Repetitive behaviour in autism: Imaging pathways and trajectories

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Banen en trajecten in beeld

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1 Introduction

Autism: phenotype and prevalence

Autism is a severe and lifelong neurodevelopmental disorder characterised by impairments in reciprocal social interaction, abnormal development and use of language, as well as by stereotypies, repetitive and rigid behaviour and restricted interests. A formal diagnosis of autism requires the presence of difficulties in each of these three domains and onset of symptoms before the age of three (American Psychiatric Association 1994).

Autism was first described by the Austrian psychiatrist Leo Kanner in 1943, but it was not until the seventies that autism was officially recognised as a separate child psychiatric disorder. In 1980 the term autism was first included in the Diagnostic and Statistical Manual of Mental Disorders (3rd edition). Nowadays, autism is the core syndrome of the Pervasive Developmental Disorders, which also include Asperger's syndrome, and PDD-Not Otherwise Specified (PDD-NOS) (often named autism spectrum disorders); and Childhood Disintegrative Disorder, and Rett's Disorder, disorders that are less prevalent and supposedly of different aetiology (for diagnostic criteria for the Pervasive Developmental Disorders, see Table 1).

The prevalence of autism was estimated at four cases per 10,000 in 1966 (Lotter et al. 1966), increasing to 30-60 per 10,000, 40 years later (Chakrabarti & Fombonne 2005; Fombonne et al. 2005). At this time, prevalence of the disorders in the full autism spectrum is estimated to be 1:150, with 1:480 for the core autism syndrome; 1:1,600 for Asperger's syndrome and 1:270 for PDD/NOS (Fombonne 2009). It is unknown how many individuals are affected with one of the autism spectrum disorders worldwide. In The Netherlands, an estimated 25,000 children are affected within the spectrum; adult prevalence is unknown. The incidence rate is much higher in boys than in girls, with an estimated gender ratio of 4:1 (Fombonne et al. 2005).

The core symptoms of autism are extremely disabling and cannot be treated, severely affecting the lives of sufferers and their caregivers. Moreover, a majority of individuals with autism (60%) and a considerable part of the population with autism spectrum disorders (30%) is characterised by mental retardation (Fombonne 2006); and a wide array of co-morbid medical conditions is associated with the disorder (Danielsson et al. 2005; LoVullo & Matson 2009), further impairing affected individuals. Epidemiological studies have shown that even most high-functioning adults with autism do not live independently, do not have permanent employment, and have no close friends (Howlin et al. 2000). Given the lifelong need for clinical, educational, and social services, societal costs of autism spectrum disorders are very high. For example, the costs of supporting children with ASDs in the United Kingdom are estimated to be £2.7 billion each year. For adults,these costs increase to £25 billion each year. The estimated life-long cost of one individual with one of the spectrum disorders is estimated at £0.8 - £1.23 million, depending on intellectual functioning (Knapp et al. 2009).

Table 1. Criteria for Pervasive Developmental Disorders, according to the DSM-IV
(Criteria for 299.80 Rett's Disorder and 299.10 Childhood Disintegrative Disorder not shown.)

299.00 Autistic Disorder

A. *A total of six (or more) items from (1), (2), and (3), with at least two from (1), and one each from (2) and (3):*

1. *qualitative impairment in social interaction, as manifested by at least two of the following:*
   - *marked impairment in the use of multiple non-verbal behaviours such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction*
   - *failure to develop peer relationships appropriate to developmental level*
   - *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)*
   - *lack of social or emotional reciprocity*
2. *qualitative impairments in communication as manifested by at least one of the following:*
   - *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)*
   - *in individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others*
   - *stereotyped and repetitive use of language or idiosyncratic language*
   - *lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level*
3. *restricted repetitive and stereotyped patterns of behaviour, interests, and activities, as manifested by at least one of the following:*
   - *encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus*
   - *apparently inflexible adherence to specific, non-functional routines or rituals*
   - *stereotyped and repetitive motor manners (e.g, hand or finger flapping or twisting, or complex whole-body movements)*
   - *persistent preoccupation with parts of objects*

B. *Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years: (1) social interaction, (2) language as used in social communication, or (3) symbolic or imaginative play.*

C. *The disturbance is not better accounted for by Rett's Disorder or Childhood Disintegrative Disorder.*

299.80 Asperger's Disorder

A. *Qualitative impairment in social interaction, as manifested by at least two of the following:*
- *marked impairment in the use of multiple non-verbal behaviours such as eye-to eye gaze, facial expression, body postures, and gestures to regulate social interaction*
- *failure to develop peer relationships appropriate to developmental level*
- *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)*
- *lack of social or emotional reciprocity*

B. *Restricted repetitive and stereotyped patterns of behaviour, interests and activities, as manifested by at least one of the following:*
- *encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity of focus*
- *apparently inflexible adherence to specific, non-functional routines or rituals*
- *stereotyped and repetitive motor mannerisms (e.g, hand or finger flapping or twisting, or complex whole-body movements)*
- *persistent preoccupation with parts of objects*

C. *The disturbance causes clinically significant impairment in social, occupational, or other important areas of functioning.*

D. *There is no clinically significant general delay in language (e.g. single words used by age 2 years, communicative phrases used by age 3 years).*

E. *There is no clinically significant delay in cognitive development or in the development of age-appropriate self-help skills, adaptive behaviour (other than in social interaction), and curiosity about the environment in childhood.*

F. *Criteria are not met for another specific Pervasive Developmental Disorder or Schizophrenia.*

299.80 Pervasive Developmental Disorder Not Otherwise Specified (Including Atypical Autism)

This category should be used when there is a severe and pervasive impairment in the development of reciprocal social interaction associated with impairment in either verbal or non-verbal communication skills or with the presence of stereotyped behaviour, interests, and activities, but the criteria are not met for a specific Pervasive Developmental Disorder, Schizophrenia, Schizotypal Personality Disorder, or Avoidant Personality Disorder. For example, this category includes 'atypical autism' - presentations that do not meet the criteria for Autistic Disorder because of late age at onset, atypical symptomatology, or sub-threshold symptomatology, or all of these.

Autism: a highly heritable neurobiological disorder

Autism has been defined a congenital condition from its first description (Kanner 1943), although other factors, such as the parent-child relationship, have also been suggested as causative factors (Bettelheim 1967). Nowadays, the concept of autism as a psycho-dynamic disorder has been left behind and the notion that autism is a neurobiologically determined developmental disorder is fully established.

Epidemiological studies have indicated that risk for autism is largely determined genetically (Folstein & Rosen-Sheidley 2001; Freitag 2007): the prevalence of autism in siblings of individuals with autism is 2-8%, which represents a 25-fold increase in risk compared to the general population. Furthermore, the concordance of autism in monozygotic twins is between 60-91% (Folstein & Rosen-Sheidley 2001). Work with relatives of autistic individuals confirms the concept of the *broader autistic phenotype*, providing further support for a genetic aetiology to the disorder (Fombonne et al. 1997; Pickles et al. 2000).

Neuroimaging in autism

Although it is widely accepted that autism has a neurobiological origin and is highly heritable, clear biological markers for autism have not yet been identified. Neuroimaging is one approach to studying the neurobiological mechanisms involved in autism. Results suggest that functional and structural abnormalities in several brain regions are related to autism (Palmen and Van Engeland 2004; Acosta and Pearl 2004; Toal et al. 2005; Stanfield et al. 2008; Amaral et al. 2008). However, no clear and consistent pathology has emerged from these studies.

One of the most prominent theories of the neuropathology of autism is that the brain undergoes a period of precocious growth during early postnatal life followed by a deceleration in age-related growth (Courchesne et al. 2003). Although cross-sectional studies have provided support for the hypothesis of accelerated brain growth, it awaits confirmation from a longitudinal MRI study (Amaral et al. 2008). Further, whether this enlargement is predominantly driven by white or grey matter remains unclear: Reports from very young children suggest that early brain enlargement is disproportionately accounted for by increased white matter (Courchesne et al. 2001; Hazlett et al. 2005), while studies in older age groups show that grey matter enlargement persists into adulthood (Courchesne et al. 2001; Herbert et al. 2003; Lotspeich et al. 2004; Hazlett et al. 2006; see Brambilla 2003; Palmen and Van Engeland 2004 and Amaral et al. 2008 for reviews).

While some studies have investigated overall brain changes, others have focused on the neural systems putatively involved in the symptoms of autism. This seems a promising approach: The syndrome is clinically heterogeneous, with a wide range of symptoms and varying severity of the impairments. This could be taken to predict a heterogeneous pattern of neuropathology in autism. Relating specific brain areas to symptom clusters may help address this heterogeneity. However, even here, results have been inconclusive. Discrepancies between studies may in part reflect differences in developmental stage between samples (McAlonan et al. 2002; Herbert et al. 2003; Hollander et al. 2005). Other

factors contributing to contradictory findings may include medication effects, small sample sizes, and heterogeneity of the samples including both high-functioning and low-functioning individuals.

Repetitive behaviour in autism: a relatively ignored class of symptoms

Stereotypies, repetitive behaviour and restricted interests form one of the three defining symptom domains of autism and have been described in detail from the first reports on the disorder (Kanner 1943; Asperger 1944). Broadly, repetitive behaviours are defined as recurring, non-functional activities or interests that occur regularly and interfere with daily functioning (Gabriels et al. 2005). This class of symptoms includes repetitive motor behaviour (hand and finger mannerisms, stereotyped body movements), the repetitive use of objects as well as intense circumscribed patterns of interests and rituals and compulsions (Lord et al. 1994). Clinically, these symptoms represent extreme challenges for individuals with autism and their caregivers, and can cause severe family distress and dysfunction due to the individual's intolerance of change and acts of aggression against themselves or others (Gabriels et al. 2005).

While a considerably body of work has investigated neurobiological mechanisms associated with the other two clusters of symptoms (impaired social interaction and language development), systematic study of the phenomenology and in particular the neurobiology of repetitive behaviour has been lacking.

This thesis addresses this issue by investigating the neurobiology of repetitive behaviours in autism, using structural magnetic resonance imaging (MRI) and diffusion tensor MRI (DTI).

Understanding the neuronal networks involved in repetitive behaviours and related problems will improve our understanding of the pathogenesis of autism spectrum disorders. This in turn will stimulate novel approaches in thinking about these behaviours and conditions, encouraging new therapeutic initiatives.

Aim and outline of this thesis

Repetitive behaviour is not specific to the autism spectrum disorders. Individuals suffering from other disorders such as obsessive - compulsive disorder and Tourette's Syndrome (TS) also display stereotypies, repetitive and rigid behaviour. Furthermore, repetitive behaviours are a normal part of early development.

In **chapters 2** and **3**, we investigate the neurobiological systems associated with various forms of repetitive behaviour by discussing findings from fundamental animal research and translational models (chapter 2), and by synthesising studies of disparate clinical syndromes (chapter 3). In these chapters, we aimed to answer the question whether repetitive behaviour across diverse neuropsychiatric disorders is caused by similar neurobiological mechanisms or whether different repetitive behaviours are neurobiologically unique.

In **chapter 4** we return to the main focus of this thesis: investigating the neurobiology of repetitive behaviour in autism. Earlier studies exploring brain mechanisms behind repetitive behaviour in other human conditions have indicated involvement of the basal ganglia in OCD (Modell et al. 1989; Scarone et al. 1992; Giedd et al. 1996) and TS (Peterson et al. 1993; Peterson et al. 2003; Albin and Mink 2006). Furthermore, fronto-striatal circuitry has been implicated in the development of autistic symptoms in individuals with 22q11 syndrome (Campbell et al. 2006). Previous research has used MRI to investigate the neurobiology of repetitive behaviour in autism and the other spectrum disorders. Whereas some studies have reported larger volumes in autism, particularly of the caudate nucleus (Sears et al. 1999; Hollander et al. 2005; Haznedar et al. 2006; Rojas et al. 2006; Voelbel et al. 2006), others have not (Gaffney et al. 1989). Two studies implicated caudate nucleus in repetitive behaviour more directly (Sears et al. 1999; Hollander et al. 2005), as they reported correlations between the volume of these structures and measures of repetitive behaviour. These findings implicate the basal ganglia, and particularly the caudate nucleus, in the pathophysiology of autism. However, it has been argued that use of neuroleptics, shown to be associated with volume changes of basal ganglia structures (Chakos et al. 1994; Keshavan et al. 1994; Shihabuddin et al. 1998; McCarley et al. 1999; Scheepers et al. 2001; Lang et al. 2004), may have confounded these studies. Therefore, we investigated basal ganglia volumes in never-medicated subjects with autism to further explore previously demonstrated enlargements of the basal ganglia and their involvement in repetitive behaviour. We hypothesised an enlargement of the basal ganglia, and particularly caudate nucleus, related to repetitive behaviour.

In **chapter 5** we touch upon a second potential 'confounder' in neuroanatomical studies of autism: developmental stage of the studied sample. Although striatum has been implicated in autism, results from MRI studies are not yet conclusive: whereas some studies have reported larger volumes in autism, particularly of the caudate nucleus (Sears et al. 1999; Hollander et al. 2005; Haznedar et al. 2006; Rojas et al. 2006; Voelbel et al. 2006), others have not (Gaffney et al. 1998). Furthermore, it is unclear whether the reported increase in volume is disproportional to an overall increase in brain volume (Sears et al. 1999; Herbert et al. 2003). In our earlier study of two smaller, independent samples of high-functioning subjects with autism, we found that the caudate nucleus was enlarged compared to typically developing individuals (Chapter 4). In this study, there was a large difference in age between the two samples (mean age for the first sample was 10 years and 20 years for the second) and the effect was greater for the older sample. This led us to hypothesise that autism may be associated with changes in striatal development, where differences become more pronounced with age. In typical development, the striatum decreases in volume over time, both in childhood (Sowell et al. 2002) and in adulthood (Gunning-Dixon et al. 1998; Jernigan et al. 2001; Walhovd et al. 2005; Toga et al. 2006).

A comprehensive longitudinal study in children and adolescents showed that the developmental trajectory of the caudate nucleus follows an inverted U-shape (Lenroot and Giedd 2006; Lenroot et al. 2007). In autism, the developmental trajectory of the striatum has not been examined. As such, differences in results between studies of striatal volume

in autism could in part reflect differences in mean age between samples (McAlonan et al. 2002; Herbert et al. 2003; Hollander et al. 2005). Therefore, we investigated structural brain development in a large and homogeneous sample of high-functioning individuals with autism and controls (n=188). We hypothesised that the caudate nucleus would be enlarged in autism and that its developmental trajectory would differ from that of controls.

In **chapter 6**, we report on findings of white matter differences between autistic individuals and controls. In addition to differences in discrete brain regions, a number of recent studies have suggested that autism may be related to differences in cortical networks (Bachevalier and Loveland 2006; Just et al. 2004). Changes in functional connectivity have been shown in ASD (Ring and Serra-Mestres 2002; Just et al. 2004; Belmonte et al. 2004; Koshino et al. 2005), especially within frontal cortex and in circuits linking frontal areas to other brain systems (Courchesne and Pierce 2005), as well as in corticostriatal circuits involving the caudate nuclei (Turner et al. 2006). To address this issue, we explored the integrity of corticostriatal white matter tracts in autism using diffusion tensor imaging (DTI) and magnetisation transfer ratio (MTR) imaging.

DTI and MTR are imaging methods that permit visualisation and quantification of different aspects of white matter. The combination of DTI and MTR allows for an assessment of white matter integrity, where information on the directionality and coherence of directionality of white matter tracts (DTI) is combined with information regarding the level of myelination (MTR). Both measures (DTI and MTR) are sensitive to white matter maturation (Baratti et al. 1999; Hüppi et al. 1998; Neil et al. 1998), although DTI has more commonly been applied to investigating development. In autism, studies of white matter integrity have typically used DTI in an exploratory fashion, with only few using tract-based approaches (Catani et al. 2008; Sundaram et al. 2008; Pugliese et al. 2009). Results suggest widespread changes in white matter integrity (Barnea-Goraly et al. 2004; Alexander et al. 2007; Keller et al. 2007; Thakkar et al. 2008; Ke et al. 2009), and reduced structural white matter integrity in autism during late childhood and the second decade of life (Ben Bashat et al. 2007). One study suggested an association between FA values in anterior cingulate cortex with repetitive behaviour (Thakkar et al. 2008). To date, no studies have focused on changes in cortico-striatal circuits. Therefore, we set out to investigate age-related changes in the microstructural integrity of corticostriatal white matter in autism, using both DTI and MTR measures. To overcome limitations of voxel-based DTI (Snook et al. 2007; Ashburner and Friston 2000; Jones et al. 2005), we used a tract-based approach. We hypothesised that development of corticostriatal white matter integrity would be compromised in autism and that these changes would relate to repetitive behaviour.

In **chapter 7** we further investigate the role of developmental stage in autism, by studying an adult population affected with the disorder. Although studying children is pivotal to investigating developmental disorders such as autism, studying adults can provide information on the endpoint of development. By studying adults we aim for a more complete picture of how brain structure and connectivity affect autism. In this chapter,

we focus on the how striatum and corticostriatal circuitry are implicated in repetitive and stereotyped behaviour. Furthermore, we explore the involvement of the frontal cortex and its connections to striatal brain regions in behavioural symptoms generally associated with repetitive behaviour, such as executive functions and in particular inhibitory control (Robinson et al. 2009; Christ et al. 2006)

There is increasing evidence that people with autism have anatomical (Carper and Courchesne 2005; McAlonan et al. 2002; Abell et al. 1999), metabolic (Horwitz et al. 1988) and functional (Murphy et al. 2002) differences in corticostriatal pathways. There are VBM based DT-MRI studies of autism (Barnea-Goraly et al. 2004; Alexander et al. 2007; Keller et al. 2007; Thakkar et al. 2008; Ke et al. 2009), reporting widespread changes in white matter integrity, but these cannot address the known issues of VBM approaches (e.g. low anatomical resolution; type I error due to multiple comparisons; problems related to spatial normalisation) Jones et al. 2005; Snook et al. 2007). There are also some prior DT-MRI tractography studies in autism (Catani et al. 2008; Sundaram et al. 2008; Pugliese et al. 2009). However, nobody has yet examined the anatomy of striatum together with the micro-structural integrity of specific white matter tracts in corticostriatal pathways in the same individuals; or related this to clinical symptoms. Therefore, in this chapter, we used sMRI and DTI to compare differences in the volume of the basal ganglia, and micro-structural integrity of corticostriatal white matter, in adults with ASD and controls. Also we related anatomical differences to repetitive behaviours and inhibitory control. To overcome limitations of voxel-based DTI (Jones et al. 2005; Snook et al. 2007), we used a tract-based approach.

Finally, in **chapter 8**, the results of these studies are summarised and the merits and limitations of the findings are discussed.

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2

The neurobiology of repetitive behaviour: of mice...

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revised manuscript submitted

Repetitive and stereotyped behaviour is a prominent element of both animal and human behaviour. Similar behaviour is seen across species, in diverse neuropsychiatric disorders and in key phases of typical development. This raises the question whether these similar classes of behaviour are caused by similar neurobiological mechanisms or whether they are neurobiologically unique?

In this paper we discuss fundamental animal research and translational models. Imbalances in corticostriatal function often result in repetitive behaviour, where different classes of behaviour appear to be supported by similar neural mechanisms. Although the exact nature of these imbalances are not understood, synthesising the literature in this area provides a framework for studying the neurobiological systems involved in repetitive behaviour.

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Introduction

During early development, children engage in a significant amount of ritualistic, stereotypic, and compulsive-like activity that is part of the normal behavioural repertoire (Evans et al. 1997). The wide variety of repetitive behaviour that can be observed in typically developing young children has striking similarities to the ritualistic, stereotypic and compulsive behaviour observed in certain neuropsychiatric syndromes such as obsessive-compulsive disorder (OCD) and autism spectrum disorders (ASD). However, whereas this behaviour is adaptive in typical development, in many psychiatric disorders repetitive behaviour forms a salient part of symptoms and causes prominent impairment in the daily life of affected individuals.

Similarly, repetition forms an important part of normal functioning in animal behaviour. In invertebrates, birds and lower mammals, fixed, repeatedly performed action patterns are vital for survival of both individuals and species, and in higher mammals, repetitive actions such as highly skilled acts acquired through practice, occur as a part of normal behaviour. However, abnormal repetitive behaviour also occurs in animals and can take numerous forms, from pacing (birds, prosimians, large carnivores), jumping and somersaulting (mice) to crib- and bar-biting (horses, pigs, mice), rocking (primates) and self-injurious behaviour (monkeys, parrots).

Scope of this review

The occurrence of similar behaviour across species, in diverse neuropsychiatric and neurodevelopmental disorders, as well as in certain phases of typical development, raises a key question: Are these similar behaviours caused by similar neurobiological mechanisms or are different repetitive behaviours neurobiologically unique? Although a single pathogenesis for all forms of repetitive behaviour seems unlikely, there is a large body of animal and human literature that points towards the involvement of both similar and specific neuronal systems in the development of repetitive behaviour (Lewis and Bodfish 1998). Understanding which neuronal networks are involved in the development of repetitive behaviour and related problems will improve insight into the pathogenesis of neuropsychiatric and neurodevelopmental disorders. This in turn will stimulate novel approaches to thinking about this behaviour in these conditions, encouraging new therapeutic initiatives.

In this paper we aim to investigate the neurobiological systems associated with various forms of repetitive behaviour and co-occurring cognitive problems by discussing findings from the animal literature. First, we place the research on repetitive behaviour and the underlying neurobiology in a historical perspective. Second, we discuss how environmental deprivation causes long-lasting changes in brain development and chemistry and how these changes may trigger and modulate stereotypies and repetitive behaviour. Third, we discuss translational studies where repetitive behaviour is induced using pharmacological agents, insults to the central nervous system or gene manipulation. Fourth, we consider the relationship between repetitive behaviour and basal ganglia circuits.

In this paper, we use the term repetitive behaviour to describe a wide range of behaviours including stereotyped movements, marked distress in response to minor changes of the environment, an insistence on following routines in precise detail, and preoccupation with narrow, circumscribed interests. Three characteristics unite these apparently disparate classes of behaviour and define them as repetitive behaviour: (1) a high frequency of repetition in the display of the behaviour; (2) the invariant way the behaviour or the activity is pursued; and (3) the behaviour is inappropriate or odd in its manifestation and display (Turner 1997). Repetitive behaviour is observed across species and manifestations range from basic motor behaviour to higher-level cognition.

Given the inconsistency in classification of repetitive behaviour, we begin by defining the terms as we use them here. We use the term 'repetitive behaviour' to refer to any kind of behaviour that fulfils the criteria above. When we discuss studies on animal or human repetitive behaviour, we use the category to which the behaviour belongs (e.g. motor stereotypy, compulsion, sameness-behaviour) or the specific repetitive behaviour that was studied (e.g. repetitive gnawing, compulsive hand-washing). In describing anatomical structures, we use the term 'basal ganglia' to refer to the complex of structures located in the midbrain (i.e. striatum (caudate nucleus, putamen, nucleus accumbens), globus pallidus, subthalamic nucleus and substantia nigra). Where possible, we specify individual structures. The feedback loops that connect cortex, basal ganglia and thalamus are referred to as corticostriatal pathways, loops or circuits; when a specific pathway is meant, the terms as defined by Alexander (Alexander et al. 1986) are used (e.g. orbitofrontal loop). In the interest of legibility, we refrain from abbreviations, except where it concerns generally acknowledged terms (e.g. MRI, GABA, D1-receptor).

1. Historical perspectives on repetitive behaviour

Initially, repetitive behaviour research was directed by fundamental animal studies and was mostly limited to motor stereotypies. Later, research advanced to developing translational animal models for human disorders, extending its scope to cognitive and emotional domains. In this section, we give an overview of what animal literature has taught us about repetitive behaviour.

Traditionally, the basal ganglia have been a candidate for explaining repetitive behaviour. In the 1920s, the striatum was directly implicated by studies of pharmacologically induced repetitive behaviour in guinea pigs (Amsler 1923) and since then many studies have used diverse techniques to confirm that damage to or dysfunction of the basal ganglia results in 'recurrent perseveration' or inappropriate response repetition (Garner 2005; Norman and Shallice 1986; Sandson and Albert 1984; Turner 1997). Many early studies focused on the development of repetitive motor behaviour and largely ignored striatal influences on other, non-motor repetitive behaviour. The reasons for this were threefold: First, motor stereotypies are more prominent than non-motor repetitive behaviour and are relatively easy to model in animals. Second, higher-order repetitive behaviour observed in animals with basal ganglia insults was thought to result from secondary neuropathological changes. Third and foremost, the leading theory of basal ganglia function at that time posed that

basal ganglia output only targeted those areas of cerebral cortex that participated in the generation and control of movement (Middleton and Strick 2000b). However, accumulating evidence led to a challenge of this belief and in a pivotal paper in 1986, Alexander and colleagues dramatically redirected basal ganglia theory and research (Alexander et al. 1986): they reviewed earlier ideas and studies of basal ganglia function (e.g. (DeLong et al. 1984; Künzle 1975, 1977, 1978; Nauta 1979; Schell and Strick 1984) and proposed that the basal ganglia should be viewed as components of multiple parallel, segregated circuits with outputs targeting not only primary motor areas, but also specific pre-motor and prefrontal cortical areas. Five parallel corticostriatal circuits were defined, although the authors noted at the time that this list was unlikely to be exhaustive. These circuits were named as (1) the motor circuit, (2) the occulomotor circuit, (3) the dorsolateral prefrontal circuit, (4) the lateral orbitofrontal circuit, and (5) the anterior cingulate circuit. The circuits were named after their cortical targets and not all circuits were initially functionally characterised. Later, Middleton & Strick (Middleton and Strick 2000a) described two additional circuits between the basal ganglia and more posterior parts of the cortex (the inferotemporal and posterior parietal circuits). Each circuit was proposed to include discrete, essentially non-overlapping parts of the striatum (caudate nucleus, putamen and nucleus accumbens), globus pallidus, substantia nigra, thalamus, and cortex.

Circuits are structured in a similar manner (Figure 1), with each circuit receiving cortical inputs to the striatum, passing the input through the basal ganglia, via output nuclei (the substantia nigra pars reticulata and the medial globus pallidus) to a restricted area of the thalamus and from there back to a single cortical area (Ring and Serra-Mestres 2002). Each corticostriatal circuit receives multiple inputs only from cortical areas that are functionally related and usually interconnected (Alexander et al. 1986).

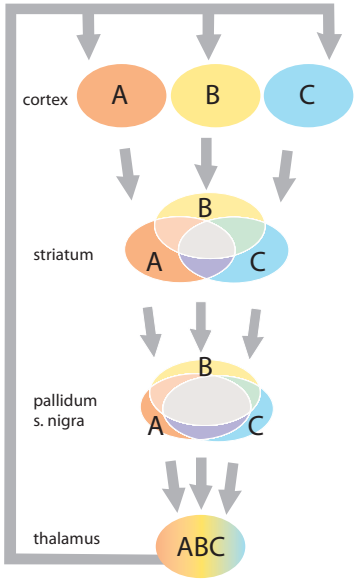

Figure 1. Corticostriatal circuits

Corticostriatal circuits as proposed by Alexander et al. (1986). Each circuit receives output from several functionally related cortical areas (A, B, C) that send partially overlapping projections to restricted parts of striatum. These striatal regions send converging projections to the globus pallidus (pallidum) and substantia nigra (s. nigra), which in turn project to specific regions of the thalamus. Each thalamic region projects back to one of the cortical areas that feed into the circuit, thereby completing the 'closed loop'.

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Furthermore, each loop consists of two distinct branches: the direct (or striatonigral) and the indirect (or striatopallidal) pathway. The net result of activity of the direct pathway is an increase in thalamic activity, whereas activity of the indirect pathway inhibits the thalamus. Thus, under normal circumstances, the direct pathway enhances behaviour, whereas the indirect pathway inhibits it (Lewis et al. 2006). This dual system is thought to allow for fine-tuning of activity in large portions of frontal cortex responsible for movement, cognitive, and limbic function (Bradshaw 2001).

Studies investigating the functional and structural architecture of corticostriatal circuits have refined, but not fundamentally changed, this original model. It is now established that corticostriatal loops can be functionally divided into three 'macro-circuits', related to the predominant cerebral cortical input to striatum. These are the sensorimotor circuit (comprising the motor and oculomotor loops), the associative circuit (dorsolateral prefrontal loop) and the limbic circuit (lateral orbitofrontal and anterior cingulate loops (Groenewegen et al. 2003). Within these macro-circuits, smaller (micro)-circuits can be recognised that subserve specific functions within the broader functional domain, i.e. sensorimotor (movements), associative (cognitive functions) or limbic (emotional-motivational behaviour) (Groenewegen et al. 2003; Mason and Rushen 2006). This level of detailed organisation has been shown most convincingly for the sensorimotor macrocircuit, where specific microcircuits are related to different parts of the body and subserve various aspects of the movement, such as the direction of a movement, or the force exerted (Groenewegen et al. 2003). Furthermore it has become clear that the various functions subserved by these circuits are not independent, but rather that they follow a spiralling organisation where information flows from higher-order circuits to lower-order ones (Haber 2003; Haber and Calzavara 2009).

Increasing understanding of corticostriatal loops has resulted in a re-evaluation of models of motor and non-motor repetitive behaviour. In the original description of the five parallel circuits, Alexander described how damage to individual loops may lead to abnormal repetitive behaviour. For example, he implicated the orbitofrontal circuit in behavioural inhibition and switching behaviour, as studies in primates had shown that bilateral lesions to the lateral orbitofrontal area or to the portion of the caudate to which it projects result in perseverative behaviour (Alexander et al. 1986). Now that corticostriatal loops have been functionally characterised, it is recognised that repetitive behaviour may reflect a disruption of co-ordinated function within the basal ganglia or between striatal and forebrain structures (Robbins et al. 1990). As such, abnormal repetitive behaviour may result from damage to any of the circuits, and the exact location of the disruption (i.e, which loop is affected) determines what type of repetitive behaviour is displayed. Both animal and human studies have suggested that the motor loop is primarily involved in abnormal stereotypical motor behaviour: continuously repeating identical movements without pursuing a goal. Involvement of the oculomotor loop in repetitive behaviour has not often been described, but oculomotor perseveration has been shown in humans and animals (e.g. repetitive eye-rolling in calves and an inability to suppress eye movements in individuals with schizophrenia) (Mason 2006). The prefrontal loop has been associated with inappropriate repetition of goal-directed behaviour, often expressed in a relatively varied behavioural repertoire (as in some obsessive-compulsive behaviour). The limbic loops

(lateral orbital loop and anterior cingulate loop) are implicated in motivational aspects of behavioural control, including impulsive behaviour (difficulty in suppressing behaviour even when consequences are negative); response to reward; and obsessive and compulsive behaviour (including compulsive drug-taking). Although this is a simplified classification of how functionality corresponds to anatomy, the literature does suggest that different frontal cortical areas and corresponding subcortical regions are involved in various and distinct aspects of motivation, cognition, and motor control (Haber and Calzavara 2009).

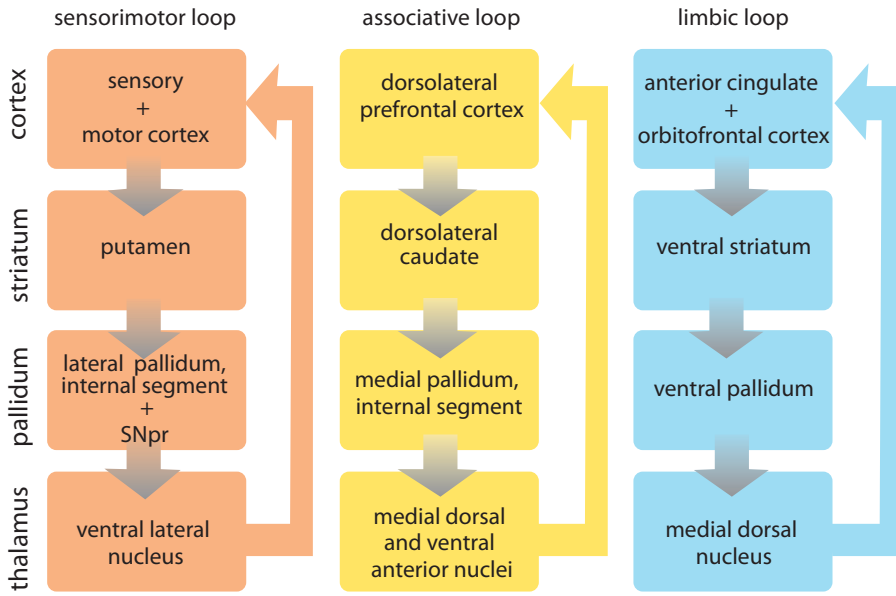

Figure 2. Parallel corticostriatal macro-circuits

Parallel corticostriatal macro-circuits with their main input, relay and output regions. Abnormal repetition of behaviour can result from damage to any of the corticostriatal circuits, where the exact location of the disruption (i.e. which loop is involved) determines what type of repetitive behaviour is seen. (SNpr = substantia nigra pars reticulata)

(See page 167 for a colour version of this figure.)

2. Repetitive behaviour induced by environmental deprivation

In animal behaviour, repetition forms an important part of normal functioning. In invertebrates, birds and lower mammals, fixed behavioural patterns are vital for survival of both the individual and species. In higher mammals, repetitive actions also occur as a part of normal behaviour and include highly skilled acts acquired through practice. However, abnormal repetitive behaviour also occurs in animals and can take numerous forms, from pacing (birds, prosimians, large carnivores), jumping and somersaulting (mice) to crib- and bar-biting (horses, pigs, mice), rocking (primates) and self-injurious behaviour (monkeys, parrots).

Adverse environmental circumstances can cause an animal to develop abnormal repetitive behaviour. Confinement and environmental restriction are well-established risk factors; indeed, repetitive behaviour is the most common category of abnormal behaviour observed in confined animals (Lewis et al. 2007). Ridley (1994) hypothesised that in confinement, the environment shapes the patterns of behaviour, as opportunities for behaviour are so limited that only a repetitive pattern of responses can be formed. Others have argued that the stress induced by confinement is an important mediating factor in developing of repetitive behaviour: Here, stereotypies are hypothesised to function as a coping mechanism to reduce the arousal level of the animal when it is exposed to stressful events or environments (for an extensive review on this theme: see Cabib 2006).

Effects of environmental deprivation on brain development and brain chemistry

In the animal literature, a distinction has been made between maladaptive and malfunctional behaviour. The first reflects a normal response to an abnormal environment, whereas the second is the product of abnormal psychology, brain development or neurochemistry and is induced by features of the restrictive environment (Garner 2005; Mills 2003). Although some authors have shown positive effects of environmental enrichment on repetitive behaviour (see Swaisgood and Shepherdson 2006), many have stressed the robustness of stereotypies in captive animals: As stereotypies develop, they become increasingly hard to abolish with environmental enrichment or neurochemical treatment (Garner 2005; Garner et al. 2003; Swaisgood and Shepherdson 2006). The difficulty in treating repetitive behaviour induced by confinement and deprivation suggests that the underlying neurobiology may be permanently altered by such environmental restrictions.

Studies investigating the neurochemical effects of deprivation substantiate this hypothesis: Numerous studies have established that rearing rats in isolation leads to substantial dysregulation of forebrain catecholamine systems (Fulford and Marsden 2007). For example, rats reared in solitude show increased stereotyped behaviour in adulthood following administration of dopamine agonists. These results suggest that environmental deprivation may have permanently affected brain biochemistry (Garner 2006; Sahakian and Robbins 1977; Sahakian et al. 1975). Taken together with other neurochemical data, this indicates that alterations in presynaptic dopaminergic function are a consistent effect of rearing animals in isolation (Powell et al. 2003). Other studies have directly demonstrated biochemical changes in the striatal system in deprived animals, including changes in dopamine and opiate metabolism (Fry et al. 1981; Kraemer et al. 1984; Kraemer et al. 1989; Lewis et al. 1996; Lewis et al. 1990; Martin et al. 1991; Ödberg et al. 1987; Robbins 1996; Sharman et al. 1982). Furthermore, some studies have been able to show structural and functional changes in striatal neurochemistry associated with environmental enrichment and relate this to prevention of developing stereotypies (Lewis et al. 2006), further implicating this system in this dysfunctional behaviour.

Early social deprivation in particular has been shown to cause irreversible repetitive behaviour (Mason and Rushen 2006). The experiments by Harlow in the 1960s are well known for demonstrating the long-lasting effects of maternal and social deprivation on the behavioural repertoire (Harlow et al. 1965; Harlow and Harlow 1962). In these studies,

primates raised in partial or total social isolation displayed severely aberrant behaviour with prominent repetitive behaviour ('compulsive' sucking and stereotyped movements). The severity and robustness of the abnormal behaviour was related to the degree of isolation and duration of the isolation period (Harlow et al. 1965; Harlow and Harlow 1962). Stereotypies induced by deprivation are more common in monkeys and apes than in lower mammals. This suggests that humans may also be particularly vulnerable to this type of behaviour. Indeed, severe effects of early social deprivation have been shown in humans, e.g. in adopted children from Romanian institutions (Groza 1999; Hoksbergen et al. 2005; Rutter et al. 1999; Rutter et al. 2007; Rutter and O'Connor 2004; Rutter et al. 2001). These children are at increased risk for behavioural and cognitive problems and show quasi-autistic features, including repetitive behaviour. Even a year after adoption from Romania, half or more of these children still displayed stereotypies and self-injurious behaviour (Beckett et al. 2002; Fisher et al. 1997; MacLean 2004). Longer periods of deprivation (six months or more) had more severe and longer lasting effects (Beckett et al. 2002; Fisher et al. 1997; Rutter et al. 2001). These observations have led to the speculation that institutionalisation may set off 'some form of programming effect or neural damage' in these children (Rutter and O'Connor 2004).

Cognitive changes following environmental deprivation

In addition to behavioural stereotypies mediated by the motor corticostriatal circuit, environmentally deprived animals show specific cognitive abnormalities. These include poor extinguishing of learnt responses (Garner et al. 2003; Lutz et al. 2004; Mason and Rushen 2006; Vickery and Mason 2003, 2005) and disinhibition of response selection (Garner and Mason 2002). These cognitive problems are related to stereotyped behaviour: One study showed a correlation between cage stereotypies and performance on a cognitive perseveration task in parrots (Garner et al. 2003). Others have shown that blue and marsh tits (Garner et al. 2003), bank voles (Garner and Mason 2002), and bears (Vickery and Mason 2005) that spontaneously exhibit stereotypic behaviour also have a perseverative response pattern on a gambling task; in reversal learning; or in the extinction of stimulus-response learning (Tanimura et al. 2008). Overall, captive animals with high levels of stereotypy show a strong tendency to repeat responses or behaviour: In every species looked at, the most stereotypic individuals also showed the most persistent, repetitive responding in a variety of cognitive tasks (Mason and Rushen 2006). These findings suggest a fundamental similarity between deprivation-induced stereotypies and specific cognitive abnormalities and suggest that a common pathway may underlie both. Some studies have used cognitive tasks to relate behavioural stereotypies directly to the basal ganglia. For example, Garner & Mason (Garner and Mason 2002) correlated stereotypies in rodents with their performance on a cognitive task that reflects basal ganglia function (a spatial extinction task). They showed a strong correlation between task performance and cage stereotypies. Their findings suggest that deprivation results in general striatal disinhibition of response selection, reflected by motor stereotypies as well as cognitive problems. Tanimura and colleagues followed a similar approach (Tanimura et al. 2008): They investigated the relationship between stereotypies and cognitive ability mediated by corticostriatal circuitry (cognitive flexibility, as assessed by reversal learning) in mice. Their results showed a strong association of high

stereotypy levels with cognitive rigidity, but not with other cognitive measures. These findings substantiate the hypothesis that distinct types of repetitive behaviour (motor stereotypies, cognitive rigidity) are inter-correlated and are mediated by corticostriatal dysfunction. However, it remains unclear what the exact mechanism behind common motor and cognitive problems is. Are problems in one system secondary to dysfunction in another? If so, what is the direction of this effect? Or are the neurobiological changes induced by environmental deprivation so widespread that they affect all corticostriatal circuitry?

In sum, repetitive behaviour is common in animals faced with environmental deprivation, particularly when they are exposed to it early in development. The repetitive behaviour induced by environmental deprivation includes motor stereotypies and cognitive rigidity, where these are related and may therefore be mediated by similar circuitry. Repetitive behaviour induced in this manner does not seem to reflect an adaptive coping mechanism. Rather, it seems to reflect robust and possibly permanent changes in brain development. This is supported by the difficulty in treating this behaviour; resulting changes in striatal neurochemistry; and by findings of cross-sensitisation between environmental factors and psycho-stimulants. However, the effects of environmental deprivation are not on an on/off scale. Rather, they are modulated by factors such as quality and duration aspects of deprivation, genetic make-up and other individual characteristics.

3. Translational studies of repetitive behaviour

In the previous paragraph, we discussed how repetitive behaviour can result from environmental conditions. In this section, we consider studies that have deliberately induced repetitive behaviour. Whereas confinement and deprivation studies implicate striatal systems in the development of repetitive behaviour indirectly, work inducing stereotypies by drugs, lesions or gene manipulation can relate this brain circuitry to repetitive behaviour more directly, as the system can be manipulated to uncover the relative contribution of its various components. Furthermore, lesions and pharmacological manipulations can be applied in young animals to assess their impact in the development of repetitive and stereotyped behaviour and gene-manipulation can be used to assess the effect of up- and down-regulating some of the genes involved in the developing animal.

Pharmacological modulation of repetitive behaviour

Figure 3 is a simplified diagram illustrating the complex nature of how the basal ganglia system is modulated by endogenous neuropeptides. As described earlier, the direct pathway enhances behaviour, whereas the indirect pathway is inhibitory. Generally speaking, activating the indirect pathway or suppressing the direct pathway will alleviate stereotypies, whereas suppressing the indirect pathway will induce them. In contrast, activating the direct pathway leads to hyperactivity, not stereotypy; and inhibiting only the direct pathway suppresses all behaviour (including stereotypy) (Garner 2006; Lewis et al. 2006). The main neurotransmitters in striatum, pallidum, and thalamus are GABA and glutamate. Corticostriatal circuitry is modulated by dopamine, opiates (dynorphin, enkephalin), serotonin and several other neurotransmitters (Albin et al. 1989; Mason and

Rushen 2006). Studies investigating the role of neurotransmitters in repetitive behaviour are faced with a number of complications: First, these systems do not function in isolation, but are interactive, meaning that manipulating one system may influence another. Second, when exogenous pharmacological agents are administered to affect these systems, it is relevant how this is done: the effects of direct injection into a brain region may be very different from the effects of oral administration or subcutaneous or intravenous injection. Third, the effects of exogenous agents are often dose-dependent, complicating the generalisation of findings relating to drug-induced behaviour (Mills and Luescher 2006).

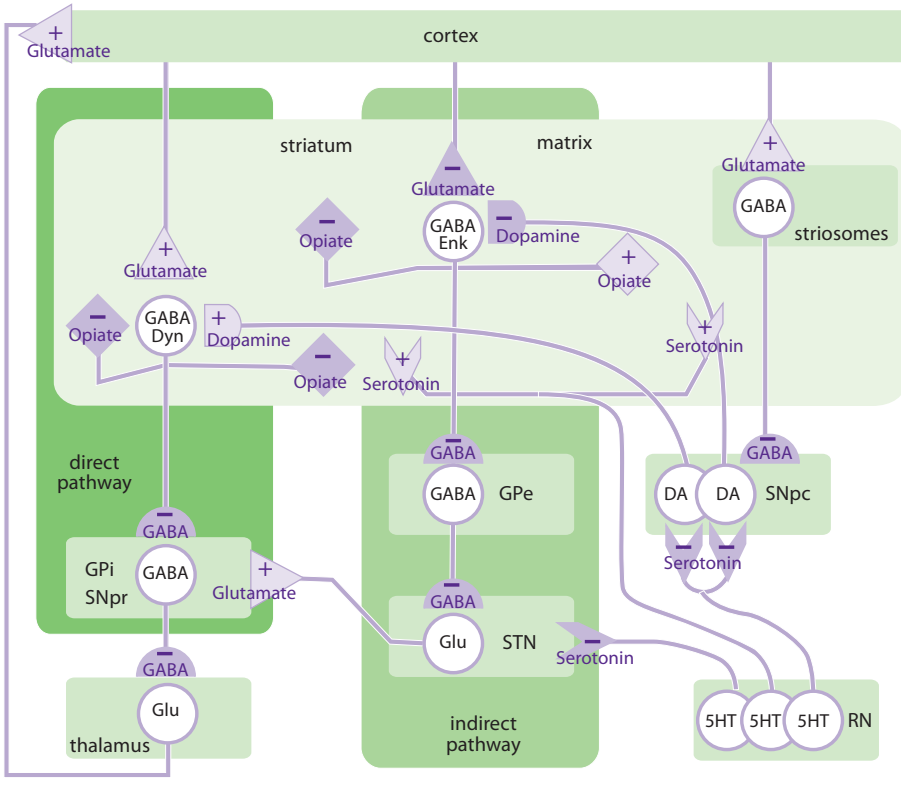

Figure 3. Schematic representation of corticostriatal circuitry, showing direct and indirect pathways and endogenous neurochemistry involved.

(Figure adapted with permission from Mason G. and Rushen J. (Eds.), 2006, Stereotypic Animal Behaviour. Fundamentals and Application to Welfare. CAB International, Wallingford, UK. See page 168 for a colour version of this figure.)

Pharmacological modulation of repetitive behaviour: GABA and glutamate

The targeted administration of agents that bind to inhibitory GABA-receptors or excitatory glutamate sites can be used to manipulate the activity of distinct elements of corticostriatal circuitry. In this way, positive feedback to the cortex can be affected to reduce or stimulate repetitive behaviour. Inhibiting the output nuclei of the basal ganglia facilitates activation of thalamo-cortical relay neurons and consequently provides positive feedback to the

cortex. As such, administering GABA agonists to the substantia nigra pars reticulata induces stereotypy in rats (Scheel-Kruger et al. 1980). Intracortical manipulation of the activity of excitatory corticostriatal projections also affects stereotypic behaviour: Administering GABA-agonists or antagonists to the frontal cortex in rats respectively attenuates or exacerbates stereotypic behaviour (Karler et al. 1995). However, distinct behavioural effects following GABAergic drug administration have been observed, affected by topographical variations (site of injection) or dose differences (Scheel-Kruger et al. 1980), stressing the neurochemical complexity of the corticostriatal feedback loops. Finally, manipulating striatal activity by administering glutamatergic agents modulates repetitive behaviour: glutamate receptor agonists, such as NMDA agonists, can induce stereotypic behaviour, whereas striatal administration of an NMDA-receptor antagonist can attenuate drug-induced stereotypy (Bedingfield et al. 1997). Similarly, transgenic mice with potentiated cortical and limbic glutamate output to the striatum show increased stereotyped behaviour after increasing glutamate release as compared to control litter-mates (McGrath et al. 2000). Elevated glutamate levels may produce a depolarising effect, eventually enabling striatal NMDA-receptors to be activated. Ultimately, neuronal activity of striatum disinhibits feedback to the cortex, inducing stereotypic behaviour (Presti 2004).

Pharmacological modulation of repetitive behaviour: Dopamine

The dopamine system was the first system to be associated with repetitive and stereotyped behaviour. In 1874, Harnack demonstrated 'compulsive gnawing' in rabbits after injection of apomorphine, an observation replicated by Amsler in 1923 and many others since. Further experiments in guinea pigs showed that repetitive gnawing after apomorphine administration originated from striatum (Amsler 1923). At the time that Harnack and Amsler conducted their studies, the concepts of chemical neurotransmission and transmitter receptors were unknown and therefore it was not until the 1960s that the neuronal mechanisms underlying apomorphine-induced stereotypy were established (Kuschinsky 2006). By then, apomorphine was recognised as a dopamine agonist, with its main site of action in the neostriatum. Apomorphine administration was shown to activate dopamine receptors in the neostriatum, resulting in compulsive gnawing behaviour (Ernst and Smelik 1966). The stereotypy-inducing effects of apomorphine and related stimulants have been replicated in numerous studies and across species since (for an extensive overview: see (Saka et al. 2004)). Striatal dopamine is thought to modulate the balance between the direct and indirect pathways and, consequently, the level of basal ganglia output (Groenewegen et al. 2003). As such, dopaminergic drugs may modulate the prevalence of stereotypy through stimulating the direct pathway and inhibiting the indirect pathway (Mason and Rushen 2006). How these agents affect these circuits is illustrated in Figure 3: In the direct pathway, post-synaptic D1 receptors are targeted by dopamine projections from the substantia nigra pars compacta. Activation of these D1 receptors increases the overall excitability of the post-synaptic neuron, resulting in amplification of excitatory corticostriatal input and subsequently increased GABA-ergic inhibition of the substantia nigra pars reticulata and the medial globus pallidus, the major inhibitory output nuclei of the basal ganglia. This in turn facilitates activation of thalamo-cortical relay neurons and consequently provides positive feedback to the cortex. Conversely, blocking these dopamine D1 receptors suppresses

the direct pathway, and decreases feedback to the cortex, resulting in less stereotypic behaviour (Joel and Doljansky 2003; Presti 2003). In the indirect pathway, activation of post-synaptic dopamine D2 receptors in the striatum reduces excitatory cortical input and thereby decreases inhibition of the globus pallidus externa. This leads to stronger inhibition of the subthalamic nucleus, thereby decreasing activation of the substantia nigra pars reticulata and the globus pallidus interna. When these major inhibitory output nuclei are inhibited, the thalamus becomes disinhibited, resulting in increased activity of the cortex (Lewis et al. 2006). Dopaminergic drugs such as apomorphine and amphetamine act on dopamine D2 receptors (Garner 2006). As such, they suppress the indirect pathway and disinhibit behaviour. Conversely, dopamine antagonists, such as haloperidol, reduce or block stereotypies by blocking dopamine D2 receptors (Kjaer et al. 2004).

Pharmacological modulation of repetitive behaviour: Serotonin

It is well established that pharmacological stimulation of post-synaptic serotonin receptors in rodents leads to complex behavioural symptoms including stereotyped and repetitive behaviour (Curzon 1990). How exactly this effect is mediated is not well understood. One hypothesis is that spontaneous stereotypic behaviour is associated with hypo-activity in serotonin (and dopamine) pathways (Korff et al. 2008). This was also suggested by a study showing stereotypy-reducing effects of citalopram, a serotonin agonist (Schoenecker and Heller 2003) in bank voles. Other studies have implicated higher serotonin release or turnover or overactivity of serotonin receptors in the development of repetitive behaviour. For example, primates reared in isolation that exhibited abnormal repetitive behaviour also had higher levels of 5-hydroxyindoleacetic acid (5-HIAA), the major brain metabolite of serotonin when compared to socially reared controls (Kraemer et al. 1989). Interestingly, environmental stress seems to be an important factor in the involvement of serotonin in repetitive behaviour: stress-induced increases in stereotypies are more dependent on serotonin than dopamine functioning (Schoenecker and Heller 2001, 2003). This may relate to why serotonergic medication is especially effective for relieving stress-related repetitive behaviour in anxiety disorders, such as OCD. However, another hypothesis states that serotonin may affect the development of stereotypies by modulating the dopamine system (Curzon 1990; Mason and Rushen 2006; Schoenecker and Heller 2001). Some findings have suggested an interaction between dopamine and serotonin systems, as dopamine-induced motor stereotypies can be alleviated by drugs that act on serotonin-receptors (Elliott et al. 1990) and motor stereotypies in rats given large doses of amphetamine have been shown to be dependent on serotonin release (Lees et al. 1979).

In sum, much of what is known about the neurobiological basis of repetitive behaviour comes from studies of drug-induced behaviour. Pharmacological experiments have established the importance of the basal ganglia in the mediation of repetitive behaviour and have elucidated the biochemical mechanisms underlying it. However, the neuroanatomy and biochemistry of corticostriatal systems is complex and how exactly repetitive behaviour is mediated by these systems is not yet fully understood.

The impact of lesions on the development of repetitive behaviour

A more limited number of studies have studied the effects of brain lesions on the development of repetitive behaviour. Unfortunately, in these studies, the stereotypies associated with such models are often not well described. Additionally, the extensive nature of the lesions caused by toxins and infectious agents and the wide variety of abnormal behaviour displayed by the animals limit the specificity of these findings. Some studies have investigated the effects of more localised, mechanically induced insults to the CNS on the development of repetitive behaviour. Some have targeted the striatum, whereas others have focused on connected cortical and subcortical structures. Enhancements in stereotyped behaviour have been associated with lesions in the substantia nigra pars reticulata, supposedly by disinhibition of dopamine neurons in the substantia nigra pars compacta (Koch et al. 2000). Interestingly, changes in stereotyped behaviour have also been reported after lesions to the hippocampal formation, with the direction of the change depending on the age at which the lesion was administered (Antoniou et al. 1998; Lipska and Weinberger 1993). Bachevalier (Bachevalier 1996) developed a primate model for autism, where conjoint lesions to amygdala, hippocampus, and adjacent cortical areas result in autistic-like symptoms, including locomotor stereotypies and self-directed activities. Applying such lesions in very young animals resulted in more pronounced behavioural abnormalities than lesions in adults (Málková et al. 1997).

In sum, studies investigating effects of brain lesions on repetitive behaviour confirm a central role for striatum in repetitive behaviour, but suggest that other areas in the medial temporal lobe may also play a role, possibly by their input to corticostriatal loops.

Genetic modulation of repetitive behaviour

In addition, to administering pharmacological agents or inducing lesions, a third way to affect central nervous system function is by genetic modification. Studying behaviour of transgenic animals (often mice), such as gene knockouts, can enhance our understanding of the role of those genes in the development of (abnormal) behaviour.

Genetic modulation of repetitive behaviour through dopaminergic genes

Consistent with pharmacological studies, genetic models have implicated the dopamine system in repetitive behaviour. These models include the dopamine transporter (DAT) and dopamine receptor D3 (DRD3) knockout mice and the dopamine receptor D1 (D1) mutant mouse. These models may be particularly informative on the spontaneous development of repetitive behaviour in that (1) they take critical developmental periods into account and (2) they mimic the complex interplay of the integrated development of associated neurobiological structures.

The dopamine transporter (DAT) regulates the extra-cellular dopamine concentration by the re-uptake of dopamine into the presynaptic terminal following release of the transmitter. The effects of knocking out the DAT-gene are two-fold. First, it results in hyperdopaminergia, increases in extracellular dopamine levels in neostriatum of up to 170% (Berridge et al. 2005). Second, it causes an imbalance between the dopamine and serotonin systems in

the basal ganglia (Pogorelov et al. 2005). The hyper-dopaminergic DAT knock-out mice display behaviour known as superstereotypy: excessively strong and rigid manifestations of complex and fixed action patterns (Berridge et al. 2005).

Unlike the profound and diverse behavioural effects observed in a DAT-knockout, the effects of knocking out the dopamine D3 (DRD3) receptor gene are more specific and lead to narrowly defined changes in behaviour. Joseph and colleagues showed an increase in spontaneous stereotypic behaviour of DRD3-knockout mice compared to the wild type (Joseph et al. 2002). Furthermore, these mice exhibited more locomotor activity, but not stereotypy, in response to amphetamine, suggesting a more limited role for the DRD3 in modulating drug-induced stereotypy (McNamara et al. 2006).

A potential problem with knock-out translational models is that modifications affect the entire organism, generating widespread, non-specific results on the one hand and possibly initiating compensatory mechanism on the other. This can complicate interpretation of the data. In contrast, if genetic modification can be targeted to specific brain regions, this may provide valuable additional information on the modulatory effects of the genes involved. Campbell and colleagues applied such an approach to investigate behavioural abnormalities in transgenic mice after they had potentiated regional subsets of dopamine D1 neurons (in cortical and limbic regions) (Campbell et al. 1999). These mice displayed episodes of perseverance and repetition of any and all normal behaviour, such as repetitive non-aggressive biting of siblings during grooming, and repetitive leaping. The cortical and limbic neurons manipulated are thought to control stimulating glutamate output to the striatum. This study suggests that genetic modification of specific elements of the dopamine system can induce complex compulsive behaviour in mice by stimulating regional activity within corticostriatal loops.

Genetic modulation of repetitive behaviour through other genes

The number of genes that may potentially affect pathological repetitive behaviour is large and the field of neuroscience is only now beginning to identify some of the players involved. Thousands of genes are expressed during brain development and are involved in regulating and shaping the function and structure of the brain. While modification of dopamine genes is clearly important to the development of repetitive behaviour, other genes may also be of interest. Examples include the GABA A-receptor beta-3 gene (GABRB3), the serotonin receptor 2C gene (HTR2C or 5-HT2c), and the disks large-associated protein-3 gene (DAP-3 or SAP90/PSD-95-associated protein 3 or SAPAP3). Translational studies have demonstrated repetitive behaviour in knockout models of these genes: The 5-HT2c knockout mouse shows intensified and stereotyped chewing and reduced habituation of responses (Chou-Green et al. 2003); SAPAP3 knockouts display increased repetitive grooming (Welch et al. 2007); and knocking out the GABRB3 gene has bee shown to result in intense circling and tail-chasing (DeLorey et al. 2008; Homanics et al. 1997). These knock-out models are of particular interest, given that (association) studies have linked all three genes to neuropsychiatric disorders, where repetitive behaviour is one of the core features: The GABRB3 gene has been linked to autism (DeLorey 2005), the HTR2C to autism and OCD (Veenstra-VanderWeele et al. 2000) and the DAP3 to trichotillomania and OCD (Züchner et al. 2009).

In sum, data from gene knock-out studies suggest that specific genes may directly and specifically affect or induce repetitive behaviour. Candidate genes include dopamine and serotonin genes but also a number of other genes, that have been implicated by whole-genome association studies. These are particularly interesting when they can inspire knock-out models where the behavioural phenotype shows repetitive behaviour. Such models may hold clues to the etiology and pathophysiology of this behaviour (Lewis et al. 2007).

4. How basal ganglia loops may modulate repetitive behaviour

Several hypotheses have been posed to explain how basal ganglia circuitry may modulate repetitive behaviour. It is important to note that these models are not mutually exclusive, but may constitute parallel processes, with additive or interactive effects. Here we describe three well-established hypotheses for the neurobiological mechanisms underlying repetitive behaviour.

The direct versus the indirect pathway

In a normally functioning system, the basal ganglia select and amplify desired movements and behavioural patterns via the direct (striatonigral) pathway, while they inhibit unwanted actions via the indirect (striatopallidal) pathway. This balancing of activity by facilitation and suppression occurs at all levels of behaviour (Bradshaw 2001). In general, activating the indirect pathway or suppressing the direct pathway will alleviate stereotypies, whereas suppressing the indirect pathway will induce them. Repetitive behaviour has been associated with an imbalance between activity in the direct and indirect pathways, and can thus be seen as a result of decreased inhibition and/ or increased facilitation of behaviour (Lewis et al. 2006; Lewis et al. 2007). Pharmacological and gene-expression studies and models of neuropsychiatric disorders have provided support for this hypothesis, as is described in section 3 (Translational studies of repetitive behaviour, page 32) and in chapter 3 of this thesis.

Dorsal versus ventral striatum

As described earlier, the striatum is comprised of sensorimotor, associative and limbic areas (Parent 1990). Sensorimotor and associative cortex projects predominantly to dorsal striatum (putamen and caudate nucleus), whereas limbic areas project predominantly to ventral striatum (including nucleus accumbens, deep layers of olfactory tubule and ventral parts of caudate and putamen). This functional-anatomical arrangement suggests a large degree of segregation between these circuits. However, several studies have shown that exchange of information between corticostriatal circuits takes place (Groenewegen et al. 1994; Haber et al. 2000; Joel and Weiner 1994; Zahm and Brog 1992) and it has been suggested that this exchange follows a ventral-to-dorsal path (Haber et al. 2000). These connections allow activity in one cortical-subcortical circuit to influence information processing in another. For example, information in ventral striatum is thought to influence the dorsal striatum, allowing emotional and motivational information to direct sensorimotor

behaviour. Dorsal striatum is known to be pivotal to habit formation. According to this model, the ventral loop, connecting ventral striatum to orbitofrontal cortex, might therefore affect the expression of habits, once they have become firmly established, or even affect the formation of habits in dorsal striatum (Groenewegen et al. 2003). In this manner, an imbalance between dorsal and ventral striatum might result in the abnormal repetition of (fragments of) behaviour or in exhibiting behaviour in inappropriate contexts (Groenewegen et al. 2003).

Striosomes versus the matrix

The corpus striatum is the main input station of the basal ganglia. Cortical input to the striatum is received through the medium spiny neurons, inhibitory neurons with large and extensive dendritic trees. Within the striatum, there are at least two different types of medium spiny neurons. Small clusters of medium spiny neurons of the first type (called 'patches' or 'striosomes') are embedded in a 'matrix', which contains medium spiny cells of the second type (Kandel et al. 1991). The matrix compartment occupies 80% - 90% of striatal volume, whereas striosome compartments represent only 20% - 10% of the volume. The two types of spiny neuron are neurochemically distinct and differ in their ontogenetic origin (van der Kooy and Fishell 1987) and cortical afferents. Projections from the striosomal and matrix compartments to the substantia nigra are organised compartmentally (Saka and Graybiel 2003).

Given the distinct anatomic connections of the striosomes and matrix, it seems likely that they are involved in different forms of information processing (Saka and Graybiel 2003). One hypothesis is that neurons in the matrix are preferentially involved in sensory-motor function and that neurons in striosomes are more involved in motivational aspects of behaviour (Leckman 2002; Saka and Graybiel 2003). Some studies have indeed suggested that a shift in activity from matrix to striosomes reflects a shift toward more motivationally driven behaviour with a consequent narrowing of focus and escalation of repetitive behaviour (Canales and Graybiel 2000; Leckman 2002; Lewis et al. 2007; Saka et al. 2004). A second hypothesis has arisen from a series of studies on the expression of immediate-early genes in animals exposed to psychomotor stimulant drugs that induce behavioural stereotypies (Saka and Graybiel 2003). Expression of these genes is a marker of neuronal activity. In one such study, activation of striosome and matrix compartments was related to the level of drug-induced stereotypy in rats: The relative hyper-activation of striosomes compared to matrix activation predicted the degree of induced motor stereotypy (Canales and Graybiel 2000). These results suggest that an imbalance between striosome and matrix activity may represent a neural correlate of motor stereotypy. Another study reported similar findings in primates: striosome predominance in activity predicted stimulant-induced stereotypy levels. This finding is particularly relevant to human behaviour, as the striatum and corticostriatal loop systems in primates are more differentiated than those in rodents (Saka et al. 2004).

In summary, the findings discussed in this section demonstrate how imbalance both within and between the motor, cognitive and limbic corticostriatal circuits can modulate repetitive behaviour. Three complementary models of (1) direct and indirect pathways, (2) ventral

and dorsal striatum and (3) striosome and matrix compartments of striatum illustrate how imbalance between these may relate to the development of these behaviours.

Conclusions

The aim of this review is to provide insight in the neurobiology of repetitive behaviour. To that end, we have provided an overview of findings from fundamental animal research and translational models.

From early on, the basal ganglia have been implicated in repetitive behaviour, although initially this was limited to motor behaviour. In the 1980s, it became clear that the basal ganglia should be viewed as components of multiple parallel, segregated feedback circuits with outputs targeting not only primary motor areas, but also pre-motor and prefrontal cortical areas. Initially, five structurally and functionally distinct parallel loops were proposed, which were later regrouped into three 'macro-circuits': the sensorimotor circuit, the associative or cognitive circuit and the limbic circuit. Respectively these involve the motor and pre-motor cortex, the dorsolateral prefrontal cortex, and the lateral orbitofrontal and anterior cingulate cortex. Recently, it has been argued that the various functions that are subserved by the macro-circuits cannot be executed independently and it has become clear that information is exchanged between circuits, likely in a ventral-to-dorsal path (Haber et al. 2000). The primary function of corticostriatal circuits is to control and select goal-directed motor, cognitive and motivational behaviour.

Disruption of co-ordinated function within the basal ganglia or between striatal and forebrain structures results in abnormal behaviour, often including repetitive behaviour. When striatal feedback to fronto-cortical areas becomes dysfunctional, it results in the inadequate repetition of a behavioural set, an inability to switch to other behaviour or the facilitation of inappropriate behavioural sets. Knowledge of the neurobiological mechanisms underlying repetitive behaviour comes from studies of environmentally deprived animals and translational studies, using pharmacological interventions, lesion approaches and gene manipulation. These studies have taught us that environmentally induced repetitive behaviour often reflect robust and perhaps even permanent changes in brain development. Furthermore, different types of repetitive behaviour are often correlated and seem to be mediated - at least partly - by similar circuitries. The main neurotransmitter systems in corticostriatal circuitry are GABA and glutamate. However, pharmacological studies show that the neuromodulators dopamine and serotonin are important for modulating repetitive behaviour. Gene manipulation is yet in its infancy, but early studies confirm the pivotal role of particularly the dopamine system.

Several hypotheses have been posited to explaining how dysfunction in basal ganglia circuits may induce abnormal repetitive behaviour. All involve imbalance between aspects of corticostriatal circuits, and they have focused on models of the direct versus indirect pathway, the ventral versus dorsal striatum or the striosomes versus matrix compartments. These models are not mutually exclusive, but more likely occur in parallel, with different additive or inter-active effects explaining different subtypes of repetitive behaviour. One topic only briefly touched upon in this review is the translation of animal work to humans,

both in typical development and neuropsychiatric conditions. This is the topic of a separate paper (chapter 3 of this thesis).

Future research will further target the integration of findings from separate research fields, across techniques and species. As such, it will enhance our understanding of the modulation of repetitive behaviour by corticostriatal systems. One of the problems faced today is that animal models of repetitive behaviour do not map one-to-one onto the complex behaviour observed in humans. Shifting focus from complex syndrome studies to inter-species trait studies may enable the definition of cross-species behavioural clusters. This will facilitate the identification of biological substrates underlying the behaviour that characterises these disorders (Kas et al. 2007). Finally, detailed phenotyping and consensus in the definitions applied is indispensable to systematic research efforts investigating repetitive behaviour across species and clinical conditions (Lewis and Bodfish 1998).

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3

The neurobiology of repetitive behaviour: ...and men

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In young, typically developing children, repetitive behaviour similar to that in certain neuropsychiatric syndromes is common. Whereas this behaviour is adaptive in typical development, in many disorders it forms a core component of symptoms and causes prominent impairment in the daily life of affected individuals.

Understanding the neurobiological mechanisms involved in repetitive behaviour will improve our understanding of the pathogenesis of developmental neuropsychiatric disorders, stimulating novel approaches to these conditions. However, studies on the neurobiology of human repetitive behaviour have often been limited to distinct conditions and generalisation has been hindered by inconsistent terminology.

In this paper, we synthesise the 'disorder-driven' literature, building on findings from fundamental animal research and translational models. These findings suggest a model for classifying repetitive behaviour by its neuro-anatomical correlates.

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Introduction

During early development, children engage in a significant amount of ritualistic, repetitive, and compulsive-like activity that is part of the normal behavioural repertoire (Evans et al. 1997). This developmental phase is characterised by perfectionism, preoccupation with ordering objects just-so, attachment to a favourite object, concerns about dirt and cleanliness, preferred household routines, actions repeated over and over or a specific number of times, rituals for eating, awareness of minute details in the home, hoarding, and bedtime rituals (Boyer and Liénard 2006). It is thought that such ritualisation and compulsions may serve to ward off anxiety (Evans et al. 1997) and may represent a mechanism for organising, accommodating to and eventually mastering the environment (Gesell et al. 1974). In other words: Childhood rituals are hypothesised to be a way to calibrate the system (Boyer and Liénard 2006). As such, early theories of child development include hypotheses of an adaptive role for repetitive behaviour (Gesell 1928; Piaget, 1952). The wide variety of ritualistic, repetitive, stereotyped and compulsive behaviour that can be observed in typically developing young children has striking similarities to the ritualistic and compulsive behaviour observed in psychiatric disorders such as obsessive-compulsive disorder (OCD), Gilles de la Tourette syndrome and autism spectrum disorders (ASD). Clearly, the adaptive element of this behaviour is lost in such neuropsychiatric conditions, where they cause prominent impairment to the daily life of affected individuals.

Repetitive behaviour was recognised as a common characteristic of mental illness from early on. In a historical overview of repetitive behaviour in schizophrenia, Frith and Done (1990) quote from 18th century publications: "We see also mad people, in whom phancy reigns, to run upon some action, as reading, or knitting of straws, without variation" (Grew 1701) and "When lunatics attempt to write, there is a perpetual recurrence of one or two favourite ideas, [...] patients will run their ideas in the very same track for many weeks together..." (Ferrier 1795). Later, Kahlbaum (1874) described repetitive behaviour in his work on catatonia, as did Kraepelin (1899) in his characterisation of dementia praecox and Asperger (Asperger 1944) and Kanner (1943) in the first reports of autism spectrum disorders. In modern-day neuropsychiatry, the term repetitive behaviour is an umbrella term, used to refer to broad and often disparate classes of behaviour linked by repetition, rigidity, invariance, and inappropriateness and observed in a wide array of developmental, psychiatric and neurological disorders (Turner 1999). Across disorders, many varieties of behaviour are included in this term, including stereotypies, rituals, compulsions, obsessions, circumscribed interests, echolalia, insisting on sameness, tics, perseveration and self-stimulation or self-injury. Even when only one particular disorder, e.g. autism, is considered, there is little consensus in the terminology used among clinicians (Bodfish et al. 2000). Furthermore, it has been argued that the use of categorisation such as the often-used subdivision into lower-level (motor) and higher-level (cognitive) repetitive behaviour may further obscure key differences between different forms of repetitive behaviour, as such broad categories may oversimplify by grouping together relatively heterogeneous behaviours (Turner 1999). In other cases, categorisation may falsely suggest differentiation between behaviours, for example, when it arises from a clinical need, whereas the distinction may not be so clear behaviourally or biologically (Garner 2006). In sum, difficulties in

classification and quantification complicate systematic research of repetitive behaviour in distinct neuropsychiatric disorder.

Scope of this review

The occurrence of similar behaviour in diverse neuropsychiatric disorders, as well as in certain phases of typical development, raises a key question: Is this similar behaviour caused by similar neurobiological mechanisms or are different repetitive behaviours neurobiologically unique? Although a single pathogenesis for all disorders of repetitive behaviour seems unlikely, there is a large body of animal and human literature that points towards involvement of both similar and specific neuronal systems in the development of repetitive behaviour (Lewis and Bodfish 1998).

Understanding the neural networks involved in repetitive behaviour and related problems will improve insight into the pathogenesis of neuropsychiatric and developmental disorders. This in turn will stimulate novel approaches in thinking about this behaviour, encouraging new therapeutic initiatives. In this paper we aim to investigate the neurobiological systems associated with various clinical manifestations of repetitive behaviour. The phenomenology and neurobiology of human repetitive behaviour has been studied from many different perspectives, but has often been limited to distinct conditions in which these phenomena occur. In this review, we aim to synthesise findings across disparate syndromes, while building on findings from fundamental animal research and translational models that are discussed in a separate review (chapter 2 of this thesis). To this aim, first we briefly discuss the anatomy of the corticostriatal circuits that are central to repetitive behaviour. Next, we discuss neurobiological findings in neurodevelopmental disorders that involve repetitive behaviour. Rather than to discuss all clinical conditions where repetitive behaviour is seen (e.g. addiction, schizophrenia, trichotillomania, anorexia, hypochondria, body dysmorphic disorder), we have chosen to focus on three neurodevelopmental disorders that include repetitive behaviour in their core symptoms: Gilles de la Tourette syndrome (section 2), obsessive compulsive disorder (section 3) and autism spectrum disorders (section 4). In section 5, we discuss research on Parkinson's disease (PD) and Huntington's disease (HD). Although the changes in motor behaviour associated with these neurological conditions are not classified as repetitive behaviour, we include these findings here, as research from these disorders has hugely contributed to our understanding of the neurobiology of repetitive behaviour.

1. Anatomy of the corticostriatal circuits

The corticostriatal circuits are multiple parallel, segregated feedback circuits with outputs from striatum targeting primary motor areas, and specific pre-motor and prefrontal cortical areas. They are typically grouped in 1) the sensorimotor circuit, 2) the associative or cognitive circuit and 3) the limbic circuit. These circuits innervate the motor and pre-motor cortex; the dorsolateral prefrontal cortex; and the lateral orbitofrontal and anterior cingulate cortex, respectively. The primary function of the corticostriatal circuits is to control and select goal-directed motor, cognitive and motivational behaviour. Disruption of co-ordinated function

within the basal ganglia or between striatal and forebrain structures results in changes in behaviour, often including repetitive or stereotyped behaviour: Feedback to fronto-cortical areas becomes dysfunctional, resulting in inadequate repetition of a behavioural set, inability to switch to other behaviour, or facilitation of inappropriate behavioural sets.

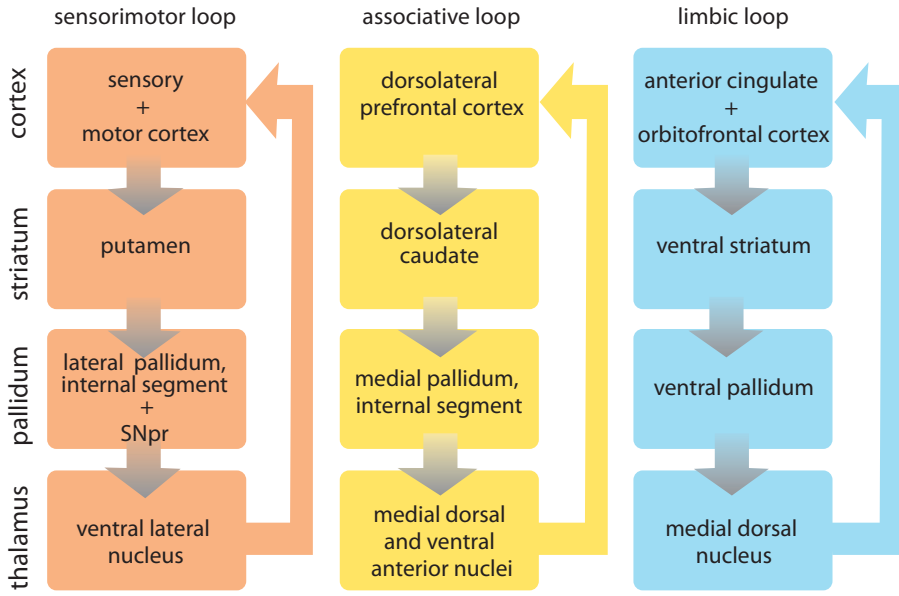

Figure 1. Parallel corticostriatal macro-circuits

Parallel corticostriatal macro-circuits with their main input, relay and output regions. Abnormal repetition of behaviour can result from damage to any of the corticostriatal circuits, where the exact location of the disruption (i.e. which loop is involved) determines what type of repetitive behaviour is seen. (SNpr = substantia nigra pars reticulata)

(See page 167 for a colour version of this figure.)

2. Gilles de la Tourette syndrome

Gilles de la Tourette syndrome (TS) is a genetically based, childhood-onset neuro-developmental disorder that is defined by the presence of phonic and motor tics (Makki et al. 2008). These tics characteristically wax and wane and often respond fairly well to treatment with dopamine antagonists. TS is defined as part of a spectrum of tic disorders, which includes transient and chronic tics. Tics usually appear around the age of 5-7 years, peak at the age of twelve and steadily decline after puberty (Swain et al. 2007). TS is associated with several co-morbid conditions including OCD and ADHD. Although the neural basis of TS is not fully understood, anatomical and functional disturbances in corticostriatal circuits are thought to be centrally involved in the pathogenesis of tics (Sowell et al. 2008).

Structural MRI studies have often focused on the basal ganglia in TS. These have typically reported reductions in volume (Bloch et al. 2005; Hyde et al. 1995; Peterson et al. 1993; Peterson et al. 2003), although both increases (Fredericksen et al. 2002) and similar volumes

(Singer et al. 1993; Zimmerman et al. 2000) have also been reported (for a review: see Albin and Mink (2006)). Some studies have also investigated related cortical areas: In a study of cortical thickness, Sowell and colleagues (Sowell et al. 2008) showed cortical thinning in the motor and sensorimotor cortex in groups of children with TS relative to controls. Furthermore, cortical thickness in these areas was correlated with tic symptom severity. Other studies have directly related the volume of striatal structures to symptoms of TS. Interestingly, these studies have typically shown an inverse relationship between caudate volume and severity of TS symptoms (Bloch et al. 2005; Hyde et al. 1995). Furthermore, Plessen and colleagues (Plessen et al. 2004) demonstrated an association between larger prefrontal cortical volumes and lower tic severity in children with TS, suggesting that increased prefrontal volumes may represent a compensatory mechanism, facilitating control of tics. A functional MRI study showed that conscious tic suppression in TS involved activation of regions of the prefrontal cortex and caudate nucleus and deactivation of putamen and globus pallidus (Albin and Mink 2006; Leckman 2002; Peterson and Leckman 1998). Other functional neuroimaging studies using fMRI, positron emisson tomography (PET) or single photon emission computed tomography (SPECT) have associated several cortical regions with both TS and tic expression in TS, including prefrontal, frontal, pre-motor, motor and cingulate areas and of basal ganglia and thalamus (Berardelli et al. 2003; Biswal et al. 1998; Braun et al. 1995; Braun et al. 1993; Chase et al. 1986; Eidelberg et al. 1997; George et al. 1992; Peterson and Leckman 1998; Sawle et al. 1993; Stern et al. 2000; Stoetter et al. 1992; Turjanski et al. 1994). A recent study using diffusion tensor imaging (DTI) to investigate the micro-structural integrity of the subcortical regions in TS showed increased mean diffusivity bilaterally in the putamen and relatively decreased anisotropy in the right thalamus. This suggests disruption to the structural organisation of corticostriatal white matter tracts (Makki et al. 2008).

Clinical symptoms of TS are often effectively treated with dopamine antagonists and selective serotonin re-uptake inhibitors implicating dopamine and serotonin systems in this disorder. This suggests that brain regions where dopamine and serotonin interact may be candidates for changes in TS, e.g. the striatum, the substantia nigra and the prefrontal cortices (Albin and Mink 2006). In addition to previously discussed neuroimaging work, neurochemical studies have also shown involvement of corticostriatal circuitry in TS. Pre-synaptic dopamine activity has been suggested to be abnormally high in TS (Albin et al. 2003; Cheon et al. 2004; Ernst et al. 1999; Peterson et al. 2001; Serra-Mestres et al. 2004; Singer et al. 1993; Srour et al. 2008). This has been taken to tentatively suggest that dopamine neurons may release greater amounts of transmitter than normal when they are activated, possibly resulting in loss of control over motor functions, and the emergence of tics (Hoekstra et al. 2004). Furthermore, in a study with twins discordant for TS, differences in dopamine D2 receptor binding in the head of caudate nucleus predicted differences in phenotypic severity (Wolf et al. 1996). This fits with scientific and anecdotal reports of efficacy of dopamine D2 receptor antagonists (such as haloperidol) in treating tics. The localisation of the finding to the head of caudate nucleus, known to be the striatal node of the dorsolateral prefrontal corticostriatal circuit, links TS symptoms to cognitive, non-motor circuits and distinguishes them from traditional hyperkinetic movement disorders that are linked more to motor corticostriatal circuitry (Wolf et al. 1996). Only limited additional

data are available to suggest the involvement of other neurotransmitter systems than the dopamine system (Hoekstra et al. 2004), although there have been some suggestions of changes in serotonin (Anderson et al. 1992; Müller-Vahl et al. 2005), noradrenaline (Leckman et al. 1995) glutamate (Anderson et al. 1992) and endogenous opioid systems (van Wattum et al. 2000).

In sum, neuroimaging studies have shown both structural and functional brain changes in TS, particularly in fronto-striatal circuits. Neuropharmacological studies have shown involvement of dopamine systems in TS, although it seems that serotonin may also play a role. In all, results have not always been consistent (Albin and Mink 2006). This may be related to differences between samples in terms of (pharmacological) treatment, co-morbidities and sample size. Furthermore, samples have differed in terms of the age range included. This may complicate results, as individuals with tics persisting into adulthood may represent an atypical group, for example (Albin and Mink 2006) and the neurobiological substrate of the disorder may change over development, meaning that changes may present differently at different ages.

3. Obsessive compulsive disorder

Obsessive compulsive disorder (OCD) is clinically characterised by two dimensions of symptoms: obsessions, which are unwanted, intrusive, recurrent thoughts; and compulsions, which consist of repetitively and ritualistically displayed behaviour (Graybiel and Rauch 2000). Repetitive thoughts and behaviour thus are core symptoms of OCD. Individuals with OCD are aware of the irrationality of their thoughts and behaviour (i.e. ego-dystonic). This distinguishes them from individuals affected with psychosis.

Early studies showed obsessive-compulsive symptoms in patients suffering from focal lesions in the striatum or globus pallidus, implicating the basal ganglia and fronto-striatal circuitry in OCD early on (Cheyette and Cummings 1995; Graybiel and Rauch 2000; Laplane et al. 1989). Modern techniques including PET and structural and functional MR imaging have confirmed frontostriatal involvement in OCD. A majority of PET studies has shown increased glucose metabolism in caudate nucleus, orbital prefrontal cortex, anterior cingulate cortex and thalamus in OCD during rest (Baxter 1990; Baxter et al. 1987; Baxter et al. 1992; Evans 2004; Rauch et al. 1994; Rosenberg et al. 1997; Saxena et al. 1999; Schwartz et al. 1996; Swedo et al. 1989), although a meta-analysis of these data showed that increased metabolism was only consistently found for orbitofrontal cortex and the head of the caudate nucleus (Whiteside et al. 2004). Eliciting OCD symptoms by exposing patients to symptom-provoking stimuli increases cerebral blood flow to the head of the caudate nucleus and orbitofrontal cortex (McGuire et al. 1994; Mitterschiffthaler et al. 2006; Rauch et al. 1994; Saxena and Rauch 2000). Successful pharmacological interventions alleviate these OCD-related activation patterns (Baxter et al. 1992; Evans 2004) whereas these metabolic changes do not occur in non-responders (Benkelfat et al. 1990; Calabresi et al. 1997; Swedo et al. 1992). Taken together, PET-studies suggest that overactivity of striatal-orbitofrontal circuitry is involved in the OCD symptoms (Insel 1992; Remijnse et al. 2006).

Overall, structural MRI findings suggest changes in basal ganglia and frontal cortex in OCD, although results have not been fully consistent: Hand-traced measures of the caudate nucleus have suggested both decreases (van den Heuvel et al. 2008; Luxenberg et al. 1988; Robinson et al. 1995), similar (Aylward et al. 1996; Bartha et al. 1998; Kellner et al. 1991; Rosenberg et al. 1997; Stein et al. 1997; Stein et al. 1993), and increases (Scarone et al. 1992) in volume from controls (Huyser et al. 2009; Saxena et al. 2001; van den Heuvel et al. 2008). Fully-automated, whole-brain, voxel-based morphometry (VBM) methods have yielded similarly variable results, although changes are often found in these circuits (van den Heuvel et al. 2008). Functional MRI studies have linked OCD symptoms to increased activity in regions in the orbitofrontal and anterior cingulate striatal loops (Fitzgerald et al. 2005; Maltby et al. 2005; Mitterschiffthaler et al. 2006; Remijnse et al. 2006; Thakkar et al. 2008; Ursu et al. 2003; van den Heuvel et al. 2005; van der Wee et al. 2003). Findings that activation in anterior cingulate cortex (ACC) increases during symptom provocation in OCD (Breiter et al. 1996), whereas cingulotomy relieves obsessions and compulsions (Dougherty et al. 2002) lends further credibility to a link between ACC function and rigid, repetitive behaviour in OCD (Thakkar et al. 2008). Recent studies have investigated neural correlates of discrete symptom dimensions of OCD. Although these studies are preliminary, they suggest that different symptoms may be mediated by distinct neural systems, and that previous discrepant findings may have resulted from phenotypic variations in the studied samples (for an overview: see (Mitterschiffthaler et al. 2006; van den Heuvel et al. 2008).

OCD was originally classified as an anxiety disorder, as the behaviour exhibited by sufferers was thought to be aimed at relieving stress and anxiety. Nevertheless, it was the effectiveness of the tricyclic antidepressants that first gave impetus to investigating the neurobiology of OCD (Stein 2000). Serotonergic antidepressants were shown to be particularly effective for reducing obsessional behaviour (Zohar and Insel 1987), stimulating research on the serotonin system in OCD. As such, a major focus of OCD research has been exploring the role of the serotonin system in this disorder (Micallef and Blin 2001): Recently, Soomro and colleagues reviewed 17 studies including over 3000 patients (Soomro et al. 2008) and confirmed the therapeutic effect of SSRIs in OCD, at least in the short-term. The longer-term efficacy and tolerability of different SSRI drugs for OCD has yet to be established. However, the mechanism by which serotonin is involved in OCD is not yet established. One hypothesis poses that serotonin transporter availability is reduced in OCD, thereby contributing to the observed overactivity of corticostriatal circuits in OCD (Reimold et al. 2007). A recent PET study showed reduced serotonin transporter availability in thalamus and midbrain of patients with OCD compared to well-matched control subjects (Reimold et al. 2007). These findings are in accordance with some (Hesse et al. 2005; Zitterl et al. 2007), but not all (Pogarell et al. 2003; Simpson et al. 2003; van der Wee et al. 2004) earlier studies on serotonin transporter availability in OCD. Other studies have related pre- and post-SSRI-treatment levels of serotonin transporter (in thalamus and hypothalamus) with pre- and post-treatment severity of OCD symptoms and showed that (1) less availability of serotonin transporter at baseline was associated with more severe OCD; and (2) higher baseline levels of serotonin transporter were associated with higher efficacy of SSRI-therapy (Zitterl et al. 2008). Taken together, these results suggest that the level of availability of serotonin transporters may be implicated in OCD.

Low doses of atypical antipsychotics such as olanzapine, quetiapine, ziprasidone and risperidone are often prescribed in severe cases of OCD and are considered a useful augmentation if SSRI treatment is not successful, putatively suggesting that in OCD dopamine-serotonin interactions may also be relevant (Bloch et al. 2006).

In sum, several neurobiological focus points have been proposed in OCD. Corticostriatal circuitry is pivotal to this disorder and a number of models have proposed how imbalances in the corticostriatal loops could induce OCD symptoms. Especially the limbic or orbitofrontal circuit (orbitofrontal cortex, anterior cingulate cortex and caudate nucleus) has consistently been shown to be involved in OCD symptoms. At the pharmacological level, the serotonin system is implicated, potentially via reduced serotonin transporter availability. However, the efficacy of augmentation using antipsychotic medication suggests there may also be a role for the dopamine system. As in TS, not all evidence on the neurobiology underlying repetitive behaviour in this disorder has been consistent. One explanation for the differences found may lie in the substantial variation between studies in methods (in- and exclusion criteria; sample size; gender; IQ; age-range; choice of paradigm in cognitive fMRI studies; MRI/PET acquisition and processing methods). Furthermore, fronto-striatal circuitry has a protracted developmental course (Durston and Casey 2006). As such, differences in OCD may change with development and grouping relatively large age-ranges together may mask differences associated with the disorder. For detailed reviews on the neurobiology and pathophysiology of OCD, see Graybiel and Rauch (2000) and Aouizerate (2004).

4. Autism

Stereotypies, repetitive behaviour and restricted interests form the third defining symptom cluster in autism. A considerably body of work has investigated neurobiological mechanisms associated with the first two clusters of symptoms in this disorder (impaired social interaction and language development), but relatively few studies have investigated the neurobiology associated with this third cluster. This is surprising, given the prominence of these symptoms and the extent to which this behaviour form a significant impairment for affected individuals and their families.

Structural MR studies of brain changes associated with repetitive behaviour in autism have often focused on the basal ganglia. Results are somewhat ambiguous, as some studies have reported larger volumes (Haznedar et al. 2006; Hollander et al. 2005; Langen et al. 2007; Rojas et al. 2006; Sears et al. 1999; Voelbel et al. 2006), whereas others have not found changes (Gaffney et al. 1989) or have only found changes in line with overall increases in brain volume (Herbert et al. 2003; Sears et al. 1999). Several studies have related brain changes directly to repetitive behaviour and have shown correlations between symptoms and striatal volumes (Hollander et al. 2005; Langen et al. 2009; Rojas et al. 2006; Sears et al. 1999), lending confidence to involvement of striatum in repetitive behaviour in this disorder. Turner and colleagues (Turner et al. 2006) investigated functional connectivity in neural networks including caudate nucleus in a small sample of adult control subjects and adolescents and adults with autism. They found atypical caudate-cortical connectivity in autism. A later study linked repetitive behaviour in autism to changes in activity of anterior

cingulate and posterior parietal cortex, but not striatum (Shafritz et al. 2008). A recent study combined fMRI with diffusion tensor imaging (DTI) and suggested that repetitive behaviour in autism may be related to deficient response monitoring in anterior cingulate cortex (ACC) in autism: Subjects with autism made more errors on an anti-saccade task and showed reduced discrimination between errors and correct responses compared to controls. Furthermore, they had increased ACC activity on correct trials, while DTI showed structural changes in ACC white matter. Both the functional and structural changes related to the behavioural responses on the task and were correlated with repetitive symptoms in the subjects with autism (Thakkar et al. 2008).

There is limited neuropsychopharmacological evidence available on autism. However, conventional antipsychotic medication is commonly prescribed to individuals with autism spectrum disorders. There is some indication that this medication may be effective for reducing hyperactivity, aggression and repetitive behaviour in this disorder, although no systematic studies are available (Barnard et al. 2002). SSRIs are also used. Here, most studies demonstrate significant improvement in global functioning and in symptoms of anxiety and repetitive behaviour (Kolevzon et al. 2006). Although the evidence available is limited, it does suggest that both antipsychotic medication and SSRIs are beneficial in autism. As such, this putatively implicates the dopamine and serotonin systems here, as in other disorders with repetitive behaviour.

In sum, although various studies have implicated corticostriatal circuitry in repetitive behaviour in autism, results are not fully conclusive. Differences between studies may reflect differences in developmental stage between samples (Herbert et al. 2003; Hollander et al. 2005; Langen et al. 2009; McAlonan et al. 2002), as well as methodological factors, such as sample size, sample composition (only high-functioning individuals meeting full criteria for autism versus lower-functioning samples and samples including other disorders in the autism spectrum). Furthermore, the heterogeneity in the autism phenotype forms a potential confound in itself: Symptoms vary across individuals, in all three symptom-domains. For example, it has been shown that clusters of repetitive behaviour in autism are associated with distinct profiles of other symptoms (Lam et al. 2008). As such, certain repetitive behaviours may be associated with distinct neural circuits. If different neural circuits indeed support different aspects of repetitive behaviour, the relationships between structure and function could feasibly take different forms (Langen et al. 2009). This highlights the importance of detailed phenotyping in this disorder as well as of taking a dimensional approach to studying the neurobiological basis of heterogeneous disorders.

5. Parkinson's and Huntington's disease

Research on the function of human corticostriatal circuitry has been strongly influenced by descriptions of the clinical phenomenology of human basal ganglia disorders, such as Huntington's and Parkinson's disease (Albin et al. 1989). Although the changes in motor behaviour associated with Parkinson's disease (PD) and Huntington's disease (HD) are not classified as repetitive behaviour, we do include them here, as they have greatly contributed to our understanding of the neurobiology of repetitive behaviour.

Parkinson's disease (PD) results from degeneration of the substantia nigra pars compacta, minimising dopamine release in the midbrain. Decreased dopamine stimulation of striatum then results in inhibition of the direct pathway and stimulation of the indirect pathway, leading to an overall decrease in motor behaviour. The opposite mechanism (overactivity in the direct pathway and under-activity in the indirect pathway) is thought to be involved in repetitive behaviour. In Huntington's disease (HD), a degeneration of neuronal cells, especially in the frontal lobes and caudate nucleus, astrogliosis and loss of medium spiny neurons occurs. This results in a reduced dopamine modulation via the indirect and direct pathways: The remaining dopamine signals within striatum are too weak to inhibit the appropriate target regions. The only exception is the globus pallidus externa, which over-inhibits when activated, and thereby alters the flow of excitation from the subthalamic nuclei, contributing to the lowered function and loss of movement control. This creates the characteristic jerky uncontrolled movement associated with HD (Kandel et al. 1991).

In addition to overall motor disturbances, individuals with Parkinson's and Huntington's disease suffer from behaviour that is characterised by repetition and perseveration. One frequently reported deficit in PD and HD concerns the ability to shift set, the ability to alter behaviour according to changes in dimensional relevance of stimuli (Cools et al. 2001). This ability has often been related to frontal lobe function (Dove et al. 2000; Mecklinger et al. 1999; Rogers et al. 1998; Sohn et al. 2000; Stablum et al. 1994), although more recent studies suggest that disrupted interactions between striatum and the frontal cortex may cause these deficits in PD and HD, ultimately resulting in perseverative behaviour (Cools et al. 2001).

Repetitive behaviour and disturbed impulse control are highly prevalent in PD patients treated with dopamine agonists (Voon and Fox 2007; Voon et al. 2007). This behaviour includes pathological gambling, hypersexuality, compulsive shopping and compulsive eating and is thought to result from aberrant or excessive dopamine receptor stimulation. A related phenomenon seen with dopamine replacement therapy is punding, an intense fascination with repetitive tasks such as collecting or arranging objects, developing from pre-potent idiosyncratic habits. This has been linked to dopamine dysregulation syndrome (Evans and Lees 2004), caused by excessive dopamine stimulation of the striatum. Punding was first described in amphetamine addicts and is thought to represent the culmination of a continuous process of psychomotor stimulation (mediated by ventral striatal structures) and behavioural competition (mediated by dorsal striatal structures). The stereotyped behaviour seen in punding is likely homologous to the complex stereotyped behaviour seen in animals with amphetamine-stereotypies, cage stereotypies and isolation-induced stereotypies (Evans et al. 2004).

In sum, studies of PD and HD have contributed greatly to our understanding of the role of corticostriatal circuitry in repetitive behaviour. Compared to the other disorders described in this review, PD and HD allow for a unique approach, as their neuroanatomical substrate is known. This field has shown us how the balance between the direct and indirect pathways in striatum modulates behaviour. Moreover, observations of the effects of dopamine replacement therapy in PD indicate the significance of the dopamine system and the interplay between the ventral and dorsal striatal system in repetitive behaviour.

Discussion and Conclusions

This paper discusses the neurobiology of repetitive behaviour in human developmental neuropsychiatric conditions that count repetitive behaviour among their core symptoms. Clearly, corticostriatal circuitry is implicated in this behaviour, across disorders and methods. However, the literature is not always consistent in terms of the direction of effects, with both smaller and larger volumes of striatal structures being reported, as well as both increases and decreases in activity in corticostriatal regions, for example. Within disorders, these discrepancies may in part reflect confounders, including (pharmacological) treatment, co-morbid disorders or symptoms, and methodological issues, such as limited sample size and non-uniformity in the classification of repetitive behaviour across studies. Furthermore, cortico-striatal circuits have a protracted developmental trajectory (Durston and Casey 2006) and neurobiological changes associated with repetitive behaviour may change over development. Discrepancies between disorders may also reflect some of these issues. However, they may additionally result from real differences in repetitive behaviour and its underlying neurobiology across clinical domains.

The term repetitive behaviour refers to broad and often disparate classes of behaviour linked by repetition, rigidity, invariance, and inappropriateness (Turner 1999). Categorisations into 'higher-order' or 'cognitive' and 'lower-order' or 'motor' repetitive behaviour do not always hold: These behaviours are often correlated and are mediated by similar or connected circuitries, at least in part. Furthermore, distinct types of repetitive behaviour are often co-morbid (e.g, obsessions and stereotypies in autism, stereotypies and obsessive/ compulsive behaviour in OCD (Garner 2006), and family studies suggest a genetic relationship between different types of repetitive behaviour (Hollander et al. 2003). In all, the evidence seems to point to a distinct but connected neurobiology for different classes of repetitive behaviour. Qualitative differences in human repetitive behaviour may then principally result from the localisation of brain changes: In primates, the anatomical distinctions between corticostriatal circuits (sensorimotor circuit, associative or cognitive circuit, and the limbic circuit) relate to different types of behaviour and lesions to each of these circuits will result in a type of repetitive behaviour that corresponds to the system affected (Haber and Calzavara 2009; Francois et al. 2004). How this translates to repetitive behaviour in humans is not fully known. The reasons for this are two-fold: First, invasive studies cannot be performed in humans, as in primates. Second, human repetitive behaviour has often been studied from the viewpoint of specific disorders and this has resulted in only limited integration of the findings. In the next section, we present a functional and neuro-anatomical classification of human repetitive behaviour, based on a synthesis of findings from developmental neuropsychiatric and neurodegenerative disorders. This classification is illustrated in Figures 2 and 3.

Repetitive behaviour may result from dysfunction at the subcortical or cortical level, or by faulty exchange of information within the circuit. Subtle variations in corticostriatal pathology across individuals may account for variations in the expression of the symptoms associated with a given disorder (Osmon 2005), as the contribution of each node in a loop to the behaviour it supports is unique (Schmahmann and Pandya 2008). For several disorders, associations between symptoms and a specific corticostriatal circuit have been suggested:

For example, neuroimaging data suggest that motor and vocal tics in TS correspond to changes in the sensorimotor loop through putamen, whereas obsession and compulsion in OCD are associated with the limbic loop (Graybiel and Rauch 2000; Menzies et al. 2008). In addition, the dorsolateral frontal circuit has been implicated in co-morbid ADHD symptoms in TS, while the orbitofrontal is implicated in co-morbid OCD symptoms in TS (Osmon 2005). In sum, both animal and human studies have suggested that the motor loop is primarily involved in abnormal stereotypical motor behaviour: continuously repeating identical movements without pursuing a goal. The prefrontal loop is likely associated with inappropriate repetition of a goal, expressed in a relatively varied behavioural repertoire (as in some obsessive-compulsive behaviour). The limbic loops (lateral orbital loop and anterior cingulate loop) are implicated in motivational aspects of behavioural control, including impulsive behaviour (difficulty in suppressing behaviour even when consequences are negative), response to reward, and obsessive and compulsive behaviour (including compulsive drug-taking; see Figure 3).

To conclude, our understanding of the neurobiology of repetitive behaviour has vastly expanded in recent years. However, there are still gaps in our knowledge. For example, what are the neurobiological mechanisms that mediate the transition from repetitive behaviour common to typically developing children to the developmentally inappropriate, persistent, fixed, and habitual repetitive behaviour in clinical disorders (Lewis et al. 2007)? We also know little about the development of repetitive behaviour over time within individuals and how the waxing and waning of symptoms is supported by changes in neurobiology (Ödberg 1993). Furthermore, the systematic comparison of repetitive behaviour between studies is complicated by inconsistent labelling and categorisation across disorders and fields. Recently, investigators have been advocating a more etiological approach of defining behaviour as domains of disorder-related traits, rather than separable categories (Hollander et al. 2007; Kas et al. 2007). By focusing on features that connect behaviours across disorders and species, rather than distinguish them from one another, we will be able to further advance our knowledge.

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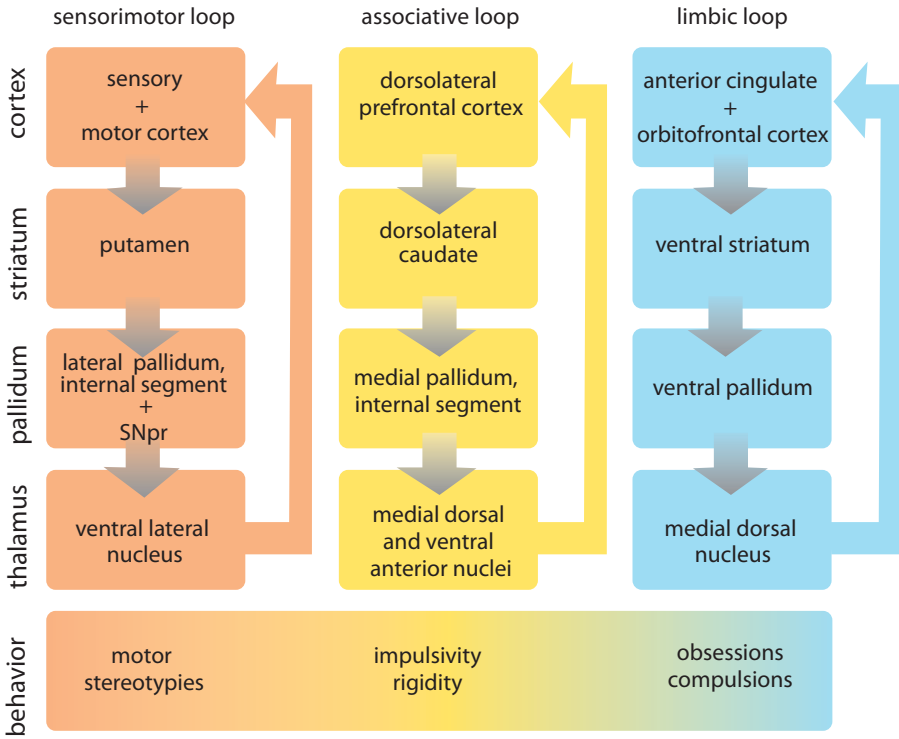

Figure 2. Involvement of parallel corticostriatal macro-circuits in repetitive behaviour

Parallel corticostriatal macro-circuits with their main input, relay and output regions. Abnormal repetition of behaviour can result from damage to any of the corticostriatal circuits, where the exact location of the disruption (i.e. which loop is involved) determines what type of repetitive behaviour is seen. Both animal and human studies have suggested that the sensorimotor loop is primarily involved in abnormal stereotypical motor behaviour: continuously repeating identical movements without pursuing a goal. The associative loop is likely to be associated with inappropriate repetition of a goal, expressed in a relatively varied behavioural repertoire (as in obsessive-compulsive behaviour). The limbic loops (lateral orbital loop and anterior cingulate loop) are implicated in motivational aspects of behavioural control, including impulsive behaviour (difficulty in suppressing behaviour even when consequences are negative); response to reward; and obsessive and compulsive behaviour (including compulsive drug-taking). (SNpr = substantia nigra pars reticulata)

(See page 169 for a colour version of this figure.)

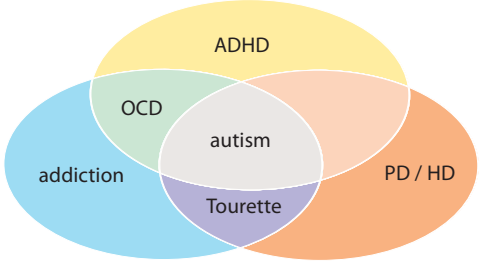

Figure 3. Schematic representation of how behaviour resulting from problems in one of the three macro-circuits (sensorimotor, associative or limbic; see Figure 1) may group together in symptom clusters as seen in various psychiatric and neurological disorders. (ADHD = Attention Deficit Hyperactivity Disorder; OCD = obsessive compulsive disorder; PD = Parkinson's disease; HD = Huntington's disease)

(See page 169 for a colour version of this figure.)

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4

Caudate nucleus is enlarged in high-functioning medication-naive subjects with autism

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Background: Autism is defined by three symptom clusters, including repetitive and stereotyped behaviour. Previous studies have implicated basal ganglia in these behaviours. Earlier studies investigating basal ganglia in autism have included subjects on neuroleptics known to affect basal ganglia volumes. Therefore, we investigated these structures in medication-naive subjects with autism.

Methods: Volumetric magnetic resonance measures of caudate, putamen, and nucleus accumbens were compared in two independent samples of medication-naive, high-functioning subjects with autism or Asperger syndrome: (1) 21 affected children and adolescents and 21 matched control subjects; and (2) 21 affected adolescents and young adults and 21 matched control subjects.

Results: Caudate nucleus was enlarged in both samples. This result remained significant after correction for total brain volume.

Conclusions: These results implicate caudate nucleus in autism, as an enlargement of this structure was disproportional to an increase in total brain volume in two independent samples of medication-naive subjects with autism.

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Introduction

Autism research has often focused more on social and communicative deficits and less on the third defining cluster of symptoms, repetitive and stereotyped behaviours (RB). Broadly, repetitive behaviours are defined as recurring, nonfunctional activities or interests that occur regularly and interfere with daily functioning, including lower-order repetitive motor behaviour, as well as intense circumscribed patterns of interests and higher-order rituals and compulsions (Gabriels et al. 2005; Lord et al. 1994). Repetitive behaviours are also associated with other neuropsychiatric disorders, such as obsessive-compulsive disorder (OCD) and Tourette syndrome (TS), although it has been argued that the type and nature of these behaviours may differ in these disorders (for a review, see Carcani-Rathwell et al. 2006; Zandt et al. 2006). Animal studies have implicated the basal ganglia in RB (Arnt 1985; Bradshaw and Sheppard 2000; Fibiger et al. 1973; Hollander et al. 2005; Purcell et al. 1998; Ridley 1994; Ring and Serra-Mestres 2002; Rosenberg et al. 1997a, 1997b; Saka et al. 2004; Segal et al. 1980). Studies in humans exploring brain mechanisms behind RB have indicated involvement of the basal ganglia in OCD (Giedd et al. 1996; Modell et al. 1989; Scarone et al. 1992) and TS (Albin and Mink 2006; Peterson et al. 1993, 2003). Furthermore, frontostriatal circuitry was recently implicated in the development of autistic symptoms in individuals with 22q11 syndrome (Campbell et al. 2006).

To date, five papers have used magnetic resonance imaging (MRI) to investigate the neurobiology of RB in autism and other spectrum disorders. Two of these reported enlarged basal ganglia volumes proportional to an increase in total brain volume replicated in an overlapping sample by Haznedar et al. 2006). McAlonan et al. (2002) found no differences in caudate volumes between groups but did report decreased gray matter density in striatal areas. Two studies implicated caudate nucleus in RB more directly (Hollander et al. 2005; Sears et al. 1999), although results were not consistent. Sears et al. (1999) found differential correlations between RB and caudate volume, depending on type of behaviour, whereas Hollander et al. (2005) reported a positive correlation between caudate volumes and overall RB scores. These findings implicate the basal ganglia, and particularly the caudate nucleus, in the pathophysiology of autism. However, it has been argued that use of neuroleptics, shown to be associated with volume changes of basal ganglia structures (Chakos et al. 1994; Keshavan et al. 1994; Lang et al. 2004; McCarley et al. 1999; Scheepers et al. 2001a, 2001b; Shihabuddin et al. 1998), may have confounded these studies.

Therefore, we investigated basal ganglia volumes in never-medicated subjects with autism to further explore previously demonstrated enlargements of the basal ganglia and their involvement in RB. We hypothesized an enlargement of the basal ganglia, and particularly caudate nucleus, would be related to RB.

Table 1. Demographic data and characteristics of the samples

Sample 1		
Variable	***Subjects with autism (n=21)***	***Normal controls (n=21)***
Gender (male/female)	*21/0*	*21/0*
Age, mean ± SD (range), yrs	*11.12 ± 2.18 (6.9 – 14.6)*	*10.37 ± 1.84 (7.3 – 14.4)*
Total IQ, mean ± SD (range)	*106.52 ± 13.68 (80 – 138)*	*102.52 ± 14.58 (80 – 151)*
Verbal IQ, mean ± SD (range)	*108.33 ± 17.54 (70 –131)*	*100.86 ± 15.81 (76 – 144)*
Performance IQ, mean ± SD (range)	*103.43 ± 16.81 (73 – 141)*	*103.62 ± 14.54 (73 – 138)*
Height, mean ± SD, cm #	*149.19 ± 16.17*	*145.83 ± 15.88*
Weight, mean ± SD, kg #	*38.81 ± 11.79*	*38.61 ± 10.11*
Handedness (right/left), n	*20/1*	*19/2*
Parental education, mean ± SD, yrs †	*14.10 ± 2.45*	*12.84 ± 2.63*
ADI-R: social deficits	*16.38 ± 4.61*	
ADI-R: abnormalities in communication	*13.00 ± 4.59*	
ADI-R: ritualistic-repetitive behaviour	*3.76 ± 2.625*	
Higher-order	*2.29 ± 1.65*	
Lower-order	*1.48 ± 1.29*	

Sample 2		
Variable	***Subjects with autism (n=21)***	***Normal controls (n=21)***
Gender (male/female)	*19/2*	*20/1*
Age, mean ± SD (range), yrs	*20.08 ± 3.10 (15.5 – 24.7)*	*20.28 ± 2.22 (17.3 – 24.8)*
Total IQ, mean ± SD (range)	*114.90 ±19.18 (81 – 126)*	*112.62 ±10.20 (96 – 130)*
Verbal IQ, mean ± SD (range)	*112.90 ±19.64 (77 – 132)*	*107.62 ±9.89 (88 – 122)*
Performance IQ, mean ± SD (range)	*114.00 ±16. 22 (84 – 129)*	*116.95 ±11.53 (94 – 134)*
Height, mean ± SD, cm	*180.62 ± 10.40*	*179.95 ± 7.37*
Weight, mean ± SD, kg	*70.14 ± 12.94*	*74.24 ± 9.10*
Handedness (right/left), n	*19/2*	*17/4*
Parental education, mean ± SD, yrs	*14.76 ± 2.00*	*13.52 ± 2.71*
ADI-R: social deficits	*19.62 ± 5.88*	
ADI-R: abnormalities in communication	*15.90 ± 3.74*	
ADI-R: ritualistic-repetitive behaviour ‡	*3.95 ± 3.15*	
Higher-order §	*2.32 ± 1.82*	
Lower-order §	*1.32 ± 1.42*	

Information was unavailable for three control subjects
† Information was unavailable for two control subjects
‡Information was unavailable for one subject
§ Information was unavailable for two subjects
ADI-R: Autism Diagnostic Interview-Revised

Methods and Materials

Participants

Sample 1. Twenty-one medication-naive, high-functioning children and adolescents meeting DSM-IV criteria for autism or Asperger syndrome (American Psychiatric Association 1994) and 21 typically developing control subjects were included. Subjects with autism and control subjects were matched for gender, age, intelligence quotient (IQ), height, weight, handedness, and socio-economic status (SES) (Table 1). The present sample was described in two earlier studies (Palmen et al. 2005, 2006).

Sample 2. Twenty-one medication-naive, high-functioning adolescents and young adults meeting DSM-IV criteria for autism or Asperger syndrome (American Psychiatric Association 1994) and 21 healthy comparison subjects were included. Subjects with autism and healthy control subjects were matched for gender, age, IQ, height, weight, handedness, and SES (Table 1). The present sample was described in two earlier studies (Palmen et al. 2004, 2006).

All procedures were approved by the Institutional Review Board at the University Medical Center and informed consent was obtained from all subjects, as well as parental consent for subjects aged under 18 years.

MRI acquisition

Magnetic resonance images were acquired on a Gyroscan (Philips Medical Systems, Best, The Netherlands) operating at 1.5 T. For volumetric measurements, T1-weighted three-dimensional (3-D) fast field echo scans and T2-weighted dual echo turbo spin echo scans were acquired. Acquisition details have been previously described (Palmen et al. 2004, 2005, 2006).

MRI processing

Magnetic resonance processing was performed at the Department of Child and Adolescent Psychiatry. All images were coded and half were randomly flipped over the y axis to ensure rater blindness to subject identity, diagnosis, and laterality. Volumetric measures of intracranial volume (ICV) and total brain volume were obtained semi-automatically (Palmen et al. 2004, 2005). Basal ganglia structures were traced manually by a single experienced rater (M.L.). Caudate nucleus, putamen, and nucleus accumbens were outlined in contiguous coronal slices in an anterior-posterior direction (Figure 1 and Figure 2). Detailed tracing guidelines are available in Supplementary Material. Ten scans were duplicated and randomly intermixed with the data set to allow for an estimation of intrarater reliability using intraclass correlation coefficients (ICCs). Intraclass correlation coefficient scores were .99 for caudate nucleus, .96 for putamen, and .97 for nucleus accumbens.

Statistical analysis

SPSS 12.0 statistical package (SPSS Inc, Chicago, Illinois) was used for all statistical analyses. Data from both samples were first analyzed independently. All clinical data and brain

volume measurements were normally distributed. Independent sample t-tests were performed to investigate differences in basal ganglia volumes between groups. To correct for multiple comparisons, a Bonferroni corrected critical p value of .017 was employed. For significant results, follow-up analyses were performed to control for age, IQ, TBV, and ICV. To investigate relationships between basal ganglia volumes and repetitive behaviour scores on the Autism Diagnostic Interview-Revised (ADI-R), Spearman correlations were calculated for the group as a whole (for 42 subjects with autism).

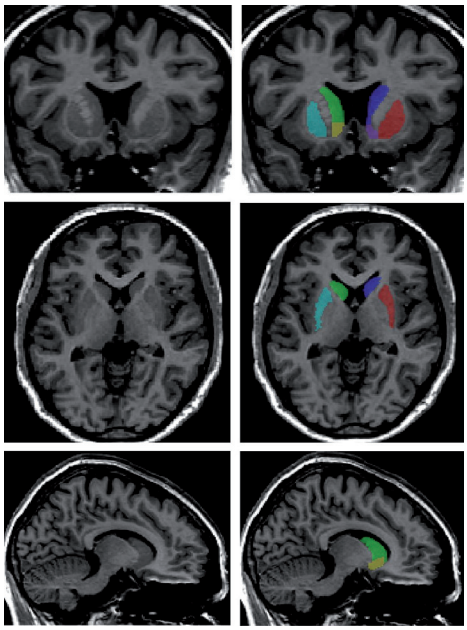

Figure 1. Segmentation of the basal ganglia in (from top to bottom) coronal, axial and sagittal view. Caudate nucleus (L/R) is displayed in green and blue, putamen in turquoise and red, nucleus accumbens in yellow and purple.

(See page 170 for a colour version of this figure.)

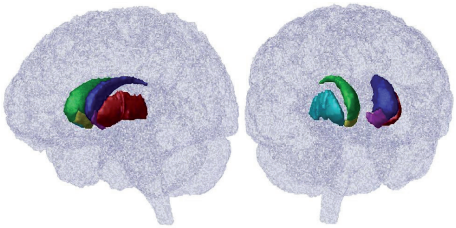

Figure 2. 3D visualizations of the basal ganglia in the brain. Caudate nucleus is displayed in green and blue, putamen in turquoise and red, nucleus accumbens in yellow and purple.

(See page 170 for a colour version of this figure.)

Results

Table 2 lists mean volumes for both independent samples. Total caudate nucleus volume was significantly enlarged for subjects with autism compared with control subjects in both samples ($|t| = 2.79$; $p = .008$). (Figure 3). In addition, bilateral putamen was significantly enlarged for subjects with autism compared with control subjects in sample 1 ($|t| = 2.27$; $p = .03$). The increase in caudate volume remained significant when covarying for TBV and ICV ($|t| > 2.67$; $p < .01$) in sample 2 and at least at trend level in sample 1 ($|t| = 1.78$; $p = .08$). All results remained significant when covarying for age and IQ. No significant correlations with ADI-R scores for higher-order or lower-order RB (Carcani-Rathwell et al. 2006; Szatmari et al. 2006) and any basal ganglia structures were found.

Table 2. Basal ganglia volumes for both samples

Brain structure	*Subjects with autism, Mean ± SD, cm3*	*Normal controls, Mean ± SD, cm3*	*|t| (df = 40)*	*p value*
Sample 1				
Intracranium	*1542.10 ± 103.02*	*1475.17 ± 69.44*	*2.47*	*0.02*
Total brain	*1422.79 ± 92.62*	*1357.85 ± 70.02*	*2.56*	*0.01*
Total caudate nucleus	*8.40 ± 0.81*	*7.70 ± 0.81*	*2.79*	*0.008*
Left	*4.13 ± 0.40*	*3.76 ± 0.40*	*3.04*	*0.004*
Right	*4.27 ± 0.47*	*3.94 ± 0.49*	*2.24*	*0.03*
Total putamen #	*10.64 ± 0.98*	*10.03 ± 0.72*	*2.27*	*0.03*
Left #	*5.39 ± 0.48*	*5.10 ± 0.35*	*2.24*	*0.03*
Right #	*5.25 ± 0.54*	*4.93 ± 0.42*	*2.11*	*0.04*
Total nucleus accumbens	*2.32 ± 0.32*	*2.24 ± 0.39*	*0.72*	*0.47*
Left	*1.19 ± 0.21*	*1.12 ± 0.21*	*1.08*	*0.29*
Right	*1.13 ± 0.16*	*1.12 ± 0.20*	*0.17*	*0.87*
Sample 2				
Intracranium	*1564.11 ± 117.08*	*1494.74 ± 87.77*	*2.17*	*0.04*
Total brain	*1393.92 ± 105.87*	*1333.33 ± 86.61*	*2.03*	*0.05*
Total caudate nucleus	*8.26 ± 1.14*	*7.20 ± 0.75*	*3.59*	*0.001*
Left	*4.12 ± 0.67*	*3.55 ± 0.36*	*3.45*	*0.001*
Right	*4.13 ± 0.51*	*3.64 ± 0.44*	*3.39*	*0.002*
Total putamen	*9.74 ± 1.23*	*9.52 ± 1.06*	*0.61*	*0.55*
Left	*4.91 ± 0.73*	*4.84 ± 0.56*	*0.31*	*0.76*
Right	*4.83 ± 0.57*	*4.68 ± 0.52*	*0.96*	*0.36*
Total nucleus accumbens	*2.07 ± 0.46*	*2.13 ± 0.31*	*0.42*	*0.67*
Left	*1.04 ± 0.24*	*1.07 ± 0.15*	*0.45*	*0.65*
Right	*1.04 ± 0.24*	*1.06 ± 0.18*	*0.35*	*0.73*

Putamen volume could not be estimated for one control subject (df = 39)

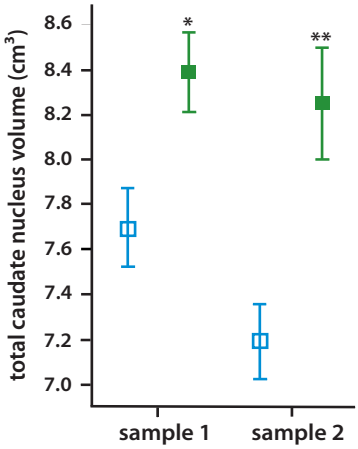

Figure 3. Total caudate nucleus volumes by group in samples 1 and 2 (Mean ± 1 SE). Green solid square = autism group; blue open square = control group; * = significant at p<0.05; ** = significant at p<0.001.

(See page 170 for a colour version of this figure.)

Discussion

This study implicates basal ganglia, and particularly caudate nucleus, in the aetiology of autism, as a significant increase in caudate volume was found in two independent samples of medication-naive subjects with autism. We report an increase in caudate nucleus volume disproportional to total brain volume in both children and adolescents with autism. As this result is found in both age groups, it suggests that caudate nucleus continues to be involved in autism over development. These findings contrast with other childhood neuropsychiatric disorders, such as attention-deficit/hyperactivity disorder (ADHD), where an initial reduction in caudate volume has been shown to normalize in adolescence (Castellanos et al. 2002), possibly suggesting a developmental decline in its involvement in the disorder.

Although our findings are in line with previous studies showing increases in basal ganglia volumes in autism (Herbert et al. 2003; Hollander et al. 2005; Sears et al. 1999), we were not able to replicate previously reported correlations between RB and caudate volume (Hollander et al. 2005; Sears et al. 1999). However, ADI-R scores on stereotyped and repetitive behaviour scales were low in both samples and variability was therefore limited. An instrument more sensitive to detecting complex RB may have been more suitable to assess the relationship between RB and brain measures.

A number of limitations to our findings should be acknowledged. First, we included only high-functioning individuals in our two samples, limiting inferences regarding low-functioning subjects with autism. A second limitation is the use of the ADI-R for measuring RB. A more sensitive instrument may have provided more scope to detect relationships between basal ganglia volumes and behavioural variables, including differentiating between subclasses of RB. Finally, the use of a cross-sectional design limits the developmental conclusions that can be drawn from these findings. A longitudinal approach would be more sensitive to detecting developmental changes in basal ganglia structures and their involvement in autism.

Conclusion

In conclusion, we report an increase in caudate nucleus volume, disproportional to an increase in total brain volume, in two independent samples of medication-naive subjects with autism. These results are consistent with evidence from other neuropsychiatric disorders implicating frontostriatal circuitry in repetitive and stereotyped behaviours, one of the defining symptom clusters of autism.

This study was financially supported by the Korczak Foundation. All authors had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. We thank all subjects and their parents for participating and the staff at the autism and psychosis clinic at the University Medical Center Utrecht for their help in subject recruitment. We gratefully acknowledge Drs. Van der Flier and Kromkamp for their advice on segmentation guidelines. Presented at the International Meeting for Autism Research (IMFAR), June 1-3, 2006, Montréal, Canada.

Supplementary Material

Tracing Guidelines

Basal ganglia structures were traced manually by a single experienced rater (ML). Caudate nucleus, putamen and accumbens nucleus were outlined in contiguous coronal slices in an anterior - posterior direction. The sagittal and axial planes were used for reference. Segmentation procedures are based on previously described guidelines (Staal et al. 2000; Scheepers et al. 2001a;b) and are detailed below. Ten scans were duplicated and randomly intermixed with the dataset, in order to allow for an estimation of intrarater reliability using correlation coefficients (ICCs). ICC-scores were 0.99 for caudate nucleus volume, 0.96 for putamen and 0.97 for accumbens nucleus.

Caudate nucleus

Caudate nucleus segmentation commenced in the first slice in which the structure was visible infero-lateral of the lateral ventricles; the last slice containing the caudate nucleus was the slice in which the posterior commissure (PC) appeared, or the last slice in which the caudate nucleus was clearly discernible in the coronal view. Medially, the caudate nucleus was bordered by the frontal horn and body of the lateral ventricle. Laterally, the caudate was bordered by the internal capsule. The interconnecting grey matter striae between the caudate nucleus and putamen visible in the internal capsule were not included in caudate nucleus or putamen volumes. The inferior border of the caudate nucleus was defined by the nucleus accumbens: the caudate nucleus was separated from the nucleus accumbens by a horizontal line from the most basal extent of the lateral ventricle to the most lateral point of the caudate.

Putamen

Putamen segmentation commenced in the first slice where the structure was clearly distinguishable; the last slice was defined as the last slice in which the boundaries were still clearly discernible. The medial border of the putamen was formed by the anterior limb of the internal capsule and the globus pallidus. Laterally, the putamen was bordered by the external capsule. Infero-medially, the putamen was bordered by the nucleus accumbens: the putamen was separated from the nucleus accumbens by a vertical line from the most latero-inferior point of the internal capsule to the inferior border of the putamen/ nucleus accumbens. In slices where the anterior commissure (AC) was visible, the most lateral point of the AC was used as the starting point of the vertical line separating the putamen and the nucleus accumbens.

Nucleus accumbens

Segmentation of the nucleus accumbens commenced in the first slice containing both caudate nucleus and putamen and ended in the slice where boundaries for the accumbens were still clearly discernible. Supero-laterally, the nucleus accumbens was bordered by the putamen and caudate. From the first slice in which the putamen was clearly visible, the nucleus accumbens was separated from the caudate nucleus by a horizontal line from the most basal extent of the lateral ventricle to the most lateral point of the caudate nucleus. The nucleus accumbens was separated from the putamen by a vertical line from the most latero-inferior point of the internal capsule to the inferior border of the putamen/ nucleus accumbens. In slices where the AC was visible, the most lateral point of the AC was used as the starting point of the vertical line separating the putamen and the nucleus accumbens. Infero-medially, the accumbens was bordered by the surrounding white matter.

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5

Changes in the developmental trajectories of striatum in autism

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Background: Repetitive and stereotyped behaviour has been associated with striatum in various neuropsychiatric disorders. However, striatal involvement has not yet been shown conclusively in autism. Issues include the use of neuroleptic medication and differences in mean age between samples, where conflicting results may reflect differences in developmental stage between samples.

Objective: To examine brain development in a homogeneous sample of subjects with high-functioning autism.

Methods: Magnetic resonance measures of brain structure of 188 individuals (99 subjects with high-functioning autism and 89 typically developing, matched controls) aged between 6 and 25 years were compared. Measurements included the volume of brain structures, including striatum, as well as voxel-based assessment of grey matter density.

Results: Developmental trajectories of the caudate nucleus, putamen and nucleus accumbens differed between subjects with autism and controls. Results were not accounted for by overall changes in brain volume or neuroleptic medication. The development of the caudate nucleus differed from typical most, as its volume increased with age in autism, while it decreased for controls. Voxel-based analysis showed that changes in striatum localised to the head of the caudate nucleus. Overall caudate nucleus volume was associated with repetitive behaviour in autism.

Conclusions: We report changes in striatal development in autism, while caudate volume is associated with repetitive behaviours. This emphasises the importance of striatum in the aetiology of autism, in particular in the development of repetitive behaviour that characterises the disorder.

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Introduction

Autism is a severe neurodevelopmental disorder that is characterised by 1) impaired social interactions, 2) impaired communication and language development and 3) stereotypies, repetitive or rigid behaviour and restricted interests. A formal diagnosis of autism requires the presence of problems in each of these three domains (American Psychiatric Association 1994). While a considerably body of work has investigated brain changes associated with the first two clusters of symptoms, relatively few studies have investigated brain changes associated with repetitive behaviour. This is surprising, given the prominence of repetitive behaviour in the disorder: in many cases these symptoms onset early in development and often form a significant impairment for affected individuals.

Repetitive behaviour has been associated with striatum, across a range of neuropsychiatric disorders, including obsessive compulsive disorder, schizophrenia and Tourette's syndrome. Striatum has also been implicated in autism, although results from Magnetic Resonance Imaging (MRI) are not yet conclusive: Whereas some studies have reported larger volumes in autism, particularly of the caudate nucleus (Sears et al. 1999; Hollander et al. 2005; Haznedar et al. 2006; Rojas et al. 2006; Voelbel et al. 2006), others have not (Gaffney et al. 1989). Furthermore, it is unclear whether the reported increase in volume is disproportional to an overall increase in brain volume (Sears et al. 1999; Herbert et al. 2003). Additionally, the subjects in these studies often used neuroleptic medication, which is associated with increases in striatal volumes (Chakos et al. 1994; Keshavan et al. 1994; Shihabuddin et al. 1998; McCarley et al. 1999; Scheepers et al. 2001a;b; Lang et al. 2004). As such, findings of increased striatal volumes in autism cannot yet be considered definitive. However, some studies have implicated striatum in the development of repetitive and stereotyped behaviour more directly: Striatal volumes have been shown to correlate with repetitive and restricted behaviour in OCD (Rosenberg et al. 1997) and in autism (Sears et al. 1999; Hollander et al. 2005; Rojas et al. 2006), lending confidence to the involvement of this area in these behaviours.

In an earlier study of two smaller, independent samples of subjects with high-functioning autism, we found that the caudate nucleus was enlarged compared to typically developing individuals (Langen et al. 2007). In this study, there was a large difference in age between the two samples (mean age for the first sample was 10 years and 20 years for the second) and the effect was greater for the older sample (14.7% increase in volume from controls, compared to 9.1% in the younger sample). This led us to hypothesise that autism may be associated with changes in striatal development, where differences become more pronounced with age.

In typical development, the striatum decreases in volume over time, both in childhood (Sowell et al. 2002) and in adulthood (Gunning-Dixon et al. 1998; Jernigan et al. 2001; Walhovd et al. 2005; Toga et al. 2006). A comprehensive longitudinal study in children and adolescents showed that the developmental trajectory of the caudate nucleus follows an inverted U-shape with peak volume at age 7.5 years in girls and 10.0 years in boys (Lenroot and Giedd 2006), although a more recent study demonstrated peaks at about 10.5 years for girls and 14.0 years for boys (Lenroot et al. 2006). In autism, the developmental trajectory of the striatum has not been examined. As such, differences in results between studies of

striatal volume in autism could in part reflect differences in mean age between samples (Hollander et al. 2005 ;Herbert et al. 2003; McAlonan et al. 2002). Other factors may include relatively small sample sizes, and differences in sample composition, as some studies have included only high-functioning individuals meeting full criteria for autism, while others have chosen to also include lower-functioning individuals and individuals with other disorders in the autism spectrum. Therefore, we set out to investigate structural brain development in a large and homogeneous sample of high-functioning individuals with autism and controls (n=188). We hypothesised that the caudate nucleus would be enlarged in autism and that its developmental trajectory would differ from that of controls.

Methods and Materials

Participants

Ninety-nine, high-functioning individuals meeting DSM-IV criteria for autism were recruited through the Department of Child and Adolescent Psychiatry at the University Medical Center in Utrecht, the National Autism Society in the Netherlands, an out-patient clinic for individuals with pervasive developmental disorders and through advertising. Diagnosis was clinically established by a Child and Adolescent psychiatrist from our department and was confirmed by trained and qualified clinicians using the Autism Diagnostic Interview Revised (ADI-R) (Lord et al. 1994). All subjects with autism had IQ greater than 75. Twelve subjects with autism were using neuroleptic medication, four more had previously received neuroleptic medication. Eighty-nine typically developing controls were recruited through schools and educational centres in the area. For control subjects under 18 years of age, a parent participated in a semi-structured interview session with a trained rater to confirm absence of any psychiatric diagnosis (Diagnostic Interview Schedule for Children; DISC-P) (Costello et al. 1985). For older subjects, the absence of psychopathological abnormalities was established using questionnaires and a short version of the Comprehensive Assessment of Symptoms and History (CASH) (Andreasen et al. 1992). For both groups, subjects with a psychiatric diagnosis (current or prior), major physical or neurological illness, history of head trauma, alcohol or other drug dependence, or full IQ below 75 were excluded. Control subjects with a family history of psychiatric illness were also excluded. Groups were matched for gender, age, IQ, height, weight, hand preference, pubertal development (assessed using Tanner scales), and for parental educational level (see Table 1).

Written informed consent was obtained for all subjects. For subjects under 18 years of age, a parent signed for consent, while assent was obtained from the subject. All subjects participated in an MRI scanning session and a neuropsychological assessment (Wechsler Adult Intelligence Scale (WAIS/ WAIS-III) (Stinissen et al. 1970; Wechsler 2000), Wechsler Intelligence Scale for Children (WISC-R/ WISC-III) (Van Haasen et al. 1986; Kort et al. 2005). Children under 13 years of age were acclimated to the scanning-procedure in a dummy-scan session prior to the actual MR-scan (Durston et al. 2009). For all subjects, MRI-scans were evaluated by independent clinical neuroradiologists. No gross abnormalities were reported for any of the subjects. The procedure was approved by the institutional review board of the University Medical Center Utrecht, the Netherlands.

Table 1. Demographic data and characteristics of the sample

Variable	*Subjects with autism (n=99)*	*Normal controls (n=89)*
Gender (male/female)	*91/8*	*82/7*
Age, mean ± SD (range), yrs	*12.89 ± 4.45 (7.04–24.67)*	*12.36 ± 4.79 (6.28–24.75)*
Total IQ, mean ± SD (range)	*107.59 ± 13.56 (81–138)*	*109.99 ± 12.81 (80–138)*
Height, mean ± SD, cm [a]	*156 ± 19*	*152 ± 20*
Weight, mean ± SD, kg [b]	*47 ± 17*	*45 ± 19*
Handedness (right/left/ambidexter), n	*85 / 9 / 5*	*76 / 12 / 1*
Parental education, mean ± SD, yrs [c]	*14.38 ± 2.2*	*13.74 ± 2.3*
Tanner A [d]	*1.22 ± 1.24*	*1.13 ± 0.87*
ADI-R: social deficits [e]	*19.12 ± 5.36*	
ADI-R: abnormalities in communication [e]	*15.32 ± 4.15*	
ADI-R: ritualistic-repetitive behaviour [e]	*5.18 ± 2.75*	

[a] Information was unavailable for five control subjects and two ASD subjects
[b] Information was unavailable for five control subjects and four ASD subjects
[c] Information was unavailable for two control subjects and two ASD subjects
[d] Information was unavailable for 26 control subjects and 25 ASD subjects
[e] Information was unavailable for two ASD subjects
ADI-R: Autism Diagnostic Interview-Revised

MRI acquisition

Magnetic resonance imaging (MRI) scans were acquired on a 1.5 T scanner (Philips, Best, the Netherlands). Data were acquired over eight years. For the definition of all brain measures, a T1-weighted 3D fast field echo scan with 130 to 150 1.5-mm contiguous coronal slices (earlier scans; 63 ASD, 55 control) or 160 to 180 contiguous coronal 1.2-mm slices (later scans; 36 ASD, 34 control) of the whole head (TE 4.6 ms, TR 30 ms, flip angle 30º, field of view (FOV) 256 mm, in plane voxel size 1 mm x 1 mm) were acquired. For one hundred-eighteen subjects (63 ASD, 55 control) T2- weighted dual echo turbo spin echo scans with 65 to 75 3.0 mm contiguous coronal slices or 120 contiguous coronal 1.6 mm slices of the whole head (TE1 14 ms, TE2 80 ms, TR 6350 ms, flip angle 90º, FOV 256 mm, in plane voxel size 1 mm x 1 mm) were acquired for the definition of the intracranial volume. For the remaining seventy subjects (36 ASD, 34 control), a single-shot EPI (Echo Planar Imaging) scan, (SENSE factor 2.5; flip angle 90o; 60 transverse slices of 2.5 mm; no gap; 128 x 96 acquisition matrix; FOV 240 mm; TE 78 ms) and a magnetisation transfer (MT) scan (60 transverse slices of 2.5 mm; no gap;128 x 96 acquisition matrix; FOV 240 mm; flip angle 8o; TE 4.5 ms; TR 37.5 ms) were combined to define the intracranial volume.

MRI processing

All images were coded to ensure rater blindness to subject identity and diagnosis. The T1-weighted images were automatically placed in Talairach orientation (Talairach and Tournoux 1988) without scaling, by registering them to a model brain in Talairach orientation. The translation and rotation parameters of this registration were then applied to the images

(Maes et al. 1997). After linear registration to the T1-weighted image, the intracranial segment served as a mask for all further segmentation steps. The T1-weighted images were corrected for field inhomogeneities using the N3 algorithm (Sled et al. 1998). An automatic image processing pipeline was used to define the volume of total brain, grey and white matter of the cerebrum, cerebellum and lateral ventricles. The software included updated versions of previously described histogram analysis, mathematical morphology operations, and anatomical knowledge based rules to connect all voxels of interest (Schnack et al. 2001a;b). The segments for intracranial volume, total brain, lateral ventricles and cerebellum were all visually checked and edited to ensure an accurate segmentation.

Manual tracing

Striatal structures were traced manually by two experienced raters (DB and ML). To ensure rater blindness to laterality, half of the images were randomly flipped over the y-axis. Caudate nucleus, putamen and nucleus accumbens were outlined in contiguous coronal slices in an anterior - posterior direction. The sagittal and axial planes were used for reference. Segmentation procedures are described in detail in (Langen et al. 2007). Intra-rater reliabilities (estimated using intraclass correlation coefficients; ICCs) were above .95 for all structures. Inter-rater reliabilities were .93 for caudate nucleus .86 for nucleus accumbens and .71 for putamen.

Voxel-based morphometry

Voxel-based morphometry was used to investigate where differences in striatum between diagnostic groups were localised. VBM included the following steps: First, a model brain was created, similar to the method used in (Hulshoff Pol et al. 2001). Second, the binary grey matter (GM) and white matter (WM) masks were blurred using a 3D Gaussian kernel (Full Width at Half Maximum (FWHM) of 8 mm). The voxel values of these blurred segments (between 0 and 1) reflect the local presence, or concentration of GM and WM respectively, and are referred to as 'density maps'. Third, in order to compare brain tissue at the same anatomical location in all subjects, the GM segments were transformed into a standardised coordinate system (i.e, the model brain). These transformations were calculated in two steps. (A) The T1-weighted images were linearly transformed to the model brain. In this linear step a joint entropy mutual information metric was optimised (Maes et al. 1997); (B) Nonlinear (elastic) transformations were calculated to register the linearly transformed images to the model brain up to a scale of 4 mm (FWHM), thus removing global shape differences between the brains, but retaining local differences. For this step, the ANIMAL algorithm was used (Collins et al. 1995). Fourth, the density maps were transformed to the model space by applying the concatenated linear and nonlinear transformations. Finally, the maps were resampled to voxels of size 2 mm x 2 mm x 2.4 mm. To allow for a region of interest analysis of striatum, a mask was created by segmenting the striatum on the model brain and dilating it by 2 mm.

Statistical analysis

SPSS 16.0.2 statistical package for Apple Mac (SPSS Inc, Chicago, IL, USA) was used for statistical analysis of the clinical and volumetric data. All clinical data, matching variables and brain volume measurements were normally distributed, except for the volume of the lateral ventricles. A natural logarithm transformation was applied to this measure before including it in further analyses. Matching data were compared between groups using independent sample T-tests.

Group differences in brain development

A multivariate analysis of variance procedure was used to investigate differences in brain development between diagnostic groups: In the primary analysis, volumetric measurements were included as dependent variables, diagnosis as a fixed factor and age as a co-variate. To control for effects of total brain volume, acquisition protocol (1.2 mm vs. 1.5 mm slice thickness) and present or past use of neuroleptic medication, additional analyses were performed including these measures as co-variates or fixed factors. For brain structures showing main effects of age, or age by diagnosis interactions, the shape of the developmental curves was investigated by examining whether a quadratic or linear model best fit the data. Linear regression analyses were performed with age and quadratic age as predictors and brain structure volumes as dependent variables. Models were calculated for the group as a whole for structures showing main effects of age and for diagnostic groups separately for structures showing interactions between age and diagnosis.

Voxel-based morphometry

In the voxel-wise analysis, linear regressions were performed through all brains for each voxel separately in the GM and WM density maps. Group (autism or control), age, gender, handedness, medication use and scan protocol were included in the analysis as regressor variables. Correction for multiple comparisons was carried out using false discovery rate (Genovese et al. 2002) ($\alpha<0.05$, two-tailed), allowing for an overall 5% chance of false positives.

Correlations with behaviour

To investigate relationships with behaviour, correlations were calculated between the three striatal volumes and three symptom clusters of repetitive behaviours, using Spearman rank-order correlations. ADI-R repetitive behaviour items were clustered into Repetitive Motor Behaviour, Insistence on Sameness and Circumscribed Interests, based on the clusters reported in a recent factor analysis on ADI-R scores in 316 subjects (Lam et al. 2008). Repetitive Motor Behaviours (RMB) included the items 'Repetitive Use of Objects', 'Hand and Finger Mannerisms', and 'Other Complex Mannerisms/Stereotyped Body Movements'. Insistence on Sameness (IS) included 'Difficulties with Minor Changes in Personal Routine and Environment', 'Resistance to Trivial Changes in the Environment', and 'Compulsions

and Rituals'. Circumscribed Interests (CI), included 'Circumscribed Interests'', Unusual Preoccupations', and 'Unusual Attachment to Objects'.

Results

Group differences in brain development

The primary multivariate analysis showed interactions between diagnosis and age for caudate nucleus volume ($F=12.8$, $p<0.001$) and lateral ventricle volume ($F=4.2$, $p=0.042$); there was a main effect of diagnosis on caudate nucleus volume ($F=7.5$, $p=0.007$); and a main effect of age on cerebellum, grey matter, white matter, nucleus accumbens, lateral ventricle and total brain volume.

After including total brain volume as a co-variate in the model, the interaction between age and diagnosis remained significant for the caudate nucleus ($F=8.1$, $p=0.005$), but not for lateral ventricle volume ($F=2.8$, $p=0.096$); an interaction between age and diagnosis was also significant for nucleus accumbens volume ($F=4.0$, $p=0.046$). The main effect of diagnosis on caudate nucleus volume remained significant ($F=4.8$, $p=0.031$) and main effects of diagnosis for putamen and nucleus accumbens volume also became significant ($F=5.0$, $p=0.026$ and $F=4.1$, $p=0.045$ respectively). Main effects of age were significant for grey matter, white matter and lateral ventricle volume.

When present or past neuroleptic use was included in the model as a fixed factor, the interaction between age and diagnosis remained significant for all three striatal structures (caudate nucleus: $F=8.5$, $p=0.004$; putamen: $F=4.1$, $p=0.043$; nucleus accumbens: $F=4.0$, $p=0.046$), as did the main effect of diagnosis (caudate: $F=5.2$, $p=0.024$; putamen: $F=6.6$, $p=0.011$; accumbens: $F=4.0$, $p=0.046$). When subjects on neuroleptic medication were excluded from the primary analysis, results were similar for both the interaction between age and diagnosis (caudate nucleus: $F=7.9$, $p=0.005$; putamen: $F=4.3$, $p=0.040$; nucleus accumbens: $F=4.5$, $p=0.036$) and the main effect of diagnosis (caudate nucleus: $F=4.8$, $p=0.030$; $F=6.7$, putamen: $p=0.011$; nucleus accumbens: $F=4.5$, $p=0.036$). Including acquisition protocol as covariate did not change the results.

Developmental trajectories

For structures showing main effects of age (grey and white matter and lateral ventricles), the shape of the developmental curve was investigated by comparing linear and quadratic fits for the group as a whole. A linear model best explained the variance for lateral ventricle volume ($t=4.81$, $p<0.001$) and total grey matter volume ($t=-5.62$, $p<0.001$). For white matter, no fits were significant. For structures showing an interaction between age and diagnosis (caudate nucleus, putamen and nucleus accumbens), linear and quadratic models were fit for diagnostic groups separately. For the caudate nucleus, a linear model best explained the variance in both the control group ($t=-3.13$, $p=0.002$) and the autism group ($t=2.06$, $p=0.042$). For the nucleus accumbens, a quadratic fit best explained the variance in the autism group ($t=-3.03$, $p=0.003$), while no fits were significant for the autism group. For putamen, no fits were significant.

Table 2. Brain volumes

Variable (mean ± SD (cm3)	*Subjects with autism*	*Normal controls*
Intracranium	*1540.56 ± 114.32*	*1520.45 ± 116.39*
Total brain	*1414.83 ± 105.28*	*1396.61 ± 114.08*
Cerebellum	*159.18 ± 12.36*	*157.64 ± 17.40*
Gray matter	*776.31 ± 68.55*	*762.48 ± 75.78*
White matter	*477.23 ± 99.01*	*463.50 ± 49.52*
Lateral ventricles [a]	*12.65 ± 6.97*	*9.69 ± 5.37*
*Nucleus accumbens *†*	*2.26 ± 0.36*	*2.23 ± 0.37*
*Caudate nucleus *††*	*7.83 ± 1.03*	*7.61 ± 0.89*
*Putamen *†*	*9.81 ± 1.09*	*9.51 ± 1.01*

Multivariate analysis, age and total brain volume were included as covariates and use of neuroleptic medication as a fixed factor: * main effect of diagnosis, p<0.05; † interaction of diagnosis and age, p<0.05; †† interaction of diagnosis and age, p<0.01; [a] A natural logarithm transformation to obtain normal distribution was applied to this measure before including it in further analyses.

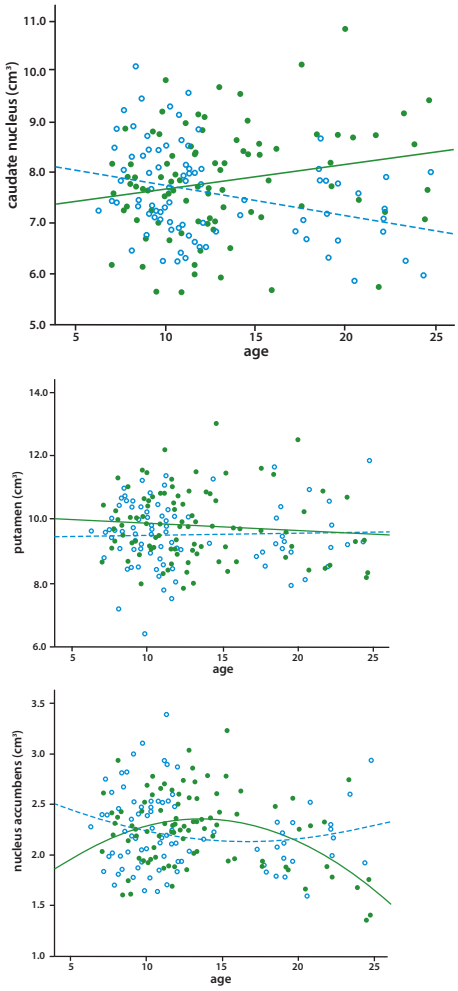

Figure 1. Scatterplots of striatal volumes in autism and controls. Top: caudate nucleus, middle: putamen, bottom: nucleus accumbens. Green solid circles and solid fit line: autism group, blue open circles and dashed fit line: control group.

(See page 171 for a colour version of these figures.)

Voxel-based morphometry

Voxel-based morphometry was used to explore where differences in striatum were localised. There was a main effect of diagnosis in the head of the caudate nucleus (max t=4.11 in right caudate nucleus; p=0.002). There were no white matter density changes in adjacent areas, such as the internal capsule.

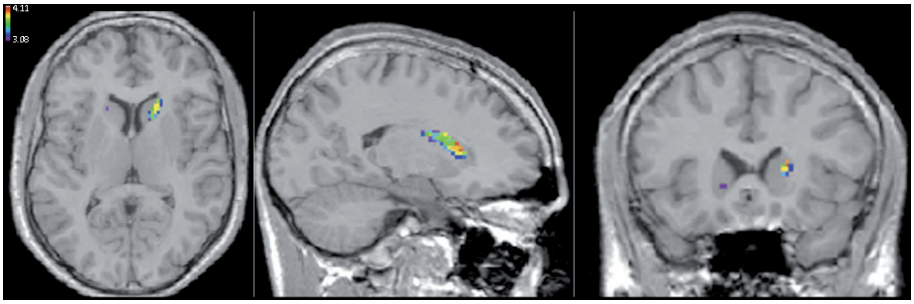

Figure 2. Focal increases in gray matter density in autism. The map is thresholded at the critical t (t>3.08) and superimposed on axial, sagittal and coronal sections through the MR image of the model brain.

(See page 171 for a colour version of this figure.)

Correlations between striatal volumes and symptom clusters

For subjects with autism, correlations were calculated between striatal volumes and three clusters of of repetitive behaviour symptoms (Repetitive Motor Behaviour, Insistence on Sameness and Circumscribed Interests). Caudate volume correlated negatively with the Insistence on Sameness cluster (r=-0.239 p=0.023). There were no significant correlations for the clusters of RMB or CI or for the other striatal structures (putamen and nucleus accumbens). To assess whether this relationship changed with development, we controlled for the effect of age in a partial correlation analysis. Secondly, we investigated this relationship in the older and younger age group separately using a median split for age. When controlling for age using partial correlations, the correlation no longer reached significance, although the direction of the correlation stayed the same (r=–0.149 p=0.160). In the median split analysis, the correlation was at trend level for the younger group (r=–0.266 p=0.068) and not significant for the older group (r=–0.149 p=0.340). As such, it appears that our brain - behaviour relationship may be mediated by age-related changes in Insistence on Sameness, where the correlation is more apparent at younger ages.

Discussion

In this study, we report changes in the trajectory of striatal development in autism. Differences in caudate development were particularly striking, as the volume of this structure increased with development in autism, while it decreased in controls. Changes were not attributable to changes in overall brain volume, or the use of neuroleptic medication and were localised to the head of the caudate nucleus. Furthermore, caudate

volume was associated with severity of repetitive behaviour (Insistence on Sameness) in subjects with autism.

We report similar developmental trajectories for total brain, grey and white matter and lateral ventricles for controls and subjects with autism. For both groups, total grey matter volume decreased, while ventricular volume increased with age. This pattern is in accordance with earlier reports of typical development (Sowell et al. 2002; Lenroot and Giedd 2006). However, the developmental trajectories of striatum, caudate nucleus in particular, differed between subjects with autism and controls. In the typically developing children, caudate nucleus volume decreased with age, similar to findings in one earlier cross-sectional study of typical development (Sowell et al. 2002). However, in autism caudate nucleus continued to increase in volume with age. This is concordant with one earlier report suggesting differential developmental trajectories for subjects with Asperger's syndrome and controls (McAlonan et al. 2002), although this finding was not specific to caudate nucleus: In this study, brain and caudate nucleus volumes were reported to remain stable over development in Asperger's syndrome, while they decreased in controls.

Voxel-based morphometry showed that striatal differences in our study localised to the head of the right caudate nucleus. This nucleus is part of an intricate system integrating multimodal information and regulating complex behaviour. It encompasses distinct cortico-striatal feedback loops that feed into each other in a ventral to dorsal pattern with outputs targeting pre-motor, prefrontal and motor cortical areas (Alexander and Crutcher 1990; Haber 2003). As such, these results are consistent with other studies showing atypical functional connectivity between caudate nucleus and cortical areas in autism (Silk et al. 2006; Turner et al. 2006; Takarae et al. 2007). The caudate nucleus is in the more dorsal part of the circuit and is linked to dorsolateral prefrontal, lateral orbitofrontal, and occulomotor loops. Given these associations, it is implicated in higher-order functions, such as planning, set shifting and cognitive control (Turner et al. 2006). This suggests that the changes in caudate nucleus development in the current study could be associated with problems in cortico-striatal feedback and may therefore be related to behavioural problems. The laterality of our voxel-based results is in line with a previous study that also found changes predominantly in the right hemisphere (Hollander et al. 2005). As discussed in that study, atypical symmetry between the two caudate heads for subjects with autism may be related to functional abnormalities (Hollander et al. 2005).

Our finding of a relationship between volume of the caudate nucleus and the Insistence on Sameness cluster is concordant with other studies that have implicated striatum in repetitive behaviour across neuropsychiatric disorders (Sears et al. 1999, Hollander et al. 2005; Rojas et al. 2006; Rosenberg et al. 1997; Scarone et al. 1992; Peterson et al. 2003). A clear hypothesis on the direction of the relationship between repetitive behaviour and caudate volume in autism is not immediately obvious from the literature. Whereas Sears et al. (1999) reported a positive correlation for low order repetitive behaviour and caudate volume and a negative correlation for high order repetitive behaviour and caudate volume, Hollander et al. (2005) reported a positive correlation for high order repetitive behaviour and caudate volume. Last, Rojas et al. (2006) demonstrated a positive correlation between repetitive behaviour (including both high and low order items) and caudate nucleus volume. Our own finding

is of a negative correlation between caudate volume and a form of high order repetitive behaviour. However, when considering which ADI-items were clustered in the different studies, it becomes apparent that these results are not as contradictory as they initially appear: There is clear overlap between the items included in the clusters which show a negative correlation with caudate volume in three of four studies (see Table 3).

Furthermore, the factor analysis of repetitive behaviour by Lam and colleagues (2008) suggests that the clusters of repetitive behaviours as used in our analyses are associated with distinct profiles of symptoms. As such, the factors could be associated with distinct neural circuits that may not necessarily all involve the basal ganglia. This could form part of explanation why we only find correlations with one of the three factors identified by Lam et al. (2008). In addition, this could contribute to the seemingly contrary results in the literature of both positive and negative correlations between symptoms of repetitive behaviour and striatal structures: If different neural circuits support different aspects of this symptom cluster, the relationships between structure and function could feasibly take different forms.

This said, it does seem counter-intuitive that in the autism group larger caudate nucleus volumes are associated with less severe repetitive behaviour. As was suggested by Sears and colleagues (1999), the negative correlation between specific high order repetitive behaviour and caudate nucleus volume may reflect abnormal functional relationships with other brain areas, resulting in behavioural inflexibility. Alternatively, larger caudate volumes in autism could reflect compensatory sparing of function in this structure, where those individuals with larger volumes are best able to compensate for rigid or stereotyped behaviours. A similar finding has been reported in Tourette's disorder: Plessen et al. (2004) demonstrated an association of larger prefrontal cortical volumes and lower tic severity in children with Tourette's syndrome and suggested that increased prefrontal volumes could represent a compensatory mechanism, facilitating control of tics.

There are some limitations to our study. First, the use of a cross-sectional design limits the developmental conclusions that can be drawn from our findings (see (Kraemer et al. 2000) for a full discussion). A longitudinal approach would be more sensitive to detecting developmental changes and would permit a more accurate assessment of the shape of developmental curves. Specifically, although our findings for typical caudate nucleus development are consistent with other cross-sectional studies (Sowell et al. 2002), longitudinal studies have reported that typical development of caudate nucleus follows an inverted U-shape trajectory (Lenroot and Giedd 2006; Lenroot et al. 2007). Second, we included only high-functioning individuals who met full criteria for autism in our sample. As such, this limits the inferences that can be made in terms of other individuals in the autism spectrum.

Conclusion

In conclusion, we report changes in striatal development in autism, as well as an association of caudate volume with repetitive behaviours. This emphasises the importance of striatum in the aetiology of autism, in particular in the development of repetitive behaviour that characterises the disorder.

Table 3. Brain - repetitive behaviour correlations

ADI-R items	*Langen et al. (present study) n=88*	*Sears et al. (1999) n=35*	*Hollander et al. (2005) n=17*	*Rojas et al. (2006) n=24*
Repetitive Use of Objects	*Repetitive Motor Behaviour (n.s.)*	*n.s.*		*Repetitive and Stereotyped Behaviour Domain +**
Hand and Finger Mannerisms		*n.s.*		
Other Complex Mannerisms/ Stereotyped Body Movements		*Low order +**		
Difficulties with Minor Changes in Routine	*Insistence on Sameness -**	*n.s.*		
Resistance to Trivial Changes in the Environment		*High order -***		
Compulsions and Rituals		*High order -***		
Circumscribed Interests	*Circumscribed Interests (n.s.)*	*n.s.*	*High order +**	
Unusual Preoccupations		*n.s.*		
Unusual Attachment to Objects		*n.s.*		

ADI-R repetitive behaviour items used in the Sears (1999), Hollander (2005), Rojas (2006) and the present study. Cells are merged to indicate when items were clustered in the analyses. n.s. = non significant; * = significant correlation with caudate volume ($p<0.05$); ** = significant correlation with caudate volume ($p<0.01$); – or + signifies direction of correlation.

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6

Changes in corticostriatal development in autism: findings from diffusion tensor and magnetisation transfer imaging

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manuscript submitted

Objective: Recent studies have suggested that autism may be associated with changes in long-range cortical networks, in addition to more focal changes in grey matter. In this study we investigated corticostriatal circuitry in autism and its involvement in repetitive behaviour.

Methods: Measures of white matter integrity from diffusion weighted and magnetisation transfer brain images were collected from 69 individuals (29 subjects with high-functioning autism and 40 typically developing, matched controls) aged between 7 and 14 years. Measurements included fractional anisotropy (FA) values and magnetisation transfer ratio (MTR) in corticostriatal white matter tracts, and total white matter.

Results: Age-dependent changes in corticostriatal MTR differed between subjects with autism and controls. Results were not accounted for by overall changes in white matter or neuroleptic medication. Furthermore, while corticostriatal FA was independent of total white matter FA in controls, it was not in autism. Finally, corticostriatal MTR was associated with the severity of repetitive behaviour.

Conclusions: This study combined information on white matter directionality (FA) and myelination (MTR) to investigate corticostriatal white matter in autism. We found changes in corticostriatal white matter development, where indirect indexes of myelin content decline between the ages of 7 and 14 years for these subjects. Furthermore, measures of corticostriatal white matter directionality and coherence were independent of total white matter values for controls, but not for autism. These changes were related to the repetitive behaviour that characterises this disorder, suggesting overall that developmental changes in corticostriatal white matter are involved in repetitive behaviour in autism.

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Introduction

Repetitive and stereotyped behaviour is one of the defining symptom clusters in Autism Spectrum Disorders (ASD) and forms a prominent impairment for many individuals with this disorder. MRI-studies have implicated striatum in the development of this behaviour: Changes in basal ganglia volume have been shown (Gaffney et al. 1987; Haznedar et al. 2006; Herbert et al. 2003; Hollander et al. 2005; Rojas et al. 2006; Sears et al. 1999; Voelbel et al. 2006) and have been associated with repetitive behaviour (Hollander et al. 2005; Langen et al. 2007; Rojas et al. 2006; Sears et al. 1999). Furthermore, changes have been shown in the developmental trajectories of striatum in autism, where typical decreases in volume after middle childhood appear not to occur (Langen et al. 2009; McAlonan et al. 2002). Recent functional MRI-studies have suggested that autism may be associated with changes in long-range cortical networks, in addition to focal changes in gray matter structures (Courchesne and Pierce 2005; Just et al. 2004). These studies have shown changes in functional connectivity in ASD (Belmonte et al. 2004; Just et al. 2004; Koshino et al. 2005; Ring and Serra-Mestres 2002), especially within frontal cortex and in circuits linking frontal areas to other brain systems (Courchesne and Pierce 2005), as well as in corticostriatal circuits involving the caudate nuclei (Turner et al. 2006).

Diffusion tensor imaging (DTI) (Le Bihan et al. 2001) and magnetisation transfer imaging (MTI) (Grossman et al. 1994) are MRI methods that permit visualisation and quantification of different aspects of white matter. DTI measures the diffusion of water molecules in tissue. As white matter consists of highly organized fiber bundles, diffusion of water is much stronger along white matter bundles than across them. DTI quantifies this anisotropic diffusion as fractional anisotropy (FA), a scalar value ranging from 0 (isotropic diffusion) to 1 (pure one-dimensional diffusion) (Basser and Jones 2002). As such, FA is a quantitative assessment of white matter directionality and coherence. With MTI, a so-called magnetisation pre-pulse is applied to measure the reduction in MRI signal due to presence of macromolecules, such as myelin, in the tissue. By comparing a scan with such a magnetisation pre-pulse to a scan without a pre-pulse, the magnetisation transfer ratio (MTR) is computed using: *(I – Impp) /I x 100%*, where Impp is the scan with magnetisation pre-pulse and I is the scan without pre-pulse. Thus MTR ranges from 0% (no reduction due to macromolecules) to 100% (full reduction) and provides a quantitative indication of macromolecule content (Kubicki et al. 2005). The combination of DTI and MTI allows for an assessment of white matter integrity, where information on the directionality and coherence of white matter tracts (FA) is combined with information regarding the macromolecule content, which is often taken as a proxy for myelination level (MTR).

Both measures (DTI and MTR) are sensitive to white matter development (Baratti et al. 1999; Hüppi et al. 1998; Neil et al. 1998), where DTI has more commonly been applied to its investigation. DTI has been used to show how structural changes with age in the brain are related to changes in behaviour, both in typical and atypical development (Durston and Casey 2006). For example, Liston and colleagues linked increases in frontostriatal white matter integrity to improved cognitive control, an ability that relies on these networks (Liston et al. 2005). MTR studies have largely been performed with typical adult populations and have shown decreases in signal with age, potentially reflecting decreasing myelination

(Hofman et al. 1999; Rovaris et al. 2003; Silver et al. 1997). Studies combining FA and MTR measures have shown significant regional variation in FA (and sometimes MTR) values (Deary et al. 2006; Silver et al. 1997), suggesting that regional variation in white matter integrity may relate to cognitive differences (Deary et al. 2006). In autism, studies of white matter integrity have typically used DTI in an exploratory fashion, with only few using tract-based approaches (Catani et al. 2008; Pugliese et al. 2009; Sundaram et al. 2008). Results suggest widespread changes (Alexander et al. 2007; Barnea-Goraly et al. 2004; Ke et al. 2009; Keller et al. 2007; Thakkar et al. 2008), with reduced structural white matter integrity in autism during late childhood and the second decade of life (Ben Bashat et al. 2007). One study suggested an association between FA values in anterior cingulate cortex with repetitive behaviour (Thakkar et al. 2008). To date, no studies have focused on changes in corticostriatal circuits.

In the current study we set out to investigate age-related changes in the microstructural integrity of corticostriatal white matter in autism, using both DTI and MTR measures. To overcome limitations of voxel-based DTI (Ashburner and Friston 2000; Jones et al. 2005; Snook et al. 2007), we used a tract-based approach. We hypothesised that development of corticostriatal white matter integrity would be compromised in autism and that these changes would relate to repetitive behaviour in this disorder.

Methods and Materials

Participants

Twenty-nine individuals meeting DSM-IV criteria for high-functioning autism, aged 7-14 years, were recruited through the Netherlands Autism Society and the Department of Child and Adolescent Psychiatry at the University Medical Center in Utrecht. Diagnosis was clinically established by a board certified Child and Adolescent psychiatrist and was confirmed by trained and qualified clinicians in direct observation of a semi-standardised situation and with the Autism Diagnostic Interview Revised (ADI-R) (Lord et al. 1994). All subjects with autism had full-scale IQ of over 80 (Wechsler Intelligence Scale for Children (WISC-III) (Kort et al. 2005). Six subjects with autism were using atypical neuroleptic medication. Forty typically developing controls, aged 7-14 years, were recruited through schools in the area. For all control subjects, a parent participated in a semi-structured interview session with a trained researcher to confirm the absence of any psychiatric diagnosis (Diagnostic Interview Schedule for Children; DISC-P) (Costello et al. 1985). Subjects with a psychiatric diagnosis (current or prior), major physical or neurological illness, history of head trauma, alcohol or other drug dependence, or full-scale IQ below 80 (WISC-III) were excluded. Control subjects with a family history of psychiatric illness were also excluded. Groups were matched for gender, age, IQ, height, hand preference, pubertal development (assessed using Tanner scales), and for parental educational level (see Table 1).

All subjects participated in an MRI scanning session and a neuropsychological assessment. Children under 13 years of age were acclimated to the scanning-procedure in a dummy-scan session prior to the actual MR-scan (Durston et al. 2008). For all subjects, MRI-scans were evaluated by independent clinical neuroradiologists. No gross abnormalities were reported

for any of the subjects. The procedure was approved by the Institutional Review Board at the University Medical Center Utrecht. Written informed consent was obtained for all subjects; a parent signed for consent, while assent was obtained from the subject.

Table 1. Demographic data and characteristics of the sample

Variable	*Subjects with autism (n=29)*	*Normal controls (n=40)*
Gender (male/female)	*26 / 3*	*36 / 4*
Age, mean ± SD (range), yrs	*10.95 ± 2.20 (7.04–13.95)*	*10.13 ± 1.90 (7.04–13.75)*
Total IQ, mean ± SD (range)	*107.45 ± 15.08 (81–137)*	*112.95 ± 13.05 (87–138)*
Height, mean ± SD, cm [a]	*147 ± 15*	*140 ± 10*
Handedness (right/left/ambidexter), n [b]	*25 / 1 / 2*	*34 / 5 / 1*
Parental education, mean ± SD, yrs [c]	*14.52 ± 2.1*	*14.65 ± 1.7*
Tanner A [d]	*1.08 ± 1.176*	*1.14 ± .891*
ADI-R: social deficits [e]	*19.68 ± 4.70*	
ADI-R: abnormalities in communication [e]	*14.93 ± 4.00*	
ADI-R: ritualistic-repetitive behaviour [e]	*5.57 ± 2.67*	

[a] Information was unavailable for ten control subjects and three ASD subjects
[b] Information was unavailable for one ASD subject
[c] Information was unavailable for nine control subjects and two ASD subjects
[d] Information was unavailable for twelve control subjects and five ASD subjects
[e] Information was unavailable for one ASD subject
ADI-R: Autism Diagnostic Interview-Revised

MRI acquisition

MRI scans were acquired on a 1.5 T scanner (Philips, Best, the Netherlands) using a 6-element SENSE receiver head coil. For each subject, a 3D T1-weighted spoiled gradient echo scan, two transverse DTI scans and a 3-dimensional magnetisation transfer scan comprising two volumes were acquired. First, a 3-dimensional T1- weighted coronal (spoiled gradient) echo scan of the whole head was acquired (256 3 256 matrix; echo time (TE) = 4.6 ms; repetition time (TR) = 30 ms; flip angle = 30 degrees; 160–180 contiguous slices; total scan duration = 405–456 s; 1 3 1 3 1.2 mm3 voxels; field of view (FOV) = 256 mm/ 70%; and parallel imaging applied in both phase-encoding directions with SENSE factor = 1.5). For white matter fiber tract reconstruction and computation of the FA value, 2 transverse DTI scans were acquired (32 diffusion-weighted volumes with different non-collinear diffusion directions with b-factor = 1000 s/mm2 and 8 diffusion-unweighted volumes with b-factor = 0 s/mm2; parallel imaging SENSE factor = 2.5; flip angle = 90 degrees; 60 slices of 2.5 mm; no slice gap; 96 3 96 acquisition matrix; reconstruction matrix 128 3 128; FOV = 240 mm; TE = 88 ms; TR = 9822 ms; no cardiac gating; and total scan duration = 296 s). The MTR was computed on the basis of a 3-dimensional magnetisation transfer scan comprising 2 volumes (transverse; 60 slices of 2.5 mm; 128 3 128 acquisition matrix; FOV = 240 mm; flip angle = 8 degrees; TE = 3.7 ms; TR = 37.5 ms; SENSE factor = 2.5). For the second volume an additional off-resonance prepulse was applied (frequency offset = 1100 Hz; 620 degrees; three-lobe sync-shaped). The total scan duration of the MTR scan was 394 s.

MRI processing

All images were coded to ensure rater blindness to subject identity and diagnosis. Methods for processing of the T1-weighted images were identical to the methods described in Langen et al. (2009). An overall (total) white matter segment was defined on the T1 image for each subject using methods described in (Brouwer et al. 2009). Preprocessing of the MTR data, DTI data, fiber reconstruction and the alignment of the individually reconstructed fiber tracts with a model brain were based on Mandl and colleagues (2008). The two DTI scans were simultaneously realigned and corrected for possible gradient-induced distortions (Andersson et al. 2002). A robust estimation of the diffusion tensors was obtained using M-estimators (Chang et al. 2005) to limit the influence of possible outliers. The FA was computed from the diffusion tensors (Basser et al. 1996). To compute the MTR, the second volume of the magnetisation transfer scan was rigidly aligned to the first volume using the ANIMAL software package (Collins et al. 1995). Mutual information was used as a similarity metric.

For both the MTR and the T1-weighted scan, rigid transformations were determined to spatially align them with the diffusion-unweighted (b = 0 s/mm2) volume of the DTI-scan using mutual information as similarity metric. For each subject, a nonlinear transformation was computed to spatially align the subject's T1-weighted scan with a T1-weighted model brain (Collins et al. 1995; Hulshoff Pol et al. 2004). This nonlinear transformation was used at a later stage to warp the reconstructed tracts from native space into the model space.

Corticostriatal tracts were selected in model space using two grey matter regions of interest (ROIs), manually delineated on the model brain: the striatum (caudate nucleus, putamen and nucleus accumbens) and pre-frontal cortex (for a detailed segmentation protocol of the striatal structures, see Langen et al. (2007)). Since grey matter does not provide sufficient directional information for fiber-tracking, ROIs were dilated with 2 voxels resulting in ROIs that penetrated white matter. Only tracts that reached both dilated ROIs were carried forward in the analysis. Tracts passing through corpus callosum and those included in the uncinate fasciculus were excluded from the isolated fiber bundles by using additional 'exclusion' ROIS placed at the centre of the genu and in the temporal lobes white matter. To increase the robustness of the method and to overcome possible problems because of relatively large variations in selected tracts, we did not measure FA and MTR values directly from the reconstructed corticostriatal tracts (Figure 1A). Rather, we defined a volume of interest (VOI) in model space: (1) For each subject all voxels in the reconstructed tracts in model space were flagged as '1'; (2) these individual binary maps were added together (Figure 1B); (3) the resulting cumulative map was thresholded at 50%; (4) the corticostriatal VOI was defined as all voxels above threshold (Figure 1C). There were no group differences in the overlap between individual and VOI corticostriatal tracts: the contribution of the autism and control group to the group VOI was similar. For each subject, the VOI was warped back from model space to the native space using the inverse from the nonlinear transformation. Individual FA and MTR were then obtained in native space. These measures were obtained for the corticostriatal VOI, as well as for a segment representing total white matter, excluding the corticostriatal tracts.

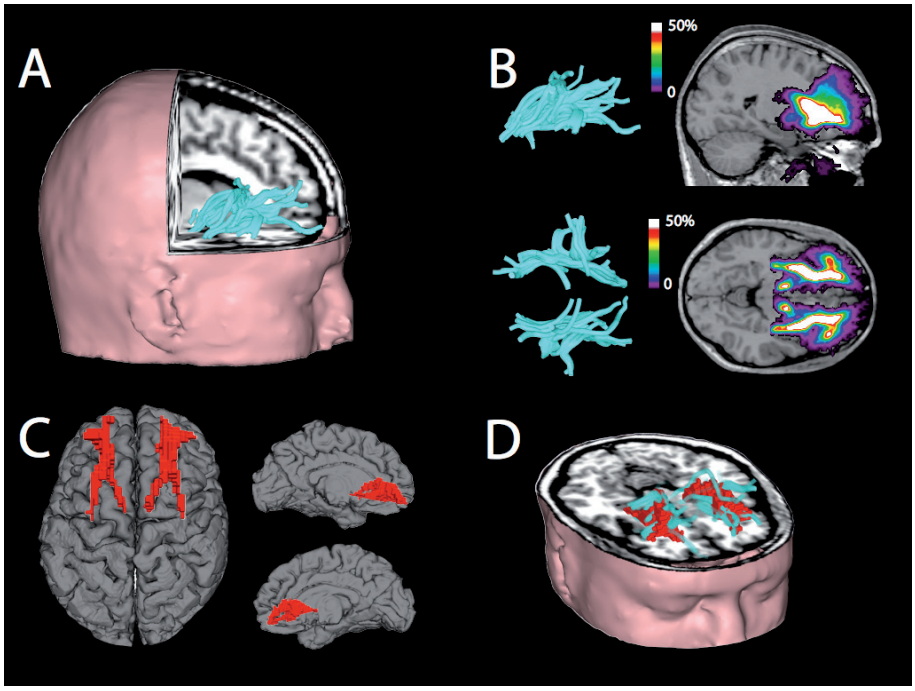

Figure 1. Schematic representation of corticostriatal tract selection

(A) Individual corticostriatal tracts, selected in model space using two manually delineated regions of interest (ROIs), striatum and frontal cortex; (B) Cumulative group map of individual binary cortico-striatal maps; (C) Corticostriatal Volume of interest (VOI) in modelspace, defined as all voxels that include 50% or more of individual corticostriatal tracts; (D) Schematic representation of corticostriatal tracts (turquoise) and corticostriatal VOI (red) for one subject in native space.

(See page 172 for a colour version of this figure.)

Statistical analysis

Overall differences in corticostriatal white matter integrity between groups (operationalised as FA and MTR-values) were investigated using t-tests. To examine differences in corticostriatal white matter development between groups and to examine the specificity of findings to corticostriatal white matter, GLM multivariate analysis of variance procedures were run. Corticostriatal FA- or MTR-values were included as the dependent variable, group was included as a fixed factor and age and overall white matter FA and MTR (excluding the corticostriatal segment) were included as co-variates. To control for possible confounding effects of medication, the analysis was repeated, (a) with medication included as a fixed factor and (b) excluding the six subjects on neuroleptic medication from the analyses.

Next, correlations using Pearson's r were calculated between (1) overall FA (excluding the corticostriatal segment) and corticostriatal FA; (2) overall MTR (excluding the corticostriatal segment) and corticostriatal MTR; (3) age and MTR- and FA-values in the cortico-striatal white matter segment. To investigate the specificity of maturational findings in

corticostriatal MTR, the potential contributing effect of overall white matter was partialled out in additional correlational analyses.

Finally, to investigate the relationship with repetitive behaviour, Spearman rank-order correlations were calculated between corticostriatal FA- and MTR-values and three symptom-clusters of ADI-R repetitive behaviours. These three symptom clusters were based on a recent factor analysis on ADI-R scores in 316 subjects (Lam et al. 2008) and included Repetitive Motor Behaviour (1), Insistence on Sameness (2) and Circumscribed Interests (3). To investigate the specificity of the brain-behaviour relationships, the potential effect of global white matter FA and MTR was controlled for using partial correlations.

Results

There were no differences between subjects with autism and control subjects in FA and MTR values for total white matter or corticostriatal tracts (FA overall white matter: $|t|>.602$; $p=.549$; FA corticostriatal white matter: $|t|>.304$; $p=.762$; MTR overall white matter: $|t|>1.018$; $p=.312$; MTR corticostriatal white matter: $|t|>.092$; $p=.927$). The GLM multivariate analyses showed (1) for corticostriatal FA: an interaction between group and FA in total white matter ($F=3.736$; $p=0.030$); (2) for corticostriatal MTR: an interaction between group and MTR in total white matter ($F=33.087$; $p<0.001$) and (3) for corticostriatal MTR: an interaction between group and age ($F=5.286$; $p=.008$). Results were similar, when medication was included in the model or when subjects on neuroleptic medication were excluded: (1) $F=3.906$; $p=0.026$, (2) $F=28.763$; $p<0.001$; (3) $F=5.867$; $p=0.005$.

Corticostriatal FA correlated with FA in total white matter for subjects with autism ($r=.425$; $p=.021$), but not in controls ($r=.221$; $p=.171$). Corticostriatal MTR correlated with MTR in total white matter for both the autism ($r=.65$; $p<0.001$) and control groups ($r=.725$;$p<0.001$). Corticostriatal MTR correlated with age for subjects with autism ($r=-.400$; $p=0.031$), but not controls ($r=-.107$; $p=0.523$). Partialling out the effect of MTR in total white matter strengthened the correlation between corticostriatal MTR and age in subjects with autism ($r=-.533$; $p=0.004$).

For subjects with autism, there were correlations between both total and corticostriatal MTR and the ADI-R cluster Insistence on Sameness ($r=.431$ $p=.028$ and $r=.516$ $p=.007$ respectively). There were no correlations for the other two symptom clusters. When the effect of age was partialled out, this brain-behaviour correlation was specific to corticostriatal MTR ($r=0.468$; $p=0.018$).

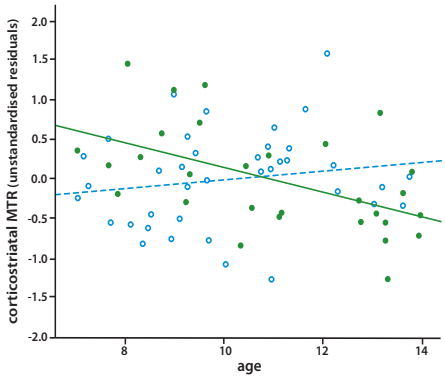

Figure 2. Corticostriatal MTR shows an age-dependent decrease in the autism group, but not in controls. MTR values are unstandardised residuals controling for the effects of MTR in total white matter MTR and medication. Green solid circles and solid line represent subjects with autism; blue open circles and dashed line represent the control group.

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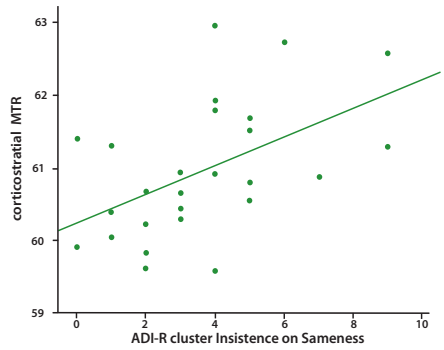

Figure 3. Correlation between corticostriatal MTR and the ADI-R Insistence on Sameness cluster (r=.516 p=.007).

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Discussion

We show age-dependent changes in corticostriatal white matter in subjects with autism between 7 and 14 years of age: In this age range, corticostriatal MTR decreased in autism, while it did not change in controls. This suggests that changes in the myelination of corticostriatal tracts may be occurring. Furthermore, while corticostriatal FA was independent of FA in total white matter for controls, it was not for subjects with autism. This suggests that corticostriatal development in this age-range may occur somewhat independently of overall white matter in controls, but less so in autism. Finally, corticostriatal MTR was associated with the severity of repetitive behaviour, suggesting that these developmental changes in autism may be related to the repetitive behaviour that characterises the disorder.

Results from typical populations have shown a gradual decrease of overall MTR with age in adults (Hofman et al. 1999; Rovaris et al. 2003; Silver et al. 1997) and adolescents (Perrin et al. 2009). Our finding of decreases in corticostriatal MTR at this young age in autism is somewhat surprising, as MTR is thought largely to reflect myelination, although it may also represent changes in other macromolecules in the brain. Studies using FA measures in typical development have suggested that frontal circuitry matures relatively late (Casey et al.

2007; Klingberg et al. 1999; Lebel et al. 2008), with adult levels not being reached until well past the age range included in this study. Our findings suggest that patterns of regionally specific white matter maturation (e.g. (Davis et al. 2009; Lebel et al. 2008)) may be a-typical in autism: The decline in MTR with age, together with an increased coupling of corticostriatal FA and MTR with other regions suggests that corticostriatal white matter maturation may follow a different developmental course than in typical development. This could reflect an imbalance of this these circuits, associated with repetitive behaviour. Certainly, it concurs with findings of aberrant connectivity of the frontal lobe in autism where connectivity between frontal cortex and other systems, including striatum (Horwitz et al. 1988)) is affected (Belmonte et al. 2004; Courchesne and Pierce 2005; Just et al. 2004).

Alternatively, the decline in MTR with age in the autism group could reflect an earlier peak in corticostriatal white matter maturation and indicate accelerated corticostriatal white matter development. This is concordant with an earlier study of FA in very young children (1.8 - 3.3 years of age) with autism, demonstrating early and accelerated maturation of white matter, predominantly in the frontal lobe (Ben Bashat et al. 2007). It also ties in to reports indicating of early brain overgrowth in autism that is largely accounted for by white matter (Courchesne et al. 2001; Hazlett et al. 2005).

Our finding of a relationship between corticostriatal white matter integrity and a measure of repetitive behaviour is concordant with other studies that have implicated corticostriatal circuitry in repetitive behaviour across neuropsychiatric disorders (Hollander et al. 2005; Peterson et al. 2003; Rojas et al. 2006; Rosenberg et al. 1997; Scarone et al. 1992; Sears et al. 1999). We find that higher levels of myelination, as measured by MTR, are associated with more severe repetitive behaviour (Insistence on Sameness) in autism. This may reflect changes in the brain driven by behaviour, where repetitive behaviour stimulates corticostriatal circuitry, resulting in increased myelination of these circuits. However, the opposite could also be true: Changes in myelination of corticostriatal tracts may negatively affect co-ordinated function between striatum and frontal cortex. The directionality of this relationship cannot be demonstrated from correlational data, such as these.

There are some limitations to this study. First, FA and MTR are indirect measures of white matter integrity. As such, results should be interpreted with caution, as other factors may contribute to MTR and FA signal (for example, neuron loss, consequent myelin loss, changes in glial tissue, altered water content, and changes in phospholipid metabolism (Silver et al. 1997)). However, we included two different measures of white matter integrity: MTR is thought to be a more direct measure of myelination than FA (Kubicki et al. 2005), whereas FA also reflects the directionality and coherence of fibers. Thus, our results lend considerable support to the idea that age-related changes in white matter are largely driven by changes in myelination rather than by changes in the axons themselves in line with other data (Davis et al. 2009; Kubicki et al. 2005). This underscores the added value of combining multiple techniques in investigating white matter integrity (Kubicki et al. 2005; Mandl et al. 2008).

A second limitation of this study, and one that applies to DTI fiber-tracking in general, is that the fiber-tracking algorithm requires sufficient directional information to successfully reconstruct the fibers. If, at a certain point, this information is not available (for instance, due to crossings with other fibers), then the algorithm cannot reconstruct the complete fiber

tract. We addressed this by the use of a VOI, which permitted us to include the same fibers in each subject. This approach is less sensitive to drops in signal, due to kissing or crossing in individual datasets, as it does not select voxels based on fiber tracking in individuals.

A third limitation of this study has to do with a general problem of definition of the corticostrial tracts in humans. Using the pre-frontal cortex as selection ROI can lead to relatively large variations in selected tracts between subjects. We addressed this by the use of a VOI. With this approach only regions that were part of the selected tracts for a large number of subjects were included in the analysis, resulting in a univocal definition of the corticostrial tracts.

Fourth, the use of a cross-sectional design limits the developmental conclusions that can be drawn from our findings. A longitudinal approach would be more sensitive to detecting developmental changes.

Conclusion

This study combined information on white matter directionality (FA) and myelination (MTR) to investigate corticostriatal white matter in autism. We found changes in corticostriatal white matter development, where indirect indexes of myelin content decline between the ages of 7 and 14 years for these subjects. Furthermore, measures of corticostriatal white matter directionality and coherence were independent of total white matter values for controls, but not for autism. These changes were related to the repetitive behaviour that characterises this disorder, suggesting overall that developmental changes in corticostriatal white matter are involved repetitive behaviour in autism.

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7

Corticostriatal circuitry and inhibitory control in autism: findings from DTI tractography

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Background: Repetitive behaviour and inhibitory control deficits are core features of autism; and it has been suggested that they result from differences in the anatomy of striatum; and/or the 'connectivity' of subcortical regions to cortex. There are few studies, however, that have measured the micro-structural organisation of white matter tracts connecting striatum and cerebral cortex.

Aims: To investigate differences in bulk volume of striatum and microstructural integrity of corticostriatal white matter in people with autism; and their association with repetitive behaviour and inhibitory control.

Methods: We compared the bulk volume of striatum (caudate nucleus, putamen and nucleus accumbens) and white matter integrity of corticostriatal tracts using (respectively) sMRI and tract specific DTI measures in 21 adults with autism and 22 controls. We also assessed performance on a cognitive inhibition (go/nogo) task.

Results: Bulk volume of striatal structures did not differ between groups. However, adults with autism had a significantly smaller total brain white matter volume, lower fractional anisotropy of white matter tracts connecting putamen to frontal cortical areas, and worse performance on the go/nogo task. Also, performance on the go/nogo task was significantly related to anatomical variation when both groups were combined; but not within the autism group alone.

Conclusions: Autism is associated with differences in the anatomy of corticostriatal white matter tracts.

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Introduction

Autism is characterised by a triad of (1) stereotyped and repetitive behaviours, and pervasive abnormalities in (2) socio-emotional and (3) communicative behaviour. While a considerable body of work has investigated brain differences associated with the last two clusters of symptoms, relatively few studies have investigated those putatively associated with stereotyped and repetitive behaviour. Hence, the biological associates of this core symptom domain are poorly understood.

Studies of people with other neuropsychiatric disorders, such as obsessive compulsive disorder and Tourette's syndrome, have highlighted the relationship between stereotyped and repetitive behaviours and abnormalities within the striatum (Albin and Mink 2006; Bloch et al. 2005; Hyde et al. 1995; van den Heuvel et al. 2008; Whiteside et al. 2004). Moreover, there is increasing evidence that autism is associated with differences in the developmental trajectory and volume of striatum (Hollander et al. 2005; Langen et al. 2007; Langen et al. 2009; McAlonan et al. 2002; Rojas et al. 2006; Sears et al. 1999) that, some have reported, are related to stereotyped and repetitive behaviour. It has also been suggested that the frontal cortex, and its connections to striatal and parietal regions, underpin repetitive behaviour and deficits in executive functions (in particular inhibitory control) (Christ et al. 2006; Garavan et al. 2002; Kana et al. 2007; Mosconi et al. 2009; Robinson et al. 2009). Hence, some investigators have suggested that differences in these brain regions play a central role in autism (e.g. see Turner et al. 2006).

Recently, there has been increasing recognition that, in addition to - or instead of - focal differences in brain anatomy, people with autism may have altered brain 'connectivity' (e.g. see Bachevalier and Loveland 2006; Courchesne and Pierce 2005; Just et al. 2004). For example, some have reported differences in intra-regional correlations of grey matter (McAlonan et al. 2004) and glucose metabolism (Horwitz et al. 1988) within corticostriatal circuits in autism, as well as altered functional connectivity (Belmonte et al. 2004; Just et al. 2004; Koshino et al. 2005; Ring and Serra-Mestres 2002) within frontal cortex and in circuits linking frontal areas to other brain systems (Courchesne and Pierce 2005; Turner et al. 2006).

Other investigators have used diffusion tensor imaging (DTI) to examine the microstructural integrity of white matter in autism. This non-invasive MRI technique measures the diffusion profile of water molecules, which, in turn, can provide valuable insights in the underlying architectural organisation of white matter fiber tracts. Water molecules diffuse more readily in the direction parallel to a tract rather than perpendicular to it (Beaulieu, 2002). The diffusion measure fractional anisotropy (FA) describes the magnitude of this directional dependence within a given voxel and has values between 0 (purely isotropic diffusion) and 1 (purely anisotropic diffusion). Most prior DTI studies of autism used VBM-based approaches (Alexander et al. 2007; Barnea-Goraly et al. 2004; Ke et al. 2009; Keller et al. 2007; Thakkar et al. 2008), in which a widespread of differences in white matter integrity have been reported. Although these studies are informative about overall changes in white matter, they do not provide direct measures of the micro-structural integrity of specific white matter tracts connecting brain regions. For example, VBM-based DTI approaches are able to describe regional differences in the anatomy of white matter – but not specific tracts - that are

affected. Hence, evidence for the involvement of specific connections (i.e., tracts) is still lacking.

A technique that can partially overcome the limitations of VBM approaches is DTI tractography (Conturo et al. 1999; Jones et al. 1999; Basser et al. 2000; Mori and Van Zijl 2002). DTI based tractography uses the orientation of the diffusion profile to reconstruct the trajectories of fiber bundles and has been used extensively to explore the micro-structural integrity of white matter in a wide range of conditions including epilepsy (Ahmadi et al. 2009; Concha et al. 2007), and schizophrenia (Oh et al. 2009; Phillips et al. 2009). DTI-tractography is the only technique that allows for the simultaneous quantification of the white matter volume and micro-structural integrity within specific tracts in the living human brain (Le Bihan 2003).

Some investigators have applied tractography in autism and reported on differences in superior cerebellar, frontal association and limbic tracts (Catani et al. 2008; Pugliese et al. 2009; Sundaram et al. 2008) and age-related differences in frontostriatal pathways in children with autism. However, no studies have yet examined the anatomy of the individual striatal structures alongside the micro-structural integrity of their corticostriatal pathways in the same individuals; or related this to clinical symptoms. Therefore, we used structural MRI (sMRI) and DTI to compare bulk volume (i.e., grey and white matter) and micro-structural organisation of the basal ganglia and connecting corticostriatal white matter in adults with autism and controls. Also we related anatomical differences to repetitive behaviours and inhibitory control.

Methods and Materials

Participants

Twenty-one right-handed males meeting International Classification of the Disease (ICD-10) criteria for autism [WHO, 1993] and twenty-two typically developing control males were included. Subjects were aged between 19 and 44 years; mean age was 26 ± 6 years for the autism group and 28 ± 6 years for the controls. All subjects had full-scale IQ over 70. None of the subjects was using neuroleptic medication. There were no between-group differences in age or IQ (details in Table 1).

Subjects with autism were recruited from a clinical research program at the South London and Maudsley NHS Foundation Trust and and Institute of Psychiatry (IoP) - part of the MRC (UK) Autism Imaging Multicentre Study (AIMS) network. Diagnosis was clinically established by a Consultant Psychiatrist from IoP's Department of Psychological Medicine and was confirmed using the Autism Diagnostic Interview Revised (ADI-R) (Lord et al. 1994) and the Autism Diagnostic Observation Schedule (ADOS) (Lord et al. 1989). None of the subjects had a history of head injury, major psychiatric disorder or medical illness affecting brain function or structure (e.g. psychosis or seizures). All had routine blood tests and a clinical examination to rule out biochemical and haematological abnormalities, or genetic disorders that may be associated with autism (including fragile X syndrome). Control subjects were recruited locally by advertisement. Control subjects with a history of head injury, major psychiatric disorder or medical illness affecting brain function or structure, or with a family

history of psychiatric illness were excluded. All subjects were right-handed, and underwent a neuropsychological test battery including assessment of general intellectual functioning using the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999), and an online version of a go/no go task that was adapted from (Rubia et al. 2001). Participants were told to expect a series of arrows, which could point to the left, right or up. They were asked to press the '1' key in response to left arrows, the '2' key in response to right arrows and not to press anything if the arrow pointed upwards. There were 300 trials, presented in the same fixed order for each participant. There were 110 trails of each of left and right arrows (go trials) and 80 up arrows (no go trials). Each trial was a maximum of 1200ms long, with a 100ms white screen between trials. As soon as the participant responded, the next trial began. Accuracy and reaction times were recorded for each trial.

For all subjects, MRI-scans were evaluated by independent clinical neuroradiologists. No gross abnormalities were reported for any of the subjects. The procedure was approved by the Joint Medical Ethical Committee of the IoP, Kings College London. Written informed consent was obtained for all subjects after complete description of the study.

Table 1. Demographic data and characteristics of the sample (all right-handed males)

Variable	*Subjects with autism (n=21)*	*Controls (n=22)*
Age, mean ± SD (range), yrs	*25.57 ± 6.08 (19–39)*	*28.45 ± 6.39 (19–44)*
Full scale IQ, mean ± SD (range)	*107.45 ± 15.08 (81–137)*	*109.82 ± 13.71 (83–133)*
ADI-R: social deficits	*18.43 ± 5.86*	
ADI-R: abnormalities in communication	*12.90 ± 4.43*	
ADI-R: ritualistic-repetitive behaviour	*4.24 ± 2.02*	

ADI-R: Autism Diagnostic Interview-Revised

MRI Acquisition

All participants were scanned at the Centre for Neuroimaging Sciences, Institute of Psychiatry, London, UK, using a 3 Tesla GE Signa System (General-Electric, Milwaukee, WI, USA). High-resolution structural T1-weighted volumetric images were acquired with full head coverage, 196 contiguous slices (1.1 mm thickness, with 1.09 mm x 1.09 mm in-plane resolution), a 256 x 256 x 196 matrix and a repetition time/echo time (TR/TE) of 7/2.8 ms (flip angle 20", FOV 28 cm). A (birdcage) head coil was used for radiofrequency transmission and reception. Consistent image quality was ensured by a semi-automated quality control procedure.

For white matter fiber tract reconstruction and computation of the FA maps, diffusion tensor MRI scans were acquired with an SE-EPI double refocused sequence providing whole head coverage with isotropic image resolution (2.4x2.4x2.4 mm3) were acquired (32 diffusion-weighted volumes with different non-collinear diffusion directions with b-factor 1300 s/mm2 and 6 non-diffusion-weighted volumes; 60 slices; no slice gap; TE 104.5 ms; TR 20 R-R intervals; 128 x 128 acquisition matrix; FOV = 30.7 cm2; peripherally gated). A more detailed description of the acquisition protocol is reported in (Jones et al. 2002).

Structural MRI processing

All scans were coded to ensure rater blindness to subject identity and diagnosis. The structural images were automatically placed in Talairach orientation, and were corrected for motion and magnetic field inhomogeneities using Freesurfer (Fischl et al. 2002; 2004a;b). The corrected scans in Talairach orientation were used for hand-tracing of striatum. For calculating volumes of total brain, grey and white matter, Freesurfer was used (Fischl et al. 2002; 2004a;b).

Manual segmentations

Striatal structures were traced manually using ITK-SNAP (Yushkevich et al. 2006). One rater (ML) was blind to subject identity, diagnosis and laterality. Caudate nucleus, putamen and nucleus accumbens were outlined in contiguous coronal slices in an anterior - posterior direction. The sagittal and axial planes were used for reference. We have previously reported the segmentation and tracing procedures in detail (Langen et al, 2007). Intra-rater reliabilities (estimated on a random duplicate set of 10 scans, using intra-class correlation coefficients; ICCs) were above .93 for all three structures.

Generation of fiber tract data

The diffusion data were analysed using ExploreDTI (Leemans et al. 2009a) and consisted of (i) correcting for eddy current distortion and subject motion (Leemans et al. 2009b); (ii) diffusion tensor estimation using a non linear least square method (Jones et al. 2004), and (iii) whole brain tractography with a step-size of 1mm, FA thresholds of 0.2 to initiate and continue tracking, and an angle threshold of 35° (Basser et al. 2000).

Visualisation and analysis of fiber tracts

For each individual subject, the high-resolution structural image and the manually segmented structures were registered to the fiber tract data using FLIRT (Jenkinson et al. 2001). TrackVis (Wang and Wedeen 2007) was used for visualising and quantifying fiber tracts. With TrackVis, tract data can be reduced to specific tracts of interest by using a region-of-interest (ROI) selection method (Conturo et a. 1999). First, inclusion and exclusion ROIs are defined on the high-resolution structural image. Second, the tract is defined by including all fibers passing through the inclusion ROI(s), and excluding all fibers passing through the exclusion ROI(s). Separate tracts were defined for fibers passing through accumbens nucleus, caudate nucleus, and putamen and motor, pre-motor, pre-frontal and limbic cortices. The hand-traced segmentations of the striatum were used as inclusion ROIs. Exclusion ROIs were defined to exclude the corticospinal tracts, artifactual inter-hemispheric fibers and tracts originating or terminating in other cortical regions. Finally, for the generated corticostriatal tracts, number of fibers and FA were calculated using the statistics tool in TrackVis.

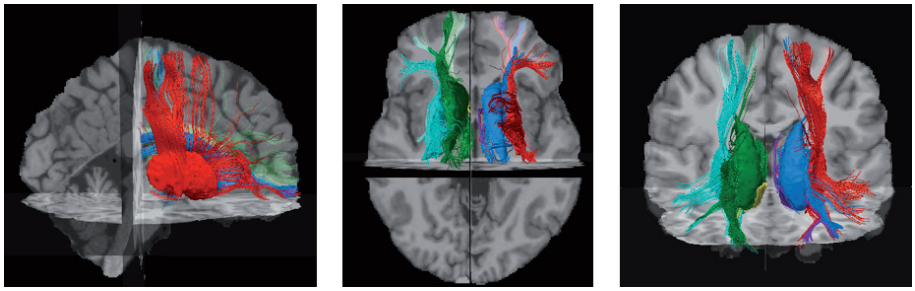

Figure 1. Sagittal, axial, and coronal views of striatal structures and corticostriatal tracts for one subject, superimposed on the T1-weighted scan. Yellow and purple = nucleus accumbens and accumbens tract; green and blue = caudate nucleus and caudate tract; turquoise and red = putamen and putamen tract.

(See page 173 for a colour version of this figure.)

Statistical analysis

Statistical comparisons of the data were performed using SPSS software for Apple Mac (SPSS Inc, Chicago, Ill).

Overall differences in age, IQ data, and performance on the go/nogo task between groups were calculated using an independent samples t-test.

To compare volumetric and tractography outcome measurements, a general linear model (GLM) multivariate analysis of variance was used. Striatal volumes, corticostriatal FA-values, and corticostriatal volume (e.g. number of tracts) were included as the dependent variables and group was included as a fixed factor. To examine the specificity of findings to striatal structures and corticostriatal white matter, intra-cranial volume and overall white matter volume were included as co-variates. Next, we explored whether results for FA-values were accounted for by other variables by including striatal bulk volumes or number of fibers in the tracts as co-variates. A post-hoc t-test was used to confirm the significant results from the GLM and to establish the direction of the effects.

To investigate the relationship between brain and behavioural measures, correlations were calculated between anatomical variables that differed significantly between groups and performance measures of the go/nogo task. Finally, within the autism-group, we related striatal volumes and corticostriatal FA-values to measures of repetitive behaviour from the ADOS and ADI-R using Spearman rank-order correlations.

Results

There were no differences in bulk volume of any striatal structure we measured (bilateral, left or right hemisphere) ($F<1.267$; $p>.267$). However the autism group had a smaller total brain white matter volume bilaterally than controls ($F>5.697$; $p<.022$).

For the DTI measures, people with autism had a significantly lower FA than controls in the left, and total (left plus right) white matter tracts originating from putamen ($F>6.002$; $p<.019$). The FA of tracts emerging from the right putamen was also lower than in controls,

and this approached (but did not reach) statistical significance (p=.055). However, there were no significant differences in FA within any other tract (F<2.109; p>.154); or in the number of fibers in any of the tracts (F<2.539; p>.119). These results remained significant when we corrected for bulk volume of putamen, putamen tract volume, total brain white matter volume or intra-cranial volume. See Table 2 for findings of brain volumes and FA, and laterality of the results.

For the behavioural measures, subjects with autism performed significantly worse than controls on nogo trials of the inhibitory control task (F=13.36; p=.001). Furthermore, performance on this task was significantly related to FA within the putamen tract over both groups (Figure 2). However, we found no significant relationship when we examined the correlations within each of the individual groups alone. Lastly, within the autism group, there was no significant relationship between ADI-R or ADOS sub scale scores for repetitive behaviours and brain measures.

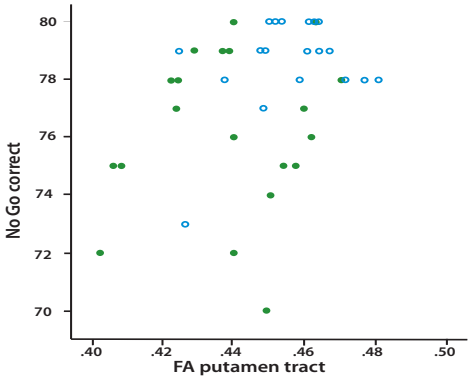

Figure 2. Associations of microstructural integrity of putamen tract (FA values) and inhibitory control (percentage correct on nogo trials of go/nogo task). Green solid circles = autism group; blue open circles = control group. Correlation analyses: total group: r = .356, p = .021; autism group: r = .151, p = .52; control group: r = 277, p = .21.

(See page 173 for a colour version of this figure.)

Table 2. Basal ganglia volumes and corticostriatal FA values for both samples

	autism group (n=21)	***control group (n=22)***	
Brain structure	***Mean ± SD, cm3***	***Mean ± SD, cm3***	***p value***
Intracranial volume	*1759.86 ± 183.21*	*1809.77 ± 169.34*	*n.s.*
Left hemisphere grey matter	*244.32 ± 22.36*	*238.90 ± 15.92*	*n.s.*
Right hemisphere grey matter	*244.30 ± 22.36*	*239.14 ± 16.60*	*n.s.*
Total brain grey matter	*488.60 ± 43.65*	*478.03 ± 32.20*	*n.s.*
Left hemisphere white matter	*255.10 ± 23.42*	*272.52 ± 24.41*	*.022*
Right hemisphere white matter	*255.88 ± 23.93*	*275.48 ± 25.27*	*.013*
Total brain white matter	*511.00 ± 47.26*	*548.00 ± 49.38*	*.016*
Left caudate nucleus	*4.59 ± .66*	*4.66 ± .46*	*n.s.*
Right caudate nucleus	*5.16 ± .65*	*5.018 ± .47*	*n.s.*
Total caudate nucleus	*9.79 ± 1.23*	*9.74 ± .90*	*n.s.*
Left putamen	*5.54 ± .42*	*5.43 ± .54*	*n.s.*
Right putamen	*5.55 ± .45*	*5.57 ± .52*	*n.s.*
Total putamen	*11.09 ± .81*	*11.00 ± 1.02*	*n.s.*
Left nucleus accumbens	*1.16 ± .20*	*1.20 ± .23*	*n.s.*
Right nucleus accumbens	*1.12 ± .18*	*1.19 ± .23*	*n.s.*
Total nucleus accumbens	*2.28 ± .35*	*2.39 ± .45*	*n.s.*
White matter tract	***Mean ± SD, FA***	***Mean ± SD, FA***	***p value***
Left caudate nucleus tract	*.43 ± .025*	*.43 ±.019*	*n.s.*
Right caudate nucleus tract	*.42 ± .018*	*.43 ± .028*	*n.s.*
Total caudate nucleus tract	*.43 ± .018*	*.43 ± .021*	*n.s.*
Left putamen tract	*.44 ± .022*	*.46 ±.017*	*.019*
Right putamen tract	*.44 ± .024*	*.45 ± .019*	*.055*
Total putamen tract	*.44 ± .020*	*.45 ± .015*	*.014*
Left nucleus accumbens tract	*.42 ± .026*	*.41 ± .023*	*n.s.*
Right nucleus accumbens tract	*.43 ± .020*	*.43 ± .025*	*n.s.*
Total nucleus accumbens tract	*.42 ± .017*	*.42 ± .021*	*n.s.*

n.s. = non-significant

Discussion

We report that subjects with autism have significantly smaller total brain white matter volume and lower FA in the white matter tract connecting putamen to cortical areas. Also, the reduction in FA of the putamen tract was not accounted for by differences in volume of whole brain white matter, bulk volume of putamen and/ or volume of the white matter tract connecting putamen and cortex. Subjects with autism had worse performance than controls on a go/nogo task. Overall, when cases and controls were examined together, performance on this task was related to microstructural integrity of corticostriatal white

matter in the putamen tract. However, within with the autism-group, there was no significant relationship between differences in FA and clinical symptoms.

Decreases in FA have been demonstrated before in autism in a number of brain regions using VBM-based approaches (Alexander et al. 2007; Barnea-Goraly et al. 2004; Ke et al. 2009; Keller et al. 2007; Thakkar et al. 2008). Also, our findings converge with other reports of abnormal anatomy of corticostriatal grey matter (Hollander et al. 2005; Langen et al. 2007; Rojas et al. 2006) in autism, as well as with findings of altered 'connectivity' in autism (Belmonte et al. 2004; Catani et al. 2008; Just et al. 2004; Koshino et al. 2005; Pugliese et al. 2009; Ring and Serra-Mestres 2002; Sundaram et al. 2008). However, differences in specific corticostriatal fiber-tracts, and/ or their relationship to problems in inhibitory control have not previously been reported in autism.

The biological cause of the differences in white matter we found are unknown; and it is unclear why we only found differences in tracts connecting putamen (but not other striatal structures) to cortex. However, results from earlier studies investigating (1) white matter development in autism, and (2) involvement of putamen networks in inhibitory control may help explaining our findings and are discussed next.

Corticostriatal white matter abnormalities in autism: Static or dynamic

Results from this preliminary study suggest that adults with autism have significantly lower FA values in white matter tracts connecting striatum to cortex. From our data, however, we cannot conclude whether these results reflect a static or dynamic difference between autism and controls. Findings from typically developing populations have shown that FA increases up to late childhood and then decreases with age (Lebel et al. 2008), where white matter maturation follows regionally specific trajectories (Barnea-Goraly et al. 2005; Salat et al. 2005). Hence our results could (1) reflect a general decrease of corticostriatal white matter integrity in autism, non-specific to a particular age-group; (2) indicate typical development in childhood, but with an accelerated decrease of white matter quality in adulthood; (3) arise from an earlier age of onset of age-related decline of white matter quality, possibly related to accelerated white matter maturation in (early) childhood.

Based on earlier findings, it seems unlikely that our results reflect the first option, i.e. a general decrease of corticostriatal white matter integrity in autism. For example, the findings by others of both increased (Ben Bashat et al. 2007) and similar FA-values (Sundaram et al. 2008) in frontal circuits in younger people with ASD than in the present study, argue against this. The currently available evidence also does not strongly support the second option, as there are no reports of an accelerated decrease of white matter in adulthood (albeit we have recently reported preliminary evidence for significantly greater age-related loss of cortical grey matter in adults with autism as compared to controls (Hallahan et al 2009). Therefore, the third option seems the most likely, i.e. our results may reflect an earlier peak in white matter maturation and an earlier age of onset for age-related decrease in FA in autism. This is in line with prior reports of early and accelerated abnormal maturation of white matter (measured with FA), predominantly in the frontal lobe, in very young children with autism (1.8 - 3.3 years) (Ben Bashat et al. 2007) and with a recent report demonstrating age-

dependent decreases in white matter (measured with myelin transfer ratio (MTR) imaging) in older children and adolescents with autism, but not in controls (Langen et al. submitted). Furthermore, other reports indicate that early overgrowth of brain volume in autism is disproportionately accounted for by increased white matter volume (Courchesne et al. 2001; Hazlett et al. 2005), potentially related to an earlier peak in white matter maturation, providing further support for this explanation. Nevertheless, further (and preferably longitudinal) work investigating development of corticostriatal white matter in younger age-groups, and specifically examining regional differences in frontal white matter integrity is required to further evaluate these issues.

Involvement of corticostriatal white matter in inhibitory control

We found that young adults with autism have reduced FA in corticostriatal circuitry localised to the tract connecting putamen to frontal cortical areas. Cortical areas in this circuit (prefrontal, parietal, anterior cingulate and pre-superior motor) are typically associated with deliberate and controlled inhibition of unwanted responses (Garavan et al. 2002). Hence we related the differences we found in white matter tracts to inhibitory control in both groups. Although we did find a significant relationship when we combined data from both groups to examine inhibitory control; there was no significant association for the autism group separately. Therefore, although previous work has implicated the putamen circuit in inhibition of responses in a go/nogo paradigm (Liddle et al. 2001; Kana et al. 2007; Garavan et al. 2002; Rubia et al. 2003; Watanabe et al. 2002), from our data, we cannot conclude that the reduced microstructural integrity in the autism group affects performance on the inhibitory control task. Our study does, however, support the suggestion that in humans (i.e. when we combined data from both groups) differences in the microstructural integrity of this tract are associated with variation of inhibitory control – at least in adults. The directionality of this association is, nevertheless, unknown. That is, the anatomy of these pathways is likely also to be modulated by life-long abnormalities in the behaviour itself. Further studies are required, and particularly of autistic individuals who have, or go on to later develop, more severe obsessional and/or repetitive symptoms.

Limitations

We only included individuals fulfilling 'gold standard' diagnostic criteria (i.e. who were above threshold in both the ADI and ADOS) and who did not differ in age or overall intellectual functioning from controls. We suggest that this lends confidence to our finding of between group differences. Nevertheless, there are some limitations to our study. First, although most DTI studies in autism have included smaller samples, the sample size of the present study is still only moderate. To better evaluate the present findings, replication of our work (in a larger sample) is required.

Second, although the anatomical localisation of our findings is consistent with a growing body of literature that implicates corticostriatal abnormalities in autism, it needs to be acknowledged that DTI-tractography cannot visualise axons directly. Rather, DTI-tractography provides a reconstruction of axonal trajectories and is therefore merely an

indirect measure of white matter tracts, (Pugliese et al. 2009), though reflecting highly reproducible features of human brain anatomy (Catani et al. 2007).

Furthermore, although FA is generally accepted as an indicator of white matter organisation, methodological considerations potentially impacting measurement of FA (partial volume effects, signal-to-noise ratio of the data) need to be acknowledged (Kubicki et al. 2005; Sundaram et al. 2008). Additional imaging techniques such as magnetisation transfer imaging (MTI), MR spectroscopy, and relaxation time measurements can be used to increase the specificity of FA findings (Kubicki 2005).

Fourth, as noted above we only included adults without learning difficulties; and so it is unknown if our findings will generalise to other groups of autistic individuals (e.g. children, or low-functioning individuals with autism). We accept that a study of children is of great interest. However brain anatomy (e.g. in grey and white matter volumes (Giedd et al. 1999)), connectivity, and function change throughout childhood and adolescence. A study of children therefore, takes place when brain maturation is incomplete, and maturational differences, including pubertal stage and cognitive developmental level, may blur the abnormalities associated with the disorder itself. Indeed, several authors have highlighted that discrepancies in results between neuroimaging studies of autism could in part reflect differences in developmental stage between samples (McAlonan et al. 2002; Herbert et al. 2003; Hollander et al. 2005; Langen et al. 2009). To reduce this confound we would need to employ either longitudinal designs, or large cross sectional studies in different age cohorts. Most importantly, we should acknowledge that studying children and adults are complementary: By studying adults we are able to identify brain systems which differ in anatomy and connectivity in the 'end-state', whereas by studying children we can detect differences in development of brain structure and function.

Conclusion

This study investigated differences in bulk volume of striatal regions and corticostriatal white matter in autism; and their involvement in repetitive behaviour and inhibitory control. We report differences in overall white matter volume and corticostriatal FA between adults with autism and matched controls, where subjects with autism have a smaller total white matter volumes and lower FA-values in the tract connecting putamen to frontal cortical areas. These findings emphasise the importance of corticostriatal circuitry in autism.

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8

Imaging repetitive behaviour in autism, what have we learned: Discussion

Repetitive behaviour in autism: scope of this thesis

Autism is a severe neurodevelopmental disorder that is characterised by (1) impaired reciprocal social interaction; (2) abnormal development and use of language; and (3) stereotypies, repetitive and rigid behaviour and restricted interests. A formal diagnosis of autism requires the presence of problems in each of these three domains (American Psychiatric 1994). While a considerably body of work has investigated brain changes associated with the first two clusters of symptoms, relatively few studies have investigated brain changes associated with repetitive behaviour. This is surprising, given the prominence of repetitive behaviour in the disorder: In many cases these symptoms onset early in development and they often form a significant impairment for affected individuals.

In this thesis we have addressed this gap by using neuroimaging techniques to investigate brain changes associated with repetitive behaviour in autism.

Structural neuroimaging studies investigating autism attempt to answer the questions whether, where and how clinical features of the disorder are reflected in brain anatomy. However, from the literature it is known that MRI studies can be confounded by issues such as medication use of the subjects and age or developmental stage of the sample. Furthermore, previous work using structural as well as functional MRI has demonstrated that autism is not reflected per se in localised brain changes in a specific region, but rather affects networks of connected brain regions. In the studies presented in this thesis we addressed these potential caveats by studying medication-naive samples or by strictly controlling for medication use; by examining developmental trajectories of brain changes; and by exploring the involvement of brain networks in autism. To begin with a clear picture of the neurobiological mechanisms underlying repetitive behaviour, we started off by reviewing animal and human literature of repetitive behaviour.

Summary of the studies presented in this thesis

In **chapter 2**, we discuss findings from fundamental animal research and translational models to provide a framework for studying the neurobiological systems involved in repetitive behaviours in human psychopathology in general and autism specifically. In this chapter, we present how it came about that the basal ganglia are now considered key to explaining repetitive motor and non-motor behaviours. We discuss the parallel corticostriatal feedback loops that connect subcortical basal ganglia structures with cortical areas and show how the anatomical organisation of these loops corresponds to their functionality. Furthermore, we discuss how disruption of co-ordinated function within the basal ganglia or between striatal and forebrain structures results in abnormal behaviours, often including repetitive behaviours.

Studies of environmentally deprived animals substantiate the notion that repetitive behaviours reflect robust and perhaps even permanent changes in brain development, and especially corticostriatal development. Work on repetitive behaviours induced by drugs or lesions and work using gene targeting technologies further explains the mechanisms

by which these behaviours are moderated, but also shows that many questions remain unanswered.

In the concluding paragraph of chapter 2, we stress that for better understanding of the multi-levelled modulation of repetitive behaviours by corticostriatal systems, future research should allow the integration of findings from separate research fields, across techniques and species. Second, by shifting focus from complex syndrome studies to inter-species trait studies, we may be able to overcome a known problem in studies using neurobiological models of abnormal behaviours in that the behaviours often do not map onto the syndromes described in humans. This will optimise genotype–phenotype relationships for research of psychiatric disorders and facilitate the identification of biological substrates underlying these disorders (Kas et al. 2007). Third, we emphasise that detailed phenotyping and consensus in definitions for repetitive behaviours are indispensable for systematic research efforts and referencing existing information about repetitive behaviours across species and clinical conditions (Lewis and Bodfish 1998).

In **chapter 3** we discuss neurobiological hypotheses of repetitive behaviours in relationship to human developmental and neuropsychiatric conditions. In this chapter, we build on the findings from animal and translational studies in chapter 2. By considering neurobiological work on repetitive behaviour in obsessive compulsive disorder, Tourette's disorder, autism and Parkinson's and Huntington's disease, we arrive a model for classifying repetitive behaviours by their anatomical correlate. However, we stress that understanding their neuroanatomical substrate does not explain the origin of repetitive behaviours.

To better understand why and how repetitive behaviours develop, we emphasise the need for multidisciplinary studies investigating the psychopathology of repetitive behaviours and, second, also for studies of repetitive behaviour in normality. Third, a rethink of diagnostic classification might be necessary to better understand the origin of repetitive behaviour. As evidence for etiological overlap between psychiatric disorders builds, it follows that disorders might better be described as domains of disorder-related traits rather than separate categories (Kas et al. 2007). In clinical practice, this entails adding a dimensional approach to the classic categorical diagnostic approach (Hollander et al. 2007); thus instead of focusing on features that distinguish disorders from one another, put attention on symptoms that connect them. Following from this, we propose that one way to improve insight in the underpinnings of repetitive behaviour in autism is by broadening the field of view to other diagnostic groups. Narrowing the field of view by subdividing individuals with heterogeneous disorders into more homogeneous well-phenotyped symptom-groups, may be a second approach.

In **chapter 4** we return to the main focus of this thesis: investigating the neurobiology of repetitive behaviour in autism, using neuroimaging techniques. We use structural MRI scans to examine volumes of caudate nucleus, putamen and accumbens nucleus in two independent samples (one younger, one older) of medication-naive subjects with autism to further explore previously demonstrated enlargements of the basal ganglia and their involvement in repetitive behaviour. We demonstrated an increase in caudate nucleus volume, disproportional to an increase in total brain volume, in the autism groups of both

the child and the adolescent sample. As this result is found in both age groups, it suggests that caudate nucleus continues to be involved in autism over development.

In **chapter 5** we further explore involvement of striatal development in autism. We compare developmental trajectories of brain volumes including volumes of caudate nucleus, putamen and nucleus accumbens between subjects with autism and controls. We report changes in the trajectory of striatal development in autism. Differences in caudate development are particularly striking, as the volume of this structure increases with development in autism, while it decreased in controls. Changes are not attributable to changes in overall brain volume, or the use of neuroleptic medication and are localised to the head of the caudate nucleus. Furthermore, caudate volume is associated with severity of repetitive behaviour (Insistence on Sameness) in subjects with autism.

In **chapter 6** we address the issue of differences in brain networks contributing to autism by exploring the involvement of corticostriatal circuitry in repetitive behaviour in children with autism using magnetic transfer imaging (used for computing the magnetic transfer ratio, MTR) and diffusion tensor imaging (DTI, used for measuring fractional anisotropy, FA). We show age-dependent changes in corticostriatal white matter in subjects with autism between 7 and 14 years of age: Corticostriatal MTR decreases, independent of overall white matter MTR, while it does not change in controls. This suggests that changes in the myelination of corticostriatal tracts may occur in this age range in autism. Furthermore, while corticostriatal FA is independent of FA in total white matter in controls, both corticostriatal FA and MTR are associated with these measures in total white matter in autism. This suggests that corticostriatal development between the ages of 7 and 14 years occurs somewhat independently of overall white matter in controls, but less so in autism. Finally, corticostriatal MTR is associated with the severity of repetitive behaviour, suggesting that the changes seen in autism are related to the repetitive behaviour that characterises the disorder.

In **chapter 7** we further examine involvement of corticostriatal circuitry in autism. We combine structural MRI and DTI and compared differences in the volume of striatum and micro-structural integrity of connecting corticostriatal white matter in adults with autism. Also we relate anatomical differences to repetitive behaviours and inhibitory control. We report that people with ASD have significantly smaller whole brain white white matter volume and a lower FA-value in the tract connecting putamen to cortical areas. The decrease in FA of the putamen tract is not accounted for by differences in whole brain white matter volume, nor by bulk volume of putamen. Also people with ASD perform significantly worse than controls on a go/nogo task. When both groups are combined, performance on the task of inhibitory control is significantly related to microstructural integrity of corticostriatal white matter. However, within the ASD group there is no significant relationship between differences in FA and clinical symptoms.

In sum, our volumetric sMRI studies demonstrate differences in striatal *grey* matter where caudate nucleus volume is increased in children, adolescents and young adults (chapter 4). Furthermore, caudate nucleus volume does not show a decrease with age as in typical development, indicating a differential developmental trajectory for striatal grey

matter (chapter 5). In our adult sample, volumes in autism are similar to controls, possibly suggesting normalisation of striatal volume (chapter 7).

The development of corticostriatal *white* matter also seems to be deviant in autism, indicating problems in brain connectivity. In children and young adolescents, micro-structural integrity indices (FA and MTR) do not differ between autistic subjects and controls, but trajectory analyses suggest age-related decrease of corticostriatal MTR in autism, while it does not change in controls (chapter 6). These findings could reflect an earlier onset of corticostriatal MTR (myeline) decrease in autism, possibly related to accelerated maturation of corticostriatal white matter in early childhood. Findings from the adult sample indicate that the age-related decrease of micro-structural white matter integrity persists into adulthood and becomes more pronounced: FA values are lower in autistic adults than in controls (chapter 7). Further, regional specificity of white matter maturational patterns seems differential in autism when compared to controls (chapter 6).

The studies in this thesis further show associations between corticostriatal grey and white matter and repetitive behaviour (Insistence on Sameness) (chapters 5 and 6) and related symptomatology (inhibition problems) (chapter 7). However, the specific nature of these relationships is difficult to interpret. Smaller caudate nuclei, as well as higher MTR values are associated with increased repetitive behaviour in children and adolescents; whereas in adults more severe inhibition problems are related to lower FA values.

Limitations

There are some limitations to the studies in this thesis. First, the findings are from cross-sectional data, limiting inferences on development. Studies of normal brain development have shown that it is a dynamic process, associated with both progressive and regressive changes in brain anatomy. However, there is as much or more inter-individual variation in brain anatomy as there is change within individuals over time (Caviness et al. 1996). As a result, developmental changes may be hard to detect in cross-sectional samples. Therefore, especially the studies described in chapters 5 and 6 should be replicated in a longitudinal study.

Second, all studies were performed in samples of high-functioning individuals who met full criteria for autism or Asperger's. As such, this limits the inferences that can be made in terms of other individuals in the autism spectrum. Further, including subjects with the full autistic phenotype might blur neuropathological differences specific to certain symptoms. This issue will be further discussed in the paragraph *New questions: Overcoming heterogeneity in autism* (page 136) in this chapter.

Third, with respect to the studies described in chapters 6 and 7: both FA and MTR are only indirect measures of white matter integrity. As such, any results from these measures should be interpreted with caution, as other factors may contribute to MTR and FA signal (for example neuron loss, consequent myelin loss, changes in glial tissue, altered water content, changes in phospholipid metabolism (Silver et al. 1997)). A further limitation to the studies in chapters 6 and 7 is that DTI fiber-tracking requires sufficient directional information to successfully reconstruct the fibers. If, at a certain point, this information is not available (for

instance, due to crossings with other fibers), then the algorithm cannot reconstruct the complete fiber tract.

The future

Two key terms emerge from the studies presented in this thesis: brain networks and development. Furthermore, our studies highlight to address clinical heterogeneity as well as complexity in interpreting brain-behaviour relationships. In the following paragraphs these issues will be touched upon.

New questions: Brain networks

Studies of the normal brain have shown that the brain should be considered a multi-network system, rather than a collection of separable structures. These findings from normative brain development have started to re-direct autism research. Focus has begun to move from investigating separate cortical or sub-cortical regions to studying networks of brain regions, including their connecting white matter tracts. Although a lesion model is experimentally convenient, it is likely to be inappropriate to the study of autism where dysfunction comprises complex networks of anatomically and functionally distant areas (Baron-Cohen and Belmonte 2005). An emerging theory in autism for example is that short-range connections may be overgrown, whereas longer-range connections between different brain lobules are reduced (Geschwind 2009). Furthermore, several recent studies have begun to identify additional problems of connectivity in autism (Catani et al. 2008; Cherkassky et al. 2006; Koshino et al. 2005; Pugliese et al. 2009; Sundaram et al. 2008); the studies described in chapters 6 and 7 of this thesis are examples.

Taken together, these results highlight that problems of connectivity in autism deserve attention. However, this also raises new questions. For example, is structural MRI suited to investigating the disconnected autistic brain or do we need to change our methods? Furthermore: Are 'networks' specific enough; in other words: does a re-focus from structure to network not imply that studies are simply becoming more vague, losing spatial resolution?

Structural and functional connectivity

In neuroimaging, structural and functional connectivity can be distinguished. In this thesis, the focus has been on structural connectivity: using DTI, the integrity of connecting white matter tracts was examined and compared with controls. With DTI, it is possible to identify structural integrity and volume of specific tracts, allowing for inferences about the structural connectivity in the circuit of interest. However, DTI does not permit conclusions on the functionality of a network. To use an analogy: DTI can visualise the subway-tracks between stations-of-interest, but it does not inform us on the frequency of trains travelling the tracks.

Functional connectivity can be measured using techniques such as functional MRI (fMRI), electroencephalography (EEG)/evoked potentials, magnetoencephalography (MEG) and

resting state fMRI. In functional connectivity analyses, functional networks are inferred from activation patterns of brain regions during task execution or rest. To return to the subway analogy: functional connectivity analyses can visualise the temporal pattern of trains arriving at different stations or the number of people at the stations, allowing for inferences about which stations are connected. However, no conclusions can be drawn about the *actual* tracks; stations can be functionally connected without a direct structural connection between them.

In conclusion, structural and functional connectivity provide different, but complementary information on networks in the brain. Combining both types of connectivity (by combining DTI and fMRI; or by using the new technique functional DTI (Mandl et al. 2008) for example) can provide additional information on brain structure and organisation of brain functions compared to traditional MRI methods (Olesen et al. 2003).

From structure to network: More than less specific?

Although the emphasis on networks and connectivity seems promising for unravelling pervasive disorders such as autism, it can be argued that shifting the focus from 'structures' to 'networks' only makes matters less specific. At this time, problems with functional connectivity in autism implicate gross and general networks (e.g. 'within the frontal lobe' or 'in fronto-striatal circuitry'), and structural connectivity studies have reported widespread white matter abnormalities (Barnea-Goraly et al. 2004; Ke et al. 2009; Keller et al. 2007; Thakkar et al. 2008). Therefore, the aim for future studies investigating connectivity problems in autism, should be to gain in spatial resolution. Possible - and complementary - ways are the use of fiber-tracking based DTI instead of DTI VBM approaches to examine structural connectivity; and connectivity-based segregation of cortical and subcortical areas (e.g. see (Behrens et al. 2003; Cohen et al. 2009; Di Martino et al. 2008)).

New questions: Development

Work of typical brain development has shown that the brain should not be considered a static entity, but rather a complex of interacting networks which continues to develop throughout childhood and into adulthood. Furthermore, these networks follow distinct developmental trajectories, although they do not so in isolation (Giedd et al. 2008; Lenroot and Giedd 2006; Shaw et al. 2008). Alterations in one network, or changes in the development of one network can have major effects on the development and functionality of other brain areas. This idea has started to win ground in autism research: Recent studies emphasise that it is the time course of brain development rather than the outcome that is most disturbed in autism (Amaral et al. 2008). Findings of altered neurodevelopment in autism, including the results described in chapters 4, 5 and 6 of this thesis, affirm this.

Taken together, this accentuates the importance of acknowledging autism as a developmental disorder. However, this also raises new questions. For example: what does 'altered development' mean? Does it imply slower or faster development, but with a similar result; or does it imply an a-typical trajectory as well as outcome? Furthermore: Which neurobiological mechanisms are involved in this abnormal development, and what are the

best methods to examine these mechanisms?

Some recent studies have started to examine brain development in autism, see for example chapters 4 and 5. However, for a more complete picture of developmental trajectories of the autistic brain, follow-up studies and replication of findings are needed. The ideal study to explore trajectories of brain development would include a very large sample size (i.e. hundreds of subjects) of well-characterised individuals of both genders, imaged at birth and followed longitudinally at least into late childhood or early adolescence (Amaral et al. 2008). However, the ideal study still awaits to be carried out.

Mechanisms driving developmental differences in autism

One finding that has been replicated and seems accepted is the observation of normative brain size at birth, followed by a rapid growth from 2 to 4 years in autism compared to typical children (Courchesne et al. 2003). What remains unknown, is what drives the accelerated brain growth: an increased rate of normal neurodevelopmental processes or entirely abnormal processes unique to the disorder (Amaral et al. 2008). Increased brain growth could indicate various causes such as disturbances in the elaboration of dendritic and axonal processes, the selective elimination of neuronal processes, and programmed cell death during brain development and later deficiency of normal neuronal pruning (Courchesne et al. 2001; Courchesne et al. 2005). Some reports have indicated that the early overgrowth of brain volume is disproportionately accounted for by increased white matter (Courchesne et al. 2001; Hazlett et al. 2005) and recent work investigating developmental trajectories of white matter (Ben Bashat et al. 2007), including the findings as described in chapters 6 and 7 of this thesis, provides evidence for this suspected acceleration of white matter maturation in autism.

The exact mechanisms behind accelerated brain growth remain unclear and additional research is needed to address these issues. Neuroimaging is not the only method to do this: whereas MRI provides a reliable method for studying gross neuropathology in a large number of subjects over time, post-mortem techniques provide a tool for understanding the underlying neurobiology of observed neuroanatomical abnormalities (Amaral et al. 2008). Other promising work comes from the fields of biochemistry and genetics. For example recent advances in autism genetics are supportive of a novel view of genetic involvement in autism in which many independent, individually rare genetic variants, together explain a large proportion of autism cases. Although these rare variants impact diverse genetic pathways, there is accumulating evidence that synaptic pathways, including those involving in synaptic maturation, connectivity and stabilisation, are disrupted in autism (see Betancur et al. 2009 and Bourgeron 2009 for a review). Furthermore, expression of other genes that have been implicated in autism, is dependent on postnatal neuronal activity (Morrow et al. 2008; Walsh et al. 2008). From this, it has been suggested that neuronal defects of (subgroups of) autism patients are likely tied to postnatal developmental stages that depend on synaptic activity and activity-dependent changes (Walsh et al. 2008).

New questions: Overcoming heterogeneity in autism

Current diagnostic criteria for autism define a clinically variable phenotype (see chapter 1, Table 1). It has become established that the clinical heterogeneity cannot be characterised by one united underlying neurobiology, but rather results from deficits in various neural networks, each with unique phenotypical features (Happe et al. 2006). From this follows that studying neurobiological differences in the full phenotype might mask differences specific to certain symptoms. One approach to overcome blurring factors introduced by studying the full phenotype is confining research to biological substrates that are associated with only part of the diagnostic criteria. Examples are the studies described in this thesis, where we have investigated involvement of corticostriatal structures and circuitry in the development of one of the defining symptom clusters of autism, stereotyped, repetitive and restricted behaviours.

A second approach could be to confine research to studying more homogeneous subgroups, defined by clustering subjects with similar phenotypes. More clear-cut pathology might be expected from future research comparing individuals with distinct phenotypes (e.g. high-scores on repetitive behaviour) with controls (Amaral et al. 2008).

Third, as evidence for etiological overlap between psychiatric disorders builds, it follows that disorders might better be described as domains of disorder-related traits rather than separate categories (Kas et al. 2007). Therefore, for research, a rethink of diagnostic classification might be necessary to better understand the origin of the diverse core symptoms of autism. This would entail research across DSM-IV categories: Instead of focusing on features that distinguish disorders from one another, put attention to symptoms that connect them.

New questions: Interpreting brain - behaviour relationships

Repetitive behaviour: Aetiology

As was briefly mentioned in the previous paragraph, the phenomenology and neurobiology of human repetitive behaviour has been studied from many different perspectives, but has often been limited to distinct conditions in which these phenomena occur. In chapter 3, we have aimed to synthesise findings across disparate syndromes and proposed a model for classifying repetitive behaviours by their anatomical correlate. However, we stressed that knowing their neuroanatomical substrate does not explain the origin of repetitive behaviours. Future research could address this issue, where a speculative hypothesis (amongst many) of the aetiology of repetitive behaviours could be that in certain cases repetitive behaviour is a result of impaired automating processes or habit formation, functions typically linked to striatum (Graybiel 1998; Packard and Knowlton 2002).

Cause or effect

A long debated topic in neuroscience is whether alterations in neuro-anatomy in psychiatric disorders are causative for the observed psychopathology or are merely a consequence of disturbed behaviour. Although findings of abnormal brain anatomy often tend to

be interpreted as prompting psychiatric problems, one must be careful when drawing such conclusions. For example, our finding of reduced FA in tracts connecting putamen and cortical regions (chapter 7) could be interpreted as causative for the observed symptomatology of the autistic subjects where reduced white matter integrity reflects sub-optimal feedback to the cortex, resulting in dysfunctional repetitive behaviour. On the other hand, it is also likely that years of altered neural activity in this circuit affects myelination of the corticostriatal tracts; in this case reduced FA would be secondary to another neural problem (e.g. disturbed development of striatal grey matter). This would also explain why findings of decreased white matter micro-structural integrity are more prominent in adult patients when compared to children. The answer to the question *what drives what* is likely not the one or the other. Findings from various fields show that brain functioning is as dynamic as the brain itself; constantly changing and maturing, driven by internal processes, thereby influenced by interactions with the environment. Future longitudinal MRI studies may help to address this issue.

Concluding remarks

In conclusion, this thesis

- Implicates changed development of corticostriatal grey and white matter in autism, especially in the repetitive behaviour which characterises the disorder.
- Emphasises the dynamics of the brain in autism: it is the time course of brain development rather than the outcome that seems to be most disturbed.
- Emphasises that the brain needs to be considered a complex of inter- and intra-communicating networks in constant interaction with the environment, rather than a collection of isolated structures or regions.
- Highlights the need for research strategies that take the heterogeneity of autism into account: Narrow the field of view by reclassifying autism as more homogeneous and well-phenotyped symptom-groups.
- Highlights the need for research strategies that take the etiologic overlap with other disorders into account: Broaden the field of view by including other diagnostic groups with similar symptomatology.

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Repetitief gedrag in autisme in beeld: Nederlandse samenvatting

S

Inleiding

Autisme is een ernstige kinderpsychiatrische ontwikkelingsstoornis die gekenmerkt wordt door 1) tekortkomingen in de wederkerige sociale interactie en communicatie, 2) een abnormale ontwikkeling en gebruik van taal en 3) rigide en stereotype gedragspatronen en beperkte interesses (zie ook Tabel 1). Voor een officiële diagnose 'autisme' moet een patiënt gedragskenmerken uit alledrie deze kerngebieden hebben. Autisme is één van de autisme spectrum stoornissen, waartoe ook het syndroom van Asperger en PDD-NOS behoren.

Cijfers over het voorkomen van autisme en aanverwante stoornissen variëren, maar op dit moment wordt de prevalentie van de autisme spectrum stoornissen op 1:150 geschat. Uitgesplitst voor de verschillende diagnoses is dit 1:480 voor autisme, 1:1.600 voor Asperger en 1:270 voor PDD-NOS. In Nederland wordt geschat dat ca. 25.000 kinderen lijden aan één van de autisme spectrum stoornissen; cijfers voor volwassenen zijn niet bekend. Autisme spectrum stoornissen komen ongeveer vier keer vaker voor bij jongens dan bij meisjes.

Vanaf de eerste beschrijvingen van autisme werd er vanuit gegaan dat het om een aangeboren aandoening gaat, hoewel een tijdje ook verklaringen zoals een verstoorde ouder-kind relatie in zwang zijn geweest. Tegenwoordig is men het erover eens dat autisme een psychiatrische aandoening is met een neurobiologische oorsprong en met een grote erfelijke component. Desondanks zijn er tot op heden geen duidelijke biologische 'markers' gevonden: autisme wordt nog steeds gedefinieerd op basis van gedragskenmerken.

Om tot zulke 'biomarkers' te komen, maar vooral om meer inzicht te krijgen in wat autisme veroorzaakt, is het belangrijk om systematisch de neurobiologie van autisme in kaart te brengen. Met dit doel wordt wereldwijd veel onderzoek gedaan. Eén van die lijnen van onderzoek is *neuroimaging*: het gebruik van beeldvormende technieken (zoals MRI) om de anatomie en functie van de hersenen beter te leren begrijpen. Uiteindelijk zal meer kennis van de onderliggende mechanismen die leiden tot autisme een belangrijke bijdrage vormen aan het beter begrijpen, diagnosticeren en behandelen van de stoornis.

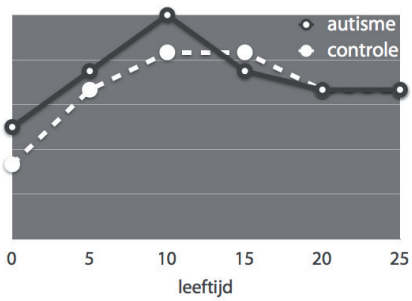
In de afgelopen 15 jaar heeft de wetenschap geprobeerd om afwijkingen in de hersenen die autistische gedragspatronen zouden kunnen verklaren op te sporen bij mensen met autisme. Het voert te ver om alle bevindingen van eerdere onderzoeken te beschrijven, maar één herhaaldelijk aangetoond resultaat is dat mensen (met name jonge kinderen) met autisme een groter hersenvolume hebben dan mensen zonder autisme (controle proefpersonen). Verbazingwekkend genoeg heeft al dit onderzoek verder weinig duidelijke *algemene* kenmerken opgeleverd: bevindingen lijken elkaar soms zelfs tegen te spreken.

Een oorzaak hiervoor zou kunnen liggen in het feit dat autisme een erg *heterogene* stoornis is: de ene patiënt verschilt enorm van de andere en gedrag dat bij de ene persoon zeer sterk aanwezig is, kan bij een ander weinig of zelfs niet voorkomen. Het is te verwachten dat deze verschillen in gedrag samenhangen en zelfs bepaald worden door een even grote variatie in de neurobiologie (zoals hersenanatomie). Onderzoeken naar algemene kenmerken die opgaan voor alle mensen met autisme lijken daarom niet zo zinnig. Naast op zoek te gaan

naar algemene verschillen in de hersenen van mensen met autisme wordt dan ook steeds meer gericht gekeken naar afwijkingen die samenhangen met specifieke gedragingen van autisme. Er wordt bijvoorbeeld gekeken naar verschillen in hersengebieden die betrokken zouden kunnen zijn bij de drie kern symptoomgebieden die aangedaan zijn (sociale interactie en communicatie, taal, en repetitief en rigide gedrag) van autisme.

Tabel 1. Voorbeelden van gedragingen behorend bij de kernsymptomen van autisme. De donkergedrukte voorbeelden zijn typerend voor repetitieve, rigide en stereotype gedrag.

- *problemen met non-verbale communicatie zoals oogcontact, gezichtsuitdrukkingen en lichaamshouding*
- *weinig of geen interesse in leeftijdgenoten; problemen in het ontwikkelen van vriendschappen met leeftijdgenoten*
- *minder of geen spontaan delen van plezier, interesses, of prestaties met andere mensen*
- *afwijkend spel (niet meedoen met groepsspelletjes, geen of beperkt fantasiespel, geen imitatie)*
- *moeite met sociale afspraken (bv. fluisteren in de bioscoop, niet in alle kamers kijken wanneer je ergens op bezoek bent)*
- *vertraagde ontwikkeling van taal, of helemaal geen ontwikkeling van gesproken taal*
- *problemen met het beginnen of onderhouden van een gesprek met anderen (praat bv. alleen over eigen interesses, houdt monologen, geeft de ander geen ruimte in het gesprek)*
- *stereotype of herhaald gebruik van taal (herhaalt bv. zinnen uit films of reclames, of papegaait stukken zin die letterlijk van anderen zijn opgevangen), of eigenaardig taalgebruik (bv. erg formeel of ouwelijk)*
- ***hardnekkige preoccupatie met gedeeltes van objecten (bv. alleen spelen met de wieltjes van een auto, of de mens-erger-je-niet pionnen alleen sorteren en op een rijtje leggen)***
- ***ongewoon sterke belangstellingen/ buitensporige interesses (bv. in lichtknopjes, cijfers, of bepaalde onderwerpen zoals sterrenstelsels, treinen)***
- ***grote moeite met met veranderingen of onverwachte gebeurtenissen (bv. een kerstboom in de woonkamer, vader een nieuwe bril, of geen zwemles op woensdagmiddag omdat de juf ziek is)***
- ***sterke neiging tot rituelen (bv. per se om 7:04 tandenpoetsen, of in bepaalde volgorde aankleden)***
- ***stereotype en repeterende lichaamsbewegingen (zoals fladderen of draaien met de handen, of complexe bewegingen met het hele lichaam maken)***
- *afwijkende motoriek en bewegingen (houterig, op de tenen lopen)*
- *ongewone reacties op zintuiglijke prikkels, bv. overdreven gevoelig (overstuur raken van geluiden of geuren die anderen als normaal ervaren), geen reactie (verminderde gevoeligheid voor kou of pijn), of fascinatie (steeds ruiken aan dingen, eindeloos lang kijken naar schaduwen op de muur)*



Figuur 1. Fictieve voorstelling van het ontwikkelingstraject van bv. hersenvolume bij een autisme- en een controlegroep. De leeftijd waarop de meting plaatsvindt is hier bepalend voor de interpretatie van de resultaten (volume is kleiner, gelijk of groter in de autisme-groep, afhankelijk van de leeftijd). Deze figuur illustreert het belang van het in acht nemen van ontwikkelingsfase bij het doen van onderzoek en het interpreteren van onderzoeksresultaten.

Dit proefschrift: aandachtspunten

In het verleden is met name aandacht besteed aan de kerngebieden sociale interactie en communicatie en taal; (de neurobiologie van) repetitief en rigide gedrag is relatief onderbelicht gebleven. Dit is vreemd, aangezien (1) dit gedrag een zeer grote impact heeft op het leven van de patiënt en zijn/ haar omgeving en (2) er uit andere ziektebeelden al aanwijzingen waren voor hersengebieden die betrokken zouden zijn bij dit gedrag.

Daarom hebben wij in dit proefschrift de neurobiologie van repetitief en rigide gedrag in autisme nader bekeken. Met name de hersenanatomie heeft hierbij onze aandacht gehad. We hebben hiervoor gebruik gemaakt van beeldvormende technieken zoals structural magnetic resonance imaging (sMRI), diffusion tensor imaging (DTI) en magnetisation transfer imaging (MTI).

Met deze structurele neuroimaging technieken hebben wij geprobeerd te beantwoorden *of*, *waar* en *hoe* klinische kenmerken van autisme zichtbaar zijn in de hersenanatomie. Uit de literatuur is echter bekend dat verschillende factoren, zoals (1) medicatie-gebruik en (2) leeftijd/ ontwikkelingsfase van de deelnemers in de steekproef, de resultaten kunnen beïnvloeden. Er wordt verondersteld dat deze factoren ook een oorzaak zijn van de inconsistentie in resultaten van verschillende MRI-studies naar autisme.

Van verschillende typen medicatie die voorgeschreven worden aan mensen met autisme is bijvoorbeeld bekend dat deze de grootte van bepaalde hersengebieden kunnen beïnvloeden: de gebieden worden kleiner bij medicatie-gebruik. Wanneer in een onderzoek een groep mensen met en een groep mensen zonder autisme vergeleken wordt en dit type medicatie alleen door mensen in de autisme groep gebruikt wordt, is het niet meer mogelijk om te onderscheiden of bepaalde verschillen in het brein veroorzaakt worden door (1) de ziekte of (2) de medicatie.

Wat betreft ontwikkelingsfase is inmiddels bekend dat de hersenanatomie zich ontwikkelt met het ouder worden. Het aantal cellen en verbindingen in de hersenen verandert en dit wordt o.a. gereflecteerd in de grootte (of beter: het *volume*) van de hersenen. Over het algemeen wordt aangenomen dat het brein doorgroeit tot een bepaalde leeftijd om na die 'piek' weer in volume af te nemen. Er is echter geen sprake van een algemeen ontwikkelingspatroon voor het totale brein: het traject van hersengroei en -krimp met leeftijd verschilt per gebied. Sommige gebieden blijven lang doorgroeien en bereiken hun piek bijvoorbeeld pas na de puberteit, terwijl andere gebieden al in de kinderjaren hun piek bereiken om vanaf die leeftijd alweer af te nemen. Daarnaast is het ontwikkelingspatroon anders voor de grijze stof (met daarin voornamelijk *neuronen*, of zenuwcellen) en de witte stof (met daarin voornamelijk met myeline beklede axonen, de paden waarover de informatieoverdracht tussen neuronen plaatsvindt). Het is onduidelijk of de hersenontwikkeling van mensen met autisme hetzelfde verloopt als bij mensen zonder autisme. Wanneer hier verschillen in zijn, kunnen verschillende resultaten gevonden worden, al naar gelang de leeftijd van de groepen die bekeken worden (zie ook Figuur 1). Daarom is het belangrijk leeftijd of ontwikkelingsfase mee te nemen in het onderzoek.

Ten derde heeft eerder onderzoek naar de structuur, maar met name ook de functie van de hersenen laten zien dat functies en gedrag (en afwijkingen in gedrag, zoals bij autisme) niet

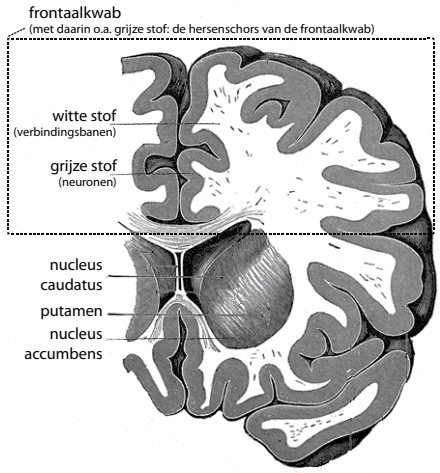
zozeer gelokaliseerd kunnen worden in één bepaald gebied, maar eerder dat netwerken van met elkaar verbonden hersengebieden betrokken zijn bij gedragspatronen.

In de studies die beschreven staan in dit proefschrift hebben wij extra aandacht besteed aan deze factoren door personen te onderzoeken die medicatie-vrij waren of zelfs nooit medicatie gebruikt hadden (ook wel: *medicatie-naïef*), of door in de statistische analyses te corrigeren voor medicatie-gebruik. Verder hebben wij specifiek naar ontwikkelingstrajecten van hersengebieden gekeken en ons daarnaast niet alleen gericht op de afzonderlijke gebieden, maar ook op de *netwerken* die verondersteld worden betrokken te zijn bij repetitief gedrag in autisme.

Samenvatting van de studies (hoofdstukken 2 t/m 7)

In de hoofdstukken 2 en 3 is een theoretische inleiding gegeven op het fenomeen 'repetitief gedrag' en is de neurobiologie van deze gedragingen besproken. Repetitief, rigide en stereotiep gedrag is niet specifiek menselijk, maar ook een zeer veel voorkomende vorm van afwijkend gedrag in dieren. Dieronderzoek heeft een grote bijdrage geleverd aan de kennis over de neurobiologische mechanismen die betrokken zijn bij repetitief gedrag en vormt daarmee een mooie opstap naar het begrijpen van de mechanismen achter repetitief gedrag (ofwel de *pathofysiologie*) in psychiatrische stoornissen zoals autisme.

Daarom is in **hoofdstuk 2** een overzicht gegeven van fundamenteel en toegepast dier-onderzoek naar de neurobiologische bases van repetitief gedrag. In dit hoofdstuk laten we zien hoe gebieden diep in het brein, de basale kernen (en met name een onderdeel daarvan, het striatum) van oudsher een belangrijke rol hebben gespeeld in de verklaringen voor het ontstaan van repetitief en stereotiep gedrag. Deze kernen staan in verbinding met de hersenschors (cortex); het complex van verbindingen tussen het striatum en de cortex wordt ook wel het 'corticostriatale systeem' genoemd. We bespreken het model van de parallelle corticostriatale 'feedback loops' dat sinds de jaren tachtig bepalend is geweest voor ons begrip van het corticostriatale systeem en laten zien hoe de introductie van dit model de functie van de basale kernen her-evalueerde. Tot dat moment werd gedacht dat de basale kernen slechts verbindingen hadden met de bewegingsgebieden van de cortex (de motor cortex) en dat de basale kernen dus alleen betrokken zouden zijn bij motorisch gedrag (bewegingen). Toen het bestaan van meerdere, parallelle loops werd aangetoond die de basale kernen met verschillende, ook niet-motorische gedeelten van de cortex verbinden, begreep men dat het corticostriatale systeem ook betrokken moest zijn bij cognitieve en emotioneel-motivationele gedragsprocessen. Heden ten dage is de opvatting dat het corticostriatale systeem verantwoordelijk is voor planning, selectie en inhibitie van gedrag en voor conditionering en automatisering van gedragingen. Het systeem wordt verondersteld te bestaan uit naast elkaar bestaande 'loops' waarvan de anatomische organisatie correspondeert met de functionaliteit.



Figuur 2. doorsnede van de hersenen met de belangrijkste structuren van het corticostriatale systeem.

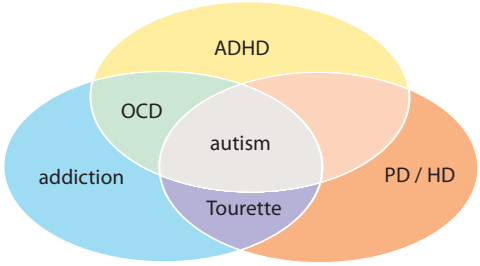
In het vervolg van hoofdstuk 2 laten we aan de hand van (1) studies van dieren in een verarmde omgeving, (2) farmacologische en lesie-studies en (3) genetisch onderzoek zien hoe de werking van het corticostriatale systeem verstoord kan worden en dat dit veelal resulteert in afwijkend, met name repetitief en stereotiep, gedrag. Vervolgens bespreken we verschillende mechanismen die kunnen verklaren waarom disfunctionaliteit van het corticostriatale systeem repetitief gedrag veroorzaakt. We laten echter ook zien dat veel vragen op dit moment nog onbeantwoord blijven en in de concluderende paragraaf van dit hoofdstuk stippen we aan hoe systematisch onderzoek naar repetitief gedrag gehinderd wordt door gebrek aan eenduidige definities en duidelijke fenotypering (het in kaart brengen van de eigenschappen van een persoon). Verder benadrukken we de noodzaak van integratie van bevindingen uit verschillende onderzoeksvelden. Tenslotte bespreken we de uitdaging hoe dier-modellen te vertalen naar complex humaan gedrag. Een oplossing hiervoor zou kunnen zijn af te stappen van het streven naar een model voor de complete stoornis, maar het blikveld te verkleinen naar een *kenmerk* van de stoornis dat beter te vergelijken is tussen dieren en mensen (*shifting focus from complex syndrome studies to inter-species trait studies*).

Hoofdstuk 3 borduurt zoals gezegd voort op de kennis uit de dierstudies van hoofdstuk 2. We verleggen de focus naar humaan repetitief gedrag en stellen de vraag of repetitief gedrag zoals dat voorkomt in de normale ontwikkeling en in verschillende psychiatrische ziektebeelden gemedieerd wordt door vergelijkbare neurobiologische systemen of dat deze gedragingen neurobiologisch uniek zijn. Repetitief gedrag is in veel ziektebeelden een weinig onderzocht fenomeen en bovendien is onderzoek naar repetitief gedrag veelal gedaan vanuit het perspectief van één aandoening. In dit hoofdstuk willen we deze verschillende zienswijzen integreren.

We bespreken achtereenvolgens onderzoeken naar de neurobiologie van repetitief gedrag in obsessieve-compulsieve stoornis, het syndroom van Gilles de la Tourette, autisme en de ziekten van Parkinson en Huntington en komen hiermee tot een model dat de verschillende

soorten repetitief gedrag classificeert op basis van de anatomie. Dit model illustreert bovendien de overlap en de verschillen tussen repetitieve gedragingen in verschillende psychiatrische stoornissen (zie Figuur 3).

Hieruit concluderen we dat voor een beter begrip van psychiatrische beelden het belangrijk is het blikveld zowel te verruimen als te beperken. Te *verruimen* omdat het zeer inzichtelijk is over ziektebeelden heen te kijken en symptomen als dimensies te beschouwen in plaats van ze alleen te bekijken binnen het kader van de categorische diagnose. In andere woorden: niet te kijken naar wat ziektebeelden van elkaar onderscheidt, maar naar wat hen verbindt. Daarnaast zal *beperken* van het blikveld ook zorgen voor een beter begrip. Psychiatrische stoornissen worden over het algemeen gekenmerkt door een grote heterogeniteit. Door niet op zoek te gaan naar één biologisch substraat voor het totale complex van symptomen, maar onderzoek te beperken tot homogene en goed gefenotypeerde subgroepen zal de zoektocht naar de neurobiologie van psychiatrische beelden meer succes opleveren.



Figuur 3. Schematische weergave van hoe gedrag, veroorzaakt door problemen in één van de drie 'macro-circuits' van het corticostriatale systeem, zich kan groeperen tot symptoomclusters die te zien zijn in verschillende psychiatrische and neurologische stoornissen. (ADHD = Attention Deficit Hyperactivity Disorder; OCD = obsessive compulsive disorder; PD = Parkinson's disease; HD = Huntington's disease)

(Zie pagina 169 voor een kleurenweergave van deze figuur.)

In **hoofdstuk 4** keren we terug naar het eerste doel van dit proefschrift: het onderzoeken van de neurobiologische kenmerken, en met name de hersenanatomie, van repetitief en rigide gedrag in autisme. In dit hoofdstuk hebben we met behulp van structurele MRI scans het volume bekeken van de drie structuren van het striatum: de nucleus caudatus, het putamen en de nucleus accumbens. We hebben dit gedaan in twee onafhankelijke steekproeven (één bestaande uit kinderen, één uit adolescenten en jong-volwassenen). Allebei de steekproeven waren opgebouwd uit een autisme- en een controlegroep. Alle deelnemers waren medicatie-naïef. De resultaten lieten in beide steekproeven een vergroting van de nucleus caudatus zien in de autisme-groep. Deze toename in volume werd gevonden bovenop de algemene vergroting van het brein.

Met deze studie wordt dus het belang van het striatum voor autisme gedemonstreerd. Het effect leek groter te zijn in de oudere steekproef (adolescenten en jong-volwassenen), hetgeen suggereert dat de nucleus caudatus betrokken blijft in de etiologie *(de oorzaak) van autisme tijdens de ontwikkeling.*

In **hoofdstuk 5** zijn we dieper ingegaan op de betrokkenheid van de ontwikkeling van het striatum in autisme. In dit hoofdstuk hebben we in één grote steekproef van kinderen, adolescenten en jong-volwassenen met en zonder autisme de ontwikkelingstrajecten vergeleken van hersenvolumes waaronder nucleus caudatus, putamen en nucleus

accumbens. Deze studie toonde verschillen aan in de ontwikkelingstrajecten van de striatale structuren in de autisme-groep. Met name de resultaten in de ontwikkeling van de de nucleus caudatus waren opvallend: het volume van deze structuur nam af met leeftijd in de controlegroep, terwijl het toenam in de autisme-groep. De verschillen in ontwikkeling waren niet toe te schrijven aan algemene verschillen in hersenvolume, of het gebruik van medicatie en waren met name gelokaliseerd in het voorste gedeelte (de *kop*) van de nucleus caudatus. Daarnaast lieten de resultaten zien dat in de autisme groep caudatus volume geassocieerd is met de ernst van repetitief en rigide gedrag.

Deze studie toont dus aan dat de ontwikkeling *van het striatum en in het bijzonder de nucleus caudatus belangrijk is in de etiologie van autisme en met name voor de repetitieve en rigide gedragssymptomen die zo kenmerkend zijn voor deze aandoening.*

In **hoofdstuk 6** richtten we ons niet op de hersen*structuren*, maar op de *netwerken* die betrokken zijn bij repetitief en rigide gedrag. In dit hoofdstuk bestudeerden we specifiek de invloed van het corticostriatale circuit in kinderen (7-14 jaar) met autisme. We maakten in dit hoofdstuk gebruik van diffusion tensor imaging (DTI) en magnetic transfer imaging (MTI). Met deze technieken kunnen maten voor de kwaliteit van de witte stof (de verbindingsbanen) in het brein berekend worden: FA (uit DTI) en MTR (uit MTI). De resultaten in dit hoofdstuk lieten zien dat in de autisme groep sprake is van leeftijdsgerelateerde veranderingen in de corticostriatale witte stof: corticostriatale MTR neemt af met leeftijd, onafhankelijk van totaal witte stof MTR, terwijl er geen veranderingen optreden in de controlegroep. Dit suggereert dat er in deze leeftijdsgroep veranderingen bestaan in de myelinisatie van corticostriatale banen in autisme. Daarnaast toonden we aan dat, terwijl corticostriatale FA onafhankelijk is van FA van de totale witte stof in de controlegroep, corticostriatale FA en MTR juist samenhangen met FA en MTR van de totale witte stof in de autisme groep. Dit suggereert dat op deze leeftijd corticostriatale witte stof ontwikkeling onafhankelijk lijkt te zijn van algemene witte stof ontwikkeling in normaal ontwikkelende kinderen, terwijl deze ontwikkelingsprocessen aan elkaar gerelateerd zijn in kinderen met autisme. Tot slot laten we in dit hoofdstuk zien dat corticostriatale witte stof kwaliteit geassocieerd is met de ernst van repetitief gedrag-symptomen.

Deze studie laat dus zien dat ook in de witte stof van het corticostriatale systeem afwijkingen te zien zijn in autisme; hetgeen gevolgen kan hebben voor de informatie-overdracht in dit systeem. Dit lijkt met name gerelateerd te zijn aan de repetitieve en rigide gedragssymptomen.

In **hoofdstuk 7** zijn we wat dieper ingegaan op de betrokkenheid van corticostriatale netwerken bij repetitief en rigide gedrag in autisme. In dit hoofdstuk gebruikten we sMRI en DTI om eventuele verschillen in striatale volumes en kwaliteit van de corticostriatale banen te detecteren in een steekproef van volwassenen met en zonder autisme. Daarnaast relateerden we de anatomische bevindingen aan maten van repetitief en rigide gedrag en prestaties op een taak die inhibitie (remming) van gedrag meet. Uit de resultaten bleek dat deelnemers met autisme een kleiner volume van totaal witte stof hebben en dat de FA (een maat voor de kwaliteit) van de witte stof van de corticostriatale banen minder was in

de autisme groep. Dit gold in het bijzonder voor de banen die het putamen met de cortex verbinden. Daarnaast presteerden de deelnemers met autisme minder goed op de inhibitie-taak. Slechter presteren op de inhibitie-taak leek gerelateerd te zijn aan een afname van corticostriatale witte stof integriteit, al verdween dit effect bij een analyse in de afzonderlijke groepen.

Deze studie laat dus zien dat ook bij volwassenen de witte stof van het corticostriatale systeem afwijkingen vertoont in autisme. De verminderde kwaliteit van de witte stof lijkt bovendien gerelateerd te zijn aan verstoringen in het vermogen gedrag te inhiberen.

Samengevat laten onze volumetrische structurele MRI studies zien dat er verschillen in *grijze* stof van het striatum bestaan tussen mensen met en zonder autisme, waarbij met name het volume van de nucleus caudatus is vergroot in kinderen, adolescenten en jong-volwassenen met deze ontwikkelingsstoornis (hoofdstuk 4). Daarnaast verschilt het ontwikkelingstraject van deze structuur: caudatus volume neemt af met leeftijd bij controlepersonen in deze leeftijdsgroep, maar niet bij mensen met autisme (hoofdstuk 5). In een derde studie met een volwassen onderzoeksgroep zijn de striatale structuren van mensen met en zonder autisme van vergelijkbare grootte (hoofdstuk 7).

Corticostriatale *witte* stof lijkt ook een afwijkend ontwikkelingspatroon te laten zien in autisme, wat zou kunnen duiden op problemen in hersenconnectiviteit in dit systeem. Er is geen verschil in maten van kwaliteit van de witte stof (FA en MTR) tussen kinderen en jonge adolescenten met en zonder autisme, maar analyses van het ontwikkelingstraject suggereren dat er sprake is van een leeftijdsafhankelijke afname van corticostriatale witte stof integriteit in autisme, terwijl er geen verandering is met leeftijd in de controlegroep (hoofdstuk 6). Deze bevindingen zouden een vroegere start van afname van corticostriatale myeline kunnen reflecteren, eventueel gerelateerd aan een versnelde toename van corticostriatale witte stof in de vroege kindertijd. Resultaten in de volwassen onderzoeksgroep tonen aan dat de leeftijdsafhankelijke afname van witte stof integriteit in de corticostriatale banen doorzet in volwassenheid en prominenter wordt (hoofdstuk 7). Tot slot lijkt de regionale specificiteit van witte stof ontwikkelingspatronen verschillend in autisme in vergelijking met controle-deelnemers (hoofdstuk 6).

De studies in dit proefschrift laten verder associaties zien tussen corticostriatale grijze en witte stof en scores op een repetitief gedragsschaal (in het bijzonder gedragingen die geclusterd kunnen worden als *Insistence on Sameness*, ofwel *Dwingende Weerstand tegen Verandering*) (hoofdstukken 5 en 6) en aanverwante symptomatologie zoals inhibitie van gedrag (hoofdstuk 7). Het dient echter gezegd te worden dat deze resultaten moeilijk te interpreteren zijn. *Kleinere* volumes van de nucleus caudatus, evenals *hogere* corticostriatale MTR waarden zijn geassocieerd met een *toename* van repetitief gedrag in kinderen en adolescenten, terwijl in volwassenen *ernstigere* inhibitie-problemen samenhangen met *lagere* FA waarden.

Beperkingen

Natuurlijk kleven er beperkingen aan de studies in dit proefschrift. In de verschillende hoofdstukken is hier uitgebreid op in gegaan; algemene punten van aandacht worden hier samengevat. Allereerst moet vermeld worden dat voorzichtigheid geboden is bij het trekken van conclusies over *ontwikkeling* uit cross-sectionele steekproeven. De studies in hoofdstukken 6 en 7 zouden daarom zeker gebaat zijn bij een replicatie met een longitudinale studieopzet.

Daarnaast hebben de deelnemers in alle onderzoeken in dit proefschrift een gemiddelde of boven-gemiddelde intelligentie, terwijl bekend is dat bij een groot deel van de autisme-populatie sprake is van laag- of zwakbegaafdheid. Hier dient rekening mee gehouden te worden bij het generaliseren van onze resultaten.

Ten derde resulteren neuroimaging methoden slechts in een indirecte afspiegeling van de daadwerkelijke anatomie of functie van de hersenen. Allereerst hebben hardware en software voor acquisitie van de scans en (pre-)processing van de data allen hun bijdrage aan het uiteindelijke resultaat. Daarnaast is er de factor van interpretatie van de data. Met name wat betreft de technieken zoals toegepast in de hoofdstukken 6 en 7 (DTI en MTI) is nog niet geheel bekend wat de maten die hiermee berekend kunnen worden (FA en MTR) nu echt betekenen. Over het algemeen worden deze parameters beschouwd als indirecte maten van de kwaliteit of integriteit van de witte stof, waarbij FA voornamelijk informatie over de richting van de witte stof-banen zou geven en MTR een index van de aanwezigheid van macro-moleculen (waaronder myeline) zou zijn. Dat ook andere factoren een bijdrage leveren aan FA en MTR en daarmee aan de resultaten dient echter niet uit het oog verloren te worden.

De toekomst: nieuwe vragen

Uit de studies van dit proefschrift komen twee kernbegrippen naar voren: (brein-) netwerken en ontwikkeling. Daarnaast benadrukken onze studies het belang van het in acht nemen van de klinische heterogeniteit van autisme, en de uitdagingen die kleven aan het interpreteren van brein-gedrag relaties. In de volgende paragrafen worden deze aandachtspunten kort besproken.

Nieuwe vragen: brein netwerken

Studies van normale brein ontwikkeling hebben laten zien dat het brein gezien moet worden als een multi-netwerk systeem, en niet als een verzameling van losse structuren. Deze bevindingen sijpelen ook steeds meer door in het autisme-onderzoek en hebben de focus van het autisme- onderzoek verlegd van het bestuderen van de afzonderlijke corticale en subcorticale structuren naar onderzoek naar de netwerken in het brein, inclusief de verbindende witte stof-banen. Uit deze recente onderzoeken blijkt inderdaad dat ook bij autisme problemen in de verbindingen van het brein lijken te bestaan, de studies in de hoofdstukken 6 en 7 zijn hier voorbeelden van. Dit roept echter ook weer nieuwe vragen op, zoals: is structurele MRI wel een goede techniek om connectiviteitsproblemen in het brein

te onderzoeken of moeten we onze methoden aanpassen? En: is het begrip 'netwerken' wel specifiek genoeg? Verliezen we niet aan spatiële resolutie door de focus te verleggen van hersenstructuren naar -netwerken?

Structurele en functionele connectiviteit

In neuroimaging kunnen de begrippen *structurele* en *functionele* connectiviteit onderscheiden worden. In dit proefschrift ligt de nadruk op structurele connectiviteit: met behulp van DTI is de integriteit van witte stof-banen onderzocht. Met DTI kunnen uitspraken gedaan worden over de aanwezigheid van verbindingspaden (structurele connectiviteit), maar niet over de functionaliteit van deze verbindingen (functionele connectiviteit). Om een analogie te gebruiken: met DTI kunnen de rails tussen de stations in een metronetwerk in beeld gebracht worden, maar het geeft ons geen informatie over de frequentie waarmee er treinen over de rails rijden of over hoeveel mensen er gebruik maken van het netwerk.

Functionele connectiviteit kan gemeten worden met technieken zoals functionele MRI (fMRI), electroencephalografie (EEG), magnetoencephalografie (MEG) en resting state fMRI. In functionele connectiviteitsanalyses wordt de organisatie van functionele netwerken afgeleid uit activatiepatronen in het brein tijdens een taak of in rust. Om terug te keren naar de metro-analogie: met functionele connectiviteitsanalyses kan de fluctuatie met de tijd weergegeven worden van treinen die aankomen op een station, of van het aantal mensen op een station. Op basis hiervan kunnen uitspraken gedaan worden over welke stations met elkaar verbonden zijn. Er kunnen echter geen conclusies getrokken worden over de daadwerkelijke metrosporen: stations kunnen met elkaar in verbinding zijn, zonder dat er een directe lijn loopt van de één naar de ander.

Concluderend kunnen we stellen dat structurele en functionele connectiviteitsanalyses verschillende, maar elkaar aanvullende typen informatie geven over netwerken in het brein. Het combineren van beide typen connectiviteitsanalyses (bijvoorbeeld door DTI en fMRI te combineren, of door de zeer recente techniek functionele DTI (fDTI) te gebruiken) belooft van toegevoegde waarde te zijn bij het verder ontrafelen van de problemen in hersenstructuur en -functie bij autisme en in het bijzonder het aandeel van netwerkproblemen.

Van structuur naar netwerk: meer dan minder specifiek?

Hoewel de nadruk op hersennetwerken en -connectiviteit een krachtige stimulans lijkt te zijn voor het onderzoek naar de neurobiologische bases van complexe psychiatrische stoornissen zoals autisme, kan ook gezegd worden dat het verleggen van de focus van structuren naar netwerken de zaak vooral minder specifiek maakt. Tot op heden leggen resultaten van structurele en functionele connectiviteits-studies zeer algemene defecten in wijdverspreide en slecht gedefinieerde netwerken bloot. Toekomstige studies zouden gebaat zijn de spatiële resolutie te verfijnen, bijvoorbeeld door gebruik van technieken zoals tract-based DTI (in plaats van voxel-based methoden) en connectivity-based differentiatie van corticale en sub-corticale structuren.

Nieuwe vragen: ontwikkeling

Studies van 'het normale brein' hebben ook laten zien dat de hersenen geen statische entiteit zijn, maar een complex van interacterende netwerken dat zich tot op volwassen leeftijd blijft ontwikkelen. Daarnaast is aangetoond dat deze netwerken afzonderlijke ontwikkelingstrajecten volgen, maar elkaar daarbij wel blijven beïnvloeden. Ook in het onderzoek naar autisme wordt dit idee van het dynamische, zich ontwikkelende brein steeds meer gemeengoed: niet zozeer het eindproduct, maar het tijdspad van hersenontwikkeling lijkt het meest verstoord bij autisme. Studies zoals de hoofdstukken 4, 5 en 6 van dit proefschrift onderschrijven dit.

De nadruk op ontwikkeling triggert echter ook nieuwe vragen. Bijvoorbeeld: wat betekent 'veranderde ontwikkeling' eigenlijk? Is de ontwikkeling sneller of langzamer, maar met een vergelijkbaar eindresultaat; of verschillen zowel ontwikkelingstraject als eindpunt bij autisme? Daarnaast: welke neurobiologische processen zijn betrokken bij en worden beïnvloed door deze veranderde ontwikkeling, en hoe kunnen wij dit het beste onderzoeken?

Mechanismen achter de verschillen in hersenontwikkeling bij autisme

Eén bevinding die vaker gerepliceerd is en inmiddels algemeen geaccepteerd wordt, is het veranderde ontwikkelingstraject van de algemene hersengrootte bij autisme. In vergelijking met kinderen zonder autisme wordt dit traject bij autisme gekenmerkt door een normaal volume bij geboorte, gevolgd door een versnelde groei in grootte tussen 2 en 4 jaar. Wat onduidelijk is, is wat deze snelle groei stuurt: een veranderd tempo van verder normale ontwikkelingsprocessen, of een totaal ander proces, specifiek voor de stoornis?

Een toename in hersenvolume zou kunnen duiden op verstoringen in verschillende neuronale processen. Om de veranderingen in hersenontwikkeling beter te begrijpen, verdient het de aandacht deze processen uitgebreider te onderzoeken. Neuroimaging is niet de enige manier om dit te doen: hoewel sMRI een betrouwbare methode is voor het bekijken van grove neuropathologie in groepen personen, vormen bijvoorbeeld post-mortem technieken een passend instrument om de onderliggende neurobiologie van de geobserveerde neuroanatomische bijzonderheden te bestuderen. Biochemisch en genetisch onderzoek zijn andere manieren.

Nieuwe vragen: de klinische heterogeniteit van autisme

De huidige diagnostische criteria voor autisme definiëren een klinisch variabel *fenotype* (het totaal van eigenschappen). Men is er inmiddels van overtuigd dat een dusdanig heterogeen beeld niet gekarakteriseerd kan worden door één onderliggende neurobiologie, maar eerder het resultaat is van afwijkingen in verschillende neurale netwerken, elk met unieke fenotypische kenmerken. Hieruit volgt dat bij het bekijken van het totale fenotype, neurobiologische verschillen die specifiek behoren bij een bepaald symptoom gemaskeerd en dus niet opgemerkt kunnen worden.

Één manier om dit te voorkomen is om onderzoek te beperken tot biologische substraten die geassocieerd zijn met een duidelijk omschreven deel van de klinische variatie. De studies in dit proefschrift zijn hier een voorbeeld van: wij hebben ons specifiek gericht op de betrokkenheid van het corticostriatale circuit bij de repetitieve en rigide gedragssymptomen van autisme.

Een tweede manier kan zijn het onderzoek te beperken tot meer homogene en goed-getypeerde subgroepen, gedefinieerd door personen met een vergelijkbaar fenotype te clusteren.

Een derde manier om het vertroebelend effect van het bestuderen van een heterogeen fenotype te minimaliseren, volgt uit recent werk waaruit blijkt dat er veel etiologische overlap bestaat tussen psychiatrische aandoeningen. Het lijkt daarom waardevol onderzoek niet te laten beperken door de grenzen van de huidige diagnostische classificaties, maar juist op zoek te gaan naar overlappende kenmerken van verschillende aandoeningen.

Concluderende opmerkingen

Dit proefschrift

- Laat zien dat een veranderde ontwikkeling van corticostriatale grijze en witte stof een rol speelt bij autisme en in het bijzonder bij het repetitieve en rigide gedrag waardoor deze ontwikkelingsstoornis gekenmerkt wordt.
- Toont de dynamiek van autisme aan: niet zozeer het eindproduct, maar het traject van hersenontwikkeling lijkt het meest verstoord. Dit vraagt om toekomstig onderzoek dat in het bijzonder aandacht besteedt aan ontwikkeling, bij voorkeur met een longitudinale studie-opzet.
- Benadrukt dat de hersenen niet beschouwd moeten worden als een ongedifferentieerde statische entiteit of als een verzameling van geïsoleerde structuren en regio's, maar als een complex van netwerken waarbinnen en waartussen uitgebreide informatie-uitwisseling plaatsvindt.
- Benadrukt dat er belang bij is in toekomstige onderzoeksstrategieën de klinische heterogeniteit van autisme in acht te nemen door het blikveld te verkleinen: het onderzoek te beperken tot homogene en goed gekarakteriseerde subgroepen.
- Benadrukt dat er belang bij is in toekomstige onderzoeksstrategieën de klinische heterogeniteit van autisme in acht te nemen door het blikveld te verruimen: het onderzoek uit te breiden naar andere ziektebeelden met een overlappende symptomatologie.

Dankwoord

Wetenschappelijk schrijven blijft een vreemd gebeuren: waarde-oordelen en sentimenten zijn uit den boze; beweringen worden afgezwakt of juist opgepoetst en de werkelijkheid wordt pas waarheid wanneer anderen haar ook gezien hebben. In dit hoofdstuk wordt het juk van de wetenschap even afgelegd; de emotie leidt de ratio, de superlatieven komen uit de kast en een referentielijst ontbreekt: mijn werkelijkheid is voor even de waarheid!

Dit proefschrift is niet alleen mijn verdienste, ik ben veel verschuldigd aan

alle deelnemers:

Jongens en meiden die mij letterlijk en figuurlijk een kijkje in hun hoofd gunden: ontzettend bedankt voor jullie vrolijkheid, dapperheid en doorzettingsvermogen. Jullie hebben me vaak positief verrast en verwonderd en hebben ervoor gezorgd dat de fase van dataverzameling bijzonder en waardevol was.

Betrokken, enthousiast en belangeloos; deze begrippen zijn typerend voor de **ouders** die hebben meegewerkt aan de verschillende studies in dit proefschrift. Zeer veel dank voor Uw inzet en tijd; zonder U was dit een heel dun boekje geworden!

mijn promotor, Herman van Engeland:

Herman: Van 'Professor van Engeland' werd je 'Herman' en van 'de promotor' werd je *'mijn* promotor'; waar een gezellig diner in Londen al niet goed voor is! Ik verbaas me iedere keer weer over de snelheid waarmee je nieuwe resultaten weet te integreren in het grote geheel en ik waardeer je betrokkenheid, humor, warmte en oog voor andere belangrijke zaken in het leven. Ik ben blij en trots dat ik nog in het rijtje van 'jouw' AIO's sta!

mijn beide co-promotoren, Sarah Durston en Wouter Staal:

Sarah: American working-spirit, British meticulousness en Hollandsche nuchterheid: jij hebt het allemaal, fantastisch. Ik heb ongelooflijk veel van je geleerd de afgelopen 4 jaar en als toetssteen ben je ontzettend belangrijk voor me geweest. Het was een voorrecht om vanonder jouw vleugels mijn eerste stappen in de wondere wereld van de wetenschap te zetten.

Wouter: Jouw doortastendheid ("We gaan nu meteen naar Herman.") zorgde ervoor dat ik 4 jaar geleden ineens AIO werd; heb ik je daar ooit wel voor bedankt? Bij deze dus. Ook tijdens m'n promotietijd ben jij op een zeer welkome manier bepalend en belangrijk geweest voor mijn ontwikkeling als onderzoeker en persoon. Ik heb genoten van de motiverende en inspirerende gedachtewisselingen en denk bij tegenvallers aan jouw opmerking: "Wees maar blij dat het zo moeilijk is, anders kon iedereen het."

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Ik ben vereerd en blij dat jullie 22 december achter mij staan!

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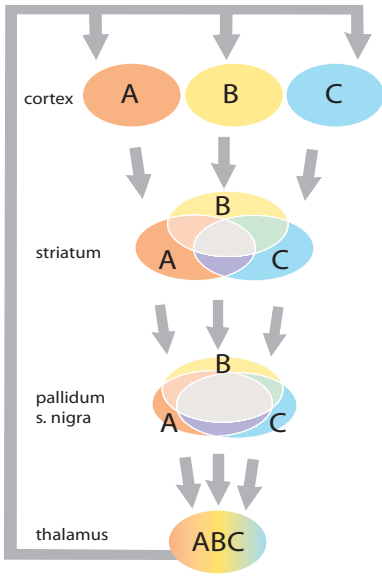
Marieke

C

Colour figures

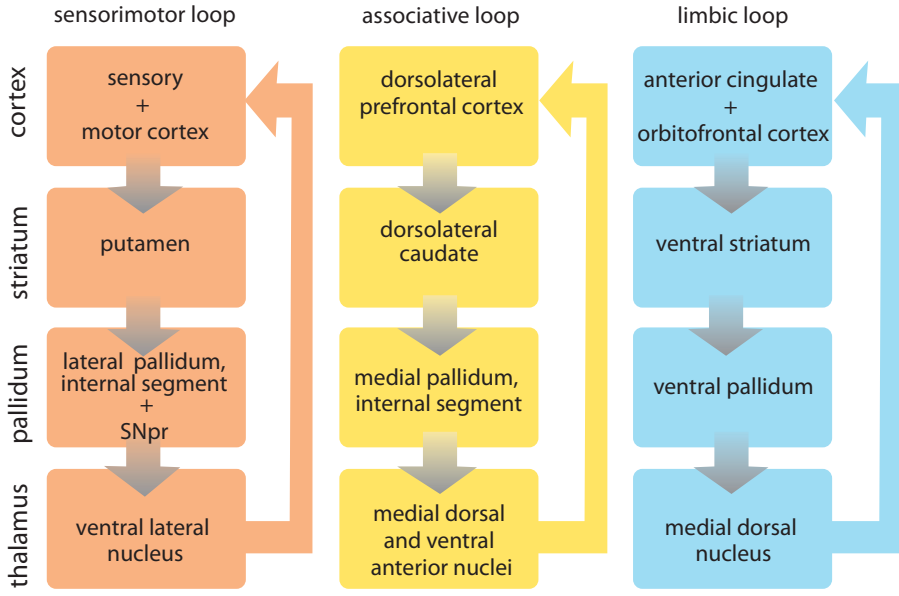
Colour figures chapters 2 and 3



Chapter 2, Figure 1. Corticostriatal circuits

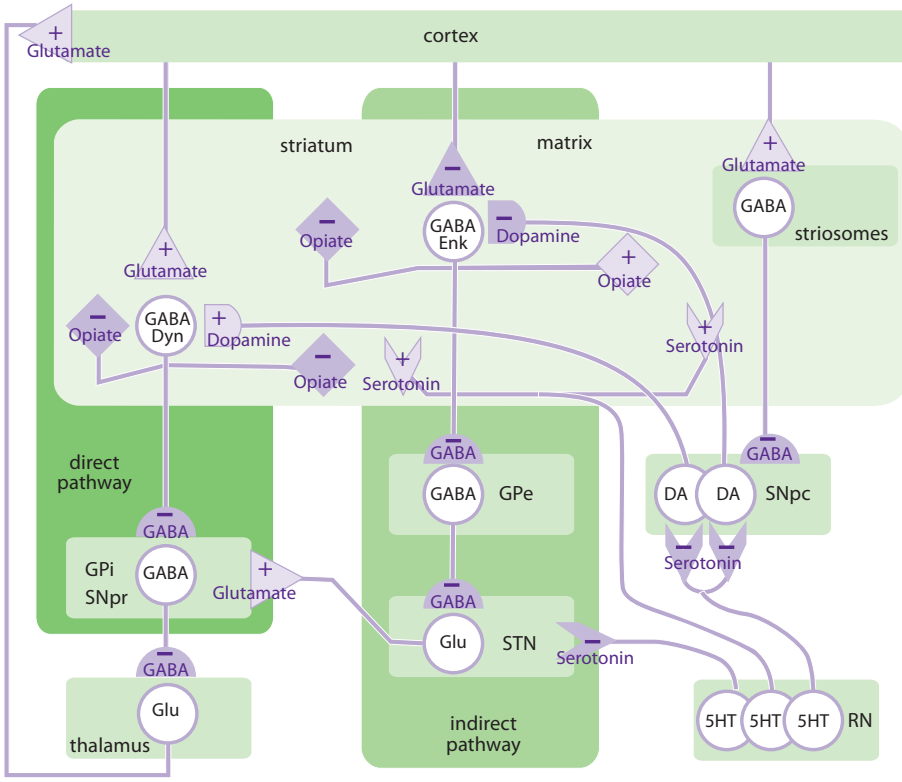
Corticostriatal circuits as proposed by Alexander et al 1986. Each circuit receives output from several functionally related cortical areas (A, B, C) that send partially overlapping projections to restricted parts of striatum. These striatal regions send converging projections to the globus pallidus (pallidum) and substantia nigra (s. nigra), which in turn project to specific regions of the thalamus. Each thalamic region projects back to one of the cortical areas that feed into the circuit, thereby completing the 'closed loop'.

(Adapted with permission from the Annual Review of Neuroscience, Volume 9, ©1986 by Annual Reviews, www.annualreviews.org)



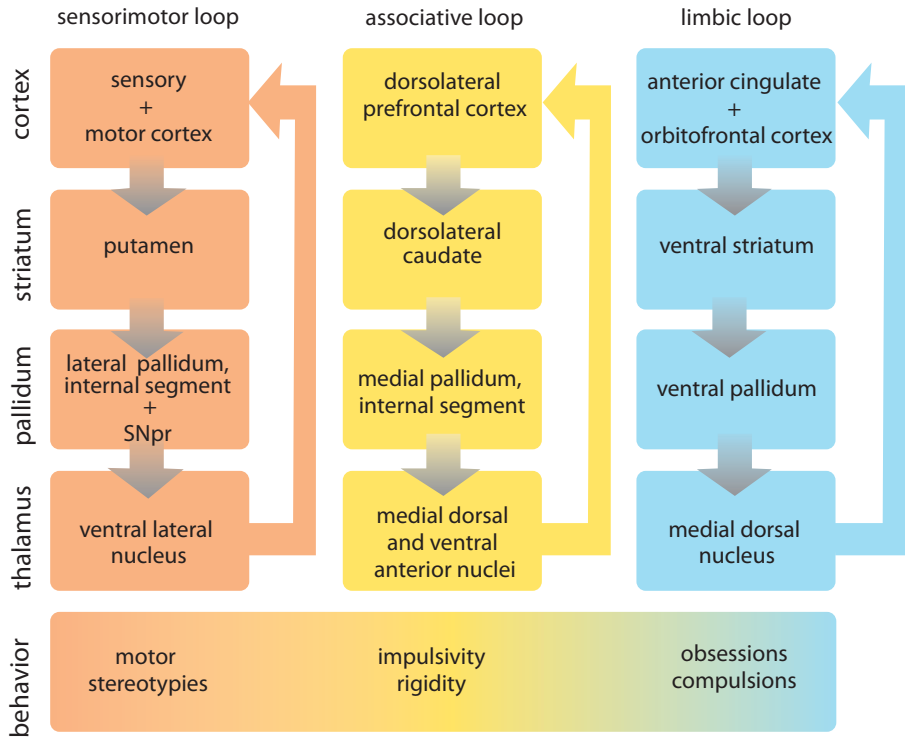
Chapter 1, Figure 2 and Chapter 2, Figure 1. Parallel corticostriatal macro-circuits

Parallel corticostriatal macro-circuits with their main input, relay and output regions. Abnormal repetition of behaviour can result from damage to any of the corticostriatal circuits, where the exact location of the disruption (i.e. which loop is involved) determines what type of repetitive behaviour is seen. (SNpr = substantia nigra pars reticulata)



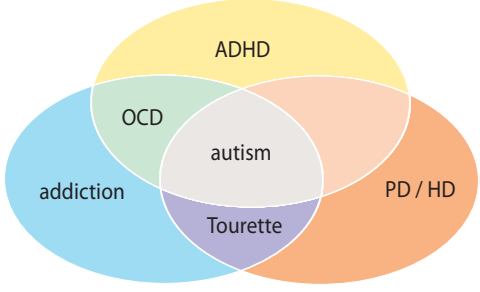
Chapter 2, Figure 3. Schematic representation of corticostriatal circuitry, showing direct and indirect pathways and endogenous neurochemistry involved.

(Adapted with permission from Mason G. and Rushen J. (Eds.), 2006, Stereotypic Animal Behaviour. Fundamentals and Application to Welfare. CAB International, Wallingford, UK.)



Chapter 3, Figure 2. Involvement of parallel corticostriatal macro-circuits in repetitive behaviour

Parallel corticostriatal macro-circuits with their main input, relay and output regions. Abnormal repetition of behaviour can result from damage to any of the corticostriatal circuits, where the exact location of the disruption (i.e. which loop is involved) determines what type of repetitive behaviour is seen. Both animal and human studies have suggested that the sensorimotor loop is primarily involved in abnormal stereotypical motor behaviour: continuously repeating identical movements without pursuing a goal. The associative loop is likely to be associated with inappropriate repetition of a goal, expressed in a relatively varied behavioural repertoire (as in obsessive-compulsive behaviour). The limbic loops (lateral orbital loop and anterior cingulate loop) are implicated in motivational aspects of behavioural control, including impulsive behaviour (difficulty in suppressing behaviour even when consequences are negative); response to reward; and obsessive and compulsive behaviour (including compulsive drug-taking). (SNpr = substantia nigra pars reticulata)



Chapter 3, Figure 3. Schematic representation of how behaviour resulting from problems in one of the three macro-circuits (sensorimotor, associative or limbic; see Figure 1) may group together in symptom clusters as seen in various psychiatric and neurological disorders. (ADHD = Attention Deficit Hyperactivity Disorder; OCD = obsessive compulsive disorder; PD = Parkinson's disease; HD = Huntington's disease)

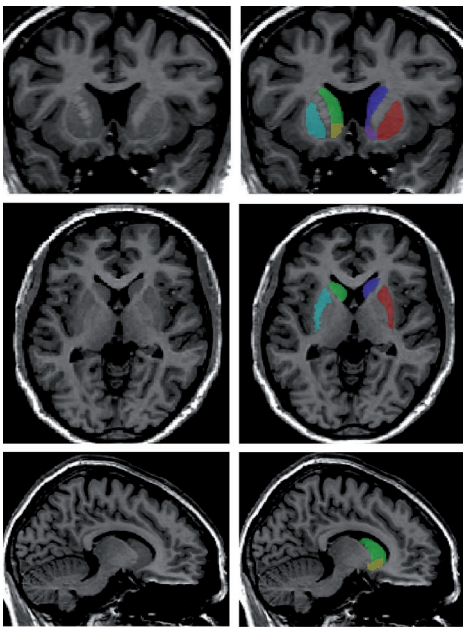


Figure 1. Segmentation of the basal ganglia in (from top to bottom) coronal, axial and sagittal view. Caudate nucleus (L/R) is displayed in green and blue, putamen in turquoise and red, nucleus accumbens in yellow and purple.

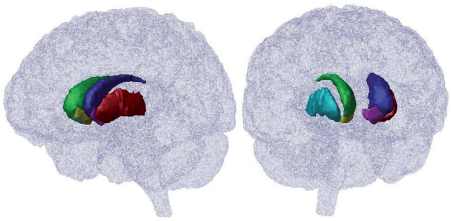


Figure 2. 3D visualizations of the basal ganglia in the brain. Caudate nucleus is displayed in green and blue, putamen in turquoise and red, nucleus accumbens in yellow and purple.

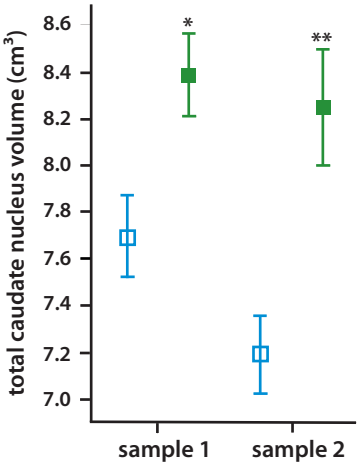


Figure 3. Total caudate nucleus volumes by group in samples 1 and 2 (Mean ± 1 SE). Green solid square = autism group; blue open square = control group; * = significant at $p<0.05$; ** = significant at $p<0.001$.

Colour figures chapter 5

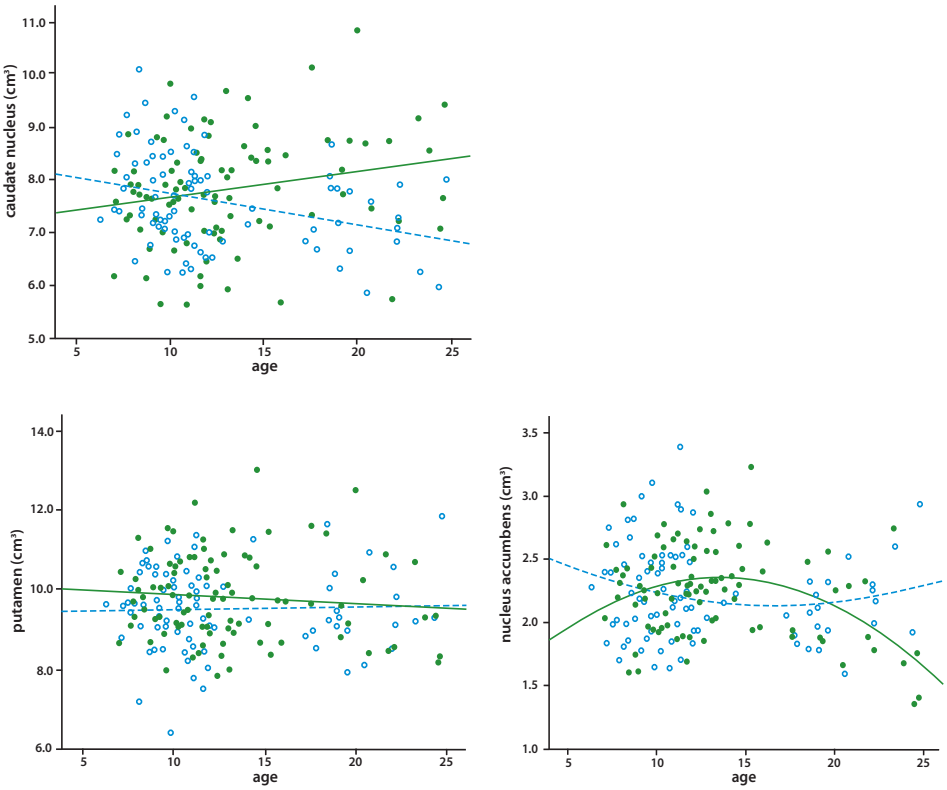


Figure 1. Scatterplots of striatal volumes in autism and controls. Top: caudate nuclues, middle left: putamen, middle right: nucleus accumbens. Green solid circles and solid fit line: autism group, blue open circles and dashed fit line: control group.

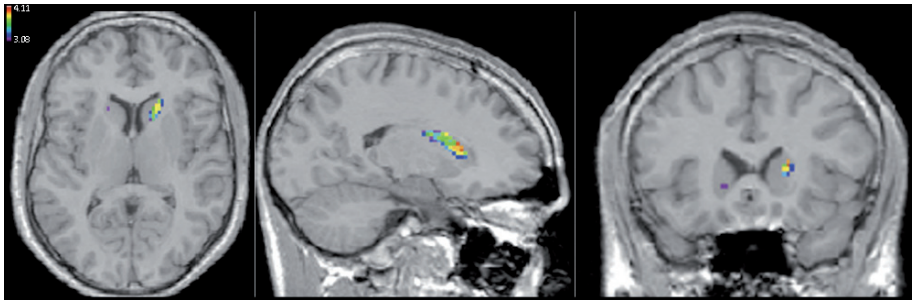


Figure 2. Focal increases in gray matter density in autism. The map is thresholded at the critical t (t>3.08) and superimposed on axial, sagittal and coronal sections through the MR image of the model brain.

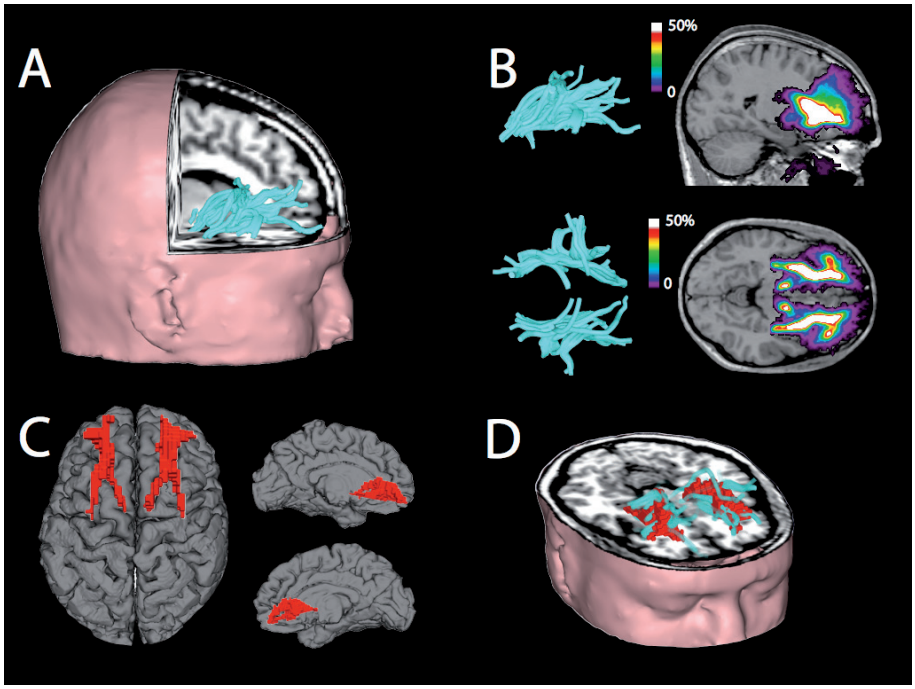


Figure 1. Schematic representation of corticostriatal tract selection

(A) Individual corticostriatal tracts, selected in model space using two manually delineated regions of interest (ROIs), striatum and frontal cortex; (B) Cumulative group map of individual binary cortico-striatal maps; (C) Corticostriatal Volume of interest (VOI) in modelspace, defined as all voxels that include 50% or more of individual corticostriatal tracts; (D) Schematic representation of corticostriatal tracts (turquoise) and corticostriatal VOI (red) for one subject in native space.

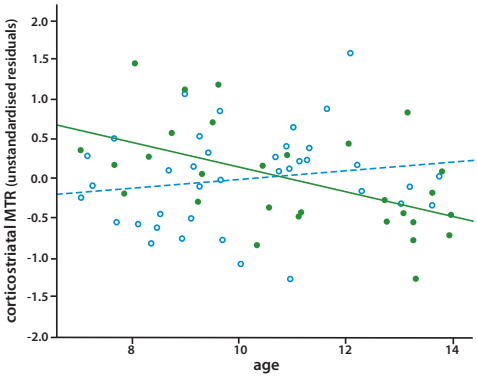


Figure 2. Corticostriatal MTR shows an age-dependent decrease in the autism group, but not in controls. MTR values are unstandardised residuals controling for the effects of MTR in total white matter MTR and medication. Green solid circles and solid line represent subjects with autism; blue open circles and dashed line represent the control group.

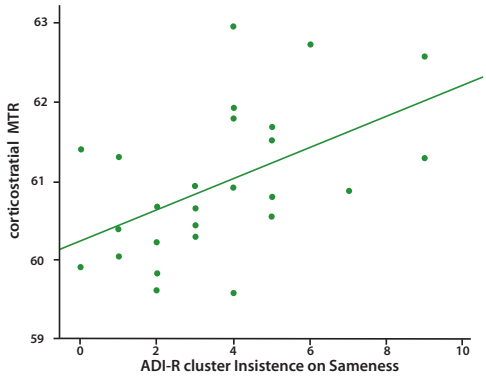


Figure 3. Correlation between corticostriatal MTR and the ADI-R Insistence on Sameness cluster (r=.516 p=.007).

Colour figures chapter 7

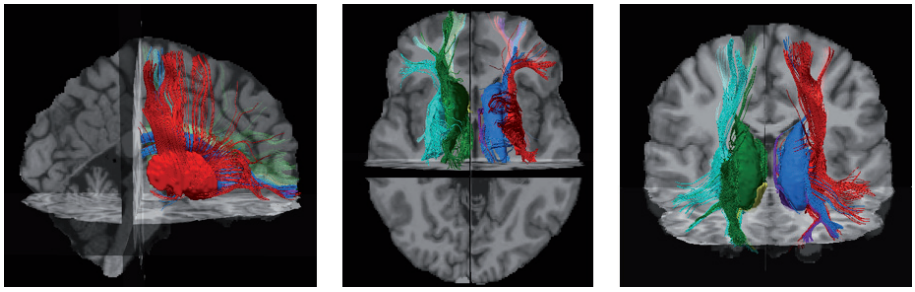


Figure 1. Sagittal, axial, and coronal views of striatal structures and corticostriatal tracts for one subject, superimposed on the T1-weighted scan. Yellow and purple = nucleus accumbens and accumbens tract; green and blue = caudate nucleus and caudate tract; turquoise and red = putamen and putamen tract.

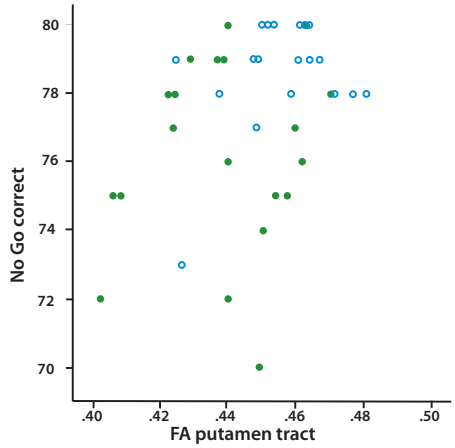


Figure 2. Associations of microstructural integrity of putamen tract (FA values) and inhibitory control (percentage correct on nogo trials of go/nogo task). Green = autism group; blue = control group. Correlation analyses: total group: r = .356, p = .021; autism group: r = .151, p = .52; control group: r = 277, p = .21.

Publications

Journal Articles

Langen M, Durston S, Staal WG, Palmen S, Van Engeland, H (2007). Caudate nucleus is enlarged in high-functioning medication-naive subjects with autism. *Biological Psychiatry*, 62(3):262-6.

Langen M, Schnack HG, Nederveen GH, Bos D, Lahuis BE, de Jonge MV, Van Engeland, H, Durston S (2009). Changes in the developmental trajectories of striatum in autism. *Biological Psychiatry*, 66(4):327-333.

Langen M, Kas MJH, Staal WG, Van Engeland H, Durston S. The neurobiology of repetitive behaviour: Of mice... *Revised manuscript submitted*.

Langen M, Durston S, Kas MJH, Van Engeland H, Staal WG. The neurobiology of repetitive behaviour: ...and men. *Revised manuscript submitted*.

Langen M, Mandl RCW, Hulshoff Pol HE, Van Engeland H, Durston S. Changes in corticostriatal development in autism: findings from diffusion tensor and magnetisation transfer imaging. *Manuscript submitted*.

Langen M, Leemans A, Johnston P, Ecker C, Daly E, Murphy CM, Catani M, dell'Acqua F, MRC (UK) AIMS Consortium, Durston S, Murphy DGM. Corticostriatal circuitry and inhibitory control in autism: findings from DTI tractography. *Manuscript submitted*.

Hulshoff Pol HE, Brans RGH, van Haren NEM, Schnack HG, **Langen M**, Baaré WFC, van Oel CJ, Kahn RS (2004). Gray and white matter volume abnormalities in monozygotic and same-sex dizygotic twins discordant for schizophrenia. *Biological Psychiatry*, 55(2):126-30.

Durston S, Nederveen GH, Van Dijk S, Van Belle J, De Zeeuw P, **Langen M**, Van Dijk A (2009). MR-simulation is effective for reducing anxiety related to MR scanning in children. *Journal of the American Academy of Child & Adolescent Psychiatry*, 48(2):206.

Abstracts

Langen M, Schnack HG, Nederveen GH, Bos D, Lahuis, BE, De Jonge, MV, Van Engeland, H, Durston S. Changes in the Developmental Trajectories of Striatum in Autism. European College of Neuropsychopharmacology (ECNP), Istanbul (Turkey), September 2009.

Langen M, Schnack HG, Nederveen GH, Bos D, Lahuis, BE, De Jonge, MV, Van Engeland, H, Durston S. Changes in the Developmental Trajectories of Striatum in Autism. International Meeting For Autism Research (IMFAR), Chicago (USA), May 2009. *Oral presentation.*

Langen M, Nederveen GH, Schnack HG, Bos D, Lahuis, BE, Van Engeland, H, Durston S. Basal Ganglia Development Follows A Different Trajectory In High-functioning Autism. American College of Neuropsychopharmacology (ACNP) Annual Meeting, Scottsdale (USA), December 2008.

Langen M, Durston S, Staal W, Palmen S, Van Engeland, H. The basal ganglia and autism: a volumetric study using MRI. International Meeting For Autism Research (IMFAR), Montréal (Canada), June 2006.

Langen M, Durston S, Staal W, Palmen S, Van Engeland, H. Structural MRI of the basal ganglia in autism. Annual Meeting Neuroimaging Section Association of European Psychiatrists (AEP), Utrecht (Netherlands), May 2006. *Oral presentation.*

Langen M, Hoogduin H, Sitskoorn MM, Rutten, GJ, Ramsey, N. Hippocampal activation in individual subjects using a verbal relational processing task. Annual Meeting of the Organization for Human Brain Mapping (OHBM), Brighton (UK), June 2001.

Marieke Langen was born on July 17, 1976 in Cuijk en Sint Agatha, The Netherlands. In 1994, she graduated from Merlet College in Cuijk (A-levels) and started her studies in Cultural Anthropology at Utrecht University. After finishing the first year, she switched to Psychology. In 2001, she successfully completed her degree, specialising in Neuropsychology, with a thesis and fMRI study investigating cognitive functioning of the hippocampus. After working as a research assistant structural neuroimaging at the Department of Psychiatry of the UMC Utrecht for 17 months, Marieke Langen started as project co-ordinator for a non-governmental organisation in the field of developmental and international co-operation in January 2003. In May 2005 she returned to neuroscience and began her PhD project at NICHE, the neuroimaging lab at the Department of Child and Adolescent Psychiatry, UMC Utrecht. She finished her project in September 2009. From August 2008 until February 2009, she worked as a research associate at the Institute of Psychiatry (IoP) at King's College, London. From May 2009, Marieke Langen has been appointed as a post-doctoral researcher at NICHE.

Marieke Langen werd op 17 juli 1976 geboren te Cuijk en Sint Agatha. Zij behaalde in 1994 haar eindexamen Atheneum aan het Merlet College te Cuijk, waarna zij startte met de studie Culturele Antropologie aan de Universiteit Utrecht. Na het behalen van de propedeuse in 1995 stapte ze over naar de Psychologie, afstudeerrichting Neuropsychologie. In april 2001 behaalde zij haar doctoraal met een afstudeerscriptie en fMRI-onderzoek naar de cognitieve functies van de hippocampus. Na van augustus 2001 tot januari 2003 werkzaam te zijn geweest als onderzoeksassistent bij de structurele neuro-imaging groep van de afdeling Volwassen Psychiatrie van het UMC Utrecht, begon zij in januari 2003 als projectcoördinator bij een non-gouvernementele organisatie op het gebied van internationale samenwerking en ontwikkelingssamenwerking. In mei 2005 keerde zij terug naar de neurowetenschappen en startte ze met haar promotietraject bij NICHE, de neuro-imaging groep van de afdeling Kinder- en Jeugdpsychiatrie van het UMC Utrecht. Zij rondde haar promotieonderzoek in september 2009 af. Van augustus 2008 tot februari 2009 werkte zij als research associate op het Institute of Psychiatry (IoP), King's College, Londen. Per mei 2009 is Marieke Langen aangesteld als post-doctoraal onderzoeker bij de Divisie Hersenen van het UMC Utrecht.

Repetitive and rigid behaviour is one of the core symptoms of autism, a severe and lifelong child psychiatric disorder. Although repetitive behaviour symptoms often form a significant impairment for affected individuals, systematic study of the phenomenology and in particular the neurobiology of repetitive behaviour has been lacking. In this thesis we address this gap by using neuroimaging techniques (structural magnetic resonance imaging (MRI), diffusion tensor imaging and magnetisation transfer imaging) to investigate brain differences associated with repetitive behaviour in autism.

We compared groups of individuals (children and adolescents, as well as adults) with and without autism and examined anatomical differences in specific structures and networks of the brain and related these to behavioural measurements. Furthermore, we explored the involvement of differences in developmental trajectories of these structures and networks in autism.

Our studies showed differences in grey and white matter of the corticostriatal system, a complex of brain regions known to be involved in motor, cognitive and emotional-motivational behaviour. These differences were found in all age-groups studied, suggesting that the corticostriatal system continues to be involved in autism over development. Closer examination of the developmental trajectories of grey matter structures in striatum and white matter pathways between striatum and the frontal cortex showed differences in development of this brain network for individuals with autism. Furthermore, the studies in this thesis showed associations between corticostriatal grey and white matter and repetitive behaviour.

In conclusion this thesis:

- Implicates changed development of corticostriatal grey and white matter in autism, especially in the repetitive behaviour which characterises the disorder.
- Emphasises the dynamics of the brain in autism: it is the time course of brain development rather than the outcome that seems to be most disturbed.
- Emphasises that the brain needs to be considered a complex of inter- and intra-communicating networks in constant interaction with the environment, rather than a collection of isolated structures or regions.
- Highlights the need for research strategies that take the heterogeneity of autism into account. Narrow the field of view by reclassifying autism as more homogeneous and well-phenotyped symptom-groups.
- Highlights the need for research strategies that take the etiologic overlap with other disorders into account. Broaden the field of view by including other diagnostic groups with similar symptomatology.

Repetitive behaviour in autism: Imaging pathways and trajectories

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