoeken hebben uitgewezen dat XXY mannen minder goed zijn in het verwerken van visuele sociale en emotionele signalen. Zoals beschreven in **hoofdstuk vier**, maakten XXY mannen meer fouten in het interpreteren van boze gezichtsuitdrukkingen, ongeacht de intensiteit van de emotionele expressie op het gezicht. Deze slechtere cognitieve prestatie was niet toe te schrijven aan een algemene cognitieve stoornis in het analyseren van gezichten, aangezien zij geen slechtere prestatie lieten zien in een taak waarbij gezichtsidentiteit geanalyseerd moest worden.

XXY mannen lieten niet alleen in het visuele domein, maar ook in het auditieve domein meer moeite zien met het analyseren van sociale signalen. Naast emotionele gezichtsexpressies is emotionele prosodie (stemgebruik) ook belangrijk voor het begrijpen van intenties van anderen, omdat variaties in stemgebruik informatie verschaft over de emotionele toestand van de spreker. Studies naar de effecten van hersenschade en studies gebruik makend van functionele Magnetische Resonantie Imaging (fMRI) hebben laten zien dat in contrast tot taal, dat voornamelijk verwerkt wordt in de linker hersenhelft, de emotionele lading in prosodie voornamelijk wordt geanalyseerd in de rechter hersenhelft. Door gebruik te maken van Transcraniële Magnetische Stimulatie (TMS) (**hoofdstuk zes**) hebben we sterke aanwijzingen gevonden dat gebieden in de rechter hersenhelft van cruciaal belang zijn voor het interpreteren van emotionele prosodie bij mensen uit de algemene populatie. In **hoofdstuk zeven** wordt beschreven dat XXY mannen meer moeite hadden met het interpreteren van emotionele prosodie. Niet alleen maakten XXY mannen meer fouten in het herkennen van emoties op basis van de betekenis van woorden (semantiek),

zoals je zou voorspellen op basis van de gerapporteerde taalstoornissen, maar specifiek met het herkennen van emoties op basis van stemgebruik maakten XXY mannen de meeste fouten.

Een andere cognitieve vaardigheid die belangrijk is voor succesvolle sociale interacties is de mogelijkheid om snel en automatisch basiselementen van een sociaal signaal op te pikken (Frith et al., 1999). Deze basiselementen kunnen bijvoorbeeld kijkrichting, hoofdoriëntatie of lichaamshouding zijn (Jellema et al., 2005). Onze onderzoeken hebben aangetoond (**hoofdstuk vijf**) dat XXY mannen minder geneigd zijn dan mannen uit de algemene populatie om automatisch te letten op basale sociale signalen zoals kijkrichting en geïmpliceerde biologische bewegingen. De prestatie van de XXY mannen was niet te onderscheiden van mannen met schizofrenie en mannelijke familieleden van mensen met schizofrenie. Deze gelijkenis in sociaal cognitieve prestatie suggereert dat verminderde sensitiviteit voor basale sociale signalen mogelijk een genetische kwetsbaarheid voor schizofrenie kan reflecteren.

Niet alleen stoornissen in de *perceptie*, maar ook in de *ervaring* en *expressie* van emoties werden gevonden bij XXY mannen (**hoofdstuk vier**). In vergelijking met mannen uit de algemene populatie waren XXY mannen minder goed in staat om hun emoties te identificeren en uit te drukken in woorden, maar rapporteerden sneller emotioneel geraakt te zijn. Een verhoogde emotionele ervaring zoals gerapporteerd door de XXY mannen was in overeenkomst met onze observaties dat zij vaker in een strategisch beslissingsspel een financieel aanbod verwierpen als dat bod oneerlijk was, ook al kon er geld mee verdient worden door het te accepteren. Andere onderzoekers hebben eerder in een fMRI studie laten zien dat het verwerpen van oneerlijke financiële aanbiedingen in deze taak gepaard gaat met meer activiteit in hersengebieden die een rol spelen in emotionele arousal en -ervaring. Bij de XXY mannen leek er dus sprake te zijn van een verhoogde invloed van emoties op strategisch beslissingsgedrag.

Sociale cognitie: neurale basis

Omdat gedacht wordt dat taalstoornissen in het Klinefelter syndroom een belangrijke bijdrage kunnen leveren aan verslechterd sociaal adaptief gedrag (Samango-Sprouse, 2001), hebben we neurale basis van taalverwerking in de hersenen onderzocht in XXY mannen (**hoofdstuk acht**). Door gebruik te maken van fMRI waren we in staat om de mate van taal-specialisatie van beide hersenhelften te bestuderen in verschillende regio's in het brein. Door de relatieve bijdrage van beide hersenhelften te meten tijdens taalopdrachten werd

aangetoond dat bij mannen uit de algemene populatie de linker hersenhelft meer gespecialiseerd was voor het verwerken van taal. Echter, bij XXY mannen was de mate van specialisatie van de hersenhelften (lateralisatie) significant minder, doordat er meer betrokkenheid van de rechter hersenhelft was tijdens het verwerken van taal. Deze verminderde asymmetrie in hersenactiviteit tijdens taal was het meest prominent in de superieure temporale gyrus (STG), een gebied dat ligt in de auditieve associatiecortex. Deze regionale verminderde specialisatie in de XXY groep was sterk gerelateerd aan de mate van desorganisatie (vage of incoherente spraak en vreemd/excentriek gedrag). Deze bevindingen zijn in overeenkomst met het idee dat het X chromosoom mogelijk een belangrijke rol speelt in de ontwikkeling van specialisatie en asymmetrie in het brein. Daarnaast suggereren deze resultaten dat verminderde specialisatie voor taal in het brein kan bijdragen aan taal- en denkstoornissen in het Klinefelter syndroom.

We hebben ook onderzocht of er effecten zijn van een extra X chromosoom op de hersensystemen die betrokken zijn bij sociale perceptie. Naast een overzicht van neuroanatomische bevindingen in de literatuur op dit gebied hebben we ook functionele netwerken in het brein die een rol spelen in sociale perceptie onderzocht met gebruik van fMRI. Omdat de amygdala een cruciale rol speelt in sociale perceptie en sociaal gedrag, zijn we nagegaan in de literatuur hoe sterk de evidentie is voor anatomische afwijkingen in dit gebied in het Klinefelter syndroom (**hoofdstuk negen**). De neuroanatomische bevindingen werden vergeleken met wat er bekend is over neuroanatomische afwijkingen van de amygdala in populaties met een verhoogde kwetsbaarheid voor schizofrenie, namelijk: mensen uit de algemene populatie die milde symptomen of kenmerken van de stoornis laten zien en genetische verwanten van patiënten met schizofrenie die een genetische predispositie bij zich kunnen dragen. Een kleiner volume van de amygdala bleek zowel voor te komen bij het Klinefelter syndroom als bij populaties met een verhoogd risico op schizofrenie, dus over een breed spectrum van kwetsbaarheid voor schizofrenie. De bevinding dat abnormale ontwikkeling van de amygdala niet alleen een kenmerk is van patiënten met schizofrenie, maar ook gevonden wordt mensen met een verhoogde gevoeligheid voor schizofrenie suggereert dat dit een uiting kan zijn in het brein van kwetsbaarheid voor deze stoornis.

Observaties van anatomische afwijkingen van de amygdala in Klinefelter syndroom roepen de vraag op of er ook functionele afwijkingen in dit gebied zijn en mogelijk in het neurale netwerk waar dit gebied deel vanuit maakt. Met behulp van fMRI hebben we het functioneren van verschillende gebieden in dit neurale netwerk onderzocht tijdens sociale evaluatie van gezichten (**hoofdstuk tien**).

We hebben hersenactiviteit tijdens het beoordelen van betrouwbaarheid van gezichten gemeten in de amygdala, fusiforme gyrus, superieure temporale sulcus en insula. Wanneer gezichten als onbetrouwbaar werden gezien, bleek er in de amygdala en fusiforme gyrus (fusiforme 'face area') minder activiteit te zijn in de XXY groep ten opzichte van de groep mannen uit de algemene populatie. De amygdala is vooral betrokken bij het signaleren van dreiging en heeft sterke connecties met de fusiforme 'face area' waar visuele kenmerken van gezichten in detail wordt geanalyseerd. Deze bevindingen geven voor het eerst inzicht in de neurobiologische basis en neurale mechanismen die mogelijk ten grondslag liggen aan sociaal cognitieve stoornissen in het Klinefelter syndroom.

Het X chromosoom en sociale cognitie

Omdat het Klinefelter syndroom gekenmerkt wordt door een X chromosomale afwijking, suggereren onze bevindingen dat het X chromosoom mogelijk een rol kan spelen in de ontwikkeling van bepaalde sociaal cognitieve vaardigheden en onderliggende neurale mechanismen. Aanvullende ondersteuning voor een mogelijke rol van het X chromosoom in de ontwikkeling van sociale informatieverwerking komt van observaties van sociaal cognitieve stoornissen en sociale disfunctioneren bij mensen met het Turner syndroom (Mazzocco et al., 1998; McCauley et al., 2006; Ross et al., 2000). Het Turner syndroom is een andere X chromosomale aandoening waarbij het X chromosoom gedeeltelijk of in geheel afwezig is bij vrouwen (45,X0). Ook bij het Turner syndroom lijkt er een neurobiologische basis ten grondslag te liggen aan beperkingen in sociaal gedrag en het verwerken van sociale informatie. Gebieden waar afwijkingen zijn gevonden zijn onder andere de amygdala, superieure temporale sulcus, anterieure cingulus, orbitofrontale cortex en insula (Cutter et al., 2006; Kesler et al., 2003; Kesler et al., 2004; Molko et al., 2004; Skuse et al., 2005).(Murphy et al., 1997).

Er lijken ook aanwijzingen te zijn dat het extra X chromosoom een effect heeft op neurale systemen in het brein die betrokken zijn bij taal. Genetische mechanismen waar het X chromosoom bij betrokken is hebben mogelijk invloed op de ontwikkeling van specialisatie van de hersenhelften voor taal, aangezien verminderde specialisatie werd gezien bij XXY mannen met behulp van fMRI. Een verband tussen het X chromosoom en verminderde specialisatie van de hersenhelften voor taal zou in overeenkomst zijn een hypothese van verminderde asymmetrie bij mensen met het Klinefelter syndroom, zoals gesuggereerd door een verhoogde prevalentie van linkshandigheid en neuropsychologische aanwijzingen van verminderde dominantie van de linker

hersenhelft in het verwerken van verbale informatie bij mensen met het Klinefelter syndroom (Geschwind et al., 1998; Netley et al., 1984). Ook bij mensen met het Turner syndroom heeft neuropsychologisch onderzoek uitgewezen dat er mogelijk verminderde dominantie van de linker hersenhelft kan zijn in het verwerken van auditief-verbale informatie (Netley et al., 1982).
Samengevat wijzen onze bevindingen op een mogelijke rol van het X chromosoom in de ontwikkeling van sociale cognitie en maturatie van hersengebieden die betrokken zijn bij sociale perceptie en taal. Echter, wat de exacte genetische mechanismen zijn die leiden tot afwijkingen in brein bij Klinefelter syndroom is nog onduidelijk. Zulke genetische mechanismen kunnen onder andere te maken hebben met overexpressie of abnormale inactivatie van genen op het X chromosoom, genomische imprinting of complexe interacties tussen genen.

De rol van testosteron deficiënties

De mate waarin cognitieve en neurobiologische afwijkingen die we geobserveerd hebben bij XXY mannen het resultaat zijn van een testosteron deficiëntie die bij XXY jongens in de puberteit lijkt te ontstaan is onduidelijk. Het verband tussen testosteron niveaus en ontwikkeling van de hersenen is complex: het moment van blootstelling aan testosteron, gevoeligheid voor testosteron gereflecteerd in androgeen receptor dichtheid en modulatie door omgevingsfactoren zijn belangrijke determinanten van de effecten van testosteron (Craig et al., 2004). Abnormale testosteron niveaus kunnen mogelijk onderdeel zijn van het mechanisme waarop afwijkingen aan het X chromosoom kunnen leiden tot verstoorde ontwikkeling van hersensystemen die een belangrijke rol spelen in sociale cognitie. Echter, dit kan één van vele mogelijke mechanismen zijn waarop sekse chromosomen invloed uitoefenen op de ontwikkeling van het brein. Recente dierstudies hebben laten zien dat sekse chromosomen ook directe, niet-hormonale effecten hebben op maturatie van de hersenen (Dewing et al., 2003). Hiermee in overeenkomst zijn bevindingen bij vrouwen met het XXX chromosomale patroon die geen hormonale afwijkingen lijken te hebben, maar bij wie wel sprake lijkt te zijn van sociale disfuncties en taalstoornissen (Bender et al., 1999; Harmon et al., 1998).

Implicaties voor studies naar autisme en schizofrenie

Onze bevindingen hebben potentiële implicaties voor studies naar ontwikkelingsstoornissen die geassocieerd worden met ernstige beperkingen in sociale adaptatie. Het X chromosoom kan mogelijk ook een rol spelen in

bepaalde sociaal cognitieve disfuncties en onderliggende hersenmechanismen in het autisme en schizofrenie spectrum. Voorzichtige ondersteuning voor deze hypothese komt van observaties van milde symptomen en kenmerken van autisme in vrouwen met het Turner syndroom en een verhoogde kwetsbaarheid voor symptomen uit het autisme- en schizofrenie spectrum in mannen met het Klinefelter syndroom. De milde kenmerken van autisme en schizofrenie in XXY mannen zoals beschreven in dit proefschrift lijken op het milde fenotype zoals gezien wordt bij mensen met een verhoogd risico op autisme of schizofrenie. Op basis van tweeling studies is gesuggereerd dat het typische klinische fenotype van autisme of schizofrenie (zoals gezien wordt bij patiënten) en het mildere sub-klinische fenotype, voort kunnen komen uit eenzelfde genetische basis (Rutter, 2000; Torgersen et al., 2002).

Moeilijkheden op het gebied van taal, sociale adaptatie en emoties worden gezien als onderdeel van de kernstoornissen in autisme en schizofrenie (Aleman et al., 2005; Crow, 2004; Fein et al., 1986). Sommige van deze disfuncties vertonen veel gelijkenis met de disfuncties die we geobserveerd hebben bij XXY mannen. Ook zijn er overeenkomsten op het niveau van onderliggende hersenmechanismen die een belangrijke rol spelen in sociale perceptie en taal. We zouden kunnen speculeren dat deze overeenkomsten duiden op een rol van het X chromosoom in sommige van de sociaal cognitieve- en neurobiologische stoornissen in deze ontwikkelingsstoornissen. Deze hypothese is in overeenkomst met het idee dat genetische factoren in de stoornis een effect hebben op specifieke onderdelen van de stoornis in plaats van de stoornis als geheel (Gottesman et al., 2003; Jablensky, 2006; Rutter, 2000). Het bestuderen van een mogelijke rol van sekse chromosomen in de ontwikkeling van het brein en cognitie is vooral relevant voor autisme en schizofrenie omdat deze stoornissen vaker bij jongens en mannen voorkomen.

Samengevat, het extra X chromosoom in Klinefelter syndroom kan mogelijk een rol spelen in afwijkende ontwikkeling van bepaalde hersenmechanismen betrokken bij taal, emotie en sociaal gedrag. Dit zijn domeinen waarin ernstige stoornissen worden gevonden bij mensen met autisme of schizofrenie. Mogelijk kan het bestuderen van X gebonden genetische mechanismen in Klinefelter syndroom inzicht bieden in routes van genen naar psychopathologie in het autisme en schizofrenie spectrum.

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Curriculum Vitae

Sophie van Rijn werd geboren op 17 maart 1978 in Amsterdam. Na het behalen van haar VWO diploma op Scholengemeenschap Damstede in Amsterdam begon zij in 1996 aan een studie Psychologie aan de Universiteit van Amsterdam. Hier specialiseerde zij zich in de psychologische functieleer en voerde haar afstudeeronderzoek uit op het Nederlands Instituut voor Hersenonderzoek in Amsterdam. Onder begeleiding van Dr. Bob Bermond (UVA) en Dr. Jan de Bruin (NIH) deed zij hier onderzoek naar de rol van dopamine D4 receptoren bij cognitieve flexibiliteit. In 2001 studeerde zij cum laude af, waarna zij in 2002 begon aan haar promotieonderzoek aan de Universiteit Utrecht. Hier was zij werkzaam op de afdeling Psychiatrie van het Universitair Medisch Centrum Utrecht en de afdeling Psychologische Functieleer van het Helmholtz Instituut van de Universiteit Utrecht. Het onderzoek dat zij deed onder begeleiding van Prof. René Kahn, Prof. André Aleman, Prof. Edward de Haan en prof. Hanna Swaab heeft geresulteerd in het voorliggende proefschrift. Momenteel is zij als onderzoeker werkzaam op de afdeling Neuropedagogiek van de Universiteit Leiden.

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Dankwoord

Na pagina's achtereen te hebben geschreven over hoe belangrijk non-verbale sociale signalen kunnen zijn voor het overbrengen en begrijpen van gevoelens, intenties en gedachten, moet ik constateren dat ik op dit moment onmogelijk een beroep kan doen op mijn eigen affectieve gezichtsexpressies en -stemgebruik om uitdrukking te geven aan mijn dankbaarheid voor alles wat mensen om mij heen de afgelopen jaren aan mij hebben gegeven: tijd, energie, expertise, enthousiasme, plezier, steun en geduld.

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Arnick,
Gelukkig heeft onze liefde geen woorden nodig.
Ook geen experimenten, testen en ingewikkelde analyses:
ik hoef alleen maar in je blauwe ogen te kijken en alles is duidelijk.